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A one-year prospective, randomized, placebo-controlled, quadruple-blinded, phase II safety pilot trial of combination therapy with interferon beta-1a and mycophenolate mofetil in early relapsing–remitting multiple sclerosis (TIME MS)

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***Abstract*:**
Background: Mycophenolate mofetil (MMF) is an oral DNA base synthesis inhibitor with immunomodulatory effects on B cells, T cells, and macrophages.
Objective: To conduct a safety and tolerability pilot study of interferon beta-1a (IFN-b1a) in combination with either placebo or oral MMF in multiple sclerosis (MS).
Methods: Twenty-four treatment-naïve R–RMS patients participated in a one-year prospective, placebo-controlled, blinded, safety pilot clinical trial. Every patient injected weekly intramuscular interferon beta-1a. The cohort was then randomized (1 : 1) to either active oral MMF or identical-appearing placebo tablets. Clinical evaluations were assessed every 3 months, along with brain MRI scans performed at baseline and repeated every 60 days for one year. Comprehensive laboratory assessments were monitored for safety, along with adverse events.
Results: In this small pilot investigation, no differences were identified between the two treatment groups with respect to patient-reported adverse events, MRI metrics, or laboratory abnormalities. Notwithstanding these observations, and the limited number of patients treated, trends appeared to favor the combination therapy regimen.
Conclusions: The combination treatment regimen of interferon beta-1a and MMF appeared to be well tolerated in this pilot study. Despite the small sample size, therapeutic trends were observed in favor of combination therapy. An adequately powered controlled trial of MMF in MS appears warranted.

***Keywords*:** CellCept, immunosuppression, mycophenolate mofetil, relapsing-remitting multiple sclerosis, treatment naïve

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Introduction

Multiple sclerosis (MS) accounts for the largest proportion of disabling neurologic diagnoses of young adults and requires lifelong comprehensive medical care [Noseworthy *et al.* 2000]. Despite the availability of disease modifying therapies (e.g. the interferons and glatiramer acetate), many patients continue to exhibit clinical and radiographic evidence of disease activity over time [Kappos *et al.* 2006; Kinkel *et al.* 2006; Goodin *et al.* 2002; Comi *et al.* 2001; Jacobs *et al.* 2000]. These observations suggest that treatment intensification may be necessary in order to limit disease progression and the corresponding sustained disability changes that compromise patients' functional capabilities. Strategies aimed at optimizing disease modifying effects include the development of more

efficacious monotherapies as well as novel combination treatment regimens that may effectively influence mechanisms of immune dysregulation and associated injury cascades that result in tissue injury [Boster *et al.* 2008; Frohman *et al.* 2005a; 2005b].

The ability to therapeutically manipulate a diversity of immunoregulatory pathways may allow the neurologist to modify humoral, cellular, oxidative, ion channel, and excitatory amino acid injury cascades in MS [Frohman *et al.* 2006; Filippi *et al.* 2003; Noseworthy *et al.* 2000]. Such a capability could potentially uncouple the coordinated interplay of pathogenic steps that ultimately culminates in inflammatory demyelination, neurodegeneration, and irreversible physical and cognitive disabilities [Weiner *et al.* 2009; Zivadinov *et al.* 2008].

A major advance in the approach to the management of MS has been the recognition that enhanced control of the disease process may be better achieved by implementing treatments that strategically target key mechanisms involved in immune activation, regulation, or trafficking of mononuclear cells into the brain and spinal cord [Polman *et al.* 2006; Rudick *et al.* 2006; Tennakoon *et al.* 2006; Hong *et al.* 2005; Aharoni *et al.* 2003; Karandikar *et al.* 2002; Stuve *et al.* 1996]. An alternative approach could involve the application of treatment regimens that utilize a combination of agents that operate through both common as well as distinctive mechanisms with resultant complementary effects on the disease process [Boster *et al.* 2008; Frohman *et al.* 2005b]. We now recognize that MS, at least in part, results from changes in both cellular and humoral mechanisms that influence both adaptive and innate immunity [Weiner, 2008]. Recently, combination therapy with methotrexate, interferon beta-1a, and corticosteroids showed favorable therapeutic trends in RRMS, but was not significantly better than monotherapy alone [Cohen *et al.* 2009]. Nevertheless, this investigation was halted early and is confounded by enrollment of only half of the projected subjects necessary in the power analysis to demonstrate superior efficacy of the combination regimen.

In the phase III trial of natalizumab and weekly intramuscular interferon beta-1a, combination therapy was associated with significant clinical and radiographic benefits when compared with interferon monotherapy [Rudick *et al.* 2006]. However, the development of progressive multifocal leukoencephalopathy (PML) has strongly militated against the use of natalizumab in combination with other disease modifying agents, notwithstanding the lack of evidence to causally link combination treatment to this opportunistic infection. In fact, intensive monotherapy approaches may be more than adequate to predispose patients to this complication, in circumstances where host and viral factors of higher predilection are operative [Berger and Houff, 2009].

A small open-label safety surveillance study involving 79 patients with MS treated with mycophenolate mofetil (MMF) was reported by our group in 2004, and suggested good tolerability and potential therapeutic benefit in those not sufficiently controlled on monotherapy with interferon or glatiramer acetate, or in those intolerant to injection therapy [Frohman *et al.* 2004].

Immunomodulatory agents that exert pleiotropic effects on adaptive and innate mechanisms of autoimmunity may have particular utility in MS. Mycophenolate mofetil is one such treatment with broad spectrum properties of immune suppression through targeting the function of B and T cells and macrophages. The application of this agent has been nearly ubiquitous for a diversity of immune-mediated disorders and for transplant recipients in particular.

Mycphenolate mofetil is a selective inhibitor of inosine 5'-monophosphate dehydrogenase (IMD) type II that is a potent immunosuppressant, principally used in transplant medicine as an antirejection agent [Jonsson and Carlsten, 2002]. This enzyme system is responsible for the *de novo* synthesis of the purine nucleotide guanine within mononuclear cells including activated T and B lymphocytes and macrophages, without affecting purine salvage pathways in body tissues.

Mycophenolate mofetil and its active metabolite, mycophenolic acid (MPA), do not interfere with early T-cell receptor-mediated activation events such as CD25 expression or IL-2 synthesis [Barten *et al.* 2002a; 2002b]. In activated lymphocytes that are dependent upon IL-2 or IL-15 for proliferation, MPA does not impair signaling events such as extracellular regulatory kinase 2 or STAT-5 phosphorylation [Quemeneur *et al.* 2002]. Alternately, MPA does inhibit the downregulation of the cyclin-dependent kinase inhibitor p27 (Kip1) [Quemeneur *et al.* 2002].

Therefore, in activated lymphocytes MPA interrupts cytokine-dependent signals that control the cell cycle and blocks activation of T-cells in the mid-G(1) phase. Mycophenolic acid does not inhibit cell survival or Bcl-x upregulation by IL-2. Further, MPA does not interfere with IL-2 dependent acquisition of susceptibility to CD95-mediated apoptosis and degradation of cellular FLIP [Quemeneur *et al.* 2002].

Mycophenolic acid has been shown to inhibit interferon gamma (IFN-γ) and LPS induced IL-6 and nitric oxide synthesis [Barten *et al.* 2002b]. This latter activity may correspondingly confer therapeutic benefits for patients with MS, given that the disease mechanism appears to involve a skewing or immune deviation toward proinflammatory immune responses, in part characterized by the inappropriate elaboration of IL-6 and nitric oxide [Frohman *et al.* 2006; Noseworthy *et al.* 2000].

Humoral effects have also been observed with MMF. For instance, the agent is effective in suppressing anti-blood type IgG antibodies in patients receiving ABO mismatched renal transplants [Ishida *et al.* 2002]. This activity may be relevant to combination treatment regimens that include MMF and interferons. In particular, MMF may potentially serve to downregulate the production of IFN-γ neutralizing antibodies and thereby facilitate the persistent benefits derived from therapy. In fact, this strategy is routinely utilized in clinical practice in order to preclude the development of human anti-chimeric antibodies (HACAs) in those receiving monoclonal antibodies (e.g. rituximab).

The unique mechanism of action for MMF and its broad spectrum effects on immune system function led us to design a randomized, blinded, safety pilot study to examine a combination treatment regimen with interferon beta-1a.

Methods

The trial was conducted in accordance with the latest edition of Declaration of Helsinki for Biomedical Research Involving Human Subjects; the United States code of Federal Regulations Title 21 Parts 50, 56, and 312.50-70; and the guidelines according to Good Clinical Practices (GCP). Informed consent was obtained for each patient prior to the initiation of any study-related assessment.

Study design

This phase II, blinded, randomized, placebo-controlled, combination safety, pilot study was approved by the University of Texas Southwestern Medical Center's Institutional Review Board. An investigational new drug (IND) exemption was granted by the FDA. With the exception of the Investigational Drug Services Pharmacist, research personnel (treating physician, examining physician, MRI staff, and other clinical staff, including the research nurse and exam technicians) were blinded to the treatment assignment (referred to as quadruple blinding).

The primary objective of this pilot study was to determine the safety and tolerability of oral MMF (CellCept) when used in combination with weekly intramuscular interferon beta-1a (Avonex) (Group A), compared with those treated with Avonex and placebo MMF (Group B) in early, treatment-naïve, RRMS. Primary safety variables on MRI were assessed by examining differences in the number of gadolinium-enhancing lesions between Groups A and B. MRI scans of the brain were obtained every 60 days with and without gadolinium contrast on a 1.5 Tesla magnet. The primary clinical outcome, adverse event severity, was defined by significant changes in both laboratory assessments and patient-reported side effects.

Exploratory outcomes investigated changes in exacerbation frequency; sustained disability as measured by the expanded disability status scale (EDSS), Hauser Ambulation Index (AI), the MS Functional Composite Score (MSFC), quality of life (MS Quality of Life-54 and Beck's Depression Index) and fatigue (Modified Fatigue Impact Scale-21).

Treating neurologists were responsible for confirming the patient's diagnosis, ensuring that inclusion/exclusion criteria were met, and treating any adverse events throughout the duration of the trial. Baseline assessments were completed on all patients, including an EKG, chest-ray, and serum laboratory tests (HIV, Hepatitis B antigen, cytomegalovirus titer and RPR). Complete blood counts and comprehensive metabolic panels were monitored for decreased white blood cell counts and/or elevated liver enzymes, primarily because of the potential risk of abnormalities associated with the use of interferons, particularly in combination with other drug agents. Cytomegalovirus (CMV) titers were monitored every 3 months

and patients were instructed to contact the clinic immediately if they experienced fever and/or diarrhea. Certified examiners performed the EDSS exam every 3 months. The Multiple Sclerosis Functional Composite (MSFC) was also completed at baseline and every 3 months thereafter in order to assess cognitive function (Paced Auditory Serial Addition Test – PASAT), ambulation status (25-foot timed walk), and upper extremity function (9-hole peg test).

Patient demographics

Twenty-four treatment-naïve patients (20 female, 4 male; age range 24–53 years, mean age 37 years) with RRMS were enrolled in this 12-month clinical trial (Table 1). All patients were recruited at the Clinical Center for MS at the University of Texas Southwestern Medical Center at Dallas. The primary investigator completed a thorough workup on each patient to confirm the diagnosis of clinically definite RRMS based on McDonald criteria [Polman *et al.* 2005] or clinically isolated syndrome (CIS) at high risk for developing MS, based on CHAMPS criteria [Jacobs *et al.* 2000]. Symptoms suggestive of MS had to have evolved less than or equal to two years before enrollment in our study. All patients were treatment-naïve, exhibited at least one exacerbation within the preceding two years of screening, and had an EDSS of 0–3.5 inclusive. A patient was excluded if he/she had a documented clinical relapse within 60 days prior to enrollment; was pregnant or breastfeeding; had a progressive form of MS (primary, secondary, or relapsing progressive); had taken immunomodulatory drugs at any time prior to enrollment; and/or had any significant medical history or laboratory abnormalities that precluded the use of either interferon or MMF. Mean EDSS at baseline was 1.5 and not significantly different between the groups (Group A mean = 1.75, std = 1.12; Group B mean = 1.17, std = 1.01; $p = 0.83$).

Study treatment

Following successful screening, all patients were trained by the research nurse at month 0 to administer weekly intramuscular interferon beta-1a. Injections were titrated by a quarter-dose each week until the patient was injecting 30 mcg each week. This dose was maintained throughout the study and a long-acting formulation of naproxyn (Naprelan) at 1000 mg taken prior to the interferon injection, was prescribed by the treating physician to prevent expected flu-like symptoms. At month 1 (upon reaching full dose interferon treatment), each patient was then randomized (1 : 1) to receive either MMF or an identically appearing placebo. The oral study drug was started at 250 mg (one tab) twice daily for one week and then escalated by 250 mg (one tab) twice daily per week until a target dose of 1000 mg (four tabs) twice daily was achieved. Patients were instructed to take the oral study drug on an empty stomach (either one hour before or two hours following a meal). Prior to study commencement, it was determined that patients would be removed early from the trial if medication compliance dropped below 80%. Compliance was assessed at all study visits. Specifically, we queried patients about any and all missed doses of interferon and/or MMF. Further, as per our study protocol, patients

Table 1. Baseline patient characteristics.

	All patients	Group A (mycophenolate mofetil & interferon beta-1a)	Group B (placebo & interferon beta-1a)	*p* value
Age (years)				
Mean, ± std	37, ± 9.2	36, ± 7.8	38, ± 10.6	NS
Range	24–53	25–46	24–53	NS
Gender				
Male	4	3	1	
Female	20	9	11	
Race				
Caucasian	23	12	11	
Hispanic	1	0	1	
Expanded Disability Status Score (EDSS) at baseline				
Mean, ± std	1.46, ± 1.08	1.75, ± 1.12	1.17, ± 1.01	NS
Range	0–3.5	0–3.5	0–3.0	NS
Annualized relapse rate	1.4	1.5	1.3	NS

*MMF: mycophenolate mofetil; IFN: interferon.

were required to return all unused drug doses to the study nurse.

A 'standard' target dose regimen strategy for CellCept does not take into account pharmacologic factors that may influence efficacy such as body weight, plasma peak and trough drug levels, and IMD enzyme inhibition effects (perhaps signifying pharmacogenomic differences among patients, possibly related to IMD gene polymorphisms). During both the planning and execution phases of our study, we had no evidence-based literature, nor available assay systems from which to determine 'appropriate' and validated dosing schemes in individual patients. Similar limitations may also apply to interferon beta and other MS therapies. Furthermore, we titrated our patients to the specified target doses over four weeks with both agents. We recognize that the onset effects for interferon and MMF may be quite different. In the future, validated measures of bioavailability (e.g. MxA levels after interferon injection), serum drug peak and trough concentrations for MMF, and enzyme inhibition effects (for MMF) will be important methodological refinements in any Class I randomized controlled comparison trials for efficacy and safety.

Treating exacerbations

Relapses were carefully documented and each patient was instructed to call the research nurse with any new symptoms and/or new medication additions (including over-the-counter agents and supplements). Patients with symptoms suggestive of a potential relapse were brought into clinic for a thorough examination by the treating and examining neurologists. Worsening of the clinical course due to an acute exacerbation prompted treatment with either 1 g of Solumedrol given intravenously or orally daily for three days, or dexamethasone at 100 mg given intravenously (or orally) twice daily for three days. Corticosteroid tapering was not utilized.

If an acute exacerbation occurred within 30 days prior to the anticipated commencement of the therapeutic phase of the study, study treatment was delayed such that there was at least 60 days between receiving the last dose of steroid and the beginning of the study drug and 60 days from the onset of the exacerbation. If an acute exacerbation occurred during the treatment phase of the study, patients received corticosteroids according to the aforementioned protocol, but MRI was delayed for 30 days following the last dose of steroids.

MRI acquisition sequences

A Philips 1.5 Tesla MRI was used throughout the trial. All images were acquired from the same magnet and standard imaging protocols were used to calibrate the MRI and account for upgrades in software at any time point. For T1-weighted images, the following parameters were used: TR-600 ms, TE: 10–20 ms, Slice number: 22, Slice thickness: 3 mm, Inter-slice gap: 3 mm, Orientation: axial, Field of view (FOV): 250 mm^2, Matrix: 140×256, Series: interleaved, Number of acquisitions: 1, Phase encoding: L > R. For T2-weighted images, a fast spin echo (FSE) sequence was used with the following parameters: TR: 1800–2800, TE: either single-echo or dual-echo (according to site preference): TE first echo: 30–50 ms; TE second echo: 60–100 ms, ETL: according to site preference, Slice number: 22, Slice thickness: 3 mm, Inter-slice gap: 3 mm, Orientation: axial, FOV: 250 mm^2, Matrix: approximately 140×256. Series: interleaved, Number of acquisitions: 1, Phase-encoding: L > R. For detection of new enhancing lesions, bolus intravenous injection of gadolinium-DPTA at a standard dose of 0.1 mmol/kg (i.e. 0.2 ml/kg) was utilized. After a post-injection delay of 5 minutes, we completed the scanning with post-gadolinium T1-weighted SE images.

MRI scan analysis

Two experienced neuroradiologists (M.F., F.A.) assessed abnormalities by consensual agreement. Every 60 days throughout the study, the following MRI measurements were calculated: total number of contrast-enhancing T1-weighted lesions, quantification of enhanced lesion volume, and quantification of hyperintense T2 lesion volume. Every 6 months, quantification of hypointense T1 lesion volume was recorded. Lesion measurements were performed using the JIM software package (Version 4.0, Xinapse Systems, Northants, UK, http://www.xinapse.com). A single enhancing lesion was defined as an area of enhancement seen on a given 3 mm axial image, which is referable neither to normally enhanced structures, nor to contrast migration within vessels. T2-weighted FSE images were used as reference for analysis. A single T2 lesion was defined as an area of increased signal on a given 3 mm axial image, which was seen on both T2- and proton density-weighted images,

and which is not referable to normally hyperintense structures. New T2 lesions had to appear in areas where on the previous scan no abnormality was detected. A single hypointense T1 lesion was defined as an area on a given 3 mm axial slice with signal intensity between those of gray matter and cerebrospinal fluid (CSF). New T1 hypointense lesions had to appear in areas where on the previous scan no discrete hypointense abnormality was detected. Lesions that were contiguous in adjacent 3 mm axial slices were counted once.

Longitudinal percentage brain volume change (PBVC) was calculated using T1-weighted images and the SIENA (Structural Imaging Evaluation of Normalized Atrophy) software [Smith *et al.* 2002].

Statistical analysis

Variables and statistical methods are outlined in each of the following respective sections. It should be noted that one patient in Group A was terminated from the trial at month 11. This patient was not able to complete early termination assessments and was therefore removed from the statistical plan when analyzing data points at month 12.

Results

Safety and tolerability

A variety of laboratory assessments (complete blood counts, liver function tests, cytomegalovirus titers, and electrolyte panels) were collected and monitored at baseline and months 0, 2, 3, 5, 6, 9, 11, and 12. Analysis of the data revealed no significant differences in the mean values between treatment groups following randomization. There was only a single clinically significant laboratory derangement, characterized by transaminitis in a patient treated with interferon and MMF, which did not require discontinuation of either agent. Following the completion of all study assessments, symptoms were tallied for each patient and recorded at each study visit. Overall, MMF was well-tolerated. The analysis did not reveal any significant differences on any safety measures between the two treatment groups when interrogating the number of patient-reported symptoms or adverse events (Table 2).

Table 2. Common adverse events.

Adverse event	Total adverse events reported* $n=115$	Group A $n=50$	Group B $n=65$
Infection	25 (22%)	14 (28%)	11 (17%)
Sinusitis*	15	7	8
URI*	7	6	1
Urinary Tract Infection	3	1	2
Fatigue	9 (8%)	4 (8%)	5 (8%)
Interferon-related	24 (21%)	11 (22%)	13 (20%)
Flu-like symptoms	9	4	5
Bruising	3	0	3
Headache	12 (10%)	7 (14%)	5 (8%)
Mood iInstability	14 (12%)	7 (14%)	7 (11%)
Depression	8	3	5
Anxiety	6	4	2
Decrease in memory/processing speed	4 (3%)	2 (4%)	2 (3%)
Gastrointestinal	15 (13%)	5 (10%)	10 (15%)
Nausea	6	2	4
Cramping/tightness	3	2	1
Loose stool/diarrhea	2	1	1
Constipation	4	0	4
Insomnia	7 (6%)	2 (4%)	5 (8%)
Oral herpetic sores	4 (3%)	1* (2%)	3 (5%)
Hair loss	2 (2%)	1 (2%)	1 (2%)
Decreased libido	2 (2%)	0 (0%)	2 (3%)
Significant lab abnormalities			
LFTs	1 (1%)	1 (2%)	0 (0%)
Serious Adverse Events	2 (2%)	1 (2%)	1 (1.5%)

*Reported more than once by some patients.

Two serious adverse events (SAEs) occurred during the trial – one patient from each of the treatment groups. Both events (appendicitis and hospitalization for migraine) were determined by the primary investigator and treating neurologist to be unrelated to study medications or procedures.

One patient from Group A was removed from the trial at month 11 due to intolerable side effects and persistent needle phobia related to interferon injections. The patient was not able to complete early termination assessments within a timely manner to include month 12 clinical and radiographic data in the final analysis.

Clinical assessments

Change in disability and progression as determined by EDSS was not different ($p = 0.8$), nor was frequency or severity of relapses ($p = 0.4$, chi square test for trend) when comparing each treatment group (Table 3). Annualized relapse rate and the proportion of relapse-free patients was also not found to be statistically different between the two treatment groups (Generalized Poisson Regression model, RR = 1.001, $p = 0.99$ and chi square test, $p = 0.99$, respectively). Five total relapses occurred during the study, as confirmed by the treating neurologist. Two of these patients were receiving combination treatment (group A) and three relapses were in the weekly interferon monotherapy treatment group (group B).

All patients with confirmed relapses were placed on steroids, per protocol. No statistical difference was observed between the two groups with regard to relapse frequency, nor with respect to categorical measures of attack severity. However, time to first relapse in the combination treatment group (group A) was prolonged in contrast to the monotherapy assignment (approximately 157 days and 122 days, respectively). Finally, no statistical difference was found between treatment groups during the 12 months of therapy when assessing validated measures of quality of life, mood, or fatigue (Table 3).

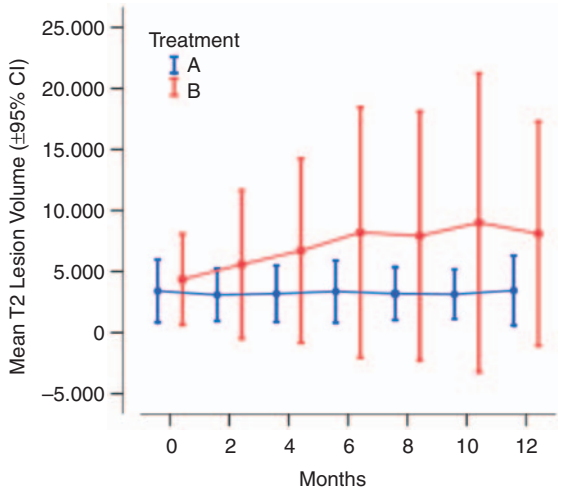
MRI assessments

MRI safety was assessed by virtue of changes in T2 lesions (number and volume) and in gadolinium enhancements (measured at baseline, 2, 4, 6, 8, 10, and 12 months after treatment initiation) compared to baseline measurements derived from one pretreatment screening scan (Figure 1). Although no statistical differences among the two treatment groups were confirmed for any of the MRI metrics analyzed, the mean difference in cumulative number of gadolinium-enhancing lesions from baseline between treatment arms favored combination treatment with weekly interferon beta-1a and MMF (Mann Whitney *U* test, 95% CI, mean difference -1.7, $p = 0.56$). Utilizing a negative binomial regression adjusted for baseline lesions, we observed twice the number of lesions over the period of ascertainment for treatment group B when compared to the combination treated group B (RR = 2.0, 95% CI = 0.4, 11.7, $p = 0.2$). Trends in favor of combination therapy were observed for the percentage change in T2, Gd, and black hole lesion volumes along with the percent change in brain volume (Table 4).

Table 3. Clinical outcome analysis between treatment groups A and B.

Mean values	Month 0		Month 6		Month 12	
Variables	A	B	A	B	A	B
EDSS*	1.75 ± 1.12	1.17 ± 1.01	2.0 ± 0.86	2.0 ± 0.52	1.7 ± 1.32	1.7 ± 1.05
Hauser AI*	0.3 ± 0.5	0.4 ± 0.5	0.25 ± 0.5	0.25 ± 0.5	0.45 ± 0.7	0.42 ± 0.5
MSFC*						
25'TW	4.3 ± 0.80	4.5 ± 0.68	4.6 ± 0.68	4.8 ± 0.65	4.5 ± 1.1	4.7 ± 0.62
9HPT	20.1 ± 2.7	18.5 ± 1.7	18.5 ± 2.5	17.8 ± 2.0	18.5 ± 2.7	17.0 ± 1.4
PASAT	48 ± 10.2	46 ± 8.2	53 ± 7.5	51 ± 6.6	55 ± 6.8	54 ± 5.1
MSQOL-54*						
Physical	66 ± 17	68 ± 18	66 ± 18	70 ± 16	70 ± 24	73 ± 17
Emotional	62 ± 22	73 ± 21	69 ± 23	77 ± 11	76 ± 16	79 ± 21
MFIS-21*	33 ± 18	24 ± 21	29 ± 14	30 ± 21	29 ± 18	24 ± 18
Beck's Depression Index-21*	12 ± 8	10 ± 8	12 ± 7	11 ± 8	8 ± 5	9 ± 9

*n/s = outcome between Group A and Group B not statistically significant.



Analysis: ANOVA for repeated measures. Time × treatment interaction: $p=0.2$ (the time trend is not significantly different between the two arms). Group A = interferon beta-1a+MMF; Group B=interferon beta-1a+placebo.

Figure 1. T2 Lesion Volume over time per treatment arm.

Discussion

Mycophenolate mofetil has been shown to exert a number of immunomodulatory activities that may be useful in the treatment of immune mediated diseases. For instance, MMF exhibits the capability to suppress lymphocyte proliferation and the expression of T-cell surface antigens in whole blood lymphocyte analysis derived from treated allograft recipients [Barten *et al.* 2002a; Jonsson and Carlsten, 2002]. This agent has already been utilized in a diversity of immune-mediated conditions in an attempt to reduce mechanisms of inflammation. These have included lupus [Karim *et al.* 2002; Lui *et al.* 2002; Schanz *et al.* 2002], ANCA vasculitis [Waiser *et al.* 1999], Takayasu's arteritis [Daina *et al.* 1999], myasthenia gravis [Chaudhry *et al.* 2001; Ciafaloni *et al.* 2001; Mowzoon *et al.* 2001; Schneider *et al.* 2001; Hauser *et al.* 1998], chronic inflammatory demyelinating polyneuropathy [Chaudhry *et al.* 2001; Mowzoon *et al.* 2001], polymyositis [Schneider *et al.* 2002; Chaudhry *et al.* 2001; Mowzoon *et al.* 2001], treatment refractory skin manifestations of dermatomyositis [Gelber *et al.* 2000], inclusion body myositis [Mowzoon *et al.* 2001], and psoriasis [Treadaway *et al.* 2009; Ameen *et al.* 2001].

In our study, the combination treatment regimen of weekly interferon beta-1a and MMF appeared to be well tolerated. Although this quadruple-blinded, controlled pilot study was not powered

Table 4. MRI metric analysis.

Treatment arm		Baseline T2 Lesion Volume (ml)	One year T2 Lesion Volume (ml)	T2 Lesion Volume change (%)	Baseline Gadolinium Volume (ml)	One year Gadolinium Volume (ml)	Gadolinium Volume change (%)	Baseline Black Hole vol (ml)	One year Black Hole vol (ml)	Black Hole vol change (%)	Baseline Normal Brain Volume (ml)	One year Normal Brain Volume (ml)	Percent Brain Volume Change (%)
A	Mean Standard deviation	3.5 (3.7)	3.3 (2.9)	16 (43)	0.1 (0.3)	0.02 (0.09)	−128 (275)	0.5 (0.9)	0.5 (0.8)	+32 (455)	1637 (764)	1625 (844)	−0.34 (0.69)
	Median (range)	1.9 (0.3–13.0)	2.1 (0.5–9.7)	16 (−39, +86)	0.02 (0–0.9)	0 (0–0.3)	0 (−917, +118)	0.1 (0–3.2)	0.1 (0–2.3)	0 (−931, +846)	1646 (1523–1751)	1613 (1502–1744)	−0.56 (−1.1, +0.97)
B	Mean Standard deviation	4.4 (5.3)	7.9 (13.0)	70 (134)	0.2 (0.5)	0.6 (1.5)	124 (457)	0.3 (0.4)	0.4 (0.6)	+119 (320)	1649 (632)	1627 (673)	−0.95 (0.93)
	Median (range)	2.6 (0.01–18.1)	4.1 (0.1–47.9)	24 (−7, +467)	0.01 (0–1.7)	0 (0–4.8)	0 (−217, +1536)	0.1 (0–1.0)	0.1 (0–2.1)	+18 (−153, +1015)	1661 (1494–1756)	1615 (1488–1750)	−0.84 (−3.1, +0.5)
p value				0.18			0.27			0.76			0.15

adequately to reject the null hypothesis for efficacy, our observations did suggest that MMF, used in combination with weekly interferon beta, exhibits a similar clinical safety profile when compared with similar patient populations administering weekly interferon beta-1a as monotherapy for 12 months. Our study was not adequately powered to detect efficacy as an outcome in this trial. However, some interesting therapeutic trends were observed in favor of combination therapy in patients with RRMS, who present with first symptoms evolving within two years of treatment initiation. We must underscore great caution in interpreting our results. Without doubt, larger, adequately powered controlled studies will be required to corroborate the trends we observed in our pilot study.

Differences in MRI measures between treatment arms were not significantly different as evidenced by changes in the T2 lesion volume ($p = 0.22$) and total number of gadolinium-enhancing lesions, compared with baseline measurements ($p = 0.56$). Nevertheless we observed that similar to the clinical outcomes, analysis of MRI parameters revealed trends that consistently favored the combination therapy group. Specifically, by the end of the ascertainment period at month 12 the mean difference in cumulative number of gadolinium-enhancing lesions from baseline between treatment arms favored combination treatment. Over the same period, twice the number of lesions evolved in those treated with weekly interferon beta compared with those randomized to treatment with interferon and MMF. Similar trends favoring combination therapy were detected for the percent change in brain volume change over time, the changes in T2 lesion volume, number of gadolinium enhancing lesions, and normalized brain volume change. These findings further underscore the merit of proceeding to larger controlled trials to determine if the application of MMF therapy in MS can mitigate clinical and radiographic evidence of disease activity.

Since 1993 with the approval of interferon beta-1b (Betaseron) by the FDA, first-line disease modifying therapy for relapsing forms of MS has principally involved the administration of parenteral therapies. The prospect of an orally active treatment for MS would represent a substantial advance in medical therapeutics with incontrovertible ramifications on ease of administration, adherence, and quality of life for our deserving patients and their families [Treadaway *et al.* 2009]. More recently MMF (Cellcept) has transitioned to generic status with important economic ramifications. For example, if MMF can be demonstrated to be an effective disease modifying therapy for MS, the use of substantially more expensive agents may be obviated in some patients. This principle is illustrated by a small retrospective study where MMF was found to have modest benefit on stabilizing chronic inflammatory demyelinating polyneuropathy (CIDP), thereby allowing the reduced utilization of steroids or very expensive intravenous immune globulin (IVIg) infusions [Gorson *et al.* 2004].

There are currently five oral agents in phase III trials for MS including cladribine, FTY-720 (fingolimod), dimethylfumarate (BG-12), laquinimod, and teriflunomide. The latter is an inhibitor of dihydrooratate dehydrogenase thereby preventing the synthesis of DNA pyrimidine bases and is thereby similar in mechanism to MMF. A phase II randomized, placebo-controlled study in 179 patients with a relapsing form of MS showed that teriflunomide was well tolerated and significantly reduced the number of combined unique MRI lesions per scan [O'Connor *et al.* 2006]. Although not powered to demonstrate efficacy on the frequency of relapses, those treated with teriflunomide exhibited a non-significant trend toward fewer relapses than those treated with placebo. While our pilot study involved only 24 RRMS patients randomized to receive interferon in conjunction with MMF *versus* interferon and placebo MMF, we observed a similar trends of benefit associated with the use of a different DNA base synthesis inhibitor. Notwithstanding the potential promise of MMF and similar agents as disease modifying therapies for MS, such enthusiasm must be counterbalanced by the corresponding risks of using powerful immunosuppressive therapies that can be associated with a variety of adverse events, some of which can be serious and even life threatening [Kieseier *et al.*, 2009].

Conclusion

Blinded, multi-center, randomized clinical trials are needed to further investigate the observations reported here. Although not powered to explore efficacy outcomes (clinical or radiographic), this safety pilot trial represents a step toward a larger investigation focused on the role of

MMF (CellCept) as a potential disease modifying therapeutic agent for MS.

Conflict of interest statement

This investigator initiated study (EMF) was funded by unrestricted grants from Biogen Idec. and Roche Pharmaceuticals. Neither entity played any role in the design, execution, or analysis of this trial. Furthermore, the sponsors had no role in the drafting or editing of the manuscript.

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