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Autologous Stem Cell Therapy for Treatment of Severe Inflammatory Autoimmune Diseases

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Introduction

Since 1982 the clinical literature contains sporadic case reports of the transfer of autoimmune disease (AID) and allergic disorders with donor bone marrow in patients treated for malignancy or aplastic anemia. These adoptively transfered AID include: thrombocytopenic purpura, thyroiditis, diabetes type I, celiac disease and myasthenia gravis (1). Similar transfer of disease with donor bone marrow has been reported in a variety autoimmune disease models in laboratory animals.

After the publication of the curative effect of bone marrow transplantation (BMT) in fully developed experimental arthritis (2), the records of long term survivors of patients with leukemia or aplastic anemia were searched for patients with coexisting AID. This revealed a total of 22 patients suffering from various different AID,who experienced a cure of their autoimmune disorder (1,35,43). The significant mortality associated with allogeneic BMT (47) has however precluded the clinical application of this modality in severe AID in spite of the high morbidity and mortality among refractory patients (3). Our finding that experimental autoimmune diseases can also be cured with autologous BMT (38,42) has paved the way for clinical studies, because the risk of autologous BMT is much less. In the following we shall discuss the experimental dat that provide useful leads for clinical treatment protocols.

Genetic factors and role of the hemopoietic stem cell

For evaluation of the predictive value of autoimmune diseases in laboratory animals, an analysis of the causes and nature of the human diseases is required. Many if not all of the human AID are T cell initiated or mediated. In the majority, the presence of auto-antibodies in the serum of the affected subjects is now considered to be an

associated- or epiphenomenon. The activation of T lymphocytes against self-antigens may be induced by the release of an excessive amount of tissue specific antigens to which the organism has not developed tolerance during development, as in the case of sympathetic ophthalmia. For the majority of the human AID however, the inducing agent is unknown. Epidemiological research has not been able to identify specific triggers suggesting perhaps that exposure to such antigens is universal and that affected individuals have an unusally high susceptibility, a predisposition that is genetically determined. Numerous reports describe the linkage of certain MHC genes of both the HLA and the D/DR regions with specific AID, but the linkages are far from absolute (4). Also, concordance in monozygotic twins is limited: in MS it is 20-30%, and in RA 11% (5), indicatig that both genetic and environmental factors are involved.

The animal models of AID are of two distinct categories: the spontaneous or hereditary types and the induced forms. In the first category the disease develops spontaneously in a large proportion or in all of the individuals of a particular inbred strain of mice or rats. The induced AID develop following immunization with certain antigens in certain inbred strains — the susceptible ones — and not in others, — the resistant ones. The best characterized models are adjuvant arthritis (AA) in rats and experimental allergic encephalitis (EAE) which can be induced in rats, mice and guinea pigs.The latter is currently considered to be the best model for human multiple sclerosis (MS). Susceptibility and resistance to induction are genetically determined as demonstrated bij cross breeding. The induced models for AID seem to be best suited for the experimental approach to human diseases because of their similar dual ethiology.

Having proposed this, we may turn our attention to the immune system which is instrumental in causing and maintaining the pathology of AID. Failure to abstain from or limit self-destruction may be regarded as a defect either of restrictive immune mechanisms or of suppression. The next question is then whether this occurs at the level of the lymphoid cell population, for example a dysregulation resulting from an abnormal formation — too much or not enough — of subpopulations as could be brought about by an unusual sequence or strength of stimuli. Examples are the diverse autoimmune syndrome that develops in neonatally thymectomized mice and Syrian hamsters (6) and the AID that occurs in rodents following treatment with cyclosporin A (52).

The alternative hypothesis as proposed by Ikehara (15) assumes a defect in the hemopoietic stem cell (HSE), a defect which is expressed specifically in certain lympoid cells. The latter hypothesis was inspired by experiments demonstrating the transfer of spontaneous AID to lethally irradiated animals from normal strains by bone marrow grafts from the affected strain and vice versa (*table 1*).

However, the conclusion that spontaneous AID are stem cell diseases is not wholy justified. Many of the spontaneous AID in animals can also be transferred by mature lymphocytes and it has not been demonstrated that these diseases can be transferred with highly purified stem cells.

As regards the inducible models, experiments have been performed with radiation chimeras of resistant recipients and susceptible donors and vice versa. (*table 2*). In

Table 1. Transfer (T) and prevention (P) of spontaneous AID by allogeneic BMT

Disease	AID strains	non-AID strains	T and/or P	ref.
SLE-like syndromes *(mice)*	NZB, (NZBxSJL/J)F1 (NZBxC57BL/6)F1	BALB/c, SJL/J, C57BL/6	T	7
	NZB	BALB/c, B10.D2, C57BL/Ks, DBA/2	T/P[1]	8,9,10
	MRL/lpr	C57BL/6J	P	11
	(NZWxBXSB)F1	C3H/HeN,C57BL/J	T	12
insulin-dependant diabetes mellitus *(mice)*	NOD	NON, (NODxNON)F1	T	13
	NOD	(NODxB10)F1	T	14
	NOD	BALB/c *nu/nu*	P	15
	NOD	C57BL/6, B10.BR/cd	T/P	16
(rats)	BB	BB diabetes-resistent subline	P	17
	BB	WF	P	18
'moth eaten' syndrome *(mice)*	me/me	+/+	T	19
skin fibrosis *(mice)*	tsk	+/+	T	20

the case of AA (various rat strain combinations) and EAE (rat, mouse and guinea pigs) the responses were determined by the bone marrow donor strain in other words both the susceptibility and the resistance could be transfered with the bone marrow. In the case of collagen induced arthritis (CIA) the susceptibility was transfered from Wag rats to resistant BUF rats, but the results of an experiment with mice were conflicting presumably because the resistant strain was C5 deficient. Interestingly, susceptibility to induction of EAE has been transfered to SCID mice with bone marrow grafts from susceptible mouse or rat strains. These results with inducible models — which have the most resemblance to human AID — do not argue a priori against the use of autologous bone marrow transplants for treating patients with severe AID, as will be demonstrated below.

Selection of an animal model of rheumatoid arthritis

A large variety of antigens and immunization schedules was known to induce various forms of inflammatory arthritis in susceptible experimental animals. Among the antigens are complete Freund adjuvant (CFA), collagens and streptococcal cell wall preparations.

The most widely studied are the Lewis rat and CFA, but in our hands, as in most other laboratories immunization with adjuvant induced a typically acute form of the disease resulting in resistance after remission. We then studied a variety of mouse, rat and guinea pig strains using CFA and collagen as sensitizing agents and compared

Table 2. Transfer of susceptibility (S) and resistance (R) to induction of AID by allogeneic BMT

AID	AID strains	non-AID strains	S and/or R	ref.
adjuvant arthritis				
(rats)	Lewis	F344	S/R	21
(rats)	BUF	WAG	S/R	30
streptococcel cell wall arthritis				
(rats)	Lewis	F344	S/R	21
collagen induced arthritis				
(rats)	WAG	BUF	S	22
(mice)	DBA/1	SWR	conflicting results	23
encephalomyelitis (EAE)				
(rats)	Lewis	BN	S/R[3]	24
(guinea pigs)	strain 13, (2x13)F1	strain 2	S/R	25
(rats)	Lewis	Le-R	S/R	26
(mice)	SJL/J	B10.S	conflicting results	27,28
(mice)	SJL/J	B10.S	S/R	29
(rats)	BUF	WAG	S/R[3]	22
(rats)	BUF	BN.1B	S/R	31
(rats, mice)	BUF, LEWIS, SJL/J	C.B-17-*scid/scid*	S	32

the clinical picture as well as the histopathology of the lesions. The conclusion from this extensive exploration was that adjuvant arthritis (AA) in the Buffalo (BUF) rat strain provided the best resemblance to rheumatoid arthritis in humans. In the BUF rat, a single intracutanous inoculation of Mycobacterium tuberculosis in incomplete Freund adjuvant induces within 3-4 weeks a chronic progressive type of polyarthritis in over 80% of the animals (2). The inflammation involves chiefly the distal extremities, as a chronic proliferative synovitis with pannus formation, destruction of cartilage and subchondral bone, vasculitis, pericapsular fibrosis and extensive reactive bone formation. In some animals the inflammation recedes after 10 weeks at the earliest, in others progressive inflammation continues for as long as 30 weeks by which time the experiments were usually terminated. Clincally there is swelling of the affected joints which is measured with calipers and expressed as the arthritic score: the sum of the increase in thickness of the 4 paws compared to preimmunization values. During the progressive chronic stage the inflamed joints are swollen, reddish and painfull. Following extinction of the inflammation, either spontaneously or as a result of treatment, the osseous deformities persist. Treatment with cyclosporin A of rats with the fully developed arthritis causes partial regression but the disease progresses again after discontinuation of the drug (2).

The EAE rat model

Similar experiments and procedures as used in the AA model were subsequently performed in the EAE model which was also developed in BUF rats. In contrast to the

widely employed Lewis rat, which in our hands developed only an acute and time-limited encephalitis, the BUF rat reacts to immunization with spinal cord tissue in CFA with relapsing neurological symptoms, accompanied by characteristic inflammatory lesions throughout the CNS. These can be found even before the appearance of clinical signs. With the immunization mixture of 6-12 mg of spinal cord in complete adjuvant supplemented with 0.14-0.28 mg of M. tuberculosis strain H37 RA, paralysis and paresis of the extremeties appear from day 11 onwards. By day 20 — when treatment was given — between 70 and 100% of the rats had full blown EAE. If left untreated, the neurological defects persist in some and gradually recede in others, to be followed by one or more spontanous relapses in 70 % of the rats. About 10 % of the animals die during this period. Between day 40 and 90 most of the rats enter a stable remission When reimmunized during that period 85% respond with a relapse, a rate similar to the incidence of EAE following primary immunization. The onset of these induced relapses is between 3 and 11 days after reimmunization as compared to a minimal latency of 11 days before symptoms occur after the initial immunization This difference suggests that induced relapses are analogous to a secondary immune response following sensitization, reactions which are ascribed to activation of memory cells.

We shall discuss the results of autologous BMT in AA and EAE in BUF rats together since the treatment protocols were basically similar.

Treatment of fully developed AID with BMT

In view of the transfer data described in the previous section it appeared logical to investigate if full blown AID can be cured by eradication of the lympho-hemopoietic system with high dose ablative treatment (in analogy with treatment of leukemia to be designated as conditioning) followed by rescue with allogeneic BM from a resistant strain donor. Such treatment was performed by Ikehara and Good in spontaneous AID models with remarkable success *(Table 3)*. When the disease had already developed in BXSB mice (SLE like disease with lympho-proliferation and glomerulonephritis) complete remissions were obtained with transplants of allogeneic marrow lasting for as long as 1 year after transplantation.(33). In similarly treated mice of the MLR/lpr strain (another model of SLE) the disease recurred after an initial remission and the relapses were associated with a reversal of the chimeric state (34). So far the efficacy of syngeneic and autologous bone marrow grafts has not been explored in the spontaneous disease models because such attempts are considered to be futile by the protagonists of the defective stem cell hypothesis.

In rats suffering from severe induced AA we obtained complete and lasting remission in all animals using a lethal dose of TBI and a graft of allogeneic bone marrow from a resistant strain. In control experiments we employed syngeneic BMT — that is bone marrow from healthy rats of the same BUF strain — expecting that the disease would relapse. However this was not the case. Much to our surprise syngeneic bone marrow was just as effective as allogeneic BM. Apparently, the complete remissions were follwed by a state of tolerance to the autoimmune antigens which may or

Table 3. Treatment of fully developed experimental AID with allogeneic BMT

A. HEREDITARY AID			
AI strain normal	effect of treatment donor strain (conditioning)		ref
B/W, BXSB	BALB/c nu/nu (TBI)	lasting reduction of glomerular damage and deposits of IgG and C, as well as reduction in circulating immune complexes	33
MRL/lpr	C57BL/6 (TBI)	complete and lasting amelioration of glomerulonephritis, arthritis and correction of immunological abnormalities	34
B. INDUCED AID			
AI strain	resistant donor strain (conditioning)	effect of treatment	ref
AA (BUF)	WAG (TBI)	complete remission of severe arthritis	2
EAE (BUF)	WAG, BN.1B (TBI or CY+BU)	remission-induction, prevention of spontaneous and induced relapse in 90% of the animals	31 36
CIA (DBA/1J)	BALB/c (TBI)	no remission-induction, but complete prevention of progression	37

abbreviations: AI, autoimmune; AA, adjuvant-induced arthritis; BU, busulfan; C, complement factor; CIA, collagen-induced arthritis; CY, cyclophosphamide; EAE, experimental autoimmune encephalomyelitis; TBI, total body irradiation

may not persist beyond the moment of treatment which was 4-5 weeks after the primary induction. Even when the rats rescued with syngeneic BM were reimmunized either at 24 hours or at 28 days following the BMT, relapses did not occur in any of the animals (1).

These findings led us to investigate the effects of autologous BMT following the same conditioning regimen (38). The bone marrow was harvested from the femur of arthritic rats by a surgical procedure, followed by TBI and i.v. return of their BM. This resulted in complete and lasting remission, similar to the results obtained with allogeneic or syngeneic BM.

In all subsequent studies on the curative effects of autologous marrow transplants both in the arthritis model and in EAE we used the pooled marrow harvested from syngeneic rats that suffered from the same fully developed AID as the animals to be treated. This procedure was adopted to avoid unnecessary suffering of the sick animals (polyarticular inflammation or in the case of EAE, paresis and paralysis) from the surgical procedure of bone marrow harvesting. For each experiment about 100 rats were immunized and 4 or 5 weeks later in the case of AA, 3 weeks later in the case of EAE, each animal was scored using a grading scale for the clinical symptoms and the animals were distributed over the various experimental groups and the donor

group of rats, assuring that the average score of all groups was similar. Animals without symptoms (10-20 %) were always excluded. The composition and properties of the marrow obtained in this way are identical to that of autologous BM of arthritic rats and will be designated as such.

Results of autologous BMT

Few results have been published on the treatment of full-blown experimental AID with syngeneic orr autologous BMT *(table 4)*. Such studies are useful for understanding the mechanisms of the curative effects and of relapses. Our group and that of Pestronk (39) are the only ones having explored autologous BMT, the results of which will be reviewed here.

Table 4. Results of treatment of fully developed experimental induced AID with syngeneic or autologous BMT

AID (strain)	Bone Marrow origin conditioning)	Effect of treatment	ref.
EAMG (Lewis)	autologous (CY + TBI)	reduction of a-AChR titer by CY, elimination of memory response by TBI; reimmunization induces a new primary response	39
AA (BUF)	autologous (TBI)	complete and lasting remission; no regression of excessive bone formation when treated at late stage of the disease	38
CIA (DBA/1)	syngeneic (TBI)	no remission-induction, but complete prevention of progression	37
EAE (SJL/J)	syngeneic (CY)	remission-induction, only 7% spontaneous relapses; 25% induced relapses (vs 100% in controls)	40
EAE (BUF)	syngeneic and autologous (TBI,CY/ALS+TBI)	remission-induction, 30% spontaneous relapses (conditioning-dose dependant); 45% induced relapses in syn BMT, 72% in aut BMT	41 42

abbreviations: AA, adjuvant-induced arthritis; a-AChR, anti-acetyl choline recptor antibodies; CIA, collagen-induced arthritis; CY, cyclophosphamide; EAE, experimental autoimmune encephalomyelitis; EAMG, experimental autoimmune myasthenia gravis; TBI, total body irradiation; syn, syngeneic; aut, autologous.

In both AA and EAE high dose TBI (8-10 Gy) followed by autologous BMT causes rapid and complete remission in all animals. Following this lethal conditioning, spontaneous relapses were extremely rare in AA, but occurred in 30% on the average in EAE *(table 5)*. Following allogeneic BMT from a resistant strain, the incidence of spontaneous relapses in EAE is only 5 % (36). The higher incidence after autologous BMT is not due to T lymphocytes in the autologous marrow graft, because with syngeneic BMT (derived from healthy rats of the same strain) the incidence is

also 30%. Furthermore, T cell depletion of the graft does not diminish the spontaneous relapse rate. Spontaneous relapses following treatment of EAE with autologous BMT are therefore attributed to residual T lymphocytes which have survived the conditioning. In analogy to leukemia we use the term 'minimal residual autoimmune disease'. Such cells capable of initiating a spontanous relapse are probably activated T cells. The notion that residual T cells are operative in spontanous relapses is supported by the increased relapse rate following less intensive conditioning and a decreased relapse rate following more intense conditioning such as adding anti-lymphocytic antibody to TBI. The near absence of spontaneous relapses of EAE following allogeneic BMT can at this time only be explained by assuming a reduction of residual host lymphocytes by a subclinical graft verus host reaction (in analogy with the term graft versus leukemia reaction, this postulated reaction was named graft versus autoimmunity by A.M. Marmont).

The finding that T cell depletion of the autologous graft does not reduce the incidence of spontaneous relapses does not imply that the use of unmanipulated autologous grafts is safe for human patients. First, rat bone marrow as harvested in our experiments contains ten times less T lymphocytes than human bone marrow grafts. Secondly, the addition of an excess of autologous spleen cells to the bone marrow graft increased the spontaneous relapse rate in EAE rats to 93% as compared to 30% with bone marrow alone. Thirdly, in clinical practice, bone marrow grafts have been largely replaced by mobilized peripheral blood cell grafts and these may contain up to 10 times more T lymphocytes.

In AA rats, less intensive conditioning — such as a lower dose of TBI or high dose cyclophosphamide — results in a high proportion of incomplete remissions (44). Some of these remain stable, others develop exacerbations after an interval of one or two months. These responses resemble those seen in patients with rheumatic disease who were treated with repeated medium to high dose cyclophosphamide or single high dose cyclophosphamide and rescue with autologous stem cells (53): complete as well as incomplete remissions and a high rate of recurrences. Thus, in arthritis, both the clinical and the animal data underscore the necessity of extensive eradication of the lymphocyte population for obtaining complete and lasting remission. As mentioned above, the results in EAE also advocate maximal lympho-myeloablation. In this model less intensive conditioning still induces complete remissions, but the relapse rate goes up. The available clincal results in MS patients do not yet provide information on this issue. The optimal conditioning seen in both animal models with 9-10 Gy TBI translates into a 3-4 log T cell reduction. Extrapolation to an assumed total T cell population of $3x10^{11}$ in an adult human, yields between $3x10^{7}$ and $3x10^{8}$ for the residual T cell number after optimal conditioning. A bone marrow graft may contain as many as $4x10^{9}$ and a mobilized peripheral blood cell graft up to $2x10^{10}$ T lymphocytes.This illustrates that it does not make much sense first to deplete the patient of his autoreactive T cells by severe conditioning and then to reinfuse 10 or 100 times his number of cells. This is the main argument for strongly recommending T cell depletion of the autologous graft in the treatment of all autoimmune diseases. In view of the uncertainties mentioned above, our recommendation for the clinic is to go for at least 3 log of T cell depletion for autologous bone marrow grafts and for 4

log when using autologous mobilized peripheral blood progenitor cells. An even better guideline is to set a maximum at 10^5 T cells /kg body weight for both types of autologous grafts in accordance with the recommendations formulated at the International Meeting on Hemopoietic Stem Cell Therapy in Autoimmune Diseases at Basel in 1996 (51).

In contrast to our findings in the EAE model,we failed to increase recurrences in AA rats by adding autologous spleen or lymph node cells to the bone marrow graft (44). This finding is so far unexplained, but should not be interpreted to mitigate the recommendation for T cell depletion of the graft. Animal models are not complete replicas of human disease. In the current exploratory phase of clinical application of autologous BMT a rational approach as outlined above should be taken. Furthermore, there is already some limited clinical experience in favour of T cell depletion.

Early recurrences have been reported in all of 5 patients who were treated with unmanipulated autologous bone marrow or mobilized peripheral blood cells for various autoimmune diseases (45). On the other hand, early relapses were not reported in two small groups of patients who recieved T cell depleted autologous stem cells (48,49) Out of 15 patients with the progressive form of MS treated by Fassas et al (46) with autologous blood cells, the only patient who relapsed had not recieved antilymphocyte antibodies (ALS) after the transplant (which is effective in depleting the graft of T cells in vivo).

We have also studied the incidence of relapses that can be induced by reimmunization of the cured animals.As long as the causes of relapses in human AID are unknown, we thought it of interest to collect information on both the spontaneous and the induced relapses in our animal models after treatment with BMT. The responses after reimmunization differ in AA and EAE. In the non-treated AA controls, the reimmunization was performed at 15 weeks after the primary immunization. At that time a varying proportion of the animals still suffer from severe stable or progressive inflammatory arthritis; in others the inflammation has remitted, but the osseous malformation does not regress. Reimmunization does not influence the course of the disease. We did not investigate the effects of reimmunization in AA rats treated with allogeneic BMT because allogeneic BMT is not a realistic option for the treatment of patients suffering from severe connective tissue autoimmune diseases. Firstly, because a proportion of the patients may develop chronic GvHD, the symptoms of which are hard to distinguish from the primary diseases. Secondly, because the transplantation associated risks of allogeneic BMT are still higher than those associated with autlogous BMT.

Reimmunization of the animals treated with autologous BM was performed at at 11 weeks after BMT, that is at 15 weeks after the primary immunization. When optimal conditioning schedules had been employed, a few (6%) mild relapses were recorded *(table 7)*. This low incidence precluded the design of experiments which can distinguish between minimal residual autoimmune disease and grafted T cells as the cause of these relapses. Following suboptimal conditioning, which resulted in partial remissions, reimmunization caused accellerated disease progression in 50% of the animals.

Here the obvious cause is inadequate elimination of host lymphocytes.

Table 5. Incidence of relapses after treatment of experimental AID with BMT in rats

	remission induction	spontaneous relapses	relapses after reimmunization	ref
AA (TBI 9 Gy)				
autologous	100 %	2 %	6%	44
allogeneic BMT	100 %	0 %	not done	
EAE (TBI 10 Gy)[1]				
autologous BMT	100 %	30 %	72 %	42
allogeneic BMT	100 %	5 %	11 %	36
EAMG (Cyclo + TBI 6 Gy)				
autologous BMT	100 %	N.A.	11 %	39

abbreviations: AA, adjuvant arthritis; EAE, experimental allergic encephalitis; EAMG, experimental autoimmune myasthenia gravis

Reimmunization of rats with relapsing EAE was performed when most of the non-treated animals had recoverd from their last spontanous relapse (day 40 onwards).

As described earlier, it induces a high incidence of new relapses, probably by activation of memory T lymphocytes. Interestingly, we observed 11% induced relapses following allogeneic BMT from resistant rats. The cause of these relapses is likely to reside in a small number of residual host type lymphocytes, the presence of which cannot be excluded even in apparently complete chimeras.

EAE rats treated with autologous BMT developed a high incidence (72%) of induced relapses, that is significantly more than following syngeneic BMT (44%), suggesting that both residual T cells of the host and grafted T cells contribute to induced relapses after autologous BMT.

Conclusions and recommendations for clinical application

Treatment with BMT following myelo-lympho ablative conditioning proved to be highly effective in both AA and EAE, justifying prudent exploration of this modality for treating selected patients with severe progressive AID. Candidates should be in the inflammatory phase, as the results in our animal models showed that extensive scar lesions do not regress. Because of its universal availability, and its lower morbidity and mortality, the use of autologous BM is to be preferred over HLA identical sibling marrow. An exception may be made for MS patients in view of the much lower incidence of relapses following allogeneic BMT as compared to autologous BMT in the EAE model. Conditioning should be as intensive as is safely tolerated, to minimize residual T lymphocytes in both the inflammatory lesions, as well as elsewhere in the body. In our animal models, supralethal TBI, the combination of cyclophosphamide with a sublethal dose of TBI (4 or 5 Gy) and the busulfan/cyclophosphamide combination were about equally effective. The BEAM regimen with ALG was employed with encourageing results by Fassas et al (46) for the treatment

of MS patients with autologous bone marrow. As it is likely to be less lympho-ablative than the other regimens, additional pretransplant administration of ALS is recommended. In selecting a conditioning regimen it should be kept in mind that Pestronk et al (39) observed in his rat model of myasthenia gravis that cyclophosphamide as the sole conditioning agent was inadequate; it had to be suplemented with sublethal TBI for eradication of memory T cells.

In all cases, extensive T cell depletion of the autologous bone marrow graft is mandatory. The use of autologous mobilized peripheral blood precursors requires technology in place to remove the larger numbers of T cells down to the low level recommended.

At the time of writing 'stem cell treatment' of AID is entering the clinic (46, 48, 49, 50). We are wittnessing the development of a highly inspiring collaborative effort of hematologists with rheumatologists, neurologists, dermatologists and gastro-enterologists in the exploratien of a new field of application of bone marrow transplantation. In view of the diversity of autoimmune disease manifestations it is important to adhere to standards of stageing, treatment protocols and reporting as are being recommended by EULAR/EBMT (51).

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