

**Hematopoietic Stem Cell Therapy for Patients with Inflammatory  
Multiple Sclerosis Failing Alternate Approved Therapy: A Randomized Study**

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## 1.0 PRECIS

Multiple sclerosis (MS) is at onset an immune-mediated demyelinating disease. In most cases, it starts as a relapsing-remitting disease with distinct attacks and no progression of symptoms between flares. Over years or decades, most cases transition into a progressive disease in which at least insidious and slow neurologic deterioration occurs with or without acute flares. Relapsing-remitting disease is often responsive to immune suppressive or modulating therapies, while immune based therapies are generally ineffective in patients with a progressive clinical course. This clinical course and response to immune suppression, as well as neuropathology and neuroimaging studies, suggest that disease progression is associated with axonal atrophy. Disability correlates better with measures of axonal atrophy than immune mediated demyelination. Therefore, immune based therapies, in order to be effective, need to be started early in the disease course while MS is predominately an immune-mediated and inflammatory disease. While current immune based therapies delay disability, no intervention has been proven to prevent progressive disability. We propose, as a randomized study, autologous unmanipulated Peripheral Blood Stem Cell Transplant (PBSCT) using a conditioning regimen of cyclophosphamide and rATG versus FDA approved standard of care (i.e. natalizumab (Tysabri), fingolimod (Gilenya) or Dimethyl fumarate (Tecfidera or BG-12) in patients with inflammatory (relapsing) MS despite treatment with alternate approved therapy.

## 2.0 OBJECTIVES

To assess the efficacy of autologous PBSCT versus FDA approved standard of care (i.e. natalizumab, fingolimod, or tecfidera) for inflammatory MS failing alternate approved therapy. The endpoints to be considered in this study are:

### 2.1 Primary Endpoint:

Disease progression, defined as a 1-point increase in the Expanded Disability Status scale (EDSS) on consecutive evaluations at least 6 months apart and not due to a non-MS disease process. Patients will be followed for 5 years after randomization.

### 2.2 Secondary Endpoints:

1. Number of relapses, defined as acute neurologic deterioration occurring after engraftment and lasting more than 24 hours, accompanied by objective worsening on neurological examination that are documented by a neurologist and not explained by fever, infection, stress or heat –related pseudoexacerbation. Supportive confirmation by enhancement on MRI is preferred but not mandatory.
2. Ambulation index
3. Twenty-five foot timed walk
4. Nine hole PEG test
5. PASAT- 3 second and PASAT - 2 second
6. MSFC
7. MRI enhancing lesions and T1 and T2 burden of disease per MRI-AC MRI protocol
8. SF-36
9. Scripps NRS
10. Survival

### 3.0 RATIONALE AND SCIENTIFIC JUSTIFICATION

Hematopoietic stem cell transplantation (HSCT) was proposed as a treatment for multiple sclerosis (MS) in 1995 based on results of HSCT for experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Since that time, more than two hundred patients with MS have undergone HSCT worldwide. These initial trials helped to clarify treatment feasibility and toxicity and to identify the subset of MS patients likely to benefit from HSCT. Three variables appear important in minimizing toxicity and predicting benefit from an autologous HSCT in patients with MS: selection of patients who are still in its inflammatory phase characterized by acute relapses and gadolinium enhancing lesions on magnetic resonance imaging (MRI), treatment early in its course before onset of significant irreversibly progressive disability, and use of an intense immune suppressive but non-myceloablative conditioning regimen.

#### *Classification and pathophysiology of multiple sclerosis*

The usual clinical course of MS is initially relapsing and subsequently progressive, although a combination of the two or progression from onset occurs in a minority of patients. Relapsing remitting MS (RRMS) is punctuated by acute neural dysfunction that resolves completely or partially without baseline deterioration between acute attacks. Secondary progressive MS (SPMS) manifests as steadily worsening neurologic baseline in patients with prior RRMS. Neural function in primary progressive MS (PPMS) deteriorates progressively from onset without identifiable acute relapses, whereas relapsing progressive MS (RPMS) has intermittent acute attacks in addition to slow progressive deterioration. At onset, approximately 15% of cases are progressive, and 85% relapsing remitting. Given a long enough time interval that varies between individuals, most cases of RRMS evolve into SPMS (1-5).

In analogy to the dual clinical presentation, relapsing or progressive, MS has dual pathology involving both demyelination and axonal degeneration (6-7). In animal models, the initial events of immune-mediated inflammatory demyelination may be triggered by immune response to autoantigens or virus infection (7) and consists of (multi)focal infiltration by activated lymphocytes and macrophages against oligodendrocytes and myelin-sheath components, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). While PPMS may be dominated by axonal degeneration and little inflammatory activity from onset, RRMS is thought to be predominantly an immune-mediated demyelinating disease that with time transforms into SPMS dominated by irreversible axonal degeneration (8). The mechanism(s) by which immune-mediated demyelination induces axonal degeneration is incompletely understood (8, 9). Since the frequency of axonal transections correlate with the degree of inflammation within a plaque (10), axons may be injured by cytokines, proteases, or nitrous oxide produced during ongoing or recurrent inflammatory demyelination (8, 11, 12). Alternatively, demyelination may also lead to axonal atrophy through loss of oligodendrocyte-mediated trophic support of axons (13).

Neurons cultured with oligodendrocytes, oligodendrocyte progenitor cells (OPC), or oligodendrocyte-cultured media have improved survival indicating that oligodendrocytes produce trophic factors, such as insulin-like growth factor 1 (IGF-1) that are required for neuronal growth (14). For invertebrates, Schwann cells in the peripheral nervous system modulate neurofilament phosphorylation and axonal transport while glial cells within the CNS transfer proteins from the surrounding glial sheath into axons (15). For vertebrates, less is known about communication between oligodendrocytes and axons, however, mice with the null allele for myelin proteins PLP and DM-20 develop axonal degeneration approximately 6-8 weeks after birth (16, 17). Since oligodendrocyte and neuron cross communication may be important for maintaining axonal integrity, failure of remyelination in diseases such as MS may predispose axons to late degeneration.

OPCs are responsible for remyelination (18). In order to remyelinate axons, OPCs must migrate into

plaques and differentiate into oligodendrocytes (19, 20). Although attempts at remyelination are frequently seen in early phases, it often ultimately fails (6). Remyelinated plaques, termed shadow plaques (21), have a diminished ratio of myelin thickness to axon diameter and shorter internodal lengths. Possible explanations for partial or absence of remyelination include immune-mediated or normal physiologic exhaustion of OPC reserve, failure of OPC recruitment or differentiation into oligodendrocytes, or disrupted axon/glia communication (19, 22-25).

Disrupted axon / oligodendrocyte communication may occur via Jagged 1 / Notch 1 or polysialylated-neural cell adhesion molecule (PSA-NCAM). Jagged 1, a ligand for Notch 1 receptor, is expressed on astrocytes in MS plaques and by binding to Notch 1 receptors on OPCs could inhibit OPC differentiation into oligodendrocytes (26). PSA-NCAM is expressed on neurons and inhibits myelination of growing axons (27). PSA-NCAM is absent in the normal adult brain but re-expressed on axons within demyelinated plaques which may also contribute to remyelination failure (28).

If immune-mediated demyelination initiates the process leading to late axonal degeneration then early and intense immune suppression to halt or at least significantly delay the natural evolution of demyelination may be essential to prevent progressive MS and permanent disabilities (10). In contrast, delayed immune suppression in predominately progressive disease driven by axonal drop out would not significantly alter the natural history of MS.

#### *Rationale of HSCT*

The current therapies for MS consist of immune modulating agents such as interferons and glatiramer acetate, and anti-inflammatory and immune suppressive drugs such as glucocorticoids, methotrexate, mitoxantrone, cladribine, and cyclophosphamide (29-34). Autologous HSCT maximizes immune suppression to the point of transient immune ablation. In theory, the transplant conditioning regimen ablates the aberrant disease causing immune cells while hematopoietic stem cells (HSC) regenerate a new and antigen naive immune system. The de novo development of the T and B cell repertoire from uncommitted progenitor cells in the presence of autoantigens is thought to reintroduce self-tolerance similar to the normal ontogeny of the immune system during fetal development.

HSC are collected prior to starting the conditioning either from the patient (autologous HSCT) or from another individual (allogeneic HSCT). Similar to autologous HSCT, after an allogeneic HSCT, reconstitution of a naive immune system will occur from the stem cell compartment. However, allogeneic HSCT by providing stem cells from a disease resistant donor will also alter the patient's genetic predisposition to disease susceptibility. Since allogeneic HSCT of malignancies has been traditionally complicated by relatively high mortality from graft versus host disease (GVHD), to date all reported HSCT for multiple sclerosis have utilized autologous HSC (35,36).

#### *Animal results*

EAE is an autoimmune demyelinating disease of the central nervous system induced by active immunization with peptides of myelin proteins such as PLP or MBP. EAE may also be adoptively transferred to a healthy recipient by injection of primed CD4<sup>+</sup> T cells from an immunized animal. In animal transplants, autologous HSCT is impractical since it would require amputation of the mouse's leg to collect sufficient marrow cells. For this reason, HSC are acquired from a euthanized animal of a different animal strain (allogeneic HSCT), of the same highly inbred strain (syngeneic HSCT) or from a syngeneic animal with the same stage of disease (pseudo-autologous HSCT). All three donor HSC sources, allogeneic, syngeneic, or pseudoautologous, are capable of inducing remission and preventing relapse when performed either before disease onset but after environmental exposure (immunization or adoptive transfer) or early after disease onset during peak of disease or 1<sup>st</sup> remission (37-42). In contrast,

HSCT is not effective therapy for late stage or chronic progressive EAE (37). HSCT is, therefore, effective in relapsing EAE (R-EAE) but not progressive or chronic EAE. In retrospect, EAE may be a reliable and predictive model for HSCT outcome in MS.

Although not directly compared against each other in the same study, in EAE, responses appear similar for a high dose immune suppressive but non-myeloablative regimen of cyclophosphamide compared to a myeloablative regimen of total body irradiation (TBI). However, these regimens may have different acute and or chronic toxicities. Van Bekkum et al reported that TBI conditioning could cause neurologic exacerbations in EAE (43). In contrast, a high dose cyclophosphamide conditioning regimen has not been reported to exacerbate EAE (43). Due to the expense of animal care, long-term follow-up of animals after HSCT has not been performed. As a result, the effect of intense immune suppressive conditioning regimens on CNS stem cell compartments, remyelination, late axonal degeneration, and long-term disability has not been evaluated in animal models.

Theiler's murine encephalomyelitis virus (TMEV) induces a CNS demyelinating disease manifest at onset as progressive neurologic deterioration (44). TMEV is a small RNA virus (picornavirus) acquired in the wild by oral inoculation while in the laboratory infection is via direct intracerebral inoculation resulting in a higher proportion of diseased animals (45). Disease resistant strains of mice clear the infection within two weeks of infection, while disease susceptible strains have a persistent CNS infection. Both virus – and myelin-specific T cell responses occur in TMEV induced demyelinating disease (45, 46). Unlike the beneficial effect of HSCT on R-EAE, syngeneic HSCT of TMEV-infected mice results in exacerbation of neurologic disability and high mortality due to CNS viral hyperinfection following immune ablation (44). HSCT using marrow from disease resistant but previously infected donors ameliorated HSCT-related neurologic mortality, presumably by transfer of virus specific cytotoxic T cells along with the marrow graft (36, 44). Therefore, a functional immune system appears important to prevent lethal neuropathic effects from a persistent viral-induced CNS demyelinating disease. Since several hundred patients with MS have undergone HSCT worldwide without experiencing viral encephalomyelitis, it is unlikely that patients with MS harbor a persistent neuropathic viral infection.

In summary, animal models such as EAE and TMEV-induced demyelinating disease suggest that: 1) MS is an autoimmune initiated disease similar to EAE and not a viral-related demyelinating disease akin to TMEV. 2) To be effective HSCT should be performed in the relapsing phase of MS while it is still an immune mediated inflammatory process rather than in its chronic progressive phase when axonal degeneration predominates. 3) An intense immune suppressive but non-myeloablative regimen without potential cytotoxicity to marrow, neural, or oligodendrocyte progenitor cells would be the preferred conditioning regimen.

#### *Mobilization of stem cells from patients with MS*

Originally, hematopoietic stem cells were collected from the posterior superior iliac crest of bone marrow by repeated aspirations performed under epidural or general anesthesia. Subsequently, in order to avoid the discomfort associated with multiple bone marrow punctures and requirement for an operating room and general anesthesia, the most common method of collecting HSC is by mobilization from the peripheral blood (47). 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 are necessary to mobilize HSC into and subsequently collect HSC from the peripheral blood.

Hematopoietic growth factors used to mobilize stem cells also have immune modulating effects and depending upon growth factor may exacerbate disease. Granulocyte colony stimulating factor (G-CSF)



may precipitate clinical flares of MS sometimes with significant and irreversible neurologic deterioration (47, 48). In analogy to MS, G-CSF as well as Flt-3 ligand and stem cell factor (SCF) exacerbate EAE while thrombopoietin (TPO) mobilizes stem cells without affecting disease severity (Verda et al. manuscript in preparation). In both EAE and MS, G-CSF induced flare may be prevented by either administration of corticosteroids or mobilization with combined cyclophosphamide and G-CSF (47-49).

HSC may be mobilized from the peripheral blood using between 5 to 16 mcg/kg/day of subcutaneous G-CSF along with 0.5 to 1.0 mg/kg/day of oral prednisone. Apheresis to collect progenitor cells begins on either day 4 or 5 of G-CSF administration. A 10-15 liter peripheral blood apheresis performed on one day is usually adequate for collection of sufficient numbers of HSC. Occasionally a consecutive second or third day of apheresis may be necessary. HSC may also be collected by administration of cyclophosphamide (2.0 to 4.0 g/m<sup>2</sup>) and daily G-CSF beginning 72 hours after cyclophosphamide. Apheresis is performed when the white blood cell count rebounds, usually 10 days after cyclophosphamide infusion (47, 50).

G-CSF mobilization is not associated with neutropenia or risk of neutropenic infections. Mobilization with cyclophosphamide and G-CSF may cause 1 to 2 days of neutropenia. Infection risk during this interval may be minimized with prophylactic antibiotics. Advantages for cyclophosphamide / G-CSF mobilization are higher stem cell yields, an in vivo purge effect by selectively killing lymphocytes in cell cycle, and a cyclophosphamide-mediated disease-ameliorating effect (47).

#### *Ex vivo stem cell selection*

The majority of mononuclear cells collected by peripheral blood apheresis (or bone marrow harvest) are immune cells such as lymphocytes and monocytes not HSCs. While the true identity of human HSCs remains elusive, purified CD34<sup>+</sup> or AC133<sup>+</sup> hematopoietic progenitor cells are sufficient for hematopoietic reconstitution. In general, a minimum number of 2 x 10<sup>6</sup> CD34<sup>+</sup> cells/kg recipient weight will ensure engraftment. HSC may be positively selected or enriched by 3 to 4 logarithms using antibodies to CD34 or AC133 or purified by negative selection to remove lymphocytes. In practice, the most common method of purging lymphocytes is via CD34<sup>+</sup> enrichment using either the Miltenyi CliniMACS or Baxter Isolex cell separator device (47). Whether enrichment of the graft for CD34<sup>+</sup> HSC is necessary or even superior to an unmanipulated graft remains unclear. CD34<sup>+</sup> selection by removing lymphocytes is perhaps best viewed as another method of immune suppression. For an intense conditioning regimen, CD34<sup>+</sup> selection may not be necessary or even detrimental by increasing the risk of post HSCT infection.

#### *Conditioning regimen*

The rationale for autologous HSCT of MS is to regenerate an antigen naïve immune system, from the patient's own hematopoietic stem cells. This will require the re-emergence of thymic educated virgin (antigen naïve) T cells from hematopoietic stem cells (35, 36). Therefore the goal of the conditioning regimen is "immune ablation" not myeloablation. The autologous transplant regimen should be based on dose escalation of immune suppressive drugs that are effective at conventional non-transplant doses. The regimen must also avoid further damage to already injured axons and oligodendrocytes. By definition, myeloablative agents are lethal to HSCs and apart from the myeloablative effect on bone marrow may be similarly lethal to tissue specific stem cells such as OPCs or neural stem cells. In animal models, 10 cGy of cranial radiation impairs mechanism of 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 (51) which raises concerns about using a TBI-based or otherwise myeloablative regimen in the treatment of MS.

Nonmyeloablative stem cell transplantation (NST) regimens that are as immune suppressive as myeloablative regimens but without myeloablative side effects may be designed by using agents or combinations of agents such as fludarabine, cyclophosphamide, anti-lymphocyte antibodies such as ATG and/or CD34<sup>+</sup> selection of the graft (50,52-57). Fever-related deterioration of neural function in MS (58), termed pseudo-exacerbations, due to conduction blocks in marginally functioning demyelinated axons should be avoided during HSCT by avoiding pyrogenic agents (59). Similarly, the risk of infection-related fever should be minimized during HSCT by use of prophylactic antibiotics.

In summary, for autologous HSCT of MS the rationale behind the conditioning regimen is to: 1) dose escalate agents that work as conventional therapy, 2) maximize immune suppression without myeloablation, 3) avoid conditioning regimen agents that may cause injury to already disease affected and damaged CNS tissue, 4) avoid injury to tissue specific stem cell compartments that may be important for CNS repair, 5) avoid pyrogenic conditioning agents, and 6) design regimens that are justified for the risk of the disease being treated.

### *Results of HSCT for MS*

Autologous HSCT appears to be an effective therapy to halt MRI lesion activity in the brain and spinal cord. In fact, there is no other therapy that may provide such a striking and long-term effect on suppressing MRI enhancing activity and new T2 lesions (60). Saiz et al. using a regimen of BCNU, cyclophosphamide, ATG, and CD34<sup>+</sup> selection of the graft reported no post HSCT enhancing lesions and a decrease in mean T2 lesion load by 11.8% (59). Mancardi et al. using a regimen of BCNU, etoposide, cytosine arabinoside, and melphalan performed triple dose gadolinium MRI monthly for 3 months before HSCT and monthly for 6 months and then every 3 months after HSCT. Complete and durably suppressed MRI activity was documented following HSCT (60).

In a European retrospective analysis on 85 patients while not yet statistically significant, (p=0.59), the progression free survival at 3 years was better in SPMS compared to PPMS (78% versus 66%) (55). In a single center study of 21 SPMS patients treated with a myeloablative HSCT regimen, disease progression in more disabled patients with a pre-treatment EDSS  $\geq$  6.0 was significantly worse compared to those with an EDSS < 6.0 (61). In fact, none of 9 patients with an EDSS < 6.0 had disease progression defined by worsening of 1.0 or more EDSS points after more than 2 years of follow-up. The one patient in this study with RRMS not only failed to progress but had a sustained improvement by 2.0 EDSS points. Equally important in supporting benefit from HSCT in inflammatory disease, two patients with pre-treatment malignant MS manifest by striking gadolinium enhancing lesions and severe deficits (non-ambulatory with EDSS scores of 7.5 and 8.0) after a short clinical duration of disease (1 and 3 years) were able to ambulate 100 and 300 meters respectively with only unilateral assistance by 6 months after HSCT (62).

Autologous HSCT is a form of intense immune suppression and thus unlikely to impact upon the non-inflammatory, i.e. degenerative aspects, of MS. In retrospect, it may be anticipated that HSCT would be most effective for early MS before onset of axonal degeneration leading to progressively higher disability scores. Results from HSCT also suggest that any form of immune suppression in patients with late progressive MS and devoid of inflammatory activity is unlikely to be effective and should only be performed in well-designed trials. HSCT is hypothesized to be axonal sparing therapy and needs to be performed before irreversible axonal degeneration predominates.

Initial studies were safety trials and as such were ethically restricted to patients with severe disability (mean EDSS score 6.5 to 7.0). Patients were also selected for rapid accumulation of irreversible disability as defined by an increase in EDSS of at least 1.0 or 1.5 points in the year prior to HSCT (50,52-55,61,63).



These trials generally utilized intense myeloablative regimens that contained busulfan or TBI in combination with other immune suppressive drugs such as ATG, bolus intravenous corticosteroids, cyclophosphamide, and/or CD34<sup>+</sup> selection or a less intense and borderline myeloablative regimen of BEAM (BCNU, etoposide, cytarabine arabinoside, melphalan) either alone or combined with ATG and / or CD34<sup>+</sup> selection. In a retrospective analysis of eighty-five patients from 20 centers across Europe, seven patients died, five from treatment related toxicity or infection and two from disease progression (55). For these phase I studies, there were no prior HSCT experience in patients with MS, and patients were selected for advanced progressive disease and significant disability. In general, the initial trials generally employed regimens previously used in HSCT for malignancies and consequently designed for myeloablation and not immune ablation.

NST regimens that are equally immune suppressive but less toxic may be performed with greater safety than myeloablative HSCT regimens. In a general review of autologous HSCT in 263 patients with various autoimmune diseases including MS, the treatment related mortality of intense myeloablative conditioning (TBI or busulfan based) regimens was four times higher than for less intense regimens with no advantage in terms of disease control (64). It is reasonable to anticipate that with appropriate patient selection and standard of care guidelines, NST for MS will have a treatment related mortality of less than 0.5 to 1.0%.

#### *Mechanism of remission*

If HSCT is only transient immune suppression, the same immune phenotype and repertoire should be preserved, although immune cell numbers will be diminished. On the other hand, if HSCT results in a new immune system, i.e. “immune reset”, the post transplant immune system should be characterized by an increase in phenotypically naïve lymphocytes, an increase in recent thymic emigrants, and differences in T and B cell repertoire distribution compared to pre-HSCT (65). Recent production of naïve lymphocytes from the thymus may be determined by T cell receptor excision circles (TREC) and/or by coexpression of phenotypic markers such as CD27 and CD45RA (or lack of CD45RO). The composition of T cell receptor (TCR) repertoires of CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets can be determined before and after HSCT by flow cytometry using a panel of TCRV $\alpha$ -specific monoclonal antibodies and/or TCR CDR3 spectrotyping analysis. Data currently in manuscript preparation demonstrate that autologous HSCT for MS results in an “immune reset” (Muraro et al. - verbal communication). These data indicate that the mechanism of action of HSCT includes immune rejuvenation or regeneration and is not a result of just transient immune suppression.

#### *Summary of autologous HSCT for MS*

Immune analysis and MRI studies confirm that HSCT is the most effective method of stopping immune-mediated inflammatory demyelination in patients with MS. Clinical trials using myeloablative regimens had little efficacy in late progressive disease (EDSS > 6.0) but stabilized those earlier in its course (EDSS < 6.0), and in the few cases of RRMS and malignant MS, manifesting as rapid and severe neurologic decline with MRI signs of inflammation, a significant neurology improvement occurred after HSCT. Therefore, future studies should focus on active inflammatory MS. Potential candidates should have relapses despite interferon and MRI markers of active lesions despite ongoing immune-modulating therapies, and mild to moderate disabilities (i.e. EDSS < 6.0). Rather than selecting for rapidly progressive disease, i.e. an increase in EDSS of 1.0 or more points in the preceding 12 months, as performed in prior studies, future patients should be selected for active inflammation. Criteria may include RRMS or RPMS with acute relapses and gadolinium enhancing MRI lesions with less accumulated disability, e.g. EDSS of 2.5 to 5.5. Patients with higher EDSS scores could be considered if they have malignant MS manifest by rapid clinical deterioration, relatively short disease duration (less than 3 years) and significant gadolinium enhancing lesion load on MRI.

NST diminishes regimen related toxicity by decreasing non-immune conditioning regimen side effects compared to conventional myeloablative transplants. NST regimens should use agents effective for inflammatory disease and without suspected axonal or OPC toxicity. One NST regimen using only drugs currently utilized to treat MS is 200 mg/kg cyclophosphamide and rATG. No CD34<sup>+</sup> selection is performed since effective in vivo purging of the graft is obtained by mobilization with 2.0 g/m<sup>2</sup> cyclophosphamide and conditioning with rATG, an antibody with a long half-life directed against T and B lymphocytes. Following NST, HSC are infused only to shorten the duration of neutropenia since immune and hematopoietic reconstitution would occur even without HSC support.

The neurology community has generally been reluctant to appreciate that stem cell therapy using HSCs to stop immune-mediated demyelination is neither theoretical nor if performed with an NST regimen unduly fraught with morbidity or mortality. NST stands on sound theoretical, scientific and empirical foundations as meaningful therapy for refractory and breakthrough MS with ominous prognosis, still showing active inflammatory demyelination and a relative absence of axonal degeneration as the cause of disabilities. While the long term durability of HSCT induced remission of active inflammation is yet to be determined, it can be performed with minimal toxicity using a NST approach, and holds promise for patients with active inflammatory disease if performed before onset of significant irreversible axonal injury. The exact role of NST in treatment of MS will require randomized trials comparing NST to standard of care for patients with active inflammatory disease without significant irreversible axonal injury.

### **Selection of High Dose Immunosuppressive Therapy and Autologous HSCT Strategy for This Trial**

#### *Selection of the Conditioning Immunosuppressive Therapy*

Cyclophosphamide (CY) is a common conditioning regimen with two decades of experience in the treatment of aplastic anemia, and has been used safely without reported mortality in the treatment of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. Cyclophosphamide is a potent immunosuppressive agent that not only has less acute toxicity, it has less chronic side effects. Cyclophosphamide is not associated with late malignancies or cataracts

To justify any new therapy such as HSCT, the risk of dying from the disease must be higher than that expected from its treatment, or the morbidities associated with the disease must justify the treatment risks. Cyclophosphamide has been used to treat several patients with systemic lupus erythematosus and approximately 80 rheumatoid arthritis patients in Australia, Europe and America, again without mortality, and has been used for longer than a decade as the conditioning regimen of choice for the non-malignant disease, aplastic anemia.

#### *Method of Harvesting Stem Cells*

Based on the experience of the pilot studies, the current protocol will mobilize stem cells with granulocyte-colony stimulating factor (G-CSF) and cyclophosphamide and collect stem cells by apheresis. A subsequent bone marrow harvest will be performed only if needed to supplement the peripheral blood stem cells (PBSC). Based on experience of autoimmune flares in patients receiving G-CSF alone for mobilization, patients will be mobilized with cyclophosphamide 2.0 g/m<sup>2</sup> and G-CSF 10 mcg /kg.

## **Cyclophosphamide**

Cyclophosphamide (CY) is an active agent in patients with a wide variety of autoimmune diseases including inflammatory MS and patients with malignancies. It is used frequently in therapy of lymphoid malignancies and has potent immunosuppressive activity. It is frequently used as a cytotoxic and immunosuppressive agent in patients undergoing marrow transplants and as a treatment for patients with autoimmune diseases. It is an alkylating agent that requires hepatic metabolism to the active metabolites, phosphoramidate mustard and acrolein. These active metabolites react with nucleophilic groups. It is available as an oral or intravenous preparation. Bioavailability is 90% when given orally. The half-life of the parent compound is 5.3 hours in adults and the half-life of the major metabolite phosphoramidate mustard is 8.5 hours. Liver or renal dysfunction will lead to prolonged serum half-life. CY is administered intravenously at a dosage of 60 mg/kg on each of two successive days (use adjusted ideal body weight if patient's actual body weight is greater than 100% ideal body weight). The major dose limiting side effect at high doses is cardiac necrosis. Hemorrhagic cystitis can occur and is mediated by the acrolein metabolite. This can be prevented by co-administration of MESNA or bladder irrigation. Other notable side effects include nausea, vomiting, alopecia, myelosuppression and SIADH. Refer to institutional manuals for more information about administration, toxicity and complications.

## **Rabbit-Derived Anti-Thymocyte Globulin (rATG)**

Rabbit-derived anti-human thymocyte globulin (ATG) is a gamma globulin preparation obtained from hyperimmune serum of rabbits immunized with human thymocytes. ATG has been used predominately in solid organ transplant immunosuppressive regimens. ATG is a predominantly lymphocyte-specific immunosuppressive agent. It contains antibodies specific to the antigens commonly found on the surface of T cells. After binding to these surface molecules, ATG promotes the depletion of T cells from the circulation through mechanisms which include opsonization and complement-assisted, antibody-dependent, cell-mediated cytotoxicity. The plasma half-life ranges from 1.5-12 days. ATG is administered intravenously at a dose of 0.5 mg/kg recipient body weight on day -5 and at a dose of 1.0 mg/kg recipient body weight on days -4 and dose 1.5 mg/kg recipient body weight on days -3, -2, -1. Unlike equine ATG, rabbit ATG does not require a pre-infusion skin test to check for hypersensitivity. Methylprednisolone 1 gram will be given before every dose of ATG. Additional medications such as diphenhydramine may be given at the discretion of the attending physician. Although rare, the major toxicity is anaphylaxis; chills, fever, pruritus or serum sickness may occur.

## **Control arm**

Patients may receive FDA approved standard therapy (natalizumab (Tysabri), fingolimod (Gilenya), Dimethyl fumarate (Tecfidera or BG-12) in the control arm. We recommend a change in therapy from that which they failed qualifying them for entry. Patients may not receive Teriflunomide (Aubagio) due to its long  $\frac{1}{2}$  life in the body (6 months to 2 years) which may complicate cross over to HSCT. The decision of specific control arm therapy will be made by attending physicians in consultation with the patient. Standard therapy choices may differ outside the United States and available formulations will also vary over time and in different countries. In all cases the standard in the country where the patient is being treated will be used.

### Tysabri® (natalizumab)

Tysabri® is a recombinant humanized IgG4K monoclonal antibody produced in murine myeloma cells. Natalizumab contains human framework regions and the complementary –determining regions of a murine antibody that binds to  $\alpha$ 4-integrin. The molecular weight of natalizumab is 149 kilodaltons.

Tysabri® binds to the  $\alpha$ 4-subunit of  $\alpha$ 4 $\beta$ 7 integrins expressed on the surface of all leukocytes except neutrophils, and inhibits the  $\alpha$ 4 family of integrins include vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated vascular endothelium, and mucosal addressin cell adhesion molecule-1 (MadCAM-1) present on vascular endothelial cells of the gastrointestinal tract. Disruption of the molecular interactions prevents transmigration of leukocytes across the endothelium into inflamed parenchymal tissue. In vitro, anti-  $\alpha$ 4-integrin antibodies also block  $\alpha$ 4-mediated cell binding to ligands such as osteopontin and an alternatively spliced domain of fibronectin, connecting segment-1 (CS-1). In vivo, Tysabri® may further act to inhibit the interaction of  $\alpha$ 4-expressing leukocytes with their ligand (s) in the extracellular matrix and on parenchymal cells, thereby inhibiting further recruitment and inflammatory activity of activated immune cells.

The specific mechanism (s) by which Tysabri® exerts its effects in multiple sclerosis have not been fully defined. In MS, lesions are believed to occur when activated inflammatory cells, including T-lymphocytes, cross the blood-brain barrier (BBB). Leukocyte migration across the BBB involves interaction between adhesion molecules on inflammatory cells and their counter-receptors present on endothelial cells of the vessel wall. The clinical effect of natalizumab in MS may be secondary to blocking the molecular interaction between  $\alpha$ 4 $\beta$ 1-integrin which is expressed by inflammatory cells and VCAM-1, which is on vascular endothelial cells. Data from an experimental autoimmune encephalitis animal model of MS demonstrate reduction of leukocyte migration into brain parenchyma and reduction of plaque formation detected by MRI following repeated administration of natalizumab. The clinical significance of these animal data is unknown.

Tysabri® was evaluated in two randomized, double blind, placebo-controlled trials in patients with multiple sclerosis. Both studies enrolled patients who experienced at least one clinical relapse during the prior year and had EDSS score between 0 and 5.0. In both studies, neurological evaluations were performed every 12 weeks and at times of suspected relapse. MRI evaluations for T1-weighted gadolinium (Gd)-enhancing lesions and T2-hyperintense lesions were performed annually.

Study 1 enrolled patients who had not received any interferon-beta or glatiramer acetate for at least the previous 6 months; approximately 94% had never been treated with these agents. Median age was 37, with a median disease duration of 5 years. Patients were randomized in a 2:1 ratio to receive Tysabri® 300 mg IV infusion every 4 weeks for up to 28 months (30 infusions).

Study 2 enrolled patients who had experienced one or more relapses while on treatment with Avonex® (Interferon beta-1a) 30 mcg IM once weekly during the year prior to study entry. Median age was 39, with a median disease duration of 7 years. Patients were evenly randomized to receive Tysabri® 300 mg or placebo every 4 weeks for up to 28 months (30 infusions). All patients continued to receive Avonex® 30 mcg IM once weekly.

The efficacy of Tysabri® alone was compared with the efficacy of Tysabri® plus Avonex®

In a Phase 3 trial of Tysabri® compared to placebo, at one year, Tysabri® was shown to reduce the annual relapse rate by 66% and new (gadolinium enhanced) MRI lesions by 92% when comparing the Tysabri® group to the placebo group of patients.

In the Phase 3 clinical trial of Tysabri® + Avonex® compared to placebo + Avonex® there was a reduction in annualized relapse rate of 54% in those patients receiving Tysabri® + Avonex® compared to those receiving placebo + Avonex®. In addition, 96% of those treated with combination of medications had no new active lesions (gadolinium enhanced) compared to 76% of those treated with Avonex® alone.

Based upon the one year data from the Phase 3 trials and previous smaller Phase 2 trials, individuals only receive benefit while receiving the monthly infusions of Tysabri®. If the infusions are stopped, the effect wears off quickly. The drug stays in the system for about 4 weeks.

### Gilenya (fingolimod)

Gilenya is an oral sphingosine 1-phosphate receptor modulator, which sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction.

In two Phase III clinical trials, Gilenya reduced the rate of relapses in RRMS over half compared both to placebo and to the active comparator interferon beta-1a.

A double-blind randomized control trial comparing Gilenya to placebo found the drug reduced the annualized frequency of relapses to 0.18 relapses per year at 0.5 mg/day or 0.16 relapses per year at 1.25 mg/day, compared to 0.40 relapses per year for those patients taking the placebo. The probability of disease progression at 24 month follow up was lower in the Gilenya groups compared to placebo. Both Gilenya doses were superior to placebo with regard to MRI-related measures (number of new or enlarged lesions on T(2)-weighted images, gadolinium-enhancing lesions, and brain-volume loss; P<0.001 for all comparisons at 24 months). A one-year study showed that Gilenya reduced relapse rates by 53% compared to beta interferon-1a.

Gilenya is generally well-tolerated. Common side effects of include headache, diarrhoea, back pain, cough and dizziness. Gilenya can rarely cause macular oedema. Its use has also been associated with potentially fatal infections and bradycardias and, more recently, a case of hemorrhaging focal encephalitis. Two patients died herpes simplex and varicella zoster infection. Fingolimod has been associated with potentially fatal infections, bradycardia and, recently, a case of hemorrhaging focal encephalitis, an inflammation of the brain with bleeding. Two subjects died: one due to brain herpes infection, and a second one due to zoster. It is unclear whether the drug was responsible for the events. Patients to be treated with Gilenya should have electrocardiogram monitoring before treatment and then continuously for the first six hours after the first dose along with measurement of blood pressure and heart rate every hour.

### Tecfidera (BG12)

Tecfidera (dimethyl fumarate) is the methyl ester of fumaric acid. It is an oral compound which has potent anti-inflammatory properties thought to be related to the induction of stress protein HO-1. There is also some evidence to suggest that Tecfidera include direct cytoprotective effects through upregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and subsequent induction of an antioxidant response.



Two phase 3 clinical trials of Tecfidera showed the treatment to be effective in patients with relapsing remitting MS. The DEFINE trial was a two year study which investigated the efficacy and safety of Tecfidera compared with a placebo. Annual relapse rates were reduced by 53% and 48% in a bid and tid dose groups respectively. Disability progression was also significantly reduced, by 38% and 34% in the bid and tid groups respectively. The study showed a considerable decrease in new or newly enlarging MRI brain lesions with those taking placebo.

The CONFIRM trial was a two year study which investigated the efficacy and safety of Tecfidera compared with Copaxone (glatiramer acetate). Annual relapse rates were reduced by 44% and 51% in the bid and tid dose groups respectively compared with a 29% in the Copaxone group. Disability progression was reduced by 21% and 24% in the bid and tid groups respectively, compared with 7% in the Copaxone group. The study showed a significant reduction (57-73%) in new or newly enlarging MRI brain lesions in those treated with Tecfidera compared with those treated with Copaxone .

## 4.0 ELIGIBILITY

### 4.1 Inclusion Criteria

1. Age between 18-55, inclusive.
2. Diagnosis of MS using revised McDonald criteria of clinically definite MS (Appendix I).
3. An EDSS score of 2.0 to 6.0 (Appendix II).
4. Inflammatory disease despite treatment with standard disease modifying therapy including at least 6 months of interferon or copaxone. Inflammatory disease is defined based on both MRI (gadolinium enhancing lesions) and clinical activity (acute relapses \*treated with IV or oral high dose corticosteroids and prescribed by a neurologist). Minimum disease activity required for failure is defined as: a) two or more \*steroid treated clinical relapses with documented new objective signs on neurological examination documented by a neurologist within the year prior to the study, or b) one \*steroid treated clinical relapse within the year prior to study and evidence on MRI of active inflammation (i.e., gadolinium enhancement) within the last 12 months on an occasion separate from the clinical relapse (3 months before or after the clinical relapse).

\*A steroid treated relapse will include a relapse that was severe enough to justify treatment but due to patient intolerance of steroids, or a history of non-response to steroids, they were offered but not used.

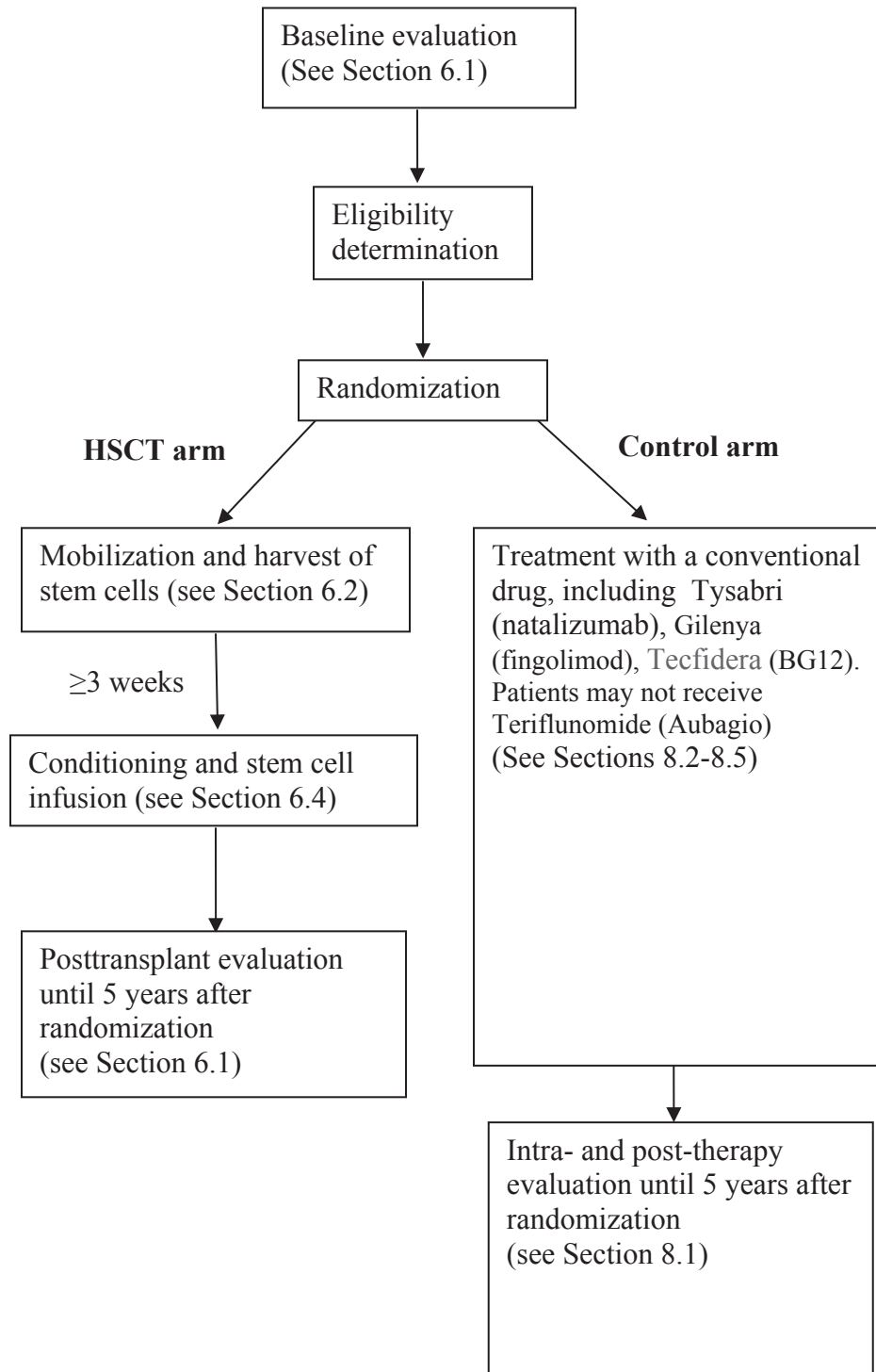
### 4.2 Exclusion Criteria\*\*

1. Any illness that in the opinion of the investigators would jeopardize the ability of the patient to tolerate aggressive chemotherapy.
2. Prior history of malignancy except localized basal cell, squamous skin cancer or carcinoma in situ of the cervix. Other malignancies for which the patient is judged to be cured, such as head and neck cancer, or breast cancer will be considered on an individual basis.
3. Positive pregnancy test
4. Inability or unwillingness to pursue effective means of birth control from the time of evaluation for eligibility until 6 months posttransplant (if on transplant) or until appropriate for non-transplant treatment (if on control arm). Effective birth control is defined as 1) abstinence defined as refraining from all acts of vaginal intercourse; 2) consistent use of birth control pills; 3) injectable birth control methods (Depo-provera, Norplant); 4) tubal sterilization or male partner who has undergone vasectomy; 5) placement of an intrauterine device (IUD); or 6) use, with every act of intercourse, of diaphragm with contraceptive jelly and/or condoms with contraceptive foam.
5. Failure to willingly accept or comprehend irreversible sterility as a side effect of therapy
6.  $FEV_1/FVC < 60\%$  of predicted after bronchodilator therapy (if necessary)
7.  $DLCO < 50\%$  of predicted (for the transplant arm)
8. Resting LVEF  $< 50\%$
9. Bilirubin  $> 2.0$  mg/dl
10. Serum creatinine  $> 2.0$  mg/dl
11. Known hypersensitivity to mouse, rabbit, or E. Coli derived proteins, or to iron compounds/medications
12. Presence of metallic objects implanted in the body that would preclude the ability of the patient to safely have MRI exams
13. Diagnosis of primary progressive MS
14. Diagnosis of secondary progressive MS

15. Platelet count < 100,000/ul, WBC < 1,500 cells/mm<sup>3</sup>
16. Psychiatric illness, mental deficiency or cognitive dysfunction making compliance with treatment or informed consent impossible
17. Active infection except asymptomatic bacteriuria
18. Use of natalizumab (Tysabri) within the previous 6 months
19. Use of fingolimod (Gilenya) within the previous 3 months
20. Use of Teriflunomide (Aubagio) within the previous 2 years unless cleared from the body (plasma concentration < 0.02mcg/ml) following elimination from the body with cholestyramine 8g three times a day for 11 days
21. Prior treatment with CAMPATH (alemtuzumab)
22. Prior treatment with mitoxantrone
23. Any hereditary neurological disease such as Charcot-Marie-Tooth disease (CMT) or Spinocerebellar ataxia (SCA) are contraindications
24. Use of tecfidera within the previous 3 months

\*\* For patients who clearly have inflammatory disease, an exception can be made if agreed upon by study PI and at least two study neurologists.

## 5.0 STUDY DESIGN AND PATIENT FLOW



**6.0 HSCT ARM**

**6.1 STUDY PROCEDURES for HSCT ARM**

	<b>@ BASELINE (Pre-Treatment)</b>	<b>During HSCT hospitalization</b>	<b>Weekly for 4 weeks then every 2 weeks for 8 weeks</b>	<b>6 months, then yearly x 5 years<sup>7</sup></b>
Complete head cover such as wig or toque or turban (Only when seen by evaluating neurologist)				X
History, MS history	X	X (daily)		X
Physical exam <sup>9</sup>	X	X (daily)		X
MS Disability Evaluation <sup>1</sup>	X			X
MSQOL-54 and SF-36 questionnaire <sup>5</sup>	X			X
MRI brain with gadolinium <sup>8</sup> by MRI-AC protocol	X			X
MRI of cervical spine	X			X(optional)
CBC with differential	X	X (daily)	X	X
PT and aPTT	X			
Serum chemistry <sup>2</sup>	X	X (daily)	X	X
CMV by PCR			X	
HIV1 and HIV2	X			
Serum or urine pregnancy test	X			
PFT w/ DLCO and FEV1/FVC	X			
Echocardiography	X			
Chest x-ray	X			
EKG	X			
Limited CT scan of sinuses w/o contrast (optional)	X (optional)			
Urinalysis	X			
Skin testing: tetanus, mumps, candida and TB <sup>6</sup>	X (optional)			
TSH, T3, T4	X			
Herpes virus serologies <sup>3</sup>	X			
Hepatitis A, B, C serologies	X			
JC virus titer	X			
Flow cytometry for absolute CD3, CD4, CD8 (optional)	X (optional)			X (optional)
Lymphopheresis <sup>4</sup>	X (optional)			X (optional)
Dental consult	X(optional)			X (optional)



<sup>1</sup> MS Disability Evaluation to include MS Functional Composite (timed 25-foot walk, 9-hole peg test and the Paced Auditory Serial Addition Test), ambulation index, EDSS and Scripps NRS performed by a blinded investigator. **THE EDSS AND NRS MUST BE PERFORMED BY A BLINDED NEUROLOGIST.**

<sup>2</sup> Serum chemistry to include electrolytes, Mg, glucose, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, creatinine, BUN.

<sup>3</sup> Herpes virus serologies to include CMV, HSV, and VZV.

<sup>4</sup> Lymphopheresis collection may be stored locally or sent to Richard Burt at Northwestern

<sup>5</sup> QOL questionnaires include the MSQOL-54.

<sup>6</sup> Skin testing is optional and only performed if clinically indicated to assess anergy.

<sup>7</sup> ALTHOUGH EVERY ATTEMPT WILL BE MADE FOR EVERY 6 MONTHS EVALUATION, SOME PATIENTS WILL NOT RETURN FOR FREQUENT VISITS IN WHICH EVENT STUDY TESTS AND MEDICAL EVALUATION WILL BE COLLECTED FROM LOCAL PHYSICIAN AS WELL AS A TELEPHONE HISTORY BY STUDY PHYSICIAN OR PROTOCOL NURSE. A FORMAL EDSS/NRS WILL NOT BE EVALUATED FROM LOCAL PHYSICIAN AND MUST BE PERFORMED AT A MINIMUM OF 6, 12, 24, 36, 48, AND 60 MONTHS AFTER HSCT BY A STUDY NEUROLOGIST.

<sup>8</sup> MRI OF BRAIN WILL BE PERFORMED PER MRI-AC PROTOCOL MANUAL

<sup>9</sup> After transplant the patient will wear a head cover whenever evaluated by a neurologist. The patient will be instructed not to tell evaluating neurologist type of treatment.

All subjects will be examined at baseline and at 6 months and yearly thereafter with neuropsychological exams which shall include the Paced Auditory Serial Addition Test (PASAT) and where facilities exist a standardized, repeatable battery of tests consisting of the Selective Reminding Test (verbal learning), 7/24 Spatial Recall Test I (visuospatial learning), and Controlled Oral Word Association (verbal fluency and semantic retrieval). These cognitive functions, in addition to the PASAT, are most often disrupted in patients with MS. Administration of these tests and MS Functional Composite (which includes timed 25-foot walk, and 9-hole peg test in addition to the PASAT) will be performed by a trained professional and should take 40 – 50 minutes. Self-administered quality of life exams (MSQOL and SF-36) will also be obtained pre-transplant, 6 and 12 months post-transplant, and then yearly until 5 years post-transplant.

The MSQOL-54 is a combination of the SF-36 (Ware 1997 and McHorney 1994) which is a generic indicator of health status derived from the 245-item Medical Outcomes Study questionnaire, and 18 MS specific questions. The SF-36 includes multi-item scales to measure the following eight dimensions; physical functioning (PF), role limitations due to physical health problems (RP), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and general mental health (MH). The scoring system for each dimension uses an approach, which recodes the answers of each question into a 0-to-100 score, oriented so that a higher score indicates a better health state. For example, functioning scales are scored such that a high score indicates better functioning and the pain scale is scored such that a higher score indicates decreased pain. The SF-36 is a commonly used quality of life measure and facilitates comparisons across treatments and disease groups as well as against population norms.

**6.2 Mobilization and Peripheral Blood Stem Cell Harvest:**

Day	0	1	2	5	ANC > 1000/ul (approximately day 10)
Cyclophosphamide 2.0 g/m <sup>2</sup>	X				
G-CSF 5-10 mcg/kg/day				X	
Apheresis					X*

\*Apheresis will begin when the ANC is greater than 1000/ul and continue until greater than 2.0 x 10<sup>6</sup> CD34<sup>+</sup> cells/kg patient weight have been collected. A maximum of three aphereses will be performed. The G-CSF will continue until apheresis is discontinued.

**6.3 Interval between Mobilization and Conditioning (Guideline)**

**In order to avoid cumulative cardiac toxicity from cyclophosphamide and to allow cultures from collected stem cells to mature for 14 days prior to infusion of the stem cells, three weeks must separate the administration of cyclophosphamide for mobilization and for conditioning.**

**6.4 Transplant Conditioning Regimen**

Since patients in this study are earlier in their disease course, a conditioning regimen that has historically been associated with less toxicity will be employed. The regimen will be cyclophosphamide 200 mg/kg and ATG (Rabbit)<sup>1</sup>. The conditioning regimen is outlined below:

Day	-6	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
Hydration	X	X	X	X	X	X						
Cyclophosphamide 50 mg/kg/day IV		X	X	X	X							
MESNA 50 mg/kg/day IV		X	X	X	X							
ATG (Rabbit) <sup>1</sup> (mg/kg/day)		0.5	1.0	1.5	1.5	1.5						
Solumedrol 1.0 g IV		X	X	X	X	X						
Stem cell reinfusion							X					
G-CSF 5-10 mcg/kg/day* SQ												X Until engraft t
Prednisone *							X 60m g	X 60m g	X 60m g	X 40m g	X 40m g	X 20mg See below *

Foley catheter * (if history of urine retention, incomplete bladder emptying)		X	X	X	X							
Broad spectrum antibiotic (e.g. cefepime or zosyn)							X	X	X	X	X	X Until engraft t

Cyclophosphamide: 50 mg/kg/day will be given IV over 1 hour in 500 cc of normal saline. If actual weight is < ideal weight, cyclophosphamide will be given based on actual weight. If actual weight is > ideal weight, cyclophosphamide will be given as adjusted weight. Adjusted weight = ideal weight + 25% (actual weight minus ideal weight).

Hydration Guideline: 100 to 175 cc/hour should be given 4 hours before cyclophosphamide and continued until 24 hours after the last cyclophosphamide dose. The rate of hydration will be aggressively adjusted. Bid weights will be obtained. Amount of fluid can be modified based on patient’s fluid status.

<sup>1</sup>ATG (rabbit): 0.5 mg/kg will be given IV on day -5, 1.0 mg/kg will be given IV on days -4, 1.5 mg/kg will be given IV on days -3,-2,-1 (no dose adjustment). It will be given over 10 hours. Premedicate with Solumedrol 1.0 gram IV, acetaminophen 650 mg po and diphenhydramine 25 mg 30 minutes before infusion. An in-line 0.22 µm filter should be used for ATG administration.

G-CSF Guideline: 5 - 10 mcg/kg/day will be started on day +4 and continued until the absolute neutrophil count reaches at least 500/µl. Dose may be rounded up or down in order to not waste medication in a vial.

**\*Prednisone Guideline**: Prednisone to prevent rATG fever is 60 mg/day for three days (day 0, 1, 2), 40 mg/day for two days (day 3, 4), then 20 mg/day until engraft, then 10 mg/day for two days, then off. **If fever occurs despite prednisone, blood cultures will be drawn and blood spectrum antibiotic will be expanded to include Vancomycin (unless allergy) and 250 mg methylprednisone will be given daily for two days.**

**\* Foley catheter Guideline**: Since neurogenic bladder with delayed emptying is common, a foley catheter is placed in patients with history of urine retention

## 6.5 Concurrent Treatment and Supportive Care Guidelines

### 6.51 Transfusion Support Guidelines

All blood products are to be irradiated (25 Gy). CMV negative patients are to receive CMV negative blood products or alternatively (leukocyte-poor) leukofiltered blood products. Prior to administration of blood products, patients may be medicated with Benadryl 10 - 25 mg IV or po and acetaminophen 650 mg po to prevent febrile reactions.  
Red cells: For Hgb < 8.0 g/dl (Hct >27) transfuse 2 units irradiated ABO/Rh matched units.

Platelets: Platelets (irradiated) are given electively for platelet count less than 20 x 10<sup>9</sup>/l. For procedures associated with a high risk of hemorrhage, including major surgical

procedures, deep tissue biopsies, lumbar puncture, placement of central vascular catheter and endoscopy of the gastrointestinal tract, maintain platelet counts greater than  $50 \times 10^9/l$ . Platelets should be infused just before an invasive procedure. In addition to the platelet count, bleeding time, PT/PTT, fibrinogen and other measures of coagulation may be helpful in some patients for defining the extent of any clotting dysfunction.

## **6.52 Infection Prophylaxis and Treatment Guidelines**

All prophylactic antibiotics may be changed or discontinued according to clinical circumstances (e.g., patient allergy) as determined by the attending physician(s).

Antibacterial Prophylaxis: On day 0 of stem cell infusion a broad spectrum intravenous antibiotic such as piperacillin/tazobactam or ceftazidime (Pseudomonal coverage is needed) regardless of temperature will be started until ANC returns to  $> 500/ul$ . Once the WBC's engraft and patient is without sign of infection and fever, intravenous antibiotics will be stopped. Administration of antibiotics will be done according to the institutional standard of practice of the participating center.

Antifungal Prophylaxis: Fluconazole 400 mg po daily from day +2 of transplant until 6 months post-transplant.

Antiviral Prophylaxis: Valacyclovir or acyclovir will be administered for HSV and VZV prevention from day of transplant until 12 months post-transplant. Administration of antiviral agents will be according to the institutional standard of practice of the participating center. If there is no documented standard of practice, then administration of antibiotics will be done.

Pneumocystis carinii pneumonia (PCP) Prophylaxis: Trimethoprim-sulfamethoxazole (TMP-SMX, Bactrim) DS tablet po every Monday, Wednesday and Friday starting after engraftment (absolute neutrophil count  $>500/ul$  for 3 days) and continued for 6 months. If the patient experiences a side effect to Bactrim (e.g., rash), aerosolized pentamidine 300 mg inhaled q month for 6 months will be substituted.

CMV prophylaxis: Patients blood will be checked weekly from time of discharge for 4 weeks then every 2 weeks until 90 days post HSCT by PCR for CMV. If positive, Acyclovir will be changed to Valcyte for two weeks or until PCR negative.

## **6.6 Hospital discharge guidelines for transplant arm**

1. Afebrile.
2. No parenteral feeding required.
3. Platelet transfusion requirement less than 3x/week.
4. Neutrophil count greater than 500/ul.
5. Patient or family member is able to provide care.
6. Arrangements for follow-up with BMT physician and primary physician.

## **7.0 Treatment of relapses or progressive disease in the HSCT arm**

### **7.1 Treatment of relapses**

Relapse is defined as acute neurologic deterioration occurring after engraftment and lasting more than 24 hours with new objective signs on neurological examination documented by a neurologist. Supportive confirmation by enhancement on MRI is preferred but not mandatory. If MRI is not used to confirm a relapse, a pseudorelapse must be excluded by confirmation of the absence of infection, fever, intercurrent illness or severe physiologic stress including transient worsening during clearly caused by the therapy. Relapse may be treated per investigator preference but 1<sup>st</sup> relapse should be treated with IV solumedrol 1.0 g a day x 3-5 day's with or without oral prednisone taper over 7 to 10 days.

## **7.2 Treatment of progressive disease**

Progressive disease is defined as an increase in the EDSS by 1.0 or more steps due to MS (any comorbid conditions affecting neurologic function excluded) obtained at a time point not associated with a clinical relapse, and confirmed on 2 separate exams by a neurologist at least 6 months apart. Patients with progressive disease will continue to be followed for the 5-year duration.



## 8.0 CONTROL ARM

Patients may receive most FDA approved standard therapy (interferons, glatiramer acetate (Copaxone), natalizumab (Tysabri), fingolimod (Gilenya), Tecfidera ( BG-12) in the control arm, we recommend change from the therapy (s) which they failed to qualify for entry into the study. **Patients in control arm may not receive teriflunomide (Aubagio).** The decision of specific control arm therapy will be made by attending physicians based on clinical circumstances and discussion with the patient. If required for patient care, the dose of MS disease modifying therapies in the control arm may be adjusted by the treating physician.

### 8.1 STUDY PROCEDURES for CONTROL ARM

	@ BASELINE (Pre-Treatment)	6, 12 months, and then yearly X 5 years <sup>7</sup>
Complete head cover such as wig or toque or turban (When seen by blinded neurologist)		X
History, MS history	X	X
Physical exam <sup>9</sup>	X	X
MS Disability Eval <sup>1</sup>	X	X
MSQOL-54 and SF-36 questionnaire <sup>5</sup>	X	X
MRI brain with gadolinium <sup>8</sup> per MRI-AC protocol	X	X
MRI of cervical spine	X	X(optional)
CBC with diff	X	X
PT and aPTT	X	
Serum chemistry <sup>2</sup> and LFTs	X	X
HIV1 and HIV2	X	
Serum or urine pregnancy test	X	
Echocardiography	X	
Chest x-ray	X	
EKG	X	
Skin testing: tetanus, mumps, candida and TB <sup>6</sup>	X (optional)	
TSH, T3, T4	X	
Herpesvirus serologies <sup>3</sup>	X	
Hepatitis A, B, C serologies	X	
Flow cytometry for absolute CD3, CD4, CD8	X (optional)	X (optional)
Lymphopheresis <sup>4</sup>	X optional	X (every 6 months, optional)

<sup>1</sup> MS Disability Evaluation to include MS Functional Composite (timed 25-foot walk, 9-hole peg test and the Paced Auditory Serial Addition Test), ambulation index, EDSS and Scripps NRS performed by a blinded investigator. **THE EDSS AND NRS MUST BE PERFORMED BY A BLINDED NEUROLOGIST.**

<sup>2</sup> Serum chemistry to include electrolytes, Mg, glucose, LDH, SGOT, SGPT, total bilirubin, alkaline  
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phosphatase, creatinine, BUN.

<sup>3</sup> Herpesvirus serologies to include CMV, HSV, and VZV.

<sup>4</sup> Lymphopheresis collection may be stored locally or sent to Richard Burt at Northwestern

<sup>5</sup> QOL questionnaires include the MSQOL-54.

<sup>6</sup> Skin testing is optional and only performed if clinically indicated to assess anergy.

<sup>7</sup> ALTHOUGH EVERY ATTEMPT WILL BE MADE FOR EVERY 6 MONTHS EVALUATION, SOME PATIENTS WILL NOT RETURN FOR FREQUENT VISITS IN WHICH EVENT STUDY TESTS AND MEDICAL EVALUATION WILL BE COLLECTED FROM LOCAL PHYSICIAN AS WELL AS A TELEPHONE HISTORY BY STUDY PHYSICIAN OR PROTOCOL NURSE. A FORMAL EDSS/NRS WILL NOT BE EVALUATED FROM LOCAL PHYSICIAN AND MUST BE PERFORMED AT A MINIMUM OF 6, 12, 24, 36, 48, AND 60 MONTHS AFTER HSCT BY A STUDY NEUROLOGIST

<sup>8</sup> **MRI OF BRAIN WILL BE PERFORMED PER MRI-AC PROTOCOL MANUAL**

<sup>9</sup> Patient will wear a head cover whenever evaluated by a neurologist. The patient will be instructed not to tell evaluating neurologist type of treatment.

## **8.2 TREATMENT PLAN FOR TYSABRI® (natalizumab)**

In the United States only prescribers registered in the TOUCH® Prescribing Program may prescribe Tysabri®. The recommended dose of Tysabri® is 300 mg IV infusion every four weeks. Tysabri® concentrate 300 mg/15 mL will be deluted in 100 mL 0.9% Sodium Chloride Injection, USP, and infused over approximately one hour. Tysabri® should not be administered as an IV push or bolus injection. Patients will be observed during the infusion and for 1 hour after the infusion is complete. The infusion will be promptly discontinued upon the first observation of any signs or symptoms consistent with a hypersensitivity-type reaction.

### **Preparation Instructions**

Aseptic technique will be used when preparing Tysabri® solution for IV infusion. Each vial is intended for single use only.

Tysabri® is a colorless, clear to slightly opalescent concentrate. The Tysabri® vial should be inspected for particulate material prior to dilution and administration. If visible particulates are observed and/or the liquid in the vial is discolored, the vial must not be used. Tysabri® must not be used beyond the expiration date stamped on the carton or vial.

To prepare the solution, 15 mL of Tysabri® concentrate should be withdrawn from the vial using a sterile needle and syringe. The concentrate should be injected into 100 mL 0.9% Sodium Chloride Injection, USP. No other IV diluents may be used to prepare the Tysabri® solution.

The Tysabri® solution should be gently inverting to mix completely. Must not be shaken. The solution should be inspected visually for particulate material prior to administration.

Following dilution, Tysabri® solution will be infused immediately, or solution may be refrigerated at 2-8° C, and used within 8 hours. If stored at 2-8° C, the solution must be allowed to warm to room temperature prior to infusion. It is not allowed to freeze solution.

### **Administration Instructions**

Tysabri® 300 mg should be infused in 100 mL 0.9% Sodium Chloride Injection, USP over approximately one hour. After the infusion is complete, flushing is recommended with 0.9% Sodium Chloride Injection, USP.

The use of filtration devices during administration has not been evaluated. Other medications should not be injected into infusion set side ports or mixed with Tysabri®.

## **8.3 TREATMENT PLAN FOR GILENYA™ (fingolimod)**

Gilenya™ is the new FDA approved drug indicated for the treatment of patients with relapsing forms of multiple sclerosis.

The recommended dose of Gilenya™ is 0.5 mg orally once daily. Patients should be observed for 6 hours after the first dose to monitor for signs and symptoms of bradycardia. Should post-dose bradyarrhythmia-related symptoms occur, appropriate management and continue observation will be initiated until the symptoms have resolved. To identify underlying risk factors for bradycardia and atrioventricular (AV) block, if a recent electrocardiogram (i.e. within 6 months) is not available, should be obtained one in patients using anti-arrhythmics including beta-blockers and calcium channel blockers, those with cardiac

risk factors, and those who on examination have a slow or irregular heart beat prior to starting GILENYA.

If Gilenya therapy is discontinued for more than two weeks the effects on heart rate and AV conduction may recur on reintroduction of Gilenya treatment and the same precautions as for initial dosing should apply.

Fingolimod doses higher than 0.5 mg are associated with a greater incidence of adverse reactions without additional benefit. Gilenya can be taken with or without food.

#### **8.4 TREATMENT PLAN FOR TECFIDERA (BG-12)**

Tecfidera (dimethyl fumarate) is the methyl ester of fumaric acid. It is an oral compound which has potent anti-inflammatory properties thought to be related to the induction of stress protein HO-1. There is also some evidence to suggest that Tecfidera include direct cytoprotective effects through upregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and subsequent induction of an antioxidant response.

Two phase 3 clinical trials of Tecfidera showed the treatment to be effective in patients with relapsing remitting MS. The DEFINE trial was a two year study which investigated the efficacy and safety of Tecfidera compared with a placebo. Annual relapse rates were reduced by 53% and 48% in a bid and tid dose groups respectively. Disability progression was also significantly reduced, by 38% and 34% in the bid and tid groups respectively. The study showed a considerable decrease in new or newly enlarging MRI brain lesions with those taking placebo.

The CONFIRM trial was a two year study which investigated the efficacy and safety of Tecfidera compared with Copaxone (glatiramer acetate). Annual relapse rates were reduced by 44% and 51% in the bid and tid dose groups respectively compared with a 29% in the Copaxone group. Disability progression was reduced by 21% and 24% in the bid and tid groups respectively, compared with 7% in the Copaxone group. The study showed a significant reduction (57-73%) in new or newly enlarging MRI brain lesions in those treated with Tecfidera compared with those treated with Copaxone.

#### **8.5 Prohibited Therapies**

It is possible that the treating physician may decide based on the patient's clinical course that additional therapy is required. In such a case, the initiation of such therapy is to be preceded by a full evaluation of the endpoints for the trial. Patients so treated will continue to be followed according to the evaluation schedule for the trial until they meet the primary endpoint of progression on the EDSS. Patients are not censored or determined failures when other MS therapies are instituted.

#### **8.6 Treatment of relapses ON CONTROL ARM**

Relapse is defined as acute neurologic deterioration lasting more than 24 hours with new objective signs on neurological examination documented by a neurologist. Supportive confirmation by enhancement on MRI is preferred but not mandatory. If MRI is not used to confirm a relapse a pseudorelapse must be excluded by confirmation of the absence of infection, fever, intercurrent illness or severe physiologic stress including transient worsening during clearly caused by the therapy. Relapse may be treated with IV solumedrol 1.0 g q day x 3-5 day's (or the equivalent oral corticosteroid dose) followed by and oral prednisone taper over 7 to 10 days

#### **8.7 Treatment of progressive disease ON CONTROL ARM**

Progressive disease is defined as an increase in the EDSS by 1.0 or more steps due to MS and not

obtained by examinations associated with acute relapses, confirmed on 2 separate exams at least 6 months apart. Patients with progressive disease may be offered to cross over to HSCT or any therapy deemed necessary by the primary care neurologist / physician. Regardless of therapy patients will continued to be followed for the 5 year duration of the study.

Progressive disease is defined as an increase in the EDSS by 1.0 or more steps due to MS (any comorbid conditions affecting neurologic function excluded) obtained at a time point not associated with a clinical relapse, and confirmed on 2 separate exams by a blinded neurologist at least 6 months apart. Patients with progressive disease will continue to be followed for the 5-year duration and will be allowed to cross over to receive a hematopoietic stem cell transplant if clinically appropriate. Patients who receive natalizumab (Tysabri) must be off of treatment for at least 6 months before crossing over.

### **8.8 Criteria to allow cross-over from control arm to hematopoietic stem cell transplantation**

Patients meeting all of the following criteria will be eligible for cross-over:

1. Patients are at least 12 months post-randomization.
2. Patients experienced EDSS worsening of at least 1.0 EDSS point that was maintained for at least 6 months AND if worsening occurred prior to month 6 of the trial EDSS worsening was sustained through to month 12.
3. If worsening occurred prior to month 6 of the trial new MS disease activity of one of the following types occurred after month 6:
  - a. Another EDSS worsening of at least 1.0 EDSS point sustained for 6 months.
  - b. A protocol defined relapse confirmed by an evaluating neurologist.
  - c. A new gadolinium enhancing MRI lesion at month 12 or later.

### **9.0 SIDE EFFECTS FOR TRANSPLANT ARM**

**Risk of hematopoietic stem cell transplantation.** The major hazard of this protocol is transplant-related morbidity and mortality. The marrow ablative regimen of cyclophosphamide will destroy the patient's immune/hematopoietic system and leave the patient susceptible to a wide variety of infections and bleeding complications until the reinfused stem cells engraft. Aggressive supportive care as described above will be used to prevent all avoidable risk. However, a small percentage of patients may die as a direct result of transplant related complications. Transplant related mortality is directly related to a patient's age, general medical condition, and prior exposure to prolonged or aggressive chemotherapy regimens. Transplant related complications include infections, bleeding, veno-occlusive disease of the liver, and failure to engraft. This protocol is designed to minimize these complications.



**Risk of central line.** Placement of an external central line catheter device is a routine procedure which may be done under local or general anesthesia. Potential complications include bleeding, pneumothorax, hemothorax, or arrhythmia. Like all artificial devices, lines may become infected and require treatment with antibiotics and/or removal.

**Risk of lymphopheresis and leukapheresis.** This procedure requires 4-6 hours (1-2 hours for lymphopheresis) and will be performed through a pheresis catheter or a 16-gauge catheter introduced into the antecubital vein. The total volume outside the body at any time does not exceed 450 ml. The most common complication is hypocalcemia arising from citrate anticoagulation, which is usually mild or rarely severe with nausea, vomiting or arrhythmias. Symptoms are avoided with replacement solutions added during apheresis, slowing the flow rate, and/or supplemental oral antacids containing calcium. Other complications are infrequent, but include hypotension, vasovagal syncope, and infection.

**Drug/chemotherapy side effects.** See Section 10 - Drug Information.

## 10.0 DRUG INFORMATION

### 10.1 Cyclophosphamide

1. Other names: Cytoxan, Neosar
2. Chemical: 2-bis (2-chloroethyl) amino tetrahydro-2H-1, 3, 2- oxazaphosphorine-2-oxide monohydrate.
3. Classification: Alkylating agent.
4. Action: Causes prevention of cell division by forming adducts with DNA.
5. Metabolism: Metabolized to active compounds by microsomal enzymes in the liver. Excreted by the kidney in both the original form and as metabolites.
6. Availability: 25 mg and 50 mg tablets (tablets cannot be split); 100 mg, 200 mg, 500 mg, 2000 mg vials Mead Johnson and Adria.
7. Storage: Stable at room temperature indefinitely before reconstitution. After reconstitution, stable for 6 days upon refrigeration or for 24 hours at room temperature.
8. Administration: Dissolved in 500 cc 0.9% NS and administered over 60 minutes IV. Must be aggressively hydrated before, during, and for 24 hours after cyclophosphamide. If the rate of required hydration is not tolerated in a patient, bladder irrigation may need to be substituted.
9. Side effects: Myelosuppression, leukopenia (nadir 8-14 days), hemorrhagic cystitis, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), bladder carcinoma, cellular dysplasias, mucositis, rash, alopecia, anorexia, nausea, vomiting, sterile phlebitis, rare pulmonary toxicity, teratogenicity, hemorrhage, myocarditis, infertility, secondary leukemia; with rapid IV push, oropharyngeal tingling, metallic taste, headache, urticaria, facial swelling. Metabolic abnormalities following cyclophosphamide induced cell lysis can require dialysis in patients with underlying renal insufficiency.

## 10.2 G-CSF

1. Other name: Neupogen®
2. Description: Hematopoietic growth factor.
3. Drug administration: Subcutaneous administration 5-15 mcg/kg/day.
4. Storage and Stability: 300 mcg and 480 mcg vials stored in refrigerator.
5. Toxicity: Myalgias, headache, flu-like symptoms, fever, bone pain in approximately 20% of patients, possible elevation of uric acid, transaminases, and LDH.

## 10.3 ATG Rabbit

1. Other names: Thymoglobulin®
2. Description: A rabbit polyclonal antibody to lymphocytes.
3. Drug administration: 2.0 mg/kg in D5W or NS infused over 10 hours.
4. Storage and Stability: 50mg/ml (5 mL ampule) vial stored in refrigerator.
5. Toxicity: Side effects of ATG are serum sickness and/or anaphylaxis: chills, arthralgias, headache, myalgia, nausea, vomiting, diarrhea, chest-pain, hypotension, dyspnea, pulmonary edema, abdominal pain. Other side effects include abnormal liver function test (SGOT, SGPT) and renal function and thrombocytopenia.

## 10.4 Tysabri®

1. Other names: natalizumab
2. Description: Tysabri is a monoclonal antibody that binds to a protein called alpha-4-integrin. Integrins are found primarily on the surface of white blood cells, and play a role in immune system activity.
3. Drug administration: The recommended dose of Tysabri® is 300 mg IV infusion every four weeks. Tysabri® concentrate 300 mg/15 mL should be diluted in 100 mL 0.9% Sodium Chloride Injection, USP, and infused over approximately one hour. Patients must remain in the office for an additional one hour for observation.
4. Storage and Stability: Tysabri® single-use vials must be refrigerated between 2-8°C (36°-46°F). Tysabri® solution for infusion must be administered within 8 hours of preparation.
5. Toxicity: Tysabri® increases the risk of progressive multifocal leukoencephalopathy (PML), an opportunistic viral infection of the brain that usually leads to death or severe disability. Tysabri® is generally recommended for patients who have had an inadequate response to, or are unable to tolerate, alternate MS therapies.

Serious side effects are signs of an allergic reaction: skin rash, hives, itching; dizziness, fever; nausea, vomiting; feeling flushed; chest pain, difficulty breathing; swelling of face, lips, tongue, or throat; feeling light-headed or fainting.

Other serious side effects are: signs of infection such as fever, chills, body aches, flu symptoms, sore throat, cough, redness, pain, swelling, or painful urination; sudden change in vision, balance, strength, or mental state; easy bruising or bleeding, unusual weakness; white patches or sores inside the mouth or on lips; vaginal itching or discharge; tooth pain, gum pain or swelling; or flare of herpes infection (cold sores, blisters or lesions of the genital or anal area).

Other, less serious side effects are more likely to occur, such as: headache; joint or muscle pain; stomach pain; depression; painful menstrual cramps; or drowsiness tiredness.

Side effects other than those listed here may also occur.

### **10.5 Gilenya™**

1. Other names: fingolimod
2. Description: Gilenya is a sphingosine 1-phosphate receptor modulator. Chemically, Gilenya is 2-amino-2-[2-(4-octylphenyl) ethyl] propan-1, 3-diol hydrochloride.
3. Drug administration: Gilenya is provided as 0.5 mg hard gelatin capsules for oral use. Each capsule contains 0.56 mg of fingolimod hydrochloride, equivalent to 0.5 mg of fingolimod.
4. Storage and Stability: Gilenya should be stored at room temperature between 59°F to 86°F (15°C to 30°C).
5. Toxicity: Gilenya can cause serious side effects including bradycardia or bradyarrhythmia, infection, macular edema, respiratory and hepatic effects. The most common side effects of Gilenya include headache, flu, diarrhea, back pain, abnormal liver tests and cough

### **10.6 Tecfidera (BG-12)**

1. Other name: Dimethyl fumarate
2. Dosage and administration: The starting dose for Tecfidera is 120mg twice a day orally. Should be swallowed whole and intact. After seven days the dose should be increased to the maintenance dose of 240 mg twice a day orally.
3. Side effects: The most common side effects were are flushing and feeling hot, gastrointestinal upset - diarrhea, nausea, abdominal pain, headache
4. Warnings: Tecfidera may cause lymphopenia and recent CBC is recommended before initiation.

## **11.0 RANDOMIZATION, MASKING, AND UNMASKING**

Treatment assignments will be provided to the clinical centers by Dr. Borko Jovanovic in the statistical department at Northwestern University by telephone call. Each patient will be followed by an unblinded treating neurologist.

## **12.0 EVALUATION OF TOXICITY**

Daily assessment will be made with regards to toxicity by one of the protocol investigators or designee, i.e. Nurse Practitioner. Common Toxicity Criteria Scale will be graded and recorded at the time of discharge summary for all non-hematologic toxicities.

## **13.0 ADVERSE EVENT REPORTING**

The Toxicity grading for Adverse Events is according to NCI common toxicity criteria for Adverse Events (CTCAE) version 2.0 at website <http://ctep.info.nih.gov> (besides being located on the website, CTC v2.0 providing an alphabetical listing of Adverse Events with associated descriptions to grade severity is attached at end of the protocol)

- 13.1 To be reported by phone (312-695-4960) or FAX (312-695-4961) to Dr. Richard Burt:  
 a) All life-threatening (Grade 4, except grade 4 myelosuppression, which is anticipated) or lethal reactions. This information is to be immediately reported to Dr Richard Burt who will report it within 72 hours of a working day to the FDA and Health Canada. The site must also advise their ethics review board. All sites will be advised of all SAEs by Dr Burt for reporting to their ethics boards.
- 13.2 To be reported in writing within 10 working days to Dr. Richard Burt via FAX (312-695 4961)  
 a) Grade 3 reactions. These will be reported by Dr Burt on annual reports to the FDA.

**14.0 EVALUATION OF RESPONSE** - To be performed every 6 months post-treatment for 5 years.

**15.0 BIOSTATISTICAL CONSIDERATIONS**

**15.1 Sample Size**

Currently there exists insufficient data to determine progression rate on the HSCT arm. This is a randomized phase II study designed to determine progression rate post HSCT compared to an accepted intervention (mitoxantrone) to determine the feasibility and numbers of patients that would be required for a phase III study. A mitoxantrone study has shown a two year progression rate of 8% for the mitoxantrone arm and 25% for the control arm. Therefore, we expect the 5 year progression rate in the mitoxantrone arm to be between 20% (no change in progression rate) and 45% (progression rate similar to placebo immediately after mitoxantrone is stopped).

If we assume an approximate estimate of the 5-year progression rate for mitoxantrone is 45%, a sample size of 110 with 55 in each arm will provide at least 90% power to detect a difference when the 5-year progression rate in the mitoxantrone arm is 45%, and the corresponding rate in the transplant arm is 15% or less. The same numbers provide at least 80% power to detect a difference exists when the 5-year progression rate in the mitoxantrone arm is 45%, and the corresponding rate in the transplant arm is 20% or less. We will therefore recruit 55 patients to each treatment arm.

**Numeric Results**

Null Hypothesis:  $P1=P2$  Alternative Hypothesis:  $P1>P2$ . Continuity Correction Used. Arcsine Used.

Power	Allocation		Ratio	Odds			Alpha	Beta
	N1	N2		P1	P2	Ratio		
0.955	55	55	1.00	0.45000	0.15000	0.22	0.050	0.045
0.845	55	55	1.00	0.45000	0.20000	0.31	0.050	0.155
0.650	55	55	1.00	0.45000	0.25000	0.41	0.050	0.350
0.420	55	55	1.00	0.45000	0.30000	0.52	0.050	0.580

**15.2 Interim Analysis and Stopping Rules**

We will estimate time to failure at several time points of interest such as 12 months, 3

years and 5 years. An approximate estimator for the standard deviation of estimated  $S(t)$  will be used to provide confidence bands. Cox regression (Cox, 1972) may be used to fit a regression model.

Interim analysis will be preformed after 25%, 50%, and 75% of patients have been entered into the study. The interim analysis was not designed to determine efficacy but rather only as a stopping rule if the treatment group was accumulating more neurologic disability compared to control. If more people were accumulating neurologic disability in the treatment group compared to control then we would do a statistical analysis to determine significance in order to determine if we needed to stop the study.

This analysis will use outcomes such as time to treatment failure and survival to assess whether loss of efficacy should warrant the study to be closed. In particular, due to the nature of the primary outcome (5 year survival) it would not be feasible to stop the study at some intermediate point and follow patients for approximately five years in order to see whether the study should be continued. Instead, we will use the following stopping rules:

- a) EDSS progression of 1.0 or more points in 5 of 10, 10 of 20, 20 of 40 or 25 of 50 patients, or
- b) Treatment mortality calculated exceeds 2 of 10, 3 of 20, 4 of 40, or 5 of 50, or
- c) Study will be held for any treatment-related or disease-related death until reviewed and approved by the IRB, DSMB, and the US FDA.

Triggering of stopping rules or any hold on the protocol will prompt cessation of new enrollment, notification of the IRB and FDA and performance of a comprehensive safety review by the DSMB and external advisor. In order to detect differences between the treatment groups, the data will be analyzed once follow-up clinical data are available on 25, 50, and 75% of the targeted number of patients. Data will be reviewed by the External Advisor, Dr Henry McFarland, Clinical Director, National Institutes of Neurologic Disease and Stroke, Bethesda, Maryland and provided to the IRB and FDA if any significant differences are detected.

### **15.3 Data Management**

Collection of data, management, data checking and verification, will be performed by the PI and his team at Northwestern University Medical School. General Clinical Research Center statistician Dr. Jovanovic will be available to assist with data management and analysis. Analysis will be performed by the research staff using S-Plus, SAS and Microsoft Excel.

### **15.4 Statistical References**

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#### **16.0 CRITERIA FOR REMOVAL FROM STUDY**

1. Pregnancy after screening but prior to starting therapy or during active treatment.
2. Patient withdrawal - before beginning conditioning regimen or after successful recovery of hematopoiesis or at any time on mitoxantrone therapy.
3. Disease progression making travel and follow-up studies of such inconvenience that they impose a significant risk or burden to the patient.

#### **17.0 REGISTRATION PROCEDURE**

Patients must not start protocol treatment prior to registration. When eligibility is confirmed by the physician and nurse, and the Enrollment Form is initialed and signed by the physician and nurse. The registrant is then added to the Protocol Registration List.

#### **18.0 RECORDS TO BE KEPT**

Enrollment, toxicity, adverse event, and follow-up study visit reports will be kept at local sites. Records of all toxicity, adverse event, and follow up data collected via Case Report Forms will be kept in the office of the Division of Immunotherapy at Northwestern University.

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**APPENDIX I**

**McDONALD CRITERIA FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS**

## **APPENDIX II**

### **EDSS-EXPANDED DISABILITY STATUS SCALE IN MULTIPLE SCLEROSIS**

**APPENDIX III**

**SCRIPPS NEUROLOGICAL RATING SCALE (SNRS)**

**APPENDIX IV**

**MS FUNCTIONAL COMPOSITE (MSFC)**



## **APPENDIX V**

### **NCI COMMON TOXICITY CRITERIA**

This study will utilize the CTC (NCI Common Toxicity Criteria) version **2.0** for toxicity and Adverse Event reporting. A copy of the CTC version **2.0** can be downloaded from the CTEP home page (<http://ctep.info.nih.gov>) and is also attached.

**APPENDIX VI**  
**SF-36**  
**MSQOL-54**

**APPENDIX VII**  
**AMBULATION INDEX**

**APPENDIX VIII**

**CombiRx MRI protocol Manual**