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Non-myeloablative stem cell transplantation for autoimmune diseases

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Abstract Treatment of life-threatening autoimmune diseases in animal models with induced or spontaneous autoimmune diseases can be accomplished by a 2-step procedure involving elimination of self-reactive lymphocytes with an immune ablative conditioning regimen followed by infusion of autologous or allogeneic stem cells, respectively. In animal models it was shown that using such a strategy, autoimmunity could be adequately controlled. It is speculated that de-novo development of the T and B cell repertoire from uncommitted progenitor cells in the presence of the autoantigens may be the best recipe for re-induction of self-tolerance, similarly to the normal ontogeny of the immune system during the induction of self tolerance in fetal stage. For both autologous and allogeneic hematopoietic stem cell transplantation, a non-myeloablative stem cell transplantation (NST) regimen may be used for safer lymphoablation rather than myeloablation. In addition, for allogeneic hematopoietic stem cell transplantation engraftment of disease resistant donor stem cells will alter the genetic predisposition towards autoimmune disease susceptibility.

Keywords Non-myeloablative stem cell transplantation · Systemic lupus erythematosus · Multiple sclerosis · Scleroderma · Rheumatoid arthritis

Introduction

Non-myeloablative hematopoietic stem cell transplantation (NST) is a rapidly expanding treatment strategy for the use of allogeneic stem cell transplantation for the treatment of both hematological malignancies and solid tumors [15, 19, 43, 49, 51, 54]. NST diminish-

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es regimen-related toxicity by decreasing conditioning regimen intensity compared to conventional myeloablative transplants. Instead of using chemoradiotherapy to achieve disease control, relapse is prevented by an immunological graft versus leukemia (GVL) or graft versus tumor (GVT) effect induced by donor lymphocytes, natural killer cells, and/or dendritic cells infused with the allogeneic graft or after hematopoietic stem cell transplantation by infusion of peripheral blood donor lymphocytes. However, the ability of a beneficial GVL effect to induce remission or prevent relapse appears to coincide with the occurrence and severity of a detrimental immunological attack upon normal host tissues known as graft versus host disease (GVHD). In terms of overall survival, the advantages of diminished regimen-related mortality and GVL might, in some cases, be offset by the morbidity and mortality of GVHD. While multiple reviews have already been published concerning allogeneic NST for malignant diseases [43, 49, 51, 54], the unique benefit of either autologous or allogeneic NST for autoimmune disease has been generally overlooked and is, therefore, the focus for this review.

Autologous NST for autoimmune disease

Autologous hematopoietic stem cell transplantation (HSCT) for autoimmune disease implies that the disease is environmentally induced and may be corrected by regenerating a new immune system from the uncommitted, newly developing stem cell compartment [15, 19]. Since the immune system is regenerated from autologous stem cells, myeloablation is an unwanted side effect of immune ablative conditioning regimens. Autoimmune diseases are, therefore, ideal candidates for intense immune suppressive or “immune ablative” but non myeloablative autologous hematopoietic stem cell transplantation. Following autologous NST, infusion of hematopoietic stem cells (HSC) is not necessary for hematopoietic reconstitution but is done for safety reasons to shorten the duration of conditioning regimen-induced neutropenia. Before treating the patient with the immunoablative conditioning regimen, autologous HSC need to be collected from the patient. Whether or not purified stem cells are superior to unseparated stem cells remains an open question, although theoretically, the former seems to be more rational as long as effective elimination of host self-reactive lymphocytes can be accomplished by the conditioning.

Mobilizing stem cells from patients with autoimmune disease

Originally, HSC were collected from the posterior superior iliac crest of bone marrow donors by repeated aspirations performed under epidural or general anesthesia. Subsequently, to facilitate hematopoietic reconstitution and avoid the discomfort associated with multiple bone punctures, as well as the need for operating room and general anesthesia, the most common method of collecting HSC has become mobilization from the peripheral blood. Since negligible HSC are detectable in the peripheral blood during steady state, either a hematopoietic growth factor such as granulocyte colony-stimulating factor (G-CSF) or chemotherapy (usually cyclophosphamide) with or without G-CSF is necessary to collect HSC from the peripheral blood [12].

To mobilize HSC from the peripheral blood, a growth factor, usually G-CSF, is administered daily at between 5 and 16 μg/kg subcutaneously. Apheresis to collect progenitor cells begins on either day 4 or 5 of G-CSF administration. A 10 to 15 litre peripheral blood

apheresis performed on 1 day is usually adequate for collection of sufficient numbers of HSC. Occasionally a consecutive 2nd or 3rd day of apheresis may be necessary. HSC may also be collected by administration of cyclophosphamide (2.0–4.0 g/m^2) and daily G-CSF beginning 72 h later. Apheresis is performed when the white blood cell count rebounds, approximately 10 days after cyclophosphamide infusion. The combined use of cyclophosphamide and G-CSF results in higher HSC yields compared to G-CSF alone [12].

Hematopoietic growth factors used to mobilize stem cells also have cytokine immune modulating effects [5, 63] and, depending on growth factor and autoimmune disease, may either exacerbate or ameliorate disease severity. In experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), growth factors such as Flt-3 ligand, stem cell factor (SCF), and G-CSF exacerbate disease, while thrombopoietin (TPO) mobilizes stem cells without affecting disease severity (manuscript in preparation). G-CSF may also cause an exacerbation of MS, sometimes with significant neurological deterioration [12, 47]. In both EAE and MS, simultaneous use of daily corticosteroids or infusion of cyclophosphamide prior to starting G-CSF prevents disease flares [47]. In rheumatoid arthritis (RA), G-CSF may cause an increase in joint swelling, tenderness and pain that responds to corticosteroids, while G-CSF has not been reported to exacerbate scleroderma. In contrast, for Crohn's disease, G-CSF alone, without use of corticosteroids, may ameliorate symptoms [61].

Based on the above, growth factors selected for mobilizing HSC from patients with autoimmune diseases need to be considered on a disease-specific basis. Hematopoietic growth factors that stimulate production of proinflammatory cytokines or alter trafficking of neutrophils, lymphocytes or dendritic cells may exacerbate some autoimmune diseases. This effect may be prevented by either administration of corticosteroids or mobilization with combined cyclophosphamide and G-CSF. Advantages of G-CSF mobilization include absence of neutropenic infections. There is little or no experience in G-CSF mobilization for autoimmune diseases other than RA, MS, and scleroderma. Mobilization with cyclophosphamide and G-CSF may cause neutropenic fevers and infection-related mortality if prophylactic antibacterial and antifungal antibiotics are not utilized. Advantages for combined cyclophosphamide G-CSF mobilization are higher stem cell yields, an in vivo purge effect by selectively killing lymphocytes in cell cycle, and a disease-ameliorating effect.

Autologous NST conditioning regimens for autoimmune diseases

For patients with malignant diseases, the autologous transplant regimen should be based on dose escalation of drugs or biologics effective at conventional non-transplant doses. Dose escalation of a non-effective agent would increase toxicity without efficacy. This same reasoning should, where possible, be applied to autoimmune disorders. Although there are exceptions, in general, autoimmune diseases, despite significant morbidity, often have a lower mortality than most malignancies and unlike malignancies may go into spontaneous remission. Therefore, due to the risk benefit of HSCT and unpredictability of a patient's natural history, conditioning regimens for autoimmune diseases should have a greater emphasis on safety than the regimens designed for malignancies. The rationale for autologous HSCT of autoimmune diseases is to regenerate a new, i.e. antigen naive, immune system, from the patient's own HSC. This will require the re-emergence of thymic-educated virgin (antigen naive) T cells from HSC. Therefore, the goal of the conditioning regimen is "immune ablation" not myeloablation [15, 31].

Unlike malignancies where any visceral organ impairment is a contraindication to HSCT, disease-related organ dysfunction is often the indication for HSCT of autoimmune disorders. For this reason, the regimen must also avoid further injury to the disease-affected organ. For example, myeloablative agents such as bleomycin, BCNU (carmustine), and radiation that are complicated by pulmonary fibrosis would not be the ideal conditioning agents for a disease such as scleroderma in which a major cause of death is related to pulmonary fibrosis and pulmonary artery hypertension [1, 9].

Since visceral organ impairment is often the indication for HSCT of an autoimmune disease, conditioning regimen design should avoid agents that could damage organ-specific stem cell compartments that may contribute to organ repair after the disease remits [9, 12]. Apart from the myeloablative effect on bone marrow, myeloablative conditioning regimens may also be lethal to other tissue stem cell compartments and, therefore, detrimental to organ repair. Radiation, delivered as 8–12 cGy of total body irradiation (TBI), is a common agent of myeloablative regimens for malignancies. While the sensitivity of non-hematopoietic tissue-specific stem cells to irradiation is generally unknown, 10 cGy of cranial radiation impairs central nervous system repair by neural stem cell apoptosis, alteration in cell cycle progression, and/or destruction of the neural stem cell niche or milieu through invasion of macrophages and microglia [44].

For autoimmune diseases, immune ablation without myeloablative side effects may be accomplished with agents such as cyclophosphamide, fludarabine, and antibodies to T cells (anti-thymocyte globulin) and/or B cells (rituximab) or T and B lymphocytes and dendritic cells (CAMPATH-1; alemtuzumab) [21]. For autoimmune diseases, advantages of autologous NST are: (1) maximal immune suppression without myeloablation, (2) avoiding injury to already disease-affected and damaged tissues, (3) minimal injury to tissue-specific stem cell compartments that may be important for tissue repair, and (4) use of regimens that are justified for the risk of the disease being treated [15, 19].

Allogeneic NST for autoimmune diseases

Unlike autologous NST in which the goal is to suppress and restart the immune system from autologous stem cells, the goal of an allogeneic NST is twofold. First, to change the genetic predisposition to disease by changing the host's susceptible to the donor's resistant stem cell compartment. Second, to introduce donor's lymphocytes with the capacity to eliminate all residual self-reactive host lymphocytes through a process known as graft versus autoimmunity (GVA) effect, in analogy to GVL in leukemia and GVT in some metastatic solid tumors. It is not clear whether a full chimera, in which all HSC are reconstituted from the donor, or mixed chimerism, with coexistence of both donor and recipient hemato- and immunopoiesis, is sufficient to control disease. In animal models, it has been shown that mixed chimerism can induce bilateral transplantation tolerance of host versus graft and graft versus host reactivity, remission and prevention of autoimmune diseases [31, 32, 56]. Therefore, unlike hematological malignancies where mixed chimerism, in contrast to full chimerism, may be associated with a higher rate of relapse, NST-induced mixed chimerism may be beneficial as well as safer in controlling autoimmune diseases. Besides, mixed chimerism may facilitate induction of full chimerism at a later stage by discontinuation of post-transplant immunosuppression.

Full donor chimerism in malignancies has been complicated by a high rate of GVHD, an immune-mediated disease in which allogeneic donor lymphocytes are directed against

the host. However, mixed chimerism may be induced with lower risk of GVHD. Due to the risk and complications of GVHD compared to the disease itself, until methods to eliminate GVHD with full donor chimerism are proven, the goal of allogeneic stem cell transplantation in autoimmune diseases should be to induce mixed chimerism by the NST approach.

Mixed chimerism may be induced without GVHD by combining an intense immune suppressive but non-myeloablative regimen with a lymphocyte-depleted donor graft [14]. The donor graft may be depleted of lymphocytes by ex vivo positive stem cell selection ($CD34^+$ or $AC133^+$) or by negative selection of T cells using immunomagnetic beads bound to anti-CD3 antibodies, or by infusion of potent in vivo lymphocyte depleting antibody such as anti-CD52 (CAMPATH-1H) [12, 21]. The principles behind selection of the conditioning agents for induction of allogeneic mixed chimerism are otherwise similar to those for autologous NST. The conditioning regimen is selected to avoid agents such as TBI that cause recipient myeloablation, damage to tissue-specific stem cell compartments, or injury to already damaged organs.

NST for autoimmune diseases

The development of stem cell transplantation for autoimmune diseases is complicated and, therefore, a simplified time line of first events is shown in Table 1. It was documented in 1977 that inadvertent life-threatening myeloablative treatment and autologous reconstitution resulted in complete elimination of all manifestations of autoimmunity and reversal of end-stage renal failure caused by mixed cryoglobulinemia in a patient followed post HSCT for more than 20 years [53]. Initial trials worldwide generally employed regimens used for HSCT of malignancies and were consequently often myeloablative [13, 17, 18, 27, 33, 41, 53, 59, 65]. In contrast, more recent protocols have generally emphasized NST regimens [16, 57]. Since immune-mediated diseases have traditionally been divided or separated within distinct subspecialties, NST for autoimmune diseases traverses traditional areas of expertise. For example, MS, myasthenia gravis, and chronic inflammatory demyelinating polyneuropathy are within the department of neurology, SLE, RA, polymyositis and scleroderma in the division of rheumatology, autoimmune bullous skin diseases such as pemphigus vulgaris in the department of dermatology, asthma and interstitial pulmonary fibrosis within the division of pulmonary diseases, and Crohn's disease and autoimmune hepatitis are part of the divisions of gastroenterology and/or hepatology. Successful NST with appropriate selection of patients and low transplant-related mortality requires a dedicated and focused team.

America's first autologous HSCT for MS, SLE, RA, relapsing polychondritis, Sjögren's disease, and Behcets, and the world's first autologous HSCT for Crohn's disease, pemphigus vulgaris, and myasthenia gravis were performed at Northwestern University in Chicago [8, 13, 16, 17, 18]. Several multi-center retrospective analyses on HSCT for autoimmune disease have been reported but do not clearly separate toxicity by conditioning regimen, and do not have commonly defined prospective endpoints. For this reason, we will focus on single center and/or single protocol data.

Table 1 Time line of first events in development of HSCT for autoimmune diseases (*ABMTR* Autologous Blood and Marrow Transplant Registry, *EAE* experimental autoimmune encephalomyelitis, *EAMG* experimental autoimmune myasthenia gravis, *EBMT* European Bone Marrow Transplant Registry, *EULAR* European League Against Rheumatism, *GVA* graft versus autoimmunity effect, *HSCT* hematopoietic stem cell transplantation, *IBMTR* International Bone Marrow Transplantation Registry, *MRL/lpr* Murphy Roth laboratory/lymphoproliferative, *MS* multiple sclerosis, *NZB* New Zealand Black, *NOD* non-obese diabetic, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus)

Date	Author [ref]	Comment
1977	Baldwin et al. [4]	First case report of remission of autoimmune disease in patients undergoing HSCT for another reason
1979	Slavin [52]	First report suggesting that elimination of the immune system and autologous reconstitution can reverse autoimmunity in (NZBxNZW)F1 mice
1979	Morton and Siegel [45]	First report of reversal of autoimmune syndrome in animals (NZB mice) by allogeneic bone marrow transplantation
1983	Pestronk et al. [48]	First report of animals cured of myasthenia gravis in EAMG model by syngeneic bone marrow transplant
1985	Ikehara et al. [31]	First report that allogeneic bone marrow transplant cures SLE-like disease in murine MRL/lpr model
1985	Ikehara et al. [32]	First report that allogeneic bone marrow transplant prevents diabetes in NOD mouse model
1989	van Bekkum et al. [60]	First report that syngeneic bone marrow transplantation cures arthritis in animal model of adjuvant-induced arthritis
1992	Karussis et al. [34]	First report that syngeneic bone marrow transplantation cures MS-like disease in EAE animal model
1993	Slavin et al. [53]	First report of cure of mixed cryoglobulinemia, describing the first patient fully cured by myeloablative treatment, suggesting that HSCT could be used for treatment of autoimmune diseases
1993	Drakos et al. [23]	First case suggesting that HSCT could be used as treatment of Crohn's disease
1993	Marmont [40]	First editorial suggesting that HSCT should be used as treatment for SLE
1995	Burt et al. [10]	First editorial suggesting that HSCT should be used as treatment for MS
1995	Committee Chair—Tyndall	EBMT and EULAR establish a working committee on stem cell transplant for autoimmune diseases for Europe and Asia
1995	Committee Chair—Burt	IBMTR and ABMTR establish a working committee on stem cell transplant for autoimmune diseases for the Americas
1997	Fassas et al. [27]	First report of HSCT for MS in the world
1997	Marmont et al. [41]	First report of autologous HSCT for SLE in the world
1997	Burt et al. [17]	First report of autologous HSCT for SLE in America
1997	Joske et al. [33]	First report of autologous HSCT for RA in the world
1997	Tyndall et al. [59]	First report of autologous HSCT for scleroderma
1998	Burt et al. [18]	First report of autologous HSCT for MS in America
1999	Wulffraat et al. [65]	First report of autologous HSCT for juvenile chronic arthritis in the world
1999	Burt et al. [13]	First report of autologous HSCT for RA in America
1999	Contract principal investigators—Burt, Burns, Sullivan	National Institutes of Health awards three contracts to develop phase III trials of HSCT for autoimmune diseases
2000	Slavin et al. [55]	First clinical evidence of allogeneic GVA
2001	Snowden, principal investigator	EBMT/EULAR opens phase III trial of autologous HSCT for RA
2001	van Laar, principal investigator	EBMT/EULAR opens phase III trial of autologous HSCT for scleroderma
2002	Burt et al. [16]	First report of autologous HSCT for Crohn's disease in the world
2004	Burt et al. [14]	World's first report of allogeneic HSCT for the indication of RA

Multiple sclerosis

MS is both an immune-mediated demyelinating disease manifest as acute relapses with gadolinium-enhancing lesions on MRI, and an axonal degenerative disease manifest as slowly progressive disability often without enhancing lesions [6, 22]. Initial HSCT protocols were focused on patients with non-relapsing progressive disease and severe neurological disability (requiring a cane, bilateral support, walker or wheelchair) [11, 18, 28, 36]. The traditional instrument used to measure MS-related neurological disability is the Kurtzke extended disability status scale (EDSS). The EDSS varies by half point increments from 0 (normal) to 10 (death due to neurological disability). Disability is mild (EDSS <4.0), moderate (4.0–6.0), or severe (EDSS >6.0). At an EDSS of 4.0 patients are able to ambulate without difficulty. At an EDSS of 6.0, support is required to ambulate, while at an EDSS of 7.0, patients can only ambulate for a few steps [37].

Centers in Greece [28], Chicago [11], Seattle [46], and Italy [39] have published phase I/II results on HSCT in patients with MS. The Greek and Italian transplant protocols used a conventional lymphoma transplant regimen of BEAM (busulfan, etoposide, adriamycin, and melphalan) [39]. This regimen is nearly myeloablative since prolonged cytopenias (approximately 50 days) will result if HSC are not infused [28, 39]. The Chicago and Seattle studies used cyclophosphamide and TBI, a common myeloablative HSCT regimen for hematological malignancies. In the Chicago and Greek studies, patients with secondary progressive MS and an EDSS >6.0 continued to have progressive neurological decline following HSCT. Patients with less disability (EDSS $\leq$6.0) have generally not progressed, although continued long-term follow-up and more patients would be necessary to confirm a change in the natural history of disease progression [11, 28]. Since MS patients with progressing disease need to be followed at least 3 years before estimating progression, post-HSCT follow-up in the Seattle study was too short to recognize progression in EDSS following treatment [46]. The Italian study focused on MRI endpoints and demonstrated a complete suppression of inflammatory disease, that is gadolinium enhancement, following Mancardi et al. [39]

From these initial studies, we concluded that HSCT should be performed earlier in disease before significant disability and in patients with continued inflammatory disease manifest by acute relapses and gadolinium enhancement on MRI [39] despite treatment with interferon (Avenox, Betaseron, or Rebif). Although this subgroup of patients may over several years develop significant and irreversible neurological impairment, a relatively non-toxic and well-tolerated conditioning regimen is required since the 10-year survival of this earlier subset of patients is similar to the general population. Therefore, at Northwestern University (Chicago), for our phase II study, we selected an NST conditioning regimen of cyclophosphamide (50 mg/kg on days −5, −4, −3, −2) and the anti-lymphocyte antibody CAMPATH-1H (20 mg on day −2). This combination was based on efficacy of both drugs in suppressing active inflammatory disease in MS. Since CAMPATH-1H has a long half-life, being a humanized antibody, and can effectively purge the graft in vivo, no ex vivo $CD34^+$ selection or manipulation of the autologous graft is performed prior to infusion.

Candidates are selected for an EDSS of 2.0–5.5 and failure of at least 6 months of interferon, failure being defined as gadolinium-enhancing lesion(s) on MRI and acute relapses within the prior 12 months. Therefore, unlike the earlier study that selected patients for progressive neurological deterioration with severe disability, this study selects patients for active relapses with mild to moderate disability. NST results in this earlier and active

inflammatory subset of patients with MS have been encouraging, with improvement and not just stabilization of EDSS scores (unpublished).

Systemic lupus erythematosus

SLE is a clinically heterogeneous disease that may predominantly affect one or combinations of organ systems, and manifestations vary between patients. Some patients have a single system which is mainly affected, e.g., nephritis, serositis, pneumonitis, cerebritis, vasculitis, anti-phospholipid antibody syndrome with venous and arterial thrombi, arthalgias, myalgias, mucocutaneous symptoms (rash, livedo reticularis, ulcerations), or immune-mediated cytopenias. The common theme is T and B cell hyper-reactivity to environmental stimuli [7, 20, 38].

One of the most effective therapies for SLE is intravenous cyclophosphamide (500–1,000 mg/m^2 monthly for 6 months then every 3 months for 18 months). Patients who fail this therapy with active visceral disease have 20% and 35% mortality within 2 and 5 years, respectively, and are candidates for NST. The Northwestern group in Chicago has reported the only single center study on SLE. The NST conditioning regimen is 200 mg/kg cyclophosphamide and 90 mg/kg equine ATG. This regimen is based on dose escalation of perhaps the most effective lupus medication, intravenous cyclophosphamide. The mobilized cells are CD34$^+$ enriched [57, 58].

This phase I/II study was remarkably successful in inducing remission in previously severe and refractory disease [17, 57, 58]. These patients have often been on long-term high-dose corticosteroids and failed multiple drugs, including corticosteroids, methotrexate, azathioprine, hydroxychloroquine, intravenous pulse cyclophosphamide, cellcept, and cyclosporine. To date 42 patients with SLE have undergone autologous NST at Northwestern University. The majority of patients entered drug-free remissions for up to several years (manuscript in preparation). We are currently reviewing and summarizing outcomes to determine if any modifications are warranted for development of randomized trials.

Crohn's disease

Crohn's disease is a Th1-skewed inflammatory delayed-type hypersensitivity that may involve any region of the gastrointestinal tract. Whether inflammation is initiated and directed against exogenous antigens such as gastrointestinal bacteria or against a gut-specific auto-antigen is unknown. Crohn's disease is complicated by chronic abdominal pain often leading to narcotic addiction, diarrhea, repeated surgeries, colostomy or ileostomy, weight loss, fistulae, abscesses, abdominal obstruction, nausea, vomiting, and failure to thrive. Repeated surgery results in short gut syndrome and chronic total parenteral nutrition (TPN) [26].

It was shown by Slavin's group [23] that treatment of an accompanying malignancy with myeloablative treatment supported by autologous stem cell transplantation resulted in amelioration of the clinical manifestations of Crohn's disease. The only report of NST for primary treatment of Crohn's disease has been from Northwestern University (Chicago). The same NST regimen with CD34$^+$ selection used for treatment of SLE was utilized, because it has been well tolerated without mucositis. We have performed NST in 12 patients with a Crohn's disease activity index (CDAI) >250 (active disease), despite treatment with

TNF inhibitor (remicade) therapy. Clinical symptoms such as diarrhea and abdominal pain generally resolve before hospital discharge. All patients have entered remission (CDAI <150). Small bowel radiographs and colonoscopy gradually improve over 2 years ([16], manuscript submitted).

Systemic sclerosis

Systemic sclerosis, also known as scleroderma, is a vasculopathy, perhaps immune-mediated, but of unknown etiology. One suggested etiology is an autoimmune process directed against unknown self antigen(s). Another hypothesis is that scleroderma is an alloimmune response due to child or parent lymphocytes that cross transplacentally, survive dormant, and years to decades later become active and cause an allogeneic GVHD [29, 30, 50]. Mortality for scleroderma with high cutaneous skin scores (Rodnan score >14) and/or visceral organ involvement is approximately 12% per year [2, 3]. There is no known effective therapy for scleroderma, although intravenous cyclophosphamide appears to improve early pulmonary manifestations [62].

McSweeney et al. [42] reported the Seattle experience in using a myeloablative cyclophosphamide and TBI regimen in 19 patients. Four patients (21%) died. TBI-based HSCT regimens for malignancy commonly utilize 1,200 cGy of TBI without undue toxicity. Due to concerns over scleroderma-related radiation toxicity, the TBI regimen was dose reduced to 800 cGy (200 cGy BID). Despite radiation-dose reduction, 2 of the first 8 patients died of TBI-related interstitial pneumonitis at day 58 and 79 post HSCT. For the last 11 patients, the study was, therefore, amended with lung shielding (97% shielding after the first dose of 200 cGy). Two of these last 11 patients died, 1 died from Epstein-Barr virus infection (day 64) and 1 from renal failure (day 123). In the remaining 9 patients, pulmonary function at the time of last disease evaluation remained clinically unchanged (less than 15% variation) in 4 patients, but declined by more than 15% in 5 patients. In these 5 patients, the decline in diffusing capacity of lung for carbon monoxide and months since HSCT were: 57% to 41% (14.5 months), 84% to 60% (12.8 months), 47% to 28% (10.8 months), 69% to 49% (9.6 months), and 72% to 52% (7.5 months) [42]. These data suggest that, while skin scores improved, pulmonary disease appears to have progressed, since TBI toxicity is indistinguishable from disease progression.

Farge et al. in Paris [25] used a myeloablative regimen of melphalan for scleroderma patients with cardiac symptoms to avoid cardiac stress due to hyper-hydration. Patients without pre-transplant cardiac symptoms received an NST regimen of cyclophosphamide and ATG. The only treatment-related death occurred with the myeloablative regimen [25]. Burt et al. in Chicago (unpublished) performed NST with a cyclophosphamide/ATG regimen in patients with scleroderma. In this Chicago study, patients with pulmonary artery hypertension are excluded from NST and no transplant-related deaths have occurred. Using cyclophosphamide at 200 mg/kg and rabbit ATG at 7.5 mg/kg without $CD34^+$ selection or manipulation of the graft, the Chicago group have seen rapid improvement in skin scores and quality of life. Post-NST improvement of skin begins prior to transplant hospital discharge and seems to continue gradually beyond 1 year after treatment.

Summary of autologous NST for autoimmune diseases

In a retrospective review of autologous HSCT in 263 patients with autoimmune diseases, the treatment-related mortality of myeloablative conditioning (TBI or busulfan based) regimens was four times higher than for non-myeloablative regimens with no advantage in terms of disease control [35]. Since non-myeloablative regimens may be as intensely immune suppressive as myeloablative regimens, unless otherwise indicated by experience in a particular disease, NST should currently be viewed as the current standard for autologous stem cell transplants in autoimmune diseases.

Allogeneic NST

As mentioned earlier, the objective of allogeneic NST in autoimmune diseases is to induce donor-recipient mixed chimerism, while avoiding GVHD. It is hoped that, similar to animal models of autoimmune disease, mixed chimerism will result in prolonged disease remission, presumably by changing genetic susceptibility. In fact, once mixed chimerism is established, alloreactive donor lymphocytes can eliminate all self-reactive lymphocytes responsible for the autoimmune disease, thus accomplishing a much more profound elimination of all self-reactive T cells and B cells on the one hand, while replacing patient's susceptible hematopoietic cells with resistant donor cells through a mechanism that resembles GVL effects in patients with hematological malignancies [55]. Recent investigations in a patient with severe and resistant SLE treated with allogeneic stem cell transplantation utilizing NST suggests that elimination of all self-reactive host lymphocytes may be successfully accomplished without the use of myeloablative conditioning (Slavin, unpublished observation), as already documented in a patient with psoriatic arthritis [55]. Similarly, improvement of autoimmune disease manifestations were seen recently in a patient with rapidly progressive multiple sclerosis treated with NST currently under observation (Slavin, unpublished observation). While several protocols are in development at Northwestern University in Chicago, allogeneic NST studies are currently open for RA and scleroderma.

Rheumatoid arthritis

RA is an inflammatory synovial disease with subsequent cartilage and joint destruction. Patients with a large number of involved joints or significant restriction of activities of daily living are at high risk for death with 40–60% mortality over 5 years [24, 64]. Patients with these manifestations, despite TNF inhibitors and methotrexate / leflunomide therapy, are candidates for allogeneic NST. To obtain mixed chimerism without GVHD, patients are treated with a non-myeloablative transplant regimen and $CD34^+$-enriched (lymphocyte-depleted) megadose ($>10^7$ $CD34^+$ cells/kg) donor HSC. The conditioning regimen is 125 mg/m^2 fludarabine, 150 mg/kg cyclophosphamide and 20 mg CAMPATH-1H. One patient has been treated. At 14 months post NST, the patient shows a stable mixed chimera [both donor and host myeloid (CD33) and T (CD3) cells], is in a complete remission, and is rheumatoid factor negative. The patient is off all immune-suppressive or modulating therapy, and has had no GVHD or infections [14].

Scleroderma

Patients with scleroderma have a disease that is clinically similar to chronic GVHD [29]. It is, therefore, important to avoid GVHD, which could exacerbate and be clinically indistinguishable from scleroderma. Again, to obtain mixed chimerism without GVHD, patients are treated with an NST regimen of cyclophosphamide (200 mg/kg) and CAMPATH-1H (90 mg) followed by an unselected HLA-matched sibling peripheral blood stem cell transplantation. CAMPATH-1H is a potent in vivo purging agent that not only eliminates donor T cells but also antigen-presenting cells (B cells and dendritic cells), markedly decreasing the risk of GVHD even for unmanipulated grafts. One patient has been treated and shows a stable mixed chimera with gradually improving skin scores over 8 months. She has had no GVHD or infections (unpublished).

Summary of allogeneic NST for autoimmune diseases

Mixed chimerism in patients with malignancies is associated with disease relapse. In contrast, in the context of autoimmune diseases, allogeneic NST trials that are designed to achieve mixed chimerism without GVHD may be capable of durable disease remission. While early results are encouraging, experience with more patients and longer follow-up is needed.

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