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Glatiramer acetate in combination with minocycline in patients with relapsing–remitting multiple sclerosis: results of a Canadian, multicenter, double-blind, placebo-controlled trial

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Abstract

Minocycline is proposed as an add-on therapy to improve the efficacy of glatiramer acetate in relapsing–remitting multiple sclerosis. The effect of minocycline plus glatiramer acetate was evaluated in this double-blind, placebo-controlled study by determining the total number of T1 gadolinium-enhanced lesions at months 8 and 9 in patients who were starting glatiramer acetate and had at least one T1 gadolinium-enhanced lesion on screening magnetic resonance imaging. Forty-four participants were randomized to either minocycline 100 mg twice daily or matching placebo for 9 months as add-on therapy. They were assessed at screening and months 1, 3, 6, 8 and 9. Forty participants completed the study. Compared with glatiramer acetate/placebo, glatiramer acetate/minocycline reduced the total number of T1 gadolinium-enhanced lesions by 63% (mean 1.47 versus 2.95; $p = 0.08$), the total number of new and enlarging T2 lesions by 65% (mean 1.84 versus 5.14; $p = 0.06$), and the total T2 disease burden ($p = 0.10$). A higher number of gadolinium-enhanced lesions were present in the glatiramer acetate/minocycline group at baseline; this was incorporated into the analysis of the primary endpoint but makes interpretation of the data more challenging. The risk of relapse tended to be lower in the combination group (0.19 versus 0.41; $p =$ NS). Treatment was safe and well tolerated. We conclude that efficacy endpoints showed a consistent trend favoring combination treatment. As minocycline is a relatively safe oral therapy, further study of this combination is warranted in relapsing–remitting multiple sclerosis.

Keywords

multiple sclerosis, glatiramer acetate, minocycline, tetracycline, clinical trial, magnetic resonance imaging

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Introduction

All approved immunomodulating agents are useful in treating multiple sclerosis (MS), however not all patients respond completely and in some, neurodegeneration and ensuing disability continue to progress. Therefore, other therapies are being investigated for the treatment of MS, including minocycline. An open-label, single-center study provided the first evidence that minocycline might be effective and safe in MS patients.[1] In this uncontrolled pilot trial, mean total gadolinium (Gd)-enhanced lesion number significantly decreased from 1.38 lesions per scan during the run-in phase to 0.22 during the treatment phase (Wilcoxon signed rank test, $p = 0.0276$).[1] Whereas 47.5% of magnetic resonance imaging (MRI) scans (19/40) were

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active during the run-in phase, only 9.3% (5/54) were active during the minocycline treatment phase. During the trial, there were no serious adverse events or laboratory abnormalities and no change in Expanded Disability Status Scale (EDSS) from its median baseline of 2.5. Follow-up after 24 months of open-label minocycline treatment suggested that MS had stabilized and that matrix metalloproteinase-9 (MMP-9) activity, a target of minocycline, decreased and remained low.[2] Whether minocycline by itself will be useful as a treatment for MS awaits confirmation.

Glatiramer acetate (GA; Copaxone; TEVA Pharmaceutical Industries, Petach Tikva, Israel) exerts anti-inflammatory effects in multiple sclerosis. These features are reflected in the efficacy of GA in reducing the rate of relapse and MRI-monitored disease activity, as demonstrated in three placebo-controlled trials.[3–6]

As mentioned previously, all current immunomodulating drugs for the treatment of relapsing–remitting MS (RRMS) are considered partially effective. A therapeutic approach that combines drugs targeting different aspects of the immune processes of MS may be reasonable; hence the development of a new treatment as a combination therapy is a possible strategy.[7,8] Minocycline has several immunomodulating activities including inhibition of MMP-9 which is believed to reduce the ability of leukocytes to cross the blood–brain barrier; GA does not effect[9] MMP-9. Preliminary animal data[9] in murine experimental autoimmune encephalomyelitis (EAE) suggest that the combination of GA and minocycline might be effective in the treatment of MS.

The phase II clinical study reported here evaluated the effect of add-on minocycline (100 mg orally twice daily) in patients with active RRMS initiating treatment with GA (20 mg subcutaneously (s.c.) once daily). The three pivotal placebo-controlled clinical trials as well as post-marketing experience have confirmed the efficacy and safety of this currently approved GA dosage. Minocycline 100 mg twice daily is the maximum recommended oral dosage and was shown to be well tolerated when administered to people with RRMS.[1]

Materials and methods

Study design

This was a Canadian, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Participants (male or female aged 18 to 50 years) with clinically definite MS (as defined by Poser et al.[10]) of relapsing–remitting course and duration of at least 6 months were recruited. They were required to have at least one Gd-enhanced lesion on T1-weighted images on the screening MRI scan and at least one documented relapse within the year prior to study entry. They also had to be relapse-free for the prior 30 days and have a Kurtzke EDSS score of between 0 and 5.0 inclusive. The following previous treatments excluded a participant from entering the study: any previous use of GA or cladribine; use of interferons or minocycline in the last 4 months; use of corticosteroids (intravenously (i.v.), intramuscularly (i.m.) and/or per os (p.o.)) within the last month or their chronic use (>28 consecutive days) in the last 6 months; use of any immunosuppressive agents or use of experimental or investigational drugs (including intravenous immunoglobulin (IVIG) and statins) in the last 6 months. Patients were ineligible if they had undergone previous total body irradiation or total lymphoid irradiation, had a contraindication to tetracyclines or a known history of sensitivity to tetracyclines, mannitol or Gd, had any condition that would preclude MRI, or if they were pregnant or lactating. Finally, the occurrence of a relapse between the screening and baseline visits disqualified the participant from further participation. During the study, the use of the following agents was not allowed: interferon, immunosuppressive or immunomodulating drugs, IVIGs, chemotherapeutic agents, 4-aminopyridine, or any other experimental drugs including any tetracycline. Concomitant use of systemic corticosteroids was prohibited with the exception of methylprednisolone for treatment of an acute relapse (allowed at a dose of 1 g/day for a maximum of 3 days). Symptomatic MS agents were however permitted.

The protocol was proposed by the investigators and developed collaboratively by the investigators and the sponsor (Teva Neuroscience Canada). It was approved by regulatory authorities and the local institutional review boards of participating centers. The study was managed by the sponsor in collaboration with the investigators; this included data management and analysis, but a complete set of study data has been provided to the investigators. This manuscript was developed collaboratively by all authors. The trial was registered at www.clinicaltrials.gov (identifier: NCT00203112). All participants gave written informed consent prior to entering this study.

Treatments

Eligible participants were randomized into one of the following two treatment arms: GA 20 mg daily plus minocycline 100 mg orally twice daily or GA 20 mg daily plus placebo. Participants were allocated to the treatment group based on a randomization procedure employing a one-to-one assignment ratio and a scheme using blocks stratified by center. The randomization

scheme was prepared by the sponsor using SAS® random number procedure. The investigators, the personnel involved in subject assessment, monitoring, analysis and data management, and the sponsor (excluding the Clinical Supplies Unit's personnel) were blinded to the subject assignment. Treatment allocation of each participant was maintained at each site in individual sealed envelopes for emergency use only.

GA (Copaxone, supplied by Teva Pharmaceutical Industries Ltd., Israel) was dispensed as pre-filled syringes to be self-administered with optional use of an automatic injection device (Auto*ject2*®). Minocycline and its placebo (both supplied by Novopharm Ltd, Canada) were manufactured, packaged, and dispensed in a way that ensured blinding to the extent that the appearance, shape, color, and smell were identical. Participants were given written instructions with the study medications and were trained on self-injection.

Compliance with the study requirements was assessed by verifying diary card data and by accounting for returned study drugs. Participants were considered non-compliant if overall compliance with either one or both study drugs during the entire study was less than 75%.

Assessments

Total study duration consisted of 9 months of double-blind treatment plus a 6–14 day screening period. Participants were evaluated at screening, baseline (month 0), and at months 1, 3, 6, 8 and 9. They underwent unenhanced T2-weighted and enhanced T1-weighted cranial MRI scans using either a 1.5 (two sites) or a 3 T (two sites) scanner at screening (10 ± 4 days prior to randomization) and during treatment at months 1, 3, 8, and 9. Scheduled complete neurological exams were performed at screening, baseline, and end of study. Safety and tolerance were monitored by physical exam and vital signs measurement, by regular adverse event reporting, and by laboratory testing.

All participants were attended by a treating neurologist and an examining neurologist who acted separately and were blinded to treatment as well as to results of MRI scans. The University of British Columbia MS-MRI Analysis Center, also blinded to treatment, analyzed all MRI scans. The treating neurologist supervised drug administration, coordinated MRI testing, recorded and treated adverse events, and, if required, confirmed the occurrence of a relapse and decided on its management. The examining neurologist conducted neurological evaluations, both those scheduled by protocol and those warranted for relapse evaluation.

Neurological exams included modified functional systems (FS) and EDSS (Neurostatus L. Kappos, Department of Neurology, University Hospital, CH-4031/Basel-Version 10/2002), and Timed 25-Foot Walk.

Outcome measures

The primary efficacy endpoint was the total number of new and persistently Gd-enhanced lesions in T1-weighted images measured at months 8 and 9. Secondary efficacy endpoints were as follows: (1) the total number of new Gd-enhanced lesions on T1-weighted images at month 9 with reference to month 8; (2) the total number of new and persistently enlarging lesions on T2-weighted images at months 8 and 9; and (3) the percentage change from baseline to last observed value in the burden of disease (total T2 volume) measured on T2 scans. Exploratory endpoints were: (1) the total number of persistent/chronic T1 hypointensities ('black holes') seen at month 9 on T1 scans relative to the number of unique newly active lesions seen at months 1 and 3; (2) the change in EDSS from baseline to month 9; (3) the total number of confirmed relapses; and (4) the change in Timed 25-Foot Walk from baseline to month 9. Unique newly active lesions at months 1 and 3 included all new enhanced T1 and non-enhanced new or newly enlarging T2 lesions plus unique persistently active (persistently enhanced and non-enhanced but persistently enlarging lesions) lesions at month 1. Tolerability was judged based on early and total treatment discontinuations due to adverse events. Safety was described using the incidence and frequency of adverse events and abnormal laboratory test results.

Statistical analyses

Sample size rationale: Power assessment was based on the expected differences between the two study arms in the primary efficacy endpoint: the total number of Gd-enhanced lesions on T1-weighted images at months 8 and 9. The expected rate of enhanced lesions in an untreated active population is 3.68 lesions/scan determined from previous Teva trial data. A rate of 1.84 lesions/scan (50% reduction relative to placebo) was anticipated in the GA/placebo arm whereas a rate of 0.552 lesions/scan was targeted for the GA/minocycline arm. Hence, this study assumed a superiority of at least 70% reduction in the primary efficacy endpoint with combination treatment relative to GA alone. The principal statistical analysis was based on an over-dispersed Poisson regression. It was expected that an untreated individual participant's number of enhanced lesions would be derived from a Poisson distribution with a rate of λ_i $(i = 1, 2, \ldots, n)$

when this individual patient rate λ_i comes from a Gamma distribution with $2/\theta = \Sigma\lambda_i/n$ and $r = 0.5$. For a two-sided α level of 5% and an assumed rate ratio of 30% between the two arms, the recruitment of 25 subjects per arm (total of 50 subjects) yielded a power of 82%.

Analysis cohorts: The primary analysis was executed on the pre-planned per protocol cohort. This cohort includes all participants who completed 9 months of treatment without a major protocol violation and who had at least one scan during months 8 and 9.

Patient characteristics at baseline: Comparability of study groups at baseline (demographic and baseline MS characteristics, and screening MRI metrics) was assessed using two-sample *t*-tests or the Wilcoxon rank test, when appropriate for continuous variables and the chi-square test or Fisher's exact test for categorical variables.

Primary endpoint analysis: Analysis of the total number of Gd-enhanced lesions in T1-weighted images at months 8 and 9 was performed using the quasi-likelihood (over-dispersed) Poisson regression (SAS® PROC GENMOD employing DIST = POI and DSCALE in the options section of the MODEL statement) with an 'offset' based on the log of the proportion of scans available (either 1/2, 2/2). The DSCALE option was used to handle over-dispersion. Screening T1 Gd-enhanced lesion count was used as a one-degree-of-freedom covariate. Treatment and center effects were also included in the model.

Secondary endpoint analyses: To control for multiplicity of testing, secondary endpoints were analyzed using the following hierarchical order: (1) total number of new Gd-enhanced lesions on T1-weighted images in month 9 with reference to month 8; (2) total number of new and enlarging T2 lesions at months 8 and 9 with reference to the previous scan; (3) percentage change from baseline to termination in burden of disease measured on T2 scans. Statistical analysis of the total number of Gd-enhanced lesions at month 9, and the number of new and enlarging lesions was similar to that for the primary endpoint. The percentage change from baseline to termination in burden of disease was analyzed by analysis of covariance (ANCOVA; SAS® GLM procedure) comparing the adjusted means of the changes observed in the two treatment groups. The model included the following fixed effects: treatment group, center, and baseline T2 volume measurement.

Exploratory endpoint analyses: The total number of 'black holes' at month 9 relative to acute lesions at months 1 and 3 was analyzed utilizing the Wilcoxon non-parametric test. The total number of confirmed relapses was analyzed using the over-dispersed Poisson regression adjusted for log of exposure. The changes from baseline to termination in the EDSS and in the Timed 25-Foot Walk test were analyzed by ANCOVA as outlined for the burden of disease analysis.

Safety and tolerability assessments: Descriptive statistics were used for abnormal laboratory tests and vital signs.

Results

Patient disposition and characteristics at baseline

A total of 44 participants were randomized to the trial and received at least one dose of study medication (Figure 1) although 104 participants were screened. Lack of a Gd-enhanced lesion at baseline was responsible for 54/60 screen failures, three participants did not meet other inclusion criteria (high EDSS, age >50, not Poser-defined MS), one withdrew consent prior to randomization, and two could not tolerate the MRI procedure (claustrophobia and inability to lie still). Also, due to a longer than expected recruitment period and impending expiration of study drug recruitment was stopped when 44, rather than 50, patients were recruited and the protocol was amended to include participants with more than 15 Gd-enhanced lesions on their screening brain MRI because the analysis plan already accounted for this baseline variable. Baseline clinical characteristics (Table 1) were comparable between the two treatment groups. However, the pre-treatment number of T1 Gd-enhanced lesions was higher in the GA/minocycline group relative to the GA/placebo group (Table 2) and this imbalance at baseline achieved nearly statistical significance (median 3.00 versus 2.00; mean 7.62 versus 2.43; Wilcoxon test, $p = 0.07$). No participants were excluded from the final analysis due to major protocol violations but four were excluded as they discontinued study drug due to an adverse event. Therefore, 40 participants (90.9% of the randomized cohort) completed the study per protocol. The readjusted power was 79%.

Primary outcome

Notwithstanding this relative imbalance in inflammatory MRI activity at baseline, and after adjusting for it as pre-planned in the statistical model, there was a trend for the group treated with combination treatment

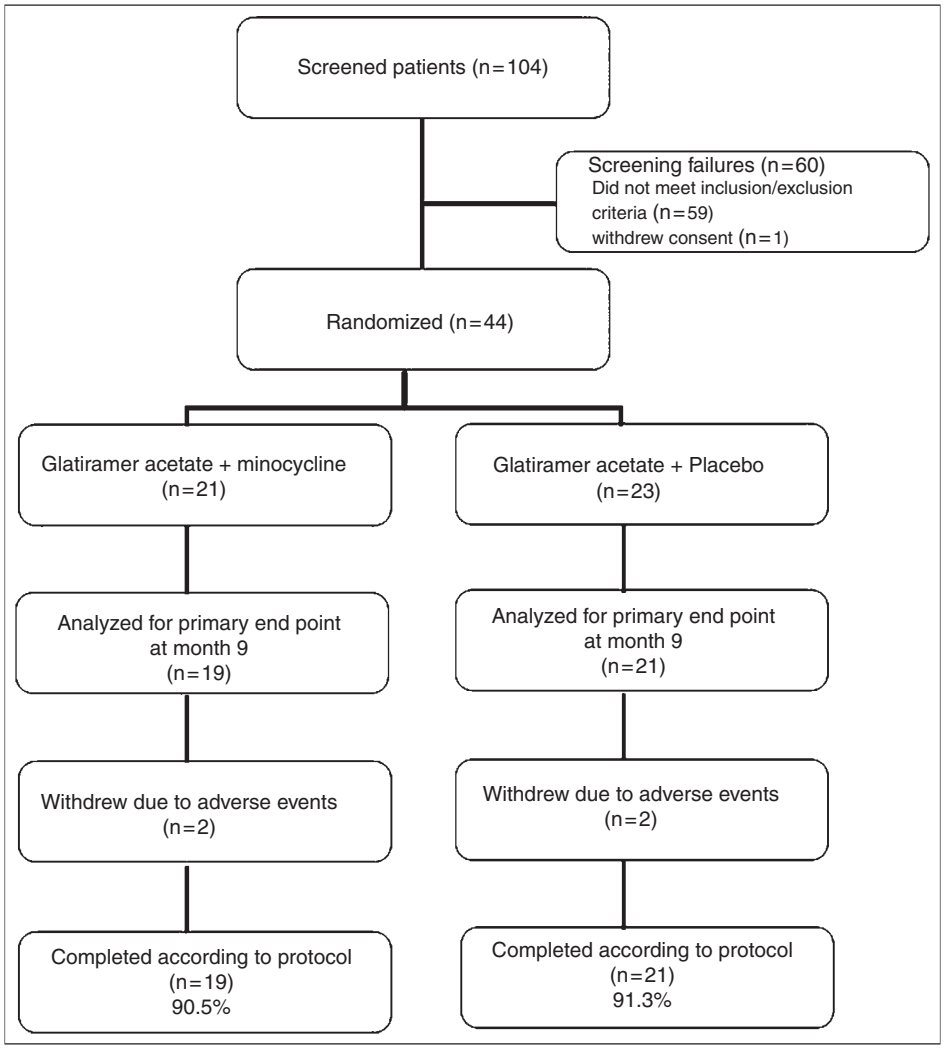

Figure 1. Patient disposition.

to exhibit fewer new total Gd-enhanced lesions at months 8 and 9. Compared with GA/placebo, treatment with GA/minocycline reduced the total number of T1 Gd-enhanced lesions by 63% (mean 2.95 versus 1.47; likelihood ratio (LR) test, $p = 0.08$) but this difference did not reach statistical significance (Table 3). The exclusion of two outliers in the combination arm, one participant with 37 and the other with 57 Gd-enhanced lesions at baseline, did not alter the results of statistical analyses (Figure 2). Over the course of the study there appeared to be a greater decrease in both the number of Gd-enhanced lesions per time point as well as in the variability at each time point in the GA/minocycline group (Figure 3).

Secondary and exploratory outcomes

A trend for fewer new and persistently enlarging T2 lesions at months 8 and 9 was also noted in the group randomized to combination treatment (Table 3). Compared with GA/placebo, treatment with GA/minocycline non-significantly reduced the total number of new and persistently enlarging T2 lesions by 65% (mean 1.84 versus 5.14; LR test, $p = 0.06$; see Table 3). The median volume of lesions on T2-weighted images (burden of disease) at termination also showed a non-significant trend in favor of the combination group with a decrease of 7.1% while the GA/placebo group had a decrease of 2% relative to baseline T2 burden of disease (Table 4). There was no difference between treatment groups in the number of enhanced T1 lesions at month 9 alone (Table 3) or in the number of black holes at month 9 that evolved from acute lesions at month 1 or 3 (Table 4).

Clinical outcomes were in line with MRI outcomes although, as expected due to the small sample size and short study duration, none were statistically significant. The mean number of relapses, adjusted for time on

Table 1. Clinical characteristics at baseline

Characteristics	Glatiramer acetate + minocycline ($n=21$)	Glatiramer acetate + placebo ($n=23$)	All ($n=44$)
Female sex, *n* (%)	16 (76.2)	16 (69.6)	32 (72.7)
Mean age ± SD, yr (range)	36.3 ± 8.7 (22.0 to 50.5)	37.7 ± 6.6 (27.0 to 48.6)	37.0 ± 7.6 (22.0 to 50.5)
Mean time from first symptoms ±SD, yr	7.9 ± 9.3	7.3 ± 6.8	7.6 ± 8
Mean time from diagnosis ± SD, yr	1.9 ± 3.5	3.5 ± 4.1	2.7 ± 3.9
Mean time from last exacerbation ±SD, yr	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
Mean number of exacerbations in the last year ±SD	1.9 ± 0.83	1.8 ± 0.85	1.84 ± 0.83
Number of participants with exacerbations in the last year			
1 exacerbation	7	10	17
2 exacerbations	10	9	19
3 exacerbations	3	3	6
4 exacerbations	1	1	2
EDSS score[a]			
Mean ± SD (range)	2.7 ± 0.9 (1.0 to 4.0)	2.4 ± 1.1 (0.0 to 5.0)	2.5 ± 1.0 (0.0 to 5.0)
Median	2.5	2.5	2.5
Number of participants with each EDSS score			
0.0	0	1	1
1.0	2	0	2
1.5	2	6	8
2.0	2	3	5
2.5	5	4	9
3.0	4	6	10
3.5	3	0	3
4.0	3	2	5
5.0	0	1	1

[a]Converted and actual EDSS scores were identical
SD = standard deviation; EDSS = Expanded Disability Status Scale

study was lower in the group treated with GA/minocycline relative to treatment with GA/placebo (0.19 versus 0.43; p = NS; Table 3). Likewise, the mean EDSS increased by 0.2 in the GA/placebo group whereas it decreased by the same extent in the GA/minocycline group and change in the Timed 25-Foot walk was also slightly better in the GA/minocycline group (Table 4).

Safety

Four participants discontinued therapy due to adverse events. Two participants discontinued at 2 months due to severe injection site reactions: one in each treatment arm. One participant discontinued minocycline at day 17 due to moderate dizziness but continued GA. One participant discontinued placebo at day 9 (headache, fatigue, nausea) and GA at day 13 (these ongoing symptoms plus dizziness).

Table 5 displays the adverse events most commonly reported (≥10%) in any of the two treatment groups. Aside from injection site reactions, headache and nausea were the most frequent adverse events. Of note, gastrointestinal side effects (nausea, diarrhea, and dyspepsia) were more common in the GA/placebo group than in the GA/minocycline group. As could be expected, the anti-infective properties of minocycline perhaps conferred a safety benefit in this patient population, as evidenced by a lower rate of infections, particularly in the upper respiratory tract (overall data not shown). The majority of reported adverse events were of mild to moderate in severity. There were no signs of autoimmune disease or liver dysfunction and there were no reported cases of skin hyperpigmentation. There were no serious adverse events.

Table 2. MRI characteristics at baseline

Characteristics	Glatiramer acetate + minocycline ($n = 21$)	Glatiramer acetate + placebo ($n = 23$)	All ($n = 44$)
Number of Gd-enhanced lesions on T1 weighed images[a]			
Mean ± SD (range)	7.62 ± 13.72 (1 to 57)	2.43 ± 2.13 (1 to 8)	4.91 ± 9.84 (1 to 57)
Median	3.00	2.00	2.00
Burden of disease (lesion volume on T2-weighted images)[a,b]			
Mean ± SD (range)	10,384.11 ± 8575.02 (718 to 30,680)	7208.00 ± 9092.06 (875 to 36,925)	8620.60 ± 8794.24 (718 to 36,925)
Median	9178.00	4142.20	4891.20
Number of participants with T1 Gd-enhanced lesions			
1	6	11	17
2	4	5	9
3	2	3	5
5	4	2	6
8	1	2	3
9	2	0	2
>9	2	0	2

[a] $p = 0.07$ for between-group difference by Wilcoxon rank test
[b] Data missing for one participant in the Glatiramer Acetate + Minocycline group
SD = standard deviation

Discussion

Combination therapy may be a suitable approach to treating MS in some patients because it may possibly have an impact on differing aspects of the immune response or pathological features of this disease.[8] In mice with EAE, the combination of minocycline with GA has been shown to reduce disease severity and to attenuate inflammation, axonal loss, and demyelination.[9] In this short-term phase II study, MRI and clinical findings suggest that adding minocycline to GA acetate may benefit patients with RRMS. Although none of the treatment differences were statistically significant, the trend across the majority of outcomes generally favored combination therapy. MRI activity tends to be highly variable due to participants with extremely high and extremely low activity. This variability is apparent in the standard deviation of the average lesion counts at each time interval. We observed that the participants on combination therapy consistently demonstrated a persistent reduction in the overall variability (reduced standard deviation) of MRI activity, consistent with a more uniform suppression of inflammation across all members in this cohort (Figure 3). It is possible that regression to the mean was also a factor because there was greater baseline inflammation in the combination group despite randomization but the outcome was not affected by removal of two outliers. This imbalance in Gd-enhanced activity seen at baseline supports the concept being adopted into many trial designs to exclude participants with a very high level of activity.

Power assumptions in our study called for a projected sample of 50 participants to demonstrate a treatment effect 70% greater than that of GA; therefore, we were aiming for a very large treatment effect in this phase IIa study. The actual recruitment of 44 participants, four of whom withdrew prematurely, only slightly reduced the statistical power to detect this difference to 79% because 90% of randomized participants completed the study on study drug. This small loss of power did not likely contribute much to the inability to detect a statistically significant treatment effect but it would have been much more satisfying to have been able to enroll all 50 participants as planned.

As with other exploratory trials in MS that target inflammatory processes, changes in MRI outcomes were selected as the first indication of a potentially clinically effective approach. MRI metrics were however complemented by clinical endpoints. Of note, this study used a rigorous definition of relapse. Indeed, the criterion for duration of neurological symptoms (>48 hours) was more stringent than that proposed by Poser et al.[10] (>24 hours) and by McDonald et al.[11] in their clinical definition of exacerbation. This requirement for a neurological deficit to be sustained for at least 48 hours allowed us to distinguish true relapses from pseudo exacerbations and took into

Table 3. Efficacy results: T1 Gd-enhanced lesions, T2 enlarging lesions, and relapses

	Results		Statistical Analyses			
Endpoints	Glatiramer acetate + minocycline ($n = 19$)	Glatiramer acetate + placebo ($n = 21$)	Rate ratio [95%CL]	Effect size (% reduction) [95%CL]	Likelihood ratio test p-value	Wilcoxon test p-value
Primary endpoint						
Total number[a] of Gd-enhanced lesions on T1-weighted images at months 8 and 9			0.37 [0.10 to 1.13]	0.63 [−0.13 to 0.90]	0.0821	0.1148
Mean ± SD (range)	1.47 ± 2.27 (0 to 7)	2.95 ± 6.49 (0 to 30)				
Median	0.00	1.00				
Secondary endpoints						
Total number[b] of new T1 Gd-enhanced lesions at Month 9			0.92 [0.30 to 2.72]	0.08 [−1.7 to 0.70]	0.8752	0.5584
Mean ± SD (range)	0.68 ± 1.29 (0 to 4)	0.57 ± 0.98 (0 to 3)				
Total number of new and enlarging T2 lesions at Months 8 and 9			0.35 [0.09 to 1.06]	0.65 [−0.06 to 0.91]	0.0642	0.0565
Mean ± SD (range)	1.84 ± 3.10 (0 to 11)	5.14 ± 8.92 (0 to 37)				
Exploratory Endpoints						
Number of confirmed relapses						
Mean	0.19	0.43				
Analysis adjusted for time in study			0.52 [0.16 to 1.68]	0.48 [−0.68 to 0.84]	0.2556	0.4697
Analysis adjusted for exposure of minocycline/placebo			0.58 [0.18 to 1.91]	0.42 [−0.91 to 0.82]	0.3585	

[a]Sum of new and persistently enhanced lesions at months 8 and 9
[b]Total number of new enhanced T1 lesions in month 9 relative to month 8
CL = confidence limits; SD = standard deviation

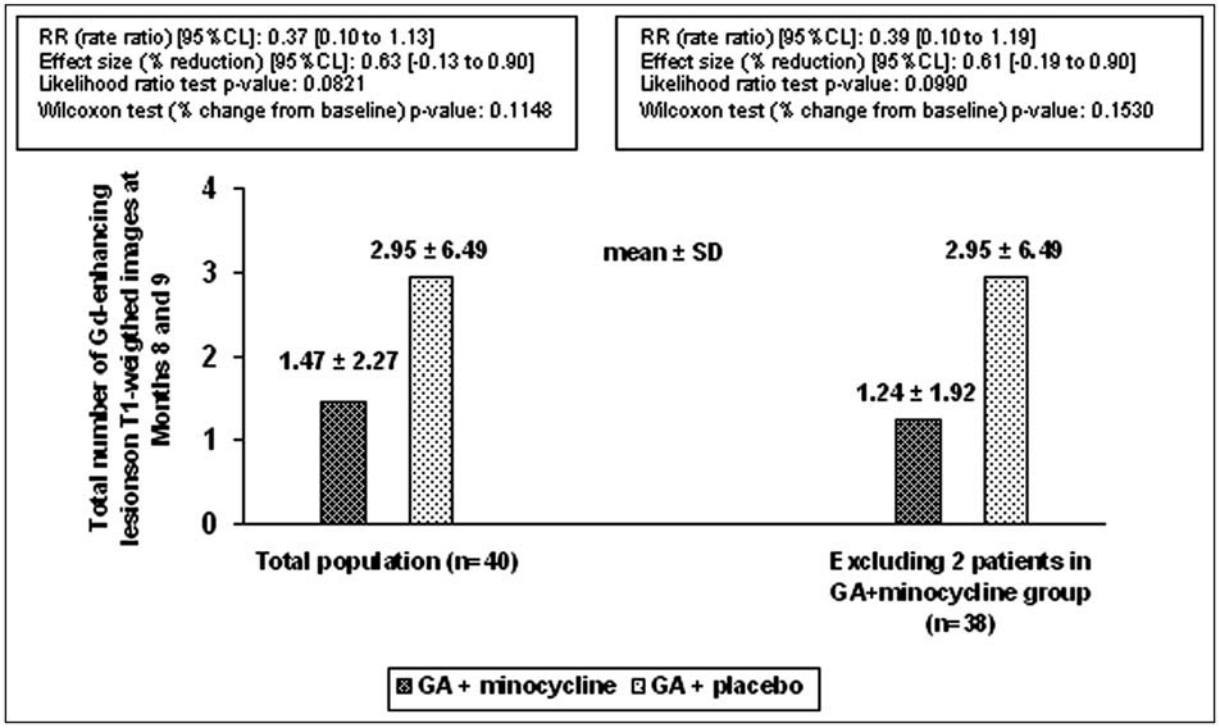

Figure 2. Primary efficacy analysis: total number of Gd-enhanced lesions on T1-weighted images at months 8 and 9 with and without data from two outliers in the GA/minocycline group.

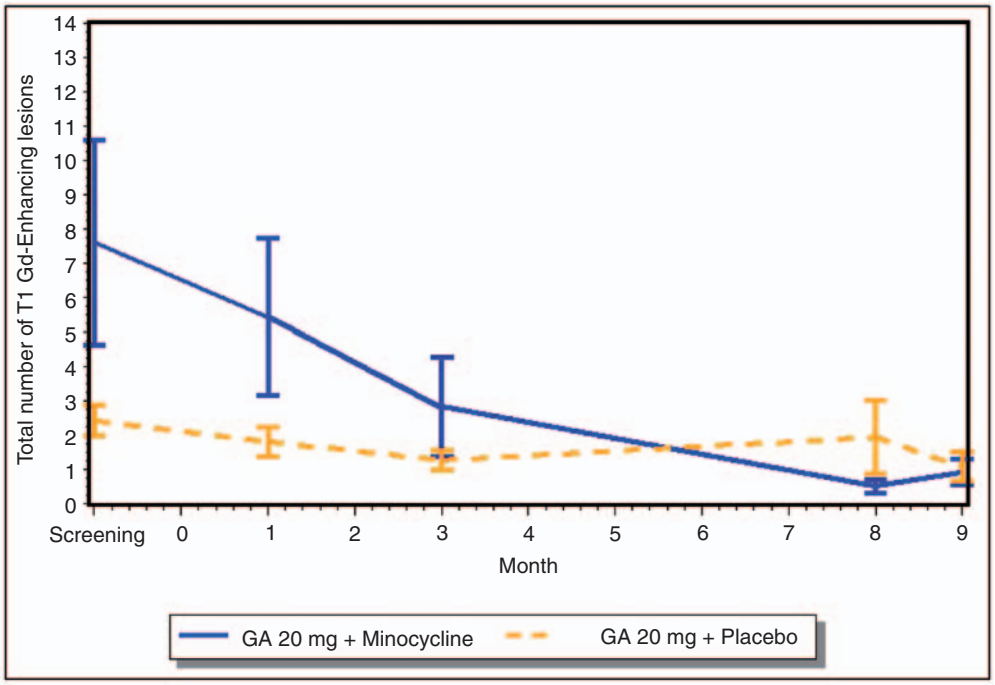

Figure 3. Total number of Gd-enhanced lesions per month (mean ± SE). Despite much greater variability early on, there is less variability at months 8 and 9 in the group receiving combination therapy.

Table 4. Efficacy results: burden of disease, Expanded Disability Status Scale (EDSS), and Timed 25-Foot walk

	Results		Statistical Analyses		
Endpoints	Glatiramer acetate + minocycline	Glatiramer acetate + placebo	Difference between adjusted means [95%CL]	*p*-value	Wilcoxon test *p*-value
Secondary endpoints					
T2 burden of disease[a]					
Number of participants patients	19	21			
Change from baseline (Mean ± SD)	−1529.0 ± 3177.9 (−10224.9 to 1747.8)	171.4 ± 2163.6 (−2375.8 to 8878.3)	−1385.47 [−2915.13 to 144.19]	0.0744	0.2474
Percentage change from baseline	−13.0 ± 22.9 (−65.4 to 19.0)	9.4 ± 51.2 (−44.4 to 214.3)	−21.40 [−48.11 to 5.32]	0.1130	0.1003
Exploratory endpoints					
Evolution of black holes at month 9 from acute lesions at months 1 and 3					
Number of participants	16	16			
Total acute lesion number at months 1 and 3	163	67			
Percent evolving into chronic black holes Mean ± SD	28.84 ± 33.99	30.00 ± 36.15			$P = 0.9858$
EDSS change from baseline					
Number of participants	20	22			
Mean change ±SD (range)	−0.2 ± 0.9 (−2.0 to 1.5)	0.2 ± 0.8 (−1.0 to 1.5)	−0.24 [−0.73 to 0.25]	0.3237	0.2751
Timed 25-Foot Walk change from baseline					
Mean change ± SD (range)	−0.02 (2.0)	−0.1 (0.9.0)	0.85 (95% CI = −1.01, 0.83)		

[a]Lesion volume in T2-weighted images
CL = confidence limits; SD = standard deviation

Table 5. Most common adverse events (incidence ≥10%), by decreasing incidence (any treatment group)

Adverse events[a]	Glatiramer acetate + minocycline *n* (%)	Glatiramer acetate + placebo *n* (%)
Injection site erythema	10 (47.6)	11 (47.8)
Injection site pain	10 (47.6)	11 (47.8)
Headache	9 (42.9)	10 (43.5)
Injection site mass	3 (14.3)	9 (39.1)
Injection site pruritus	8 (38.1)	10 (43.5)
Nausea	5 (23.8)	8 (34.8)
Injection site bruising	7 (33.3)	2 (8.7)
Upper respiratory tract infection	3 (14.3)	7 (30.4)
Injection site reaction	6 (28.6)	4 (17.4)
Nasopharyngitis	3 (14.3)	6 (26.1)
Dizziness	5 (23.8)	3 (13.0)
Diarrhea	1 (4.8)	5 (21.7)
Dyspepsia	0 (0.0)	5 (21.7)
Fatigue	4 (19.0)	1 (4.3)
Injection site irritation	3 (14.3)	4 (17.4)
Influenza	2 (9.5)	4 (17.4)
Injection site urticaria	0 (0.0)	4 (17.4)
Sinusitis	0 (0.0)	4 (17.4)
Dyspnea	3 (14.3)	2 (8.7)
Injection site swelling	3 (14.3)	1 (4.3)
Chest discomfort	3 (14.3)	0 (0.0)
Pollakiuria	1 (4.8)	3 (13.0)
Alanine aminotransferase increased	0 (0.0)	3 (13.0)
Blood alkaline phosphatase increased	0 (0.0)	3 (13.0)
Herpes simplex	0 (0.0)	3 (13.0)
Insomnia	0 (0.0)	3 (13.0)
Pharyngolaryngeal pain	0 (0.0)	3 (13.0)

[a]Irrespective of causality

account the need for patients to undergo an independent neurological evaluation.

Several efficacy and safety aspects of combining MS therapies must be considered. First, it is possible that the combination of useful immunomodulators might not lead to improvements or might even decrease efficacy as a result of unforeseen antagonism. Second, one should consider the possibility that excessive suppression of the immune response may increase the risk of central nervous system (CNS) infections or inhibit repair processes such as remyelination[8] that require some degree of inflammation to be successful. However, a predictably low adverse event pattern was observed in this study and there was no evidence that combination therapy worsened any outcome or that it might trigger CNS infections. Finally, there was no apparent impairment of remyelination based on exploration of the evolution of enhanced lesions into black holes.

With regards to the recent observation/publication that minocycline worsens disease outcomes in amyotrophic lateral sclerosis (ALS),[12] it is important to note that ALS and MS are very different both pathologically and in their pathogenesis. In particular, there is a significant peripheral inflammatory response in MS and immune cells then enter into the CNS to promote disease. Moreover, the subsequent T-cell activation of microglia, and a chronic widespread activated microglia response, are thought to further promote neuroinflammation and injury in MS.[13–15] In ALS the peripheral inflammatory response is not a major contributor to the disease process, if at all and it appears that the microglia activation in ALS may be a protective response to prevent further neuronal degeneration.[16] Thus, the deactivation of microglia in ALS by minocycline could worsen the disease while microglia inactivation in MS would be a favorable response. Another key difference is that minocycline is expected to inhibit leukocyte adhesion onto endothelium and their transmigration into the CNS by interfering with adhesion molecules and MMP-9 activity[13,17,18] so this should be a beneficial mechanism of minocycline in MS while of no likely consequence in ALS. It is also notable that studies of the impact of treatment on Gd-enhanced activity in MS have proven to be a useful method of screening potential anti-inflammatory MS therapies to detect both disease improvement as well as disease worsening. Such a proof-of-concept model that focuses on mechanism of treatment impact is not available in ALS. It is also notable that much higher doses of minocycline (400 mg/day) were used in the ALS trial[12] compared with that in MS (200 mg/day). The chronic use of such high doses of minocycline in ALS may result in quite different outcomes. Finally, studies of minocycline in MS[1,2,19] support the safety of minocycline in MS. This combination study provides no hint that minocycline is likely to result in worsening of MS.

The overall results of this study show trends in favor of combination therapy with minocycline and GA for RRMS. Although there is insufficient data to support the addition of minocycline to GA in clinical practice the data from this study allow sample size estimates for further trials with MRI outcomes. Given that minocycline is a relatively safe, well-tolerated and inexpensive therapy, further study of this combination is warranted.

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