Hematopoietic stem cell therapy for autoimmune diseases – clinical experience and mechanisms

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Abstract

With accumulating evidence and improved outcomes along with recognition that modern biological therapies are not universally effective, require chronic administration and have high acquisition costs, hematopoietic stem cell transplantation (HSCT) has become an emerging direction for cell therapy in autoimmune diseases (ADs). The goal of this therapy is to induce medication-free remissions by resetting the immune system into a naïve and self-tolerant state through eradication of the autoreactive immunologic memory and profound re-configuration of the immune system induced by the transplant procedure. Safety of HSCT has generally improved by implementing internal quality management and external accreditation. Inter-disciplinary guidelines for patient selection, transplant technique and supportive care along with greater center experience should optimize safe and appropriate delivery of HSCT in specific ADs. In this review, we discuss the current role and future perspectives of HSCT in AD, focusing on recent published clinical and scientific studies and recommendations in the field.

1. Introduction

Autoimmune diseases (AD) are a heterogeneous group of conditions that affect around 10% of the population in Western countries with increasing incidence [1]. Such diseases constitute a heavy burden to society and are in many instances a debilitating health problem to the individual patient affected. AD are characterized by a breakdown of self-tolerance, triggered by certain environmental factors in a genetically predisposed population, with activation of normally quiescent autoreactive cells that escape self-regulation, resulting in chronic inflammation in target organs or multiple organ systems [2, 3]. Although these conditions share common immunopathogenic mechanisms [4], the clinical phenotype widely varies depending on the type or tissue distribution of autoreactive clones involved.

Almost all current therapeutic concepts in AD are based on systemic suppression of immune functions, which ameliorate symptoms and halt progression in the vast majority of patients, but usually require continuous administration and may be associated with long-term side effects. Frequently, patients develop refractory disease states that are associated with significantly reduced quality of life and increased comorbidity.

The recent introduction of biologics, like cytokine blocking agents or B-cell depleting therapies, has added more specificity to an efficient disease management by targeted suppression of inflammation. It has become evident however, that only the eradication of the cells, secreting inflammatory mediators, rather than the blockade of secreted cytokines, will offer treatment-free remissions, i.e. cure. This therapeutic approach is based on the recent identification of an autoreactive immunologic memory as major driver of chronic autoimmune responses in ADs. Such memory cells, like long-lived plasma cells (PCs), are generated early during disease development and may even be present years before clinical symptoms occur [5]. They are harbored in dedicated survival niches in bone marrow or inflamed tissues, contributing to chronic autoimmune responses by the continuous secretion of autoantibodies, but are unresponsive to state-of-the-art immunosuppressive as well as targeted B-cell therapies [6, 7].

Alternative cellular therapies have, therefore, emerged to reset or rebalance the immune system to restore self-tolerance. Among those, hematopoietic stem cell transplantation (HSCT) following high dose chemotherapy has developed as a promising treatment option for patients with ADs responding poorly or refractory to conventional treatments [8]. In this review, we summarize the clinical experience accumulating over the past 20 years and highlight most relevant mechanistic studies unraveling the immunological mechanisms of HSCT in various ADs.

2. Rationale and preclinical models of HSCT for autoimmune diseases

Hematopoietic stem cell transplantation (HSCT) is a standard of care for hematological malignancies and congenital or acquired disorders of the hematopoietic system yielding at eradication of malignant cell clones by a conditioning regimen, usually a combination of chemotherapy agents, with or without radiation therapy, salvaged by transplantation of hematopoietic stem cells (HSC) previously isolated from the bone marrow (Figure 1). The source of HSC can either be autologous, which allows the administration of high-dose chemotherapy without prolonged bone marrow aplasia or allogeneic, which is associated with the risk of graft versus host disease (GvHD) but has the advantage of providing alloimmunity with graft-versus-tumor effect to eradicate residual disease.

The experimental basis for the treatment of AD with HSCT derives from the pioneering translational research in murine models of autoimmunity of Ikehara in 1985, who first evidenced that the origin of autoimmune diseases is in the bone marrow and that bone marrow and not thymus transplantation can restore tolerance [9]. Subsequently, van Bekkum and co-workers demonstrated that conditioning followed by transplantation of syngeneic (i.e., pseudoautologous) HSC resulted in cure of induced models of autoimmunity, in particular collagen-induced arthritis (CIA, a model for rheumatoid arthritis, RA) and experimental autoimmune encephalomyelitis (EAE, as model for MS), suggesting that tolerance induced by HSCT can prevent autoimmunity even after antigenic re-encounter [10-12]. Similar results were obtained in models of spontaneous (i.e., genetically determined) AD, such as lupus-prone mice, where both allogeneic and syngeneic HSCT could induce prolonged remissions [13].

In human autoimmunity, the role of genetic predetermination in disease development is still uncertain. Although varying across different ADs, genome wide association studies (GWAS) as well as twin studies indicated that genomic variants predispose to autoimmunity, but environmental factors seem to have a major impact in disease development [14, 15], suggesting that autologous HSCT may potentially provide a curative treatment option in ADs. This notion is supported by serendipitous case reports demonstrating that patients with coincident autoimmune disease and hematological malignancy may remain in long-term remission of both diseases after autologous HSCT [16-18].

3. Proposed mode of action of HSCT in autoimmune diseases

Early pilot trials in severe forms of AD, including multiple sclerosis (MS), systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis (JIA), demonstrated that autologous HSCT could induce sustained treatment-free remissions, confirming the potential benefits of this type of stem cell therapy in appropriately selected patients [19-21]. Meanwhile, considerable advances were made in understanding the mode of action of HSCT. Mechanistic studies demonstrated that HSCT exerts not only prolonged downstream immunosuppressive effects, but also induces fundamental changes in chronically dysfunctional immune systems, restoring a naïve and self-tolerant state [8, 22, 23]. Such 're-booting' of self-tolerance is achieved through two principal pathways: (i) purging of the pathogenic autoreactive immune repertoire and (ii) profound immunologic renewal, i.e. immune reset (Figure 2).

By analogy to HSCT for hematologic cancers (with eradication of malignant clones), immunoablation for ADs is performed on the premise of removal of autoreactive immunologic memory cells. This notion is supported by serologic data and T-cell receptor (TCR) repertoire analyses demonstrating disappearance of pre-existing serum autoantibodies [24] and prominent T-cell clones [25, 26] post-transplantation, respectively. Further studies of immune reconstitution have provided evidence for substantial post-transplant modification of the adaptive immune system in various AD, including a vast diversification of the TCR repertoire [25, 27, 28] and functional renewal of the T regulatory (Treg) cell compartment [29-31].

Immune reconstitution occurs at different paces in different lineages and subsets (Figure 1). Following engraftment, the first phase of immune reconstitution is characterized by a significant though transient increase in the proportion of natural killer (NK) cells and clonal expansion of residual memory T-cells due to antigen encounter during early infections or driven by lymphopenia-induced homeostatic proliferation. Subsequently, regeneration of a new, naïve T- and B-cell repertoire occurs, emerging from thymic reactivation, which represents a form of true thymic hyperplasia that is associated with the continuous influx of recent thymic emigrants (RTE), providing numbers in HSCT-treated patients comparable to those of young children [24, 26, 27, 31]. Altogether, these data support the notion that HSCT has the potential to rebalance immune regulation and control aberrant autoimmune responses, in other words, reset the immunologic clock (Figure 2).

4. Current data from the EBMT Autoimmune Diseases Working Party (ADWP) database

In the mid-1990s the first transplants performed specifically for ADs were followed by the formation of the Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT) in 1997. The EBMT database was established and multidisciplinary recommendations were published to advise on selection and management of patients [32] and these have been updated by the EBMT [33]. Similar developments happened worldwide and there have been a large number of retrospective and prospective studies.

The EBMT ADWP database has now accumulated over 2500 patients during the past 20 years [34]. Since 1994, a total of 247 centers in 40 countries have reported data relating to HSCT in ADs. Figure 3 presents the current status of HSCT performed in Europe for AD as of January 2018. Among 2606 HSCT procedures reported to the ADWP databank, there were 2417 patients who have undergone autologous HSCT and 133 patients allogeneic HSCT. The main indications for autologous HSCT were MS (n=1181), systemic sclerosis (n=519), Crohn´s disease (n=169), inflammatory arthritis (n=166), SLE (n=108), hematological immune cytopenia (n=47), vasculitis (n=45), and type 1 diabetes (n=20). Other neurological diseases have included chronic inflammatory demyelinating polyneuropathy (CIDP, n=44), neuromyelitis optica (NMO, n=18) and myasthenia gravis (n=7) (Figures 3A).

There were two peaks of activity, initially driven by HSCT for inflammatory arthritis, followed by increasing number of HSCT for MS, systemic sclerosis and Crohn's disease, which are the commonest indications nowadays. Overall, annual numbers of HSCT are continuously increasing despite the widespread use of modern biologic therapies in ADs, reflecting the persistent medical need of effective therapies in some indications or individual cases, accumulating positive results from randomized controlled trials (RCTs) in the major indications and generally improved HSCT outcomes due to optimized supportive care, educational programs and updated recommendations in the field (Figure 3B) [34].

5. HSCT in rheumatic autoimmune diseases

5.1. HSCT in systemic sclerosis (SSc)

5.1.1. Clinical experiences in SSc

In severe forms of diffuse systemic sclerosis (SSc), where 3-5 year survival is between 50-70%, early European [35, 36] and North-American [37, 38] open-label phase I-II studies and several retrospective registry studies [39] demonstrated that HSCT induced major regression of both skin and lung fibrosis in SSc patients [40, 41]. These results were the basis for three randomized trials, namely ASSIST [42], ASTIS [43] and SCOT [44] conducted with comparable inclusion criteria and control arms, which all confirmed the superiority of HSCT over continued intravenous cyclophosphamide (CY).

The ASSIST trial, performed at the Northwestern University, Chicago, including 19 patients randomized to receive either unpurified autologous HSCT after non-myeloablative conditioning with CY and rabbit anti-thymocyte globulin (ATG) or monthly CY demonstrated that autologous HSCT resulted in significant improvement in both, respiratory and cutaneous manifestations [42]. One year after HSCT, clinical benefits were such that the ASSIST trial closed earlier, after enrollment of 19 instead of 60 SSc patients initially powered, due to failure to reach equipoise between HSCT and the control group [42]. This study did not show any treatment-related mortality.

Subsequently, the international, multicenter, investigator-initiated, open-label phase III trial ASTIS comparing CD34-selected HSCT after conditioning with CY and ATG

versus monthly intravenous pulse of CY 750 mg/m^2 for 12 months has been a unique ground breaking EBMT-EULAR academic collaborative project. From 2001 to 2009, 156 patients from 10 countries with early diffuse SSc were recruited and followed up until the end of October 2013. ASTIS results first demonstrated that HSCT confers better long-term survival than 12 monthly intravenous pulses of CY in the long-term. Despite increased treatment-related mortality (TRM) of 10% during the first year, treatment responses in SSc clinical outcome variables 2 years after HSCT were higher than controls, allowing superior event-free and overall survival rates during the 10 years following HSCT [43].

Finally, the multicenter SCOT trial investigated CD34-selected autologous HSCT after a myeloablative conditioning with total body irradiation and reduced CY (120 mg/kg instead of 200 mg/kg) (n=36) versus 12 monthly infusions of CY (n=39). The primary end-point was the global rank composite score (GRCS), a hierarchical scoring system that compares outcome variables, including death, EFS, FVC, the health assessment questionnaire-disability index and modified Rodnan Skin Score (mRSS). In the intention-to-treat (ITT) population, global rank composite scores at 54 months showed the superiority of transplantation (67% of pairwise comparisons favored transplantation and 33% favored CY, p=0.01) [44]. The EFS also differed between the two groups in favor of HSCT (74% vs. 47%, p=0.03), but not before 72 months post-HSCT. Notably, in contrast to the ASSIST and ASTIS trials, lung function, as measured by forced vital capacity (FVC), did not significantly improve (p=0.3 by ITT and p=0.5 by PP), and malignancies were observed in 9% of patients after HSCT (two instances of MDS and one instance of thyroid cancer), which seemed to be related to the use of total body irradiation (TBI).

Although HSCT has been demonstrated efficacy for patients with SSc, concerns remain over the safety of the treatment, in particular regarding cardiovascular risks and malignancies. Over the past 5 years the mortality associated with the procedure has decreased from 10% in the early days of the ASTIS trial to 5 % since 2010 within the EBMT registry and is considered at 3 % in SCOT trial. This is related to a better patient selection before transplant especially with regard to cardiac involvement including pulmonary arterial hypertension, which were not rigorously excluded in the ASTIS trial. Extended cardiopulmonary work-up including right-heart catheterization

with fluid challenge and MRI has progressively improved safety [38, 45]. With increasing knowledge, a cardiopulmonary assessment guideline updating previous consensus [46] and incorporating ten years of additional international experience, at Northwestern University, Chicago [47] and within the EBMT Autoimmune Diseases Working Party, was published in 2017 [48].

Since 2012, HSCT for SSc is performed in expert centers in Europe with a grade I level of evidence according to EBMT guidelines [33] and transplant activity has doubled over the last 5 years with around 35 transplants being reported yearly to the EBMT registry [49]. In 2017, according to the revised EBMT guidelines, HSCT should be considered for patients with severe SSc progressing despite standard established therapy. More recently, updated guidelines from the European League against Rheumatology (EULAR) recommend HSCT as therapeutic option in refractory cases of the disease [50]. Currently, HSCT is indicated for SSc patients earlier in disease course after complete cardiopulmonary evaluations. A future challenge will be to optimize the safety of HSCT in SSc. Further trials of autologous HSCT for SSc are ongoing with the aim to refine non-myeloablative regimens by providing a "less toxic" reduced intensity protocol or by applying individualized approaches according to patient's cardiac function [51].

5.1.2. Mechanistic studies in SSc

Initial studies in SSc patients demonstrated that HSCT acts differently from standard immunosuppression, which modulates specific components of the autoimmunity process, whereas HSCT allows resetting of immune system into a self-tolerance state. In a French cohort of 7 patients, a reduction in anti-topoisomerase autoantibody levels post-transplantation was achieved in all patients [26]. The decrease was more prominent in good clinical responders and high $CD19^+$ B-cell counts positively correlated with anti-topoisomerase antibody levels, suggesting that pathogenic B-cell clones might preferentially expand in the patients with a less favorable outcome [26]. Such observation was confirmed in two other studies with progressive decrease in the autoantibody levels in 11 SSc patients over 3-years follow-up [52], and in 9/10 transplanted SSc patients, respectively, while the total immunoglobulin levels in serum remained within normal range [53].

Analysis of the early determinants of the immune reconstitution profile after high dose CY and CD34-selected HSCT in SSc patients allows to detect meaningful differences between the long-term responders and non-responders/relapsing patients according to the trends in early SSc clinical response and to global trends of immune reconstitution [26]. A more recent study of ten SSc patients followed after HSCT demonstrated increased T-cell receptor excision (TREC) levels in regenerating T-cells along with a normalization of an initially restricted TCR repertoire in responding patients, while a pronounced increase in effector T-cells was observed in non-responders [28]. This may reflect the persistence of autoimmune responses in these patients and call for new therapeutic post-transplant protocols or use of adjuvant cellular therapies to dampen the autoimmune and inflammatory response.

These notions were supported by a recent study investigating immune reconstitution in 31 SSc patients undergoing HSCT, which demonstrated that thymic rebound was associated with increased TCR diversity, while regeneration of naïve B-cells led to an increase in levels of B regulatory (Breg) cells that produced higher interleukin-10 levels than before transplantation [31]. When non-responding patients were evaluated separately, Treg and Breg counts did not adequately increase after HSCT, and high TCR repertoire overlap between pre- and post-transplantation periods indicating maintenance of underlying disease mechanisms in those patients [31]. Collectively, these data suggest that clinical improvement of SSc patients is related to increased counts of newly generated Tregs and Bregs after HSCT as a result of coordinated thymic and bone marrow rebound.

5.2. HSCT in systemic lupus erythematosus (SLE)
5.2.1. Clinical experience in SLE

The first successful autologous HSCT for SLE was reported by Alberto Marmont and colleagues in a 46-year old woman with severe long-lasting disease in 1997 [54]. Since then, several phase I/II clinical trials have been reported covering approximately 300 patients worldwide [55]. The two largest experiences on HSCT for SLE so far come from EBMT data registry (n=53; mean follow-up 25 months, range: 2–123 months) [56] and from the single center pilot trial by Northwestern University (n=50; mean follow-up: 29 months, range: 6–90 months) [57]. The probability of five-year disease free survival was 50% in both studies, confirming results from smaller

pilot studies [24, 58]. Notably, disease manifestations completely resolved in responding patients and autoantibody levels mostly normalized. In a follow-up report from the EBMT registry, summarizing the outcome of HSCT in SLE with various regimens between 2001 and 2008 (n=28, median follow-up: 38 months, range: 1-110 months), PFS was only 29% at 5y, but TRM had gradually improved. Conditioning regimens applied were mostly intermediate-intensity (64%) and CD34-selection performed only in minority of patients (36%). Notably, multivariate analyses revealed a significant benefit of *ex vivo* stem cell purging on flare-prevention post-transplantation, as already assumed from experimental considerations [59].

Following these initial trials, recent reports from two independent Chinese groups, both with 10-year follow-up, demonstrated impressive clinical responses with PFS of 86% [60] and 68% [61], respectively, while TRM across both studies was reduced to 2% (1/46). In the first study from Leng and colleagues from the Medical College Hospital Beijing reporting outcomes of 24 patients, responding patients had no signs of clinical disease activity, level of anti-dsDNA antibodies normalized and proteinuria significantly improved in 15 patients with lupus nephritis form a median of 4 g/day to normal levels [60]. In the second study from the Nanfang Medical University reporting the outcome of 22 patients, lupus nephritis was controlled in the majority of patients, reflected by a significantly decrease of proteinuria to almost normal levels and a marked reduction of immune complex depositions in renal re-biopsies performed in 6 out of 22 patients [61].

These data demonstrate that, once achieved, clinical remissions may be sustained for many years post-transplantation in SLE even in the absence of chronic immunosuppression, but the encouraging results of phase I/II trials have to be weighed against the increased risk of short-term mortality associated with HSCT, which needs further to be investigated in RCTs. The only controlled trial for HSCT in SLE is currently performed in an ongoing multicenter study in Germany, comparing HSCT with best available standard of care, including rituximab (NCT00750971).

Based on these clinical experiences, current expert consensus recommendations suggest that candidates for HSCT would reasonably include those with sustained or relapsed active BILAG (British Isles Lupus Assessment Group) category A remaining

steroid dependent after at least 6 months of the best standard therapy, using mycophenolate mofetil or cyclophosphamide with or without anti-CD20 and other monoclonal antibodies, with documented evidence of visceral involvement or refractory SLE [33, 62].

5.2.2. Mechanistic studies in SLE

Over the past years, clinical trials have been complemented by mechanistic studies to correlate the remarkable clinical effects achieved with HSCT in SLE with corresponding changes of immunologic abnormities described in this prototypical autoimmune disease [2]. Initial studies investigating the effect of HSCT on immune depletion confirmed the assumption of a vast eradication of the autoreactive immunological memory, reflected by the normalization of serum autoantibodies, including anti-Ro/SSA, anti-RNP, and antiphospholipid-antibodies [24, 57, 63], which are presumably secreted by long-lived plasma cells and detectable in serum many years before clinical symptoms occur [5]. Accordingly, $CD138^+CD38^+$ PCs in bone marrow were vastly diminished in investigated patients [24], confirming the notion that conditioning with polyclonal antithymocyte globulin (ATG) depletes PCs [64]. Likewise, TCR repertoire analyses demonstrated a disappearance of pre-existing prominent T-cell clones and, using *in vitro* short-term stimulation assays, T-cells specific for autoepitopes became undetectable in peripheral blood after HSCT [24].

Subsequent studies focusing on aspects of immune renewal, demonstrated a stable thymic reactivation, even in older patients, reflected by recurrence of naïve $CD4^+$ T-cells with markers of recent thymic emigrants, i.e. high levels of TRECs and/or surface expression of CD31 [24, 65]. Thymic re-education was also associated with a functional renewal of the Treg compartment, including $CD4^+CD25^{high}Foxp3^+$ and an unusual $CD8^+Foxp3^+$ subset, respectively, accompanied by inhibition of pathogenic T-cell responses to critical peptide autoepitopes [24, 66]. A subsequent study demonstrated that recurring $Foxp3^+$ Tregs post-transplantation co-express high levels of CD31 and Helios, a marker for naturally occurring Tregs that develop in the thymus, and their previously restricted TCR repertoire completely normalized, suggesting that thymic reactivation provides a new and diverse repertoire of Tregs in SLE [29], similar to what has been observed in other ADs [30]. In addition, a dramatic shift in B cell subpopulations from memory phenotype towards a naïve B cell

predominance after HSCT was confirmed and the initial expansion of peripheral blood plasmablasts, a hallmark of lupus immunopathology, completely resolved [24]. Collectively, such data confirm the notion that HSCT has the potential to reset the chronic autoreactive immune system in SLE into a naïve and self-tolerant state.

5.3. HSCT in rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA)
5.3.1. Clinical experience in RA and JIA

RA and JIA are relatively common causes of major disability in adults and children. When studies of HSCT in AD commenced, they were considered attractive candidate diseases backed up by pre-clinical data and serendipitous case reports [17, 18, 67]. Several centers in Australia, the Netherlands, UK and USA embarked upon studies to define the potential of not only of autologous, but also syngeneic and allogeneic HSCT [68-79]. In association mechanistic studies correlated the clinical effects of autologous HSCT with peripheral blood, synovial immunology, arthroscopy and erosive joint damage and quality of life [80-83].

The EBMT registry showed inflammatory arthritis to be the commonest indication in 1999, but with the roll-out of the 'biological era' reporting of cases has dwindled massively. Even so, HSCT in RA remains of historical interest and summarized in a review [84]. The outcomes of autologous HSCT are best reflected in the joint EBMT and ABMTR registry analysis of 76 patients from 15 centers, who were largely treated before the availability of anti-TNF and other biological agents [85]. Autologous HSCT was followed by a high frequency of persistent or relapse disease, but reintroduction of new or previously used DMARDs resulted in disease control in most patients, with two-thirds of patients sustaining a medium term ACR (American College of Rheumatology) 50 response out to 18 months. Patients with seronegative RA had a significantly better response than those with seropositive disease. No transplant related mortality was reported.

A similar retrospective European analysis of JIA analysed 34 patients treated with T-cell depleted autologous HSCT using ATG, and cyclophosphamide with or without 4Gy low-dose total body irradiation [86]. Responses were more impressive than in RA, with the majority of patients achieving long term complete or partial drug-free

remissions. However, treatment related toxicity was higher and idiosyncratic, with three episodes of fatal macrophage activation syndrome.

EBMT recommendations still cover the potential of HSCT in occasional patients with RA, JIA and other forms of inflammatory arthritis refractory to modern biological therapies [33]. In addition to autologous HSCT, two patients have had the rare opportunity to undergo syngeneic HSCT for severe RA, with one having a prolonged remission and possible cure, the other having active disease [76, 77, 87]. There has been one report of allogeneic HSCT for severe RA, which remitted completely, although RA is rarely sufficiently severe to justify the associated treatment related mortality and morbidity risks [78].

5.3.2. Mechanistic studies in RA and JIA

Modern immune reconstitution studies following HSCT in adult RA are relatively limited, largely because HSCT activity in RA declined after the late 1990s. However, observations related to JIA are much more developed, and, combined with studies in other pediatric autoimmune diseases, have provided some important insights in age groups where thymic function is better preserved and potentially more responsive to recovery from lymphopenia.

Dutch groups have explored the patterns of immune reconstitution in JIA patients undergoing autologous HSCT. Restoration of initially reduced Foxp3$^+$ Treg levels was observed with change in autoreactive T-cells from a pro-inflammatory (IFN-gamma and transcription factor T-bet (Tbox protein expressed in T cells) high) to a tolerant phenotype (IL-10 and GATA-3 high) [88]. Delemarre *et al* [30] built on these observations to associate successful clinical outcomes in JIA and pediatric rheumatologic diseases with re-diversification of T-cell repertoires in the thymic driven T regulatory cell compartment.

In adult RA active disease is associated with thymic defects such as reduced T-cell receptor excision circles (TREC) production, telomere shortening and loss of receptor diversity with expansion of oligoclonal Vβ T-cell populations [89, 90]. Compared with cancer patients undergoing autologous HSCT, recovery from lymphopenia is significantly perturbed in RA patients. Although RA patients can produce naive T-

cells containing TRECs there is also a failure to recover peripheral T-cells, particularly memory cells. In this context serum IL-7 levels appear to be deficient in RA compared with controls and such relative IL-7 deficiency may contribute to poor early T-cell reconstitution. The inability to correct intrinsic defects in immune reconstitution may explain the short-lived responses seen in many adult RA patients post-autologous HSCT [90].

In summary, although relatively few patients with RA and JIA are being treated with autologous HSCT since the advent of anti-TNF and other cytokine blockade, the differential of clinical responses and immune reconstitution patterns following autologous HSCT provide some interesting insights between the two disease categories. Failure to correct intrinsic thymic functional defects in adult RA may partly explain why autologous HSCT is limited in controlling clinical disease activity, whereas the re-diversification of the Treg compartment may contribute to the profound clinical responses observed in JIA and other pediatric diseases. How much this is a reflection of different disease processes is unclear but may explain better responses in patients with seronegative RA [85], but thymic function in younger patients may also be a significant factor. If the opportunity arises, further studies using modern approaches for assessing post-transplant immune reconstitution in further patients with RA and JIA provide further insights.

Rheumatic disease	No.	Conditioning regimens	Graft manipulation	Outcome	Ref.
SSc	10	CY 200mg/kg, rATG 6.5mg/kg	Unselected graft	Clinical improvement at 1y (mRSS or FVC) (P<0.001), TRM 0%	[42]
SSc	75	CY 200mg/kg, rATG 7.5mg/kg	CD34-selection	EFS (P= 0.006), mRSS (P<0.001), FVC (P = 0.004), TRM 10%	[43]
SSc	36	CY 120mg/kg, eATG 90mg/kg TBI 800Gy	CD34-selection	GRCS at 5y (P=0.01), mRSS (P=0.05), FVC (P=0.3), TRM 3%	[44]
SLE	53	Various, CY/ATG based	Various, mostly CD34-selection	PFS 44% after 5y, TRM 13%	[56]
SLE	50	CY 200mg/kg + rATG 6.5mg/kg	CD34-selection	PFS 50% after 5y, TRM 4%	[57]
SLE	28	Various, CY/ATG based	Various, mostly Unselected graft	PFS 29% after 5y, TRM 10%	[59]
SLE	24	CY 200mg/kg +ATG or TBI	CD34-selection	PFS 86% at 10y, TRM 4%	[60]

SLE	22	Various, CY/ATG based	CD34-selection	PFS 68% at 5y, TRM 0%	[61]
RA	14	CY 200mg/kg	CD34-selection	Marked improvement of disease activity in 8/12 pts	[79]
RA	18 15	CY 200mg/kg CY 200mg/kg	CD34-selection Unselected graft	no difference between groups, median time to relapse 147 vs. 180d	[74]
RA	76	CYC 200mg/kg	various	Response 67% ACR 50, DMARDs mostly started within 6 months	[85]

Table 1: Clinical trials of hematopoietic stem cell transplantation (HSCT) for inflammatory rheumatic diseases. ACR, American College of Rheumatology, CY, cyclophosphamide, eATG, equine antithymocyte globulin, EFS, event free survival, FVC, forced vital capacity, GRCS, global rank composite score, mRSS, modified Rodnan skin score, PFS, progression free survival, rATG, rabbit antithymocyte globulin, TBI, total body irradiation, TRM, treatment related mortality.

6. HSCT in neurologic autoimmune disease

6.1. HSCT in multiple sclerosis (MS)

6.1.1. Clinical experience in MS

Twenty years ago Athanasios Fassas and colleagues reported their seminal study in patients with MS treated with autologous HSCT demonstrating feasibility and some hint of benefit in the short term available follow up [91]. During the following two decades the evidence has grown massively and supports the selective use and further investigation of HSCT for treatment of patients with aggressive forms of MS. Clinical results are particularly favorable in patients transplanted in the early, inflammatory phase of the disease (relapsing-remitting, RR) and in particularly in aggressive MS [92-95]. Indeed, remission of relapses and suppression of subclinical inflammation without maintenance therapy following autologous HSCT has been achieved in a majority of patients with MS even though they previously failed to respond to conventional treatment [92, 94, 96-99]. Confirmed improvement in disability after HSCT was also reported in patients with relapsing remitting MS [94, 98].

One recent randomised phase 2 trial showed superior efficacy of HSCT on the development of MRI lesions compared to mitoxantrone, which is considered a highly effective control treatment, yet the trial was underpowered to detect clinical outcomes [99]. One study employing high-intensity myeloablative conditioning has demonstrated virtually complete suppression of MS activity lasting several years [100]; however, the occurrence of busulfan-related liver toxicity has raised safety

concerns. Less complete but still profound effects on inflammatory MS activity in patients with RRMS have been obtained with less intensive conditioning regimens such as BEAM/ATG [101, 102].

Important knowledge has emerged from studies in small numbers of patients, but larger studies with long-term follow up and meta-analyses provide higher power to detect or validate variables that influence outcomes. A recent long-term retrospective study that included unselectively any transplants performed between 1996-2005 (n=281) and included all relapsing and progressive MS subtypes has documented the long-term durability of clinical stabilization, which was significantly longer in patients with RRMS, younger and who received less previous immunotherapies as compared to their progressive MS, older and more previously treated counterparts [103]. A meta-analysis of efficacy and safety of HSCT for treatment of MS has also recently been published and has confirmed the significant association of RRMS with lower progression rate of MS [104]. In the same study, a higher disability score at baseline was associated with a higher TRM. TRM was considerably lower in the pool of patients transplanted after 2005 (TRM 0.3%) than in the older studies. Also in this meta-analysis the pooled proportion of patients with no evidence of disease activity (NEDA) at 2 years was 83% (range 70%–92%) and at 5 years was 67% (range 59%–70%).

Considering that the data suggest potential superior efficacy of HSCT [105], there is a need for RCTs comparing HSCT with contemporary high-efficacy immunotherapy. The MIST phase 3 trial randomizing patients to either HSCT utilizing CY/ATG a lower intensity (non-myeloablative) conditioning regimen, or FDA-approved standard of care has recently completed recruitment (ClinicalTrials.gov Identifier: NCT00273364) and will be reported in preliminary form at the EBMT annual meeting 2018. New trials comparing HSCT with the more recently introduced high-efficacy disease modifying treatments such as alemtuzumab are warranted and are being planned, although lack of support has slowed their development [106].

According to current practice, EBMT guidelines and experts' consensus enable considering HSCT as a treatment option in patients with inflammatory active MS and aggressive course [33, 106]. The profile of a patient who may be an appropriate

candidate for HSCT has been defined as a subject with RRMS, or progressive MS for a short period of time, who has presented recent clinical and/or MRI inflammatory activity, is young (<45), has had MS for <10 years and remains ambulatory. An EDSS of 6-6.5 is often regarded as the upper limit of disability acceptable for HSCT, based on data demonstrating higher risks in patients with more advanced disability scores. Higher EDSS was significantly associated with poorer overall survival with a hazard ratio of 2.03 per EDSS point [107]. In the recent meta-analysis, baseline EDSS>6 was significantly associated (p=0.013) to a higher treatment related mortality [104].

Regarding prior treatments, presently the majority of clinicians would consider failure of a high-efficacy disease modifying treatment a requirement for considering HSCT in patients with RRMS, however it has been argued that HSCT could be offered as second-line treatment in patients with highly active MS [108]. Perhaps only evidence from randomised controlled trial will settle this controversy. In the meantime patients with the appropriate MS profile who have no contraindications from comorbidities or prior treatments and who fully understand risks and benefit may be offered HSCT at centers with optimal neurological and hematological expertise [106].

6.1.2. Mechanistic studies in MS

The clinical experience in MS has been enhanced by immunological studies that provided more than one decade ago the proof-of-principle of 'immune resetting' [27, 109]. More recently deep sequencing of TCR repertoires has been performed in blood samples from patients in the HALT-MS trial [102] to investigate the T-cell clonal renewal postulated after the "immune resetting" treatment. The analysis of TCR repertoires demonstrated that autologous HSCT induces a massive regeneration of circulating T-cell clones [25]. Importantly, an association was also detected between early post-transplant T repertoire diversity with complete clinical responses.

In another study, the effect of HSCT on relevant immune cell subsets was examined resulting in the demonstration of near complete depletion from blood of CD161^{high}CD8$^+$ cells, pro-inflammatory T-cells that produce IFN-γ and IL-17, two cytokines that promote inflammatory processes in MS [110]. These cells were identified as mucosal-associated invariant T (MAIT) cells, a novel cell population

originating in the gut mucosa but circulating in blood and expressing the central nervous system-homing receptor CCR6. Their presence in MS post-mortem brain lesion tissue was demonstrated, strongly suggesting their implication in the inflammatory disease process. Several additional immune mechanisms that may contribute to the efficacy of HSCT in MS have been reported and have been recently reviewed [106, 111].

7. HSCT in inflammatory bowel disease
7.1. HSCT in Crohn´s disease (CD)
7.1.1. Clinical experience in CD

Early evidence to support use of HSCT was derived from case reports of patients undergoing allogeneic or autologous transplants for malignancy with serendipitous remission of the underlying IBD. Short and medium term outcome data form case series and single center cohort studies reported sustained clinical disease regression but few data on endoscopic endpoints [112-116]. There has been one randomised controlled trial (ASTIC) that assessed the value of HSCT in refractory CD [117]. The trial protocol was designed to assess whether HSCT delivered long term clinical and endoscopic disease remission. It also assessed whether the same outcome at one year could be achieved with CY alone or required immune ablation and stem cell rescue. Therefore, patients underwent stem cell mobilization with $4g/m^2$ cyclophosphamide and G-CSF and were then randomised to immediate stem cell transplant (n=23), or conventional therapy (n=22). The ambitious primary endpoint was clinical remission off all medication for 3 months with no evidence of mucosal disease activity on imaging and endoscopy one year after randomization. This was a negative trial with few patients in either group achieving the primary endpoint at one year. In addition, there was a heavy burden of serious adverse events associated with both the dose of cyclophosphamide used at mobilization and stem cell transplantation; one patient undergoing HSCT died. CY mobilization alone resulted in a significant but transient reduction in clinical disease activity. Despite these negative results, significant benefits were seen in patients undergoing HSCT compared to mobilization alone. For example, 61% HSCT vs 23% control patients were off all therapy for 3 months (p<0.01), 35% HSCT vs 9% control were in clinical remission (p=0.053) and 35% HSCT vs 9% control had no objective evidence of active disease on endoscopy and radiology (p=0.053). Patients randomised to the control group in

ASTIC were eligible to proceed to HSCT after the primary endpoint had been assessed and underwent the same schedule of assessments over the subsequent year. A subsequent manuscript reported the outcome of the combined cohort of patients [118]. Compared to baseline, there were highly significant improvements in clinical disease activity, quality of life and endoscopic disease activity at one year after HSCT. Furthermore, 43% patients achieved steroid free clinical remission and 50% had mucosal healing with an SES CD ulcer sub-score of 0 in all segments examined. HSCT did not result in perianal fistula healing perianal disease at baseline.

Long-term clinical and endoscopic outcome after HSCT comes from a single center cohort of 29 patients (some of whom participated in the ASTIC trial) [119]. Drug-free clinical and endoscopic remission (CDAI <150, SES-CD< 7) was seen in 61% at one year, 52% at 2 years, 47% at 3 years, 39% at 4 years, and 15% at 5 years. However, clinical or endoscopic evidence of relapse was seen in one half of patients after a median duration of 12 months. Despite this, reintroduction of anti-TNF therapy to which patients had previously been refractory, induced clinical remission in 80% patients with disease relapse.

Therefore, it would appear that although HSCT results in marked reduction in clinical and mucosal disease activity in patients with treatment refractory Crohn's disease, although this is not sustained in many patients. This is confirmed by a recent publication from the EBMT registry that reported outcome from 82 patients undergoing stem cell transplants for Crohn's disease. Clinical remission was reported in 43% at one year, but 73% required re-introduction of Crohn's disease therapy after a median of 10 months [120]. The chemotherapeutic regimen used in ASTIC resulted in an unattractive burden of adverse events. Recent reports demonstrate that appropriate supportive care can reduce adverse events, highlighting the necessity that HSCT for CD is only performed in centers with appropriate experience [121].

Current guidelines for the use of HSCT in patients with Crohn's disease are outlined in a position paper from EBMT and the European Crohn's and Colitis Organization (ECCO) [49]. Patients considered for HSCT should have clear evidence of a diagnosis of Crohn's disease, a severe disease course with objective evidence of

active intestinal inflammation, that is refractory to currently available medical therapies, and in whom surgery is inappropriate. In addition patients considered for HSCT should be discussed in a multidisciplinary team meeting prior to undertaking the procedure.

Further research is required to assess the place of HSCT in the management of patients with treatment refractory and poor prognosis CD. The exact benefit and risk of HSCT compared to ongoing partially effective therapy will require a controlled clinical trial using endpoints such as patient reported outcome and endoscopic healing that are traditional for trials in this disease area. It will be important to see whether the use of low intensity mobilization and conditioning regimens can maintain any observed benefit whilst enhancing safety. Given that a proportion of patients will relapse having initially responded to HSCT, it will be important to assess the efficacy and safety of the re-introduction of therapies to which patients were previous refractory. ASTIClite is a multicenter NIHR funded UK randomised controlled trial of HSCT in patients with refractory Crohn's disease that commenced in 2018 and is designed to answer many of these outstanding questions (ISRCTN 17160440).

7.1.2. Mechanistic studies in CD

Mechanistic data relating to HSCT in CD is limited compared with other autoimmune diseases. A small pilot study demonstrated an abrogation of dysregulated T effector cell responses with a reduction of specifically bacterial lipopolysaccharide recognizing TLR4-expressing as well as TNF-alpha and IFN-gamma expressing monocytes together with an increase in Foxp3$^+$ Tregs in patients undergoing HSCT [122].

Next generation sequencing (NGS) of the TCRβ locus in ileal and colonic biopsies from 16 patients with CD collected at baseline (pre-mobilization) and after auto-HSCT (6 months and/or 1 year after transplantation) correlated the TCR diversity with clinical and endoscopic outcome. As previously demonstrated, monoclonal expansions in the mucosal T cell compartment were present at baseline. TCR clonality was significantly increased in the mucosa of patients after HSCT and the T cell repertoire appears reset, as the similarity index between baseline and after the procedure was low (Le Bourhis, Gastroenterology 2017; 152:S613-4). Further studies to assess immune reconstitution after HSCT in patients with Crohn's disease are

required to explain why some patients benefit from a prolonged disease remission whilst others have a relapse of disease activity. Such studies could also assess the mechanism by which patients regain responsiveness to anti-TNF therapy after HSCT and are included in the ASTIClite protocol [123].

8. Conclusion and future perspectives

The improved understanding of the immunopathology of AD has lead to the development of novel targeted immunotherapies by pharmaceutical companies, but although providing more specificity to disease management, they are not curative. Meanwhile, hematopoietic stem cell therapies have emerged as promising treatment options in severe AD. Driven by academic research, both clinical and immunologic studies provided the proof-of-concept that immunoablation followed by transplantation of HSC can reset the immune system into a naïve and self-tolerant state potentially providing cure in ADs. Initially associated with increased transplant related mortality (TRM), HSCT was considered as salvage therapy in severely affected patients with poor outcomes. Over the past years, TRM has significantly improved due to greater center experience, better patient selection and supportive care [34]. In addition, large RCTs of HSCT have demonstrated superior outcome and prolonged survival compared to standard-of-care in the major indications.

Moving forward, further efforts are needed to drive HSCT and other cellular therapies into routine clinical care. Although evidence and clinical experience for HSCT in ADs are accumulating, most of the results obtained and reviewed in this paper do not reflect necessarily real world experience, but are largely achieved in highly specialized centers by multidisciplinary teams with appropriate expertise and access to clinical trials. As for hematologic malignancies, there have been center-specific outcomes for HSCT in ADs with gradual improvement due to a learning curve. Such "center-effect" reflects all aspects of required infrastructure, adjustment to appropriate standard operation procedures and current guidelines as well as patient selection and supportive care [34]. In particular, caution is required in transplanting AD patients, as they have idiosyncrasies, which often require more attention and individualized, patient-centered care than patients with hematologic diseases. Therefore, HSCT should only be performed at centers in which staff are experienced, including specifically trained medical, nursing and other allied professionals, and

where a close interdisciplinary working between the disease specialty (i.e. rheumatology, gastroenterology or neurology) and hematology is guaranteed.

In most autoimmune diseases there is normally limited toxicity from conventional treatments, at least in the short-term. Disease specialists may be unfamiliar with the intensity and range of toxicities of the high-dose cytotoxic therapy and HSCT, which are routinely encountered and managed by hematologists practicing HSCT on a daily basis. The hematology community have long recognized the need to maximize safety in HSCT and have widely implemented internal quality management and externally validated accreditation processes and external across all aspects of their practice (clinical, stem cell collection and laboratory processing). The potential benefits of accreditation by the Joint Accreditation Committee of the International Society for Cellular Therapy and EBMT (JACIE), and its American counterpart (Foundation for the Accreditation of Cellular Therapy, FACT) are supported by better survival outcomes in HSCT [124]. Recently, data supporting improved outcomes in JACIE-accredited units performing autologous HSCT for autoimmune diseases has been published [34] validating EBMT recommendations that AD patients should be treated in transplant centers that have been JACIE-accredited (or equivalent) whilst follow screening procedures for patient selection and models of care promoting safety and quality [33, 48, 49]. Close and 'routine' collaborative working between hematologists and disease specialists is essential for every stage; from patient selection and screening, through the HSCT procedure to the long-term follow up and management of the 'late effects' of the transplant and any active disease. In the future, quality of care may be underpinned by disease-specific 'benchmarking' exercises to provide reassurance that transplant center survival outcomes for various autoimmune diseases are maintained within expected ranges, with ideally this information available to the referring specialists and their patient communities.

Where possible, patients should be treated on prospective clinical trials and linked with basic scientific academic studies to further evaluate the various mechanism of action. A future goal is to determine optimized conditioning regimens and graft selection and to investigate the potential role of maintenance therapies per disease to further improve the safety and efficacy of HSCT. In addition, comprehensive data reporting, harmonization and exploitation of existing biobanking infrastructure [125],

education at individual center and network level, and health economic evaluations along with evidence-based recommendations coordinated by national and European societies will all help to determine the future place of HSCT in the treatment algorithm for ADs.

Competing interests statement

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Figure Legends:

Graphical abstract | Concept of hematopoietic stem cell therapy in autoimmune diseases.

A) An autoreactive immunologic memory may drive chronic autoimmune responses and represents a major barrier for curative therapeutic approaches in autoimmunity. Once developed, autoreactive memory cells migrate into inflamed tissues where they reside as tissue-resident memory cells, e.g. CNS-infiltrating T-cells in MS after crossing the blood-brain-barrier, kidney-infiltrating T-cells in SLE, skin-resident T-cells in systemic sclerosis or synovial T-cells in inflammatory arthritis. Likewise, autoreactive memory T-cells and plasma cells migrate into the bone marrow where they survive in dedicated survival niches. B) Current therapeutic concepts are historically based on chronic suppression of immune functions either with conventional immunosuppression or by targeting inflammatory cytokines, costimulatory signals or adhesion molecules. However, the autoreactive immunologic memory is largely unresponsive to these approaches. C) In contrast, hematopoietic stem cell transplantation is performed with the premise to eradicate autoreactive memory clones using chemotherapeutic agents usually in combination with polyclonal antibodies such as anti-thymocyte globulin (ATG) for *in vivo* T-cell depletion. D) Transplanted autologous HSC promote an extensive immune renewal providing a new and polyclonal repertoire of naïve T- and B-cells and a novel protective immunologic memory is generated. As a consequence, restoration of self-tolerance may be achieved resulting in long-term remissions that are not further dependent on chronic immune suppression

Figure 1 | Hematopoietic stem cell transplantation in patients with autoimmune disease. Autologous hematopoietic stem cells (auto-HSC) are harvested from peripheral blood, purified (mostly using CD34 selection with magnetic separation) and cryopreserved after being mobilized from bone marrow by treatment with cyclophosphamide (CY) and granulocyte colony stimulating factor (G-CSF). Approximately 4 weeks later patients receive a conditioning to eradicate autoreactive immunologic memory cells, usually a combination of intravenous CY and anti-thymocyte globulin (ATG). Subsequently, HSC are infused to allow the regeneration of a new immune system that is reset to a self-tolerant state. After engraftment and neutrophil recovery, the first phase of immune reconstitution is characterized by

clonal expansion of residual memory lymphocytes in response to early infections and/or lymphopenia-induced proliferation. Finally, lymphocyte renewal occurs through thymic reactivation leading to restoration of a polyclonal repertoire of T regulatory and conventional T-cells.

Figure 2 | Resetting the immunologic clock with autologous hematopoietic stem cell transplantation. Autoimmune diseases develop in a genetically predisposed population when, upon certain environmental triggers, such as viral infections, smoking, obesity, vitamin D deficiency and possibly high sodium intake, normally quiescent autoreactive cell clones become aberrantly activated and escape regulation. Following their repeated or chronic activation, effector memory cells may form an immunologic memory, similar to immune responses during infections or after vaccinations to provide protection for antigenic reencounter. With some latency, such memory cells together with pro-inflammatory cytokines and/or autoantibodies may eventually lead to chronic inflammation in target organs or multiple organ systems resulting in clinical manifestations of ADs. For affected individuals, HSCT could reset the immune system to an earlier phase of disease development, reverting to the initial state of genetic predisposition but erasing the subsequent lifetime exposure to immune activating events. This 'reset of the immunological clock' could underlie the prolonged clinical remissions, which may be life-long in some individuals. However, under some circumstances, pre-activated autoreactive memory cells may escape their depletion or immune renewal of the regulatory immune network is hampered or delayed, predisposing individuals to relapses post-transplantation and reducing the efficacy of the procedure.

Figure 3 | Reported cases of autologous hematopoietic stem cell transplantation for autoimmune diseases to the EBMT Autoimmune working party (ADWP) data registry. A) As of January 2018, 2417 autologous HSCT for ADs have been reported to the EBMT since 1994. Major indications for HSCT are multiple sclerosis (MS), systemic sclerosis (SSc) and Crohn´s disease (CD), together covering more than two thirds of cases. B) Number of autologous HSCT reported to the EBMT registry per year and indication as of January 2018.
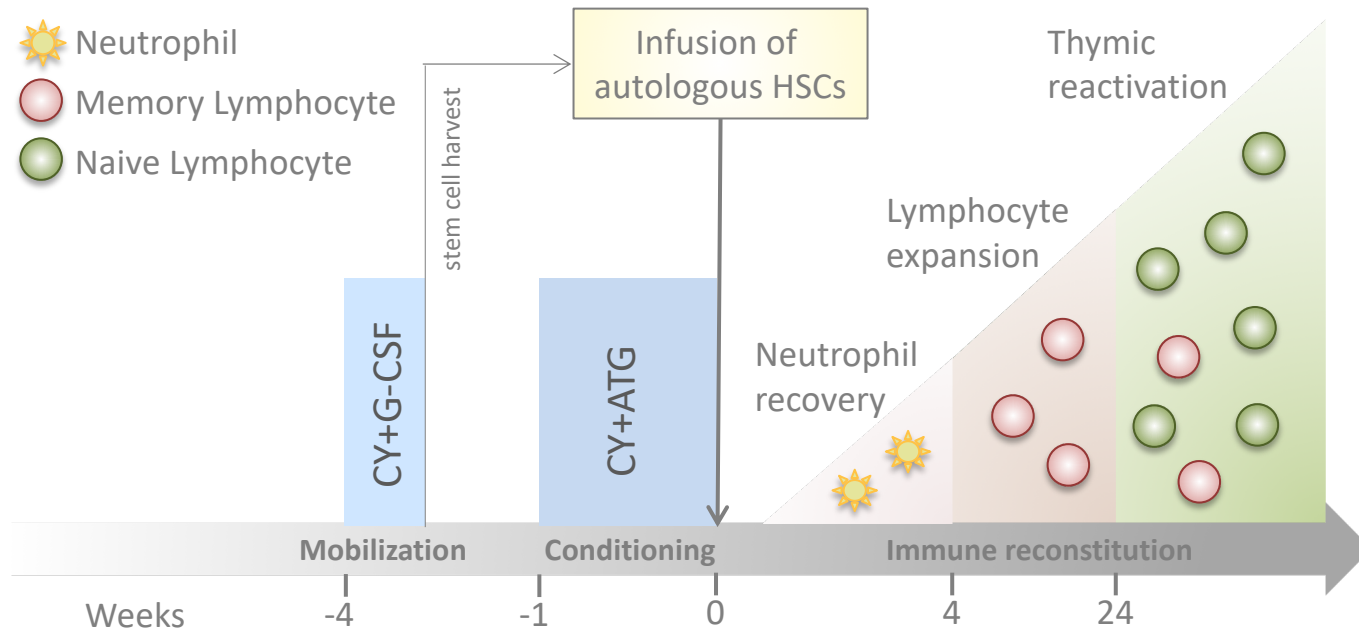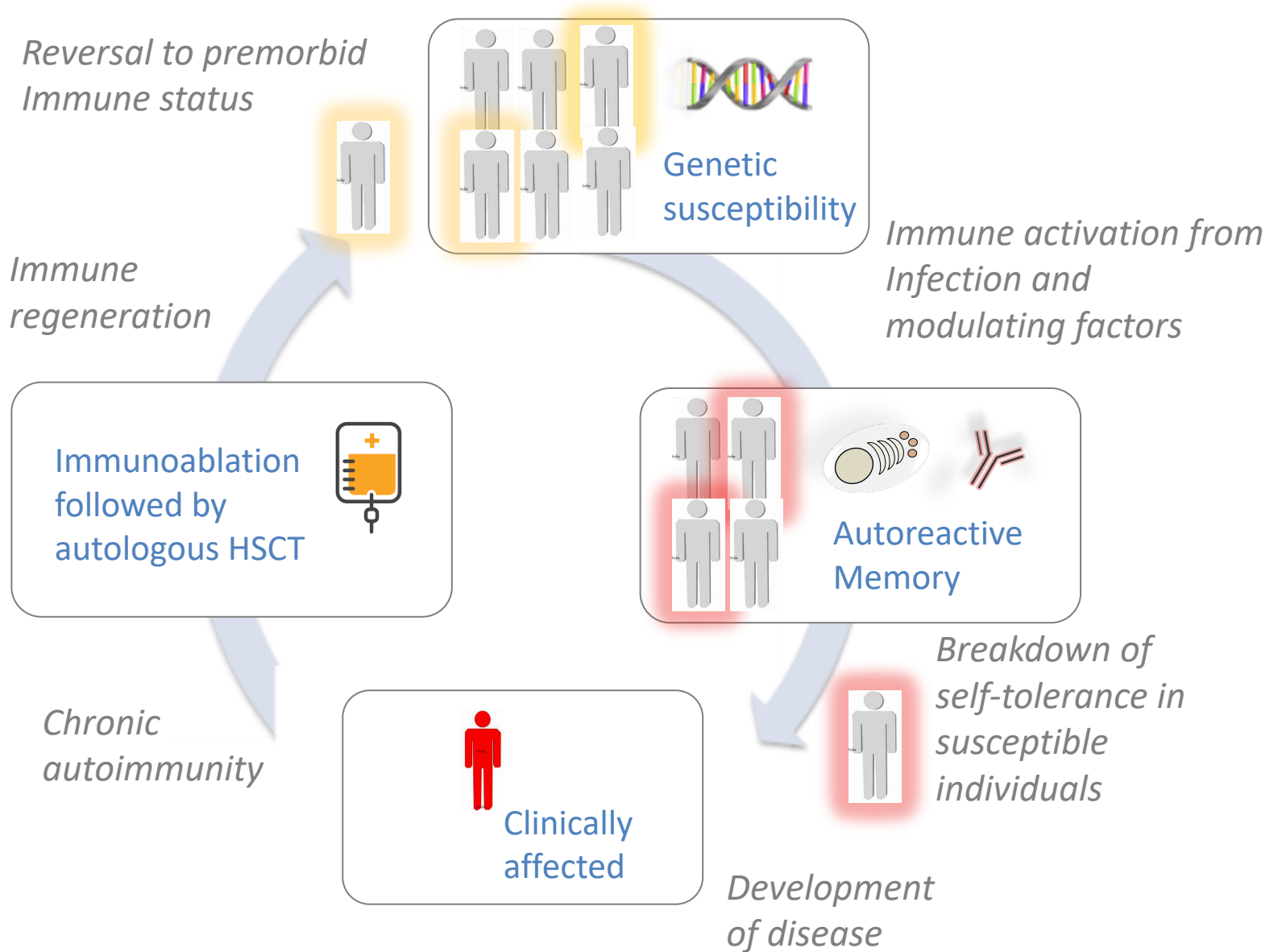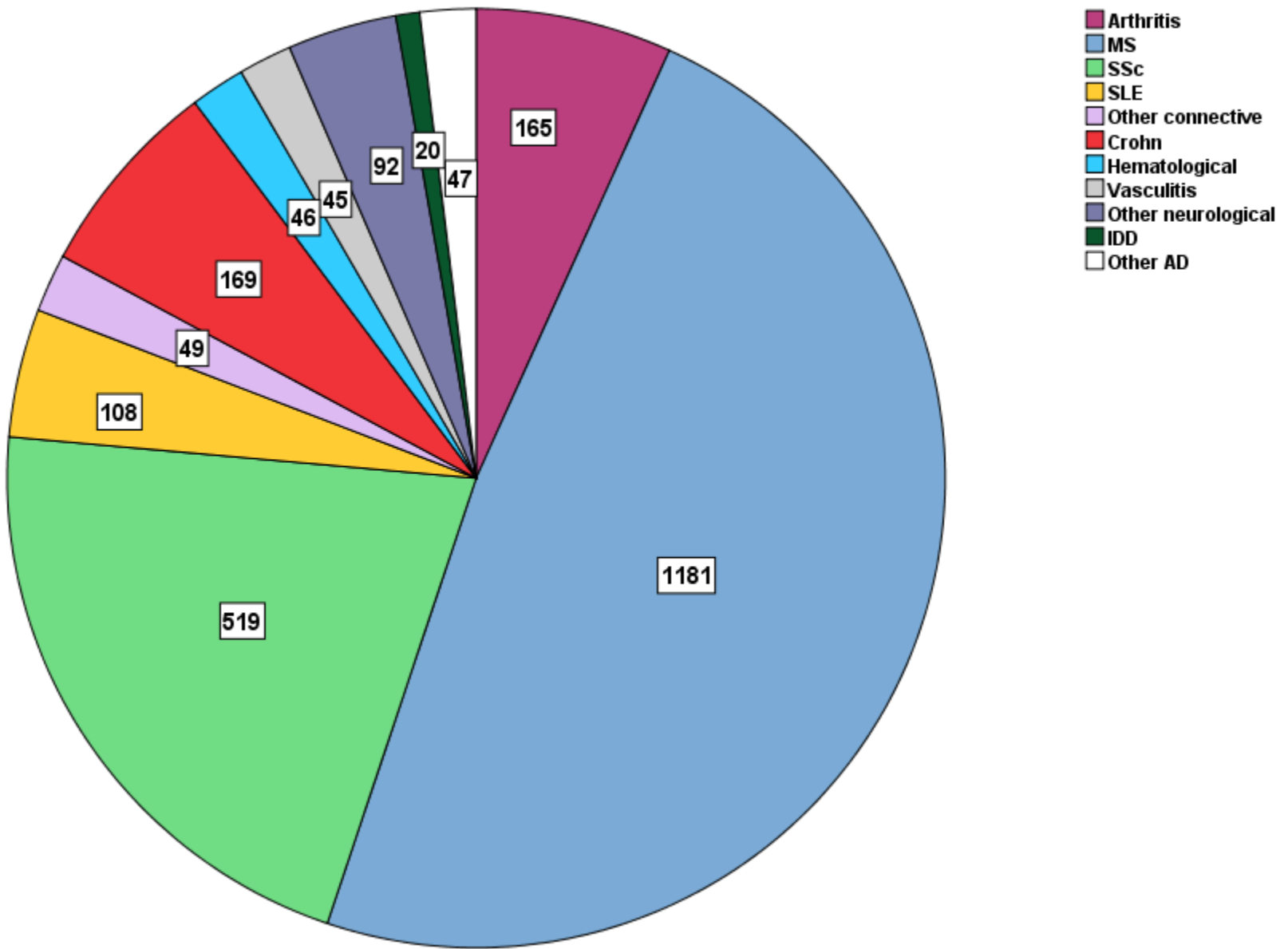* incomplete data for 2017.
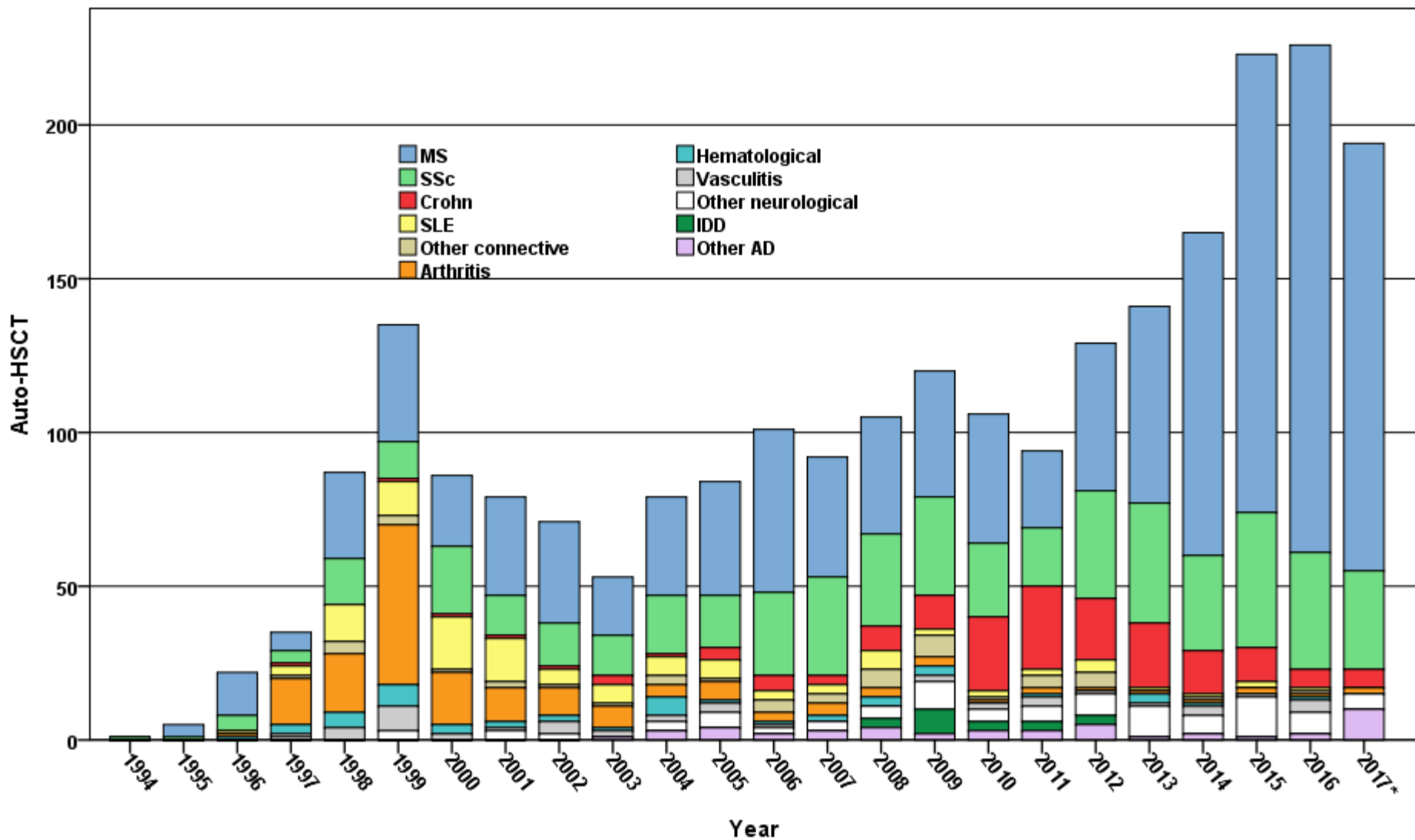
Figure 1

Figure 2

Figure3A

Figure3B
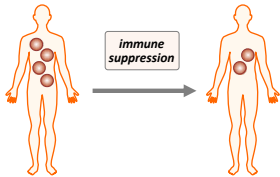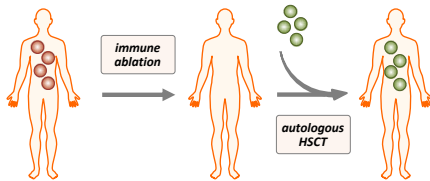
A)
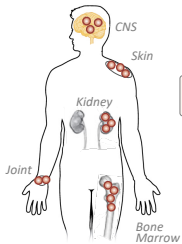
immune suppression

B)

immune ablation
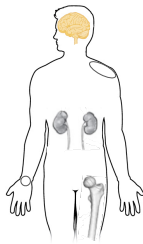
autologous HSCT

A) CNS Skin Kidney Joint Bone Marrow

B) immune suppression

C) immune ablation

D) autologous HSCT