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Autologous T-Cell Vaccination for Multiple Sclerosis: A Perspective on Progress

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Abstract

T-cell vaccination (TCV) is a unique approach to induce regulation that may have importance in the treatment of autoimmune diseases, including multiple sclerosis (MS). TCV employs a classic vaccine strategy of injecting an attenuated form of the disease-causing agent – in this case myelin-reactive T-cells - that have been selected and expanded from each MS donor and then re-injected after irradiation to induce protective immunity. This anti-T-cell immunity causes deletion or regulation of the pathogenic T-cells. TCV has been used successfully to prevent or treat autoimmune diseases in a number of animal models and now appears to be promising for treatment of MS.

Keywords

T-cell vaccination; multiple sclerosis (MS); clinical trials

Immunopathogenesis of MS?

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) that is characterized by progressive loss of motor and sensory nerve function and immune-mediated inflammation and demyelination [1]. Clinically, most MS patients experience multiple episodes of neurological impairment or symptoms that involve multiple regions of CNS damage. Early MS may spontaneously resolve partially or completely, but often the disease course becomes chronic and unremitting, leading to loss of ambulation and dexterity, and cognative and psychological deficits.

The most important unresolved issue in MS today is the lack of a clear understanding of what causes MS. Although Charcot described MS more than 150 years ago, its etiology remains a mystery. It can safely be said that the cause of MS remains a mystery, and insights into causation would represent a major advance in the field. Without knowledge of its cause, there can be no real cure for MS - only treatments that may diminish its progression. The major research effort in MS currently is directed at such disease-modifying strategies. Taken together, the current available therapies for MS have had some benefit on lesion formation and relapse rates, to be sure, but do not appear to significantly impede disease progression.

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From an imaging standpoint, MS is characterized by focal MRI changes suggesting local inflammation that can occur throughout the CNS. Histologically, perivenular inflammatory lesions involving infiltrating mononuclear cells are evident in the earlier phases of the disease, and these regions may coalesce into scarified macroscopic plaques concomitant with CNS tissue damage and disease progression. Demyelination is a hallmark feature of MS, clearly involving damage or loss of oligodendrocytes and disruption of nerve conduction and signal integration through affected regions. It is now recognized that the MS disease process results in damage and eventual death of neurons themselves [2]. Early effects on nerve cells may be reversible and functional damage may initiate compensatory conduction pathways. However, as the insult continues and nerve cells die, lost function cannot be restored resulting in disease progression [3].

In the absence of known agents or factors that are known to cause MS, the field has gravitated towards testing the assumption that MS is caused or propagated by an autoimmune process involving inflammatory T-cells directed at myelin self antigens. A working hypothesis known as molecular mimicry is that cross-reactive antigens expressed by an as-yet-unknown virus or other microorganism activate the myelin-specific T-cells that initiate an encephalitogenic cascade similar to inducible or spontaneous forms of experimental autoimmune encephalomyelitis (EAE) [4,5]. An alternative and potentially overlapping immune hypothesis is that MS results when naturally occurring myelin-specific T-cells expand to a critical pathogenic frequency due to diminished immunoregulatory mechanisms (ie. Th2, Th3, Tr1, Treg, or CD8+ T-cells)[6].

Although the autoimmune hypothesis has many attractive features, it does not fully explain the pathophysiology of MS. In particular, the frequency of T-cells responding to myelin antigens in MS patients is approximately 10-fold lower than T-cell frequencies responding to recall antigens such as Herpes simplex virus or tetanus toxoid, and are lower than would be expected based on many EAE studies. Moreover, it now appears that within each MS patient, active lesions have one of four distinct profiles [7]. Most lesions apparently result from immune-mediated myelin destruction as expected, whereas others may involve an oligodendrocyte dystrophy, perhaps induced by toxins or infection and without a strong autoimmune component. As mentioned above, it was only recently appreciated that MS involves progressive neuronal loss and a reduction in total brain volume [2]. Thus, one cannot rule out the possibility that MS is primarily a neurodegenerative disease that may or may not include a superimposed autoimmune-mediated inflammatory process directed at myelin or other CNS antigens. Viewed from this perspective, loss of neurons could be the force driving disease progression, leading to associated myelin damage. Whether neuronal damage might eminate from neurotrophic agents such as viruses or non-lethal prions is debatable, but it is now clear from the EAE models that inflammation itself (in the absence of such neurotrophic agents) can result in nerve death similar to that observed in MS [8]. This is a key observation that provides the rationale to continue the quest for effective anti-inflammatory therapies for MS, including immunomodulators.

MS may involve myelin reactive T-cells

It is probable that Th1 cells specific for encephalitogenic myelin antigens, including myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) contribute to the pathogenesis of MS [9]. Collectively, data supporting a role for myelin antigens in the pathogenesis of MS include: 1) increased frequency of MBP-, PLP-, MOG-, and α-B-crystallin-specific T-cells in the blood or cerebrospinal fluid (CSF) of MS versus control patients [10–17]; 2) increased state of activation of MBP- and PLP-specific, or other oligoclonal T-cells in the blood and CSF of MS patients [12,18–23]; 3) sporadic increases in the frequency of MBP-specific T-cells that correlate with disease activity in MS patients [24,25];

4) possible therapeutic efficacy of intervention strategies directed at MBP-specific T-cells [25–27]; 5) exacerbation of disease activity in MS patients treated with an altered peptide ligand for MBP [28]; 6) demonstrated encephalitogenicity of MBP, PLP and MOG epitopes in EAE that are also immunogenic in MS patients. Because of the extensive literature available, and the recently reported ability of MBP-specific clones to be stimulated by a variety of microbial and self antigens [29], MBP must be viewed as a useful prototypic myelin target antigen in MS, but it is now widely recognized that PLP, MOG, or other as-yet-unrecognized myelin antigens may also participate as encephalitogens. In MS patients of northern European descent, there is a disease association with HLA-DR2 and -DQw6, with different class II associations (i.e. DR3, DR4, and DR6) in other populations [30–33]. Although the role of the MHC in the MS disease process has not been fully defined, it well accepted that class II molecules, particularly HLA-DR2 [34], can present encephalitogenic myelin epitopes to potentially pathogenic T-cells. These findings provide the rationale for using TCV to target the TCRs of myelin antigen-specific T cells in MS, the subject of this review. Alternatively, disease-associated MHC class II molecules could be inefficient at presenting regulatory peptides to inhibitory T-cells that normally limit the activation of autoreactive T-cells.

Therapeutic approaches for MS

In addition to the anti-inflammatory drugs such as corticosteroids that may temporarily relieve acute symptoms of MS, treatment options for MS are currently limited to three different forms of beta-interferon, glatiramer acetate, [35] mitoxantrone and Tysabri, a humanized monoclonal antibody to VLA-4. The interferon therapies have only moderate anti-inflammatory properties, but have the added feature of inhibiting viral infections, which conceivably could play a role in MS. However, in the long term the interferons may have only limited impact on nerve damage and disease progression. Glatiramer acetate is a random oligopeptide copolymer that can induce a set of peptide-reactive T-cells with an anti-inflammatory cytokine profile, and the moderate success of this drug may derive from a bystander suppression mechanism mediated by secreted cytokines, as well as recent claims of neuroprotective and neurogenic activity. Mitoxantrone has strong anti-proliferative activity and may mediate its moderate effect in advanced MS patients by inhibiting broad-spectrum T- and B-cell expansion.

The most recent drug available to MS patients who have failed on other therapies is Tysabri® [36,37]. This drug blocks attachment of peripheral immune cells to the CNS vascular endothelium, and thus greatly inhibits entry of myelin-specific T-cells and recruited inflammatory cells from the periphery into the CNS. Tysabri® has more potent therapeutic activity compared to the other available therapies, especially in preventing relapses, but its long-term impact on MS remains to be determined. Exclusion of immune cells into the CNS in MS patients treated with a combination of Tysabri® and Avonex® resulted in progressive multifocal leukoencephalitis (PML) in ~1/1000 patients, thus limiting its applicability to a subset of patients. This being said, the increased efficacy shown by Tysabri® is yet another indication that blockade of immune-mediated inflammation is likely to be a fruitful avenue for future drug development in MS. Notably, success in early phase MS trials with the drug fingolimod (FTY720), a spingosine-1-phosphate receptor modulator, may be due to its relatively unique ability to inhibit the number of circulating CD4+ T-cells and B cells that escape from the spleen and other peripheral lymphoid organs [38].

There now appear to be a number of different approaches in development that target T-cell activation through the TCR, including tolerance induced with soluble antigen, peptide/MHC complexes, altered peptide ligands, antigen-based DNA vaccines, monoclonal antibodies to costimulatory molecules and inflammatory cytokines, TCR peptide therapy, and the focus of this review - T-cell vaccination (TCV) to attenuated myelin-reactive T-cells, recently reviewed [39]. Collectively, these approaches hold the promise of selective inhibition of

inflammation initiated by key T-cell specificities without broad-based immunosuppressive side effects.

Rationale for TCV

Like the classic notion of inducing immune recognition of attenuated pathogenic organisms (ie. smallpox), TCV involves vaccination with attenuated T-cells that are specific for myelin peptides and as such would potentially be encephalitogenic in humans without the attenuation step. Although such cells bear self-proteins, it is now well-accepted that tolerance to self can be broken relatively easily after exposure of the immune system to rare or altered peptide sequences. In many cases, self proteins are already being recognized by immunocytes or antibodies, but with a non-inflammatory outcome. Thus, injection of attenuated myelin-specific T-cells might be expected to induce processing of self components by macrophages, dendritic cells and B cells, leading potentially to a wide variety of T-cell specificities that might regulate the targeted T-cells. This concept was introduced by Cohen and colleagues in 1981, who demonstrated protection and therapy of EAE using activated and irradiated MBP-specific T-cell clones [40]. The responsive regulatory cells included those directed at the unique T-cell receptor on the targeted pathogenic T-cells (anti-idiotypic)[27] and those reactive to activation antigens (anti-ergotypic)[41]. The specific importance of anti-TCR regulation was demonstrated by us and others using defined TCR peptides from MBP-specific T-cells to inhibit and treat EAE and MS [25,42–44], and involvement of ergotypic regulation to the activation molecule, CD25 (IL-2R), was demonstrated after TCV by Zhang and colleagues [45].

Vaccine preparation

The basic approach for preparing the TCV is to stimulate blood PBMC's or CSF mononuclear cells with antigen, and then expand the cells specific only for the selecting protein or peptide until sufficient numbers of cloned T-cells are available to vaccinate [46]. PBMC or CSF cells are seeded at-cell concentrations sufficient to produce positive proliferation responses in the presence of the chosen antigen. Wells which are proliferating (a split well approach is used to assess uptake of 3H-Tdy) to antigen are expanded in IL-2 and then periodically restimulated with the same antigen into T-cell lines that are then cloned by limiting dilution. These cloned cells are stimulated by phytohemagglutinin (PHA) in the presence of additional autologous antigen presenting cells (typically irradiated PBMC), followed by more IL-2 expansions. When a sufficient number of cells are recovered, cells are activated (with antigen or PHA) and attenuated using radiation exposure (6–12,000 Rads) to prevent proliferation after injection into the patient. The beauty of the TCV approach is that the methods for preparing the cell vaccine are highly refined and generally consistent from laboratory to laboratory. One exception, however, is that some studies employ pure T cell clones, whereas others use short term T cell lines that may retain T cell clonotypes reactive to non-selected but potentially important host protective determinants. It is possible to determine at each step if the cells in each responsive well are specific for the selecting antigen, the composition of CD4+ vs. CD8 + responder cells, the identity of the TCR BV and AV chains and the actual sequences of the CDR3 that define clonality, the degree of expression of activation markers, the cytokine profile present in culture supernatants, and other discriminatory features of interest. Moreover, comparison of several different clonal populations can determine if a response is oligoclonal to the peptide antigen of choice. Finally, this sort of analysis lends itself well for determining if vaccination reduces the frequency of the targeted antigen-specific T-cells in the recipient and what are the effects of TCV on non-targeted clonal specificities that were not included in the vaccine.

Initial studies typically used one or two dominant T-cell clones to a defined antigenic sequence of MBP. However, given the number of myelin reactive specificities that might be present in a given MS patient, later procedures incorporated multiple antigenic peptides, whole purified myelin proteins or whole myelin in the antigen cocktail used for selection and expansion of lines and clones to be included in the vaccine (See Table 1). These strategies broadened coverage of potentially encephalitogenic T-cell clones reactive to many myelin determinants. With this approach, it will be important to verify which of the included determinants induce significant responses with pre-vaccination screening, a particular concern when using DR2-binding peptides in DR2 negative patients. Yet another strategy was to expand activated CD4 + T-cells present in CSF with IL-2, in order to allow emergence of naturally induced T-cell specificities that resided closer to the target organ. The expanded T-cells could then be characterized for response to known myelin antigens, TCR expression, and cytokine profiles.

Clinical outcome measures

There are now reports from at least seven different early stage clinical trials using TCV in MS patients, with six additional trials in progress or recently completed but not yet reported (recently reviewed by Hellings, Raus and Stinissen [46]). These drug development trials have largely been focused on treatment variables (cell number, dosage and composition of the T-cell vaccine, source of myelin-reactive T-cells from blood or CSF, patient subtypes), with only the most recent ones being designed to demonstrate definitive proof of the efficacy of the approach in treating MS. However, from the published reports, there are many hopeful signs to suggest that at least some subtypes of MS patients are being helped by the TCV approach.

The Initial landmark pilot studies conducted in Belgium involved 8 MS patients (5 RR-MS, 2 SP-MS, 1 PP-MS) that received 3 s.c. injections of spaced by 2–4 months of 15 million activated, irradiated MBP-specific T-cell clones [47]. The vaccination was well tolerated, with no adverse effects except skin redness at the injection site. Remarkably, responses to MBP decreased to background in all vaccinated patients, concomitant with a reduction in the rate of relapses and stabilization of disease scores and brain lesions. In three of the vaccinated patients, MBP-reactive T-cells of different clonal origins reappeared after 18–22 months coincident with relapses and increased brain lesions in two patients [48]. Later studies determined that the new MBP specificities could be regulated successfully by additional vaccinations [49]. These studies were followed by an evaluation of TCV effects on 49 MS patients from Belgium and 54 patients (28 RR-MS and 26 SP-MS) from Houston, Texas [50,51]. These studies confirmed the consistent reduction of MBP-specific T-cells after TCV and prolonged time to progression in both RR-MS and SP-MS patients compared to pre-vaccination rates.

Using bovine myelin selected T-cells for vaccination every three months over 2 years, Correale and colleagues in Los Angeles, CA, followed four SP-MS patients for an additional year for clinical and immunological changes [52]. Clinically, two vaccinated patients remained stable by EDSS and ambulation index, one improved and one progressed. Immunologically, T-cells specific for whole myelin or selected myelin peptides decreased 80–90% in all patients after 8–15 months of vaccination. This trial was followed by a blinded study using bovine myelin-stimulated T-cells to vaccinate 84 SP-MS patients in Los Angeles (completed but not yet reported), and an ongoing trial using hydrolyzed bovine brain white matter as antigen in 40 SP-MS patients in Buenos Aires, Argentina.

In Israel, Achiron and colleagues vaccinated 20 RR-MS patients who were non-responsive to beta-interferon, glatiramer acetate or intravenous immunoglobulin therapy with 3 doses of ≤60 million irradiated T-cells that had been selected against a panel of MBP and MOG peptides [53]. The results of this study showed that vaccinated patients had a significant reduction in annual relapse rate and MRI activity one year after the last TCV injection when

compared with one and two-year pretreatment values. These promising results spurred additional TCV trials using T-cells selected with MBP, PLP and MOG peptides to treat 80 probable MS patients in Tel Hashomer, Israel (in progress). In another phase I/II study initiated in Jerusalem [54], 30 RR-MS and PR-MS patients were enrolled, 20 receiving TCV and 10, placebo. TCV was composed of MBP, MOG and PLP specific T-cell lines. Patients received 30 million cells, 10 million of each line (see Table 1 for details).

Finally, three clinical studies using CSF-derived T-cells have been carried out. The initial study using CSF-derived T-cells from four progressive MS patients as a vaccine observed that vaccination was safe and induced short term immunosuppression [55]. The second study by Van der Aa et al. involved TCV with three injections of 10 million IL-2 expanded CSF cells from 4 RR-MS and 1 SP-MS patient [56]. All five patients in this pilot trial remained clinically stable or improved by EDSS, with no relapses over a one-year followup period. Anti-myelin T-cell responses remained low or were reduced in all patients. These encouraging preliminary results led to a double-blind placebo-controlled trial involving 29 early stage RR-MS patients that has now been completed but not yet reported (see Table 1).

Characterization of vaccine-reactive T-cells

Vaccination with at least 10 million whole attenuated T-cells results in the expansion of a second set of T-cells specific for both clonotypic and ergotypic determinants. Anti-clonotypic T-cells have been shown to consist of both CD4+ and CD8+ T-cells, with fine specificity for hypervariable TCR regions, including both CDR2 and CDR3 determinants that likely are self presented by the targeted myelin reactive clones [44]. The CD8+ T-cells may be cytotoxic and/or inhibitory for CD4+ target T-cells, thus causing deletion or inhibition of the vaccinating T-cell clones [57]. The CD4+ T-cells are the major cytokine producers, and secrete a variety of cytokines, including IL-4 and IL-10, that may exert regulatory effects on the potentially pathogenic Th1 cells selected for the TCV [50,58]. Moreover, the regulatory CD4+ subset specific for the vaccinating T-cells may include FoxP3+ Treg cells that inhibit the activation of target T-cells non-specifically through T-T interactions [45,59,60].

Given the use of whole cell vaccines comprised of activated T-cells, there may be many potential ergotypic determinants and many anti-ergotypic cell types involved. Both heat-shock proteins and the cytokine receptors, IL-2R and TNFR, have been identified as two important ergotypic targets, but conceivably there may be many more such cellular determinants that become immunogenic under TCV conditions [41]. In addition to CD4+ and MHC class I-restricted CD8+ cells, HLA-E-restricted T-cells, TCRγδ+ T-cells, CD4-CD8- T-cells, FoxP3 + Treg cells and NK cells have been potentially implicated in recognition and reactivity to ergotypic determinants on activated T-cells [46]. In one report by Hong and colleagues [45], all anti-vaccine reactivity mediated by CD4+ TCRαβ+ T-cells was directed against epitopes derived from the CD25 (IL-2R) molecule, indeed implicating ergotopes as major contributors to the TCV mechanism. It is noteworthy that antibodies to clonotypic or ergotypic determinants have rarely been observed in TCV-treated MS patients [50], with the exception of a single report by Hong and colleagues [61].

Effects on target and bystander cells

The major effect of TCV is to delete or down-regulate activation of the targeted T-cells. The disappearance of T-cell clones used in the vaccination procedure has been one of the most consistent findings of the various studies [46]. Regulatory properties of non-CD8+ T-cells can be demonstrated in vitro, including TCR-reactive CD4+ regulatory T cells, and it is likely that such regulation also takes place to some degree in vivo after TCV [57]. The secretion of regulatory cytokines and the recognition of ergotypic determinants would clearly be expected to have substantial inhibitory effects on bystander T-cells that express activation markers but

different clonotypic sequences in their TCR. This bystander suppression may be important in modulating disease particularly during the vaccination period and shortly thereafter, but the evidence showing appearance of new specificities of myelin reactive T-cells after 1–3 years argues that at best, regulatory effects of TCV on bystander T-cells is temporal. This limited effect may be related to the reported poor growth characteristics of anti-ergotypic T-cells.

Strengths and weaknesses of the TCV approach

Studies of TCV have produced major advances in our understanding of the frequencies and functions of myelin-reactive T-cells in MS. The process of identifying potentially encephalitogenic T-cell clones for use in the TCV preparation has elevated the art of growing and expanding low frequency (on the order of 1 cell/million or less) T-cell clones from blood and CSF. Most importantly, longitudinal studies of a number of TCV treated MS patients have established that the disappearance or regulation of the targeted clones often results in a reduced relapse rate and clinical stability or improvement, at least temporarily, in a majority of patients. On the other hand, not all TCV-treated patients experience clinical benefit from the procedure, suggesting the wrong choice of vaccinating clones or multiple pathogenic processes that do not include a strong inflammatory component in those particular MS patients. Thus, it may be that TCV will be most effective when administered during the inflammatory phase of MS in patients with a clear T cell response to immunodominant determinants. Generally, the TCV procedure appears to be quite safe, with the attenuation step used in both animal and human studies effectively preventing encephalitogenic activity of injected myelin-reactive T cells. However, a potential down-side of using activated whole T-cells is the possible recognition of ergotopes that may induce immuno-enhancing rather than regulatory effects on the targeted T-cells. If indeed, there is a net effect that depends on enhancing vs. inhibitory effects of TCV, it may be necessary to utilize component molecules or epitopes, such as T-cell receptor determinants, that consistently inhibit autoimmune responses.

Overall, however, these are indeed impressive studies that lend direct support to the involvement of inflammatory myelin-reactive T-cells to the MS disease process in some patients. However, the trends reported in the many pilot studies reviewed above need to be validated by similar results in double-blind, placebo-controlled trials. These trials are now in progress and will soon reveal whether the current state-of-the-art in TCV can exert a net positive effect on the course of MS. It is also conceivable that the double blind trials will show a significant but not a pronounced effect on the progression of MS. If this should occur, one must then consider that there may be additional destructive processes at work that are relatively unaffected by this elegant approach to specifically regulate inflammation directed at myelin antigens.

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Table 1

T-Cell Vaccination Trials for MS

	TCV center	Subjects	Clinical phase	Cell type	Cell number	Regime	Outcome
1	Harvard Medical School, Boston, USA. [55]	P-MS (n=4)	I	CSF-derived $CD4^+$ T-cell clones	Not found	7 injections	Partial short-term immunosuppression
2	LUC, Diepenbeek, Belgium. [47]	RR-MS (n=5) PP-MS (n=1) SP-MS (n=2)	I	Blood derived T-cell clones specific for MBP	2–4 clones (Each clone 15×10^6)	3 injections every 2–4 months	Reduced relapse rate, reduced MRI lesion load as compared to matched controls
3	LUC, Diepenbeek, Belgium. [50]	RR-MS (n=49)	I	Blood derived T-cell clones specific for MBP	Maximum 6 clones (Each clone 10×10^6)	3 injections every 2 months	
4	Baylor College, Houston, USA. [51]	RR-MS (n=28) SP-MS (n=26)	II	MBP peptides	2–4 clones (Each clone 30×10^6 – 60×10^6)	3 injections every 2 months	40% reduction in relapse rate; stabilization by MRI and EDSS (RR>SP)
5	HSC, Los Angeles, USA. [52]	SP-MS (n=4) SP-MS (n=80)	I II	Whole bovine myelin selected T-cells	40×10^6	During 24 months injection every 3 months	EDSS, 2-stable; 1-worse; 1-better
6	Buenos Aires, Argentina. (Correale et al.)	CP-MS (n=40)	I	Hydrolyzed bovine brain white matter as antigens			ongoing
7	Hadassah Hospital, HBRC, Jerusalem, Israel. [54]	PR-MS, RR-MS (n=30)	I/II	Lines of myelin peptides: MBP, MOG, PLP	30×10^6 ($10\times10^{6/}$line)	4 injections at months 0,1,3,6	Completed Summary in progress
8	Tel Hashomer Hospital, Ramat-Gan, Israel. [53]	RR-MS (n=20)	I	MBP and MOG peptide lines	1.5×10^7 cells/line, up to 5 lines	5 injections every 2 months	Reduction in relapse rate, decreased rate of progression to disability, stabilization of MRI
9	LUC, Diepenbeek, Belgium. [56]	RR-MS (n=4) SP-MS (n=1)	I	CSF-derived activated $CD4^+$ T-cells, IL-2 expanded	$*10\times10^6$	*3 injections every 2 months	No relapses during or after treatment; clinically stable or reduced EDSS
10	LUC, Diepenbeek, Belgium. (Stinissen et. al.)	RR-MS (n=29)	II	CSF-derived activated $CD4^+$ T-cells	*as above	*as above	Ongoing

	TCV center	Subjects	Clinical phase	Cell type	Cell number	Regime	Outcome
11	Tel Hashomer Hospital, Ramat-Gan, Israel. (Achiron et. al.)	Probable-MS (n=80)	I/II	MBP, PLP and MOG peptide derived lines	1–1.5×10^7 cells/line, up to 5 lines	5 injections, 3 every 6 weeks +2 boosts on weeks 24 & 28	Almost completed