A Randomized Controlled Double-Masked Trial of Albuterol Add-on Therapy in Patients With Multiple Sclerosis

Samia J. Khoury, MD; Brian C. Healy, PhD; Pia Kivisäkk, PhD, MD; Vissia Viglietta, PhD, MD; Svetlana Egorova, PhD; Charles R. G. Guttmann, MD; Josiah F. Wedgwood, MD, PhD†; David A. Hafler, MD; Howard L. Weiner, PhD; Guy Buckle, MD; Sandra Cook, RN; Susheel Reddy, PhD

Background: Interleukin 12 (IL-12), a cytokine that promotes generation of helper T cells subtype 1, is increased in multiple sclerosis. Albuterol sulfate, a β2-adrenergic agonist, reduces IL-12 expression, so we tested the effect of albuterol as an add-on treatment to glatiramer acetate therapy.

Objectives: To investigate the clinical and immunologic effects of albuterol treatment as an add-on therapy in patients starting glatiramer acetate treatment.


Setting: Academic research.

Patients: Subjects with relapsing-remitting multiple sclerosis.

Main Outcome Measures: In this single-center double-masked clinical trial, subjects with relapsing-remitting multiple sclerosis were randomized to receive a subcutaneous injection of glatiramer acetate (20 mg) plus an oral dose of placebo daily for 2 years or a subcutaneous injection of glatiramer acetate (20 mg) plus an oral dose of albuterol daily for 2 years. The primary clinical efficacy measurement was the change in Multiple Sclerosis Functional Composite at 2 years, and the primary immunologic end point was the change in expression of IL-13 and interferon-γ at each study time point. The classification level of evidence from this trial is C for each question, as this is the first class II clinical trial addressing the efficacy of glatiramer acetate plus albuterol.

Results: Forty-four subjects were randomized to receive glatiramer acetate plus albuterol or glatiramer acetate plus placebo, and 39 subjects contributed to the analysis. Improvement in the Multiple Sclerosis Functional Composite was observed in the glatiramer acetate plus albuterol group at the 6-month (P = .005) and 12-month (P = .04) time points but not at the 24-month time point. A delay in the time to first relapse was also observed in the glatiramer acetate plus albuterol group (P = .03). Immunologically, IL-13 and interferon-γ production decreased in both treatment groups, and a treatment effect on IL-13 production was observed at the 12-month time point (P < .05). Adverse events were generally mild, and only 3 moderate or severe events were considered related to the treatment.

Conclusion: Treatment with glatiramer acetate plus albuterol is well tolerated and improves clinical outcomes in patients with multiple sclerosis.

Trial Registration: clinicaltrials.gov Identifier: NCT00039988

Arch Neurol. 2010;67(9):1055-1061

MULTIPLE SCLEROSIS (MS) is a chronic inflammatory disease characterized by the presence of perivascular infiltrates composed of mononuclear cells that lead to demyelination of central nervous system white matter. T cells recognizing myelin antigens seem to be involved in the pathogenesis and perpetuation of the disease. It is hypothesized that MS is a helper T cell subtype 1 (Th1)-mediated autoimmune disease. Interleukin 12 (IL-12) is a heterodimeric cytokine produced by phagocytic cells and promotes Th1 generation. There are increased IL-12 messenger RNA transcripts in acute inflammatory lesions found in MS brains and increased anti-CD3-induced IL-12 secretion in patients with secondary progressive (SP) MS. Elevated serum levels of IL-12 have been reported in SP MS. It was previously reported that IL-12 production is elevated in monocytes from patients with MS and that monthly treatment with combined cyclophosphamide-methylprednisolone boosts normalized IL-12 production. Albuterol sulfate, a β2-adrenergic agonist commonly used in the
Disease-modifying therapy, especially disease-modifying therapy with interferon, patients with SP MS induced a significant decrease in IL-12 was earlier reported that oral albuterol treatment in pa-

tier was compared with glatiramer acetate therapy alone. Each component of the first 8 subjects [4 in each study arm]. The primary clinical end point was the MSFC change at 6, 12, 18, and 24 months. Prebaseline MSFC sessions were performed in all subjects except the first 8 (the protocol was amended to include a prebaseline assessment after examination of the first 8 subjects [4 in each study arm]). The primary research question was whether adding albuterol to glatiramer acetate therapy would lead to a higher MSFC at 24 months compared with glatiramer acetate therapy alone. Each component of the study was IL-13 and IFN-γ secretion by glatiramer acetate–reactive T cells, corresponding to immune deviation toward TH2-cytokine secretion. We investigated the clinical and immunologic effects of albuterol treatment as an add-on therapy in patients starting glatiramer acetate treatment.

### STUDY METHODS

**STUDY DESIGN**

The Institutional Review Board at the Brigham and Women's Hospital, Boston, Massachusetts, approved this study. The trial was a randomized double-masked 2-arm pilot study of glatiramer acetate plus albuterol vs glatiramer acetate plus placebo for the treatment of relapsing-remitting MS. Subjects were randomized to receive a subcutaneous injection of glatiramer acetate (20 mg) plus an oral dose of albuterol (4 mg) daily and were followed up for 2 years (eFigure 1; http://www.archneur.com). The inclusion criteria were a definite diagnosis of relapsing-remitting MS, no prior immunomodulatory or immunosuppressive treatment, age 18 to 55 years, an Expanded Disability Status Scale (EDSS) score of 0 to 3.5, no corticosteroid use within 1 month of enrollment, and no active infections or malignant neoplasms. Exclusion criteria were normal brain magnetic resonance (MR) imaging, primary progressive MS, systemic diseases, or concomitant therapy with β2-adrenergic agonists or antagonists, diuretics, tricyclic antidepressants, or monoamine oxidase inhibitors. Subjects were examined by a neurologist (S.J.K., H.L.W. and G.B.) at baseline and at 6, 12, 18, and 24 months, and blood samples were collected at baseline and at 3, 6, and 12 months. Medication compliance and adverse events were reported to the physician at each study visit and were recorded after physician examinations. Brain MR imaging with and without gadolinium enhancement was performed at enrollment, at 12 months, and at 24 months.

The mean (SD) expected change in the Multiple Sclerosis Functional Composite (MSFC) over 24 months in the glatiramer acetate plus placebo group was 0.6 (0.6). Therefore, our study was 87% powered to detect an expected MSFC improvement of 0.6 in the glatiramer acetate plus albuterol treatment arm compared with the glatiramer acetate plus placebo treatment arm using our target sample size of 20 per group and a 2-sample t test. Twenty-three subjects were randomized to glatiramer acetate plus albuterol, and 21 subjects were randomized to glatiramer acetate plus placebo (Table 1). Given our study design, enrollment criteria, and dropout rate (eFigure 1), our study is a class II clinical trial as defined previously for primary clinical and immunologic outcomes, and our results provide level C evidence in favor of our conclusions.

### STUDY END POINTS

The primary clinical end point was the MSFC change at 6, 12, 18, and 24 months. Prebaseline MSFC sessions were performed in all subjects except the first 8 (the protocol was amended to include a prebaseline assessment after examination of the first 8 subjects [4 in each study arm]). The primary research question was whether adding albuterol to glatiramer acetate therapy would lead to a higher MSFC at 24 months compared with glatiramer acetate therapy alone. Each component of the study was IL-13 and IFN-γ secretion by glatiramer acetate–reactive T-cell lines at the 3-, 6-, or 12-month measurements.

Secondary immunologic, clinical, and MR imaging end points were also investigated. The secondary immunologic end points were change in proliferation and IL-5 secretion after glatiramer acetate stimulation. The secondary clinical end points were change in EDSS score, ambulation index, time to first exacerbation, and number of exacerbations. Finally, the secondary brain imaging end points included the number of enhancing lesions on T1-weighted images and the change in the brain parenchymal fraction (BPF). Data management was conducted by Rho, Inc, Chapel Hill, North Carolina, and statistical analyses were performed in collaboration with Rho, Inc. An independent data safety monitoring board was responsible for overseeing the data and the safety of subjects participating in the study.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Glatiramer Acetate Plus Albuterol Group (n=23)</th>
<th>Glatiramer Acetate Plus Placebo Group (n=21)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>8</td>
<td>.18</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>13</td>
<td>.40</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>39.3 (8.0)</td>
<td>37.0 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis Functional Composite, mean (SD)</td>
<td>0.03 (0.93)</td>
<td>-0.04 (0.67)</td>
<td>.54</td>
</tr>
<tr>
<td>Paced Auditory Serial Addition Test score, mean (SD)</td>
<td>51.2 (9.3)</td>
<td>49.6 (8.7)</td>
<td>.36</td>
</tr>
<tr>
<td>Timed 25-Foot Walk, mean (SD), s</td>
<td>4.37 (0.87)</td>
<td>4.25 (0.66)</td>
<td>.75</td>
</tr>
<tr>
<td>9-Hole Peg Test, mean (SD), s</td>
<td>18.8 (2.6)</td>
<td>19.1 (2.1)</td>
<td>.48</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain parenchymal fraction, mean (SD)</td>
<td>88.2 (3.7)</td>
<td>88.6 (3.0)</td>
<td>.72</td>
</tr>
<tr>
<td>Gadolinium-enhancing lesions, mean (SD) [range]</td>
<td>1.99 (1.48) [0-4]</td>
<td>1.90 (3.56) [0-12]</td>
<td>.86</td>
</tr>
</tbody>
</table>

**METHO**

**TABLE 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>39.3 (8.0)</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis Functional Composite</td>
<td>0.03 (0.93)</td>
</tr>
<tr>
<td>Paced Auditory Serial Addition Test score</td>
<td>51.2 (9.3)</td>
</tr>
<tr>
<td>Timed 25-Foot Walk, mean (SD), s</td>
<td>4.37 (0.87)</td>
</tr>
<tr>
<td>9-Hole Peg Test, mean (SD), s</td>
<td>18.8 (2.6)</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>Brain parenchymal fraction, mean (SD)</td>
<td>88.2 (3.7)</td>
</tr>
<tr>
<td>Gadolinium-enhancing lesions, mean (SD) [range]</td>
<td>1.99 (1.48) [0-4]</td>
</tr>
</tbody>
</table>
MR IMAGING

Head MR imaging was obtained using a 1.5-T system (GE Medical Systems, Milwaukee, Wisconsin). Section thickness was 3 mm (no gap and interleaved acquisitions), and nominal in-plane image resolution was 0.9375 × 0.9375 mm. Dual-echo long-repetition time conventional spin-echo images were acquired with a repetition time of 3000 milliseconds, echo times of 30 milliseconds (first echo) and 80 milliseconds (second echo), and half-Fourier acquisitions. Precontrast and postcontrast T1-weighted conventional spin-echo images were acquired with a repetition time of 740 milliseconds and an echo time of 19 milliseconds. Postcontrast T1-weighted acquisitions were acquired 5 minutes after a bolus intravenous injection of gadopentetate dimeglumine (Magnevist; Bayer HealthCare Pharmaceuticals Inc, Wayne, New Jersey) containing 0.1 mmol/kg of body weight. The number of gadolinium-enhancing lesions was assessed independently by 2 experienced readers, who subsequently reached consensus in a joint session. The T2-weighted lesion volumes were estimated using an automated template-driven image segmentation approach combined with a partial volume-effect correction algorithm.11

PROLIFERATION ASSAY
AND MEASUREMENT OF CYTOKINES

Peripheral blood mononuclear cells were purified (Ficoll-Paque; Amersham Pharmacia, Uppsala, Sweden) according to the manufacturer’s protocol within 4 hours of blood drawing. Primary in vitro cultures were established by plating 1 × 10⁶ peripheral blood mononuclear cells per well in RPMI 1640 (Roswell Park Memorial Institute, Buffalo, New York) containing 5% heat-inactivated pooled human serum, sodium pyruvate, nonessential amino acids, and HEPES with no antigen and with 1, 10, 50, or 100 µg/mL of glatiramer acetate in a final volume of 0.2 mL in round-bottomed 96-well microtiter plates, with 10 wells of each condition. The plates were incubated for 6 days, and then the supernatant was removed and analyzed by enzyme-linked immunosorbent assay according to the manufacturer’s instructions for the presence of IFN-γ (Thermo Fisher Scientific, Houston, Texas), IL-5 (R&D Systems, Minneapolis, Minnesota), and IL-13 (BD Biosciences, San Jose, California). The cells were pulsed with tritium-labeled thymidine (1 µCi/well) and harvested after 24 hours of incubation to measure the amount of isotope uptake.

STATISTICAL ANALYSIS

The baseline demographic, clinical, and imaging characteristics of the subjects were compared using the Wilcoxon rank sum test or Fisher exact test as appropriate to evaluate whether randomization led to similar groups. The treatment effect on the MSFC at each posttreatment time point was examined using a mixed-effects model with a categorical time effect, a subject-specific random effect, and control for the baseline MSFC.12 Robust standard errors were used to accommodate departures from the assumed correlation structure. In addition, each component of the MSFC was examined using the same model. The time to first relapse was investigated using a log-rank test. Although 3 subjects had more than 1 relapse, additional relapses were not considered because some subjects dropped out of the study after a relapse. For the EDSS score and ambulation index analyses, the value at the last available scheduled clinical visit was compared between the 2 groups, stratifying on the baseline EDSS score using the test by van Elteren.13

For the immunologic outcomes, the expression level for each stimulation and immunologic factor was measured using 10 separate wells, and the results from these 10 wells were modeled using 2 approaches. The mean expression level over the 10 wells was calculated and corrected by subtracting the mean of unstimulated wells. Three analyses were applied to each factor and stimulation level. The first modeled the probability that expression of the immunologic factor was below the level of detection using a generalized estimating equation model with an exchangeable correlation structure.14 Two other models were used (eAppendix) to confirm the results of the first model. Data from line stimulation at 1 µg and 10 µg of glatiramer acetate were not used in the analysis because little cytokine response was observed at these dosage levels even at baseline. In all longitudinal models for immunologic data, the baseline measurement was included as a response, and a common intercept was assumed.

For the analysis of imaging outcomes, the treatment effect on the BPF was assessed using linear regression with control for baseline and Wilcoxon rank sum test for the change from baseline. The change in the gadolinium-enhancing lesion count was determined using Wilcoxon rank sum test for the difference in number compared with baseline. All analyses were completed on the intent-to-treat and per-protocol populations. The intent-to-treat population was defined as subjects who had at least 1 posttreatment measurement (n = 39), and the per-protocol population was defined as subjects who had been receiving treatment for at least 1 year (n = 34). Missing data were treated as missing at random as defined by Little and Rubin15 for models involving the MSFC and its components and as missing completely at random for models involving immunologic end points.

RESULTS

STUDY POPULATION

Forty-four subjects were enrolled; 23 were randomized to the glatiramer acetate plus albuterol treatment arm, and 21 were randomized to the glatiramer acetate plus placebo treatment arm. The baseline demographic, clinical, and MR imaging characteristics were well balanced between the 2 groups (Table 1). Of 44 subjects, 27 (61%) completed 2 years of clinical follow-up, 34 (77%) had 1 year of complete clinical follow-up, and 39 (89%) contributed to the analysis (eFigure 1). The proportion of subjects who discontinued study participation was similar in each treatment arm (9 of 23 in the glatiramer acetate plus albuterol treatment arm and 8 of 21 in the glatiramer acetate plus placebo treatment arm, P > 50), and the mean time on study was 79.1 weeks for the glatiramer acetate plus albuterol treatment arm and 82.0 weeks for the glatiramer acetate plus placebo treatment arm.

EFFICACY

The primary clinical end point was the MSFC at 6, 12, 18, and 24 months (Table 2). No significant treatment effect was observed at 24 months (mean difference between groups, 0.09; 95% confidence interval [CI], −0.17 to 0.35), but subjects receiving glatiramer acetate plus albuterol had a significantly greater MSFC compared with subjects receiving glatiramer acetate plus placebo at 6 months (mean, 0.26; 95% CI, 0.08-0.43) and at 12 months (0.210; 0.008-0.420) in the intent-to-treat population (Figure). The improvement in the MSFC was largely driven by different results between the groups on the timed 25-foot walk, which improved for the glatiramer acetate...
plus albuterol group and worsened for the glatiramer acetate plus placebo group. Similar results were obtained in the per-protocol analysis (data not shown).

Ten subjects experienced relapses during the study, 2 in the glatiramer acetate plus albuterol group and 8 in the glatiramer acetate plus placebo group. Subjects in the glatiramer acetate plus placebo group experienced a first relapse faster than subjects in the glatiramer acetate plus albuterol group ($P=0.03$) (eFigure 2). The annualized relapse rate was 0.09 relapses per year for the glatiramer acetate plus albuterol group and 0.37 relapses per year for the glatiramer acetate plus placebo group. Finally, the changes in the EDSS score and ambulation index were not significantly different between the 2 treatment groups.

The second primary end point was the change in the immunologic markers IFN-$\gamma$ and IL-13. We measured the frequency of glatiramer acetate–specific lines that produced IFN-$\gamma$ or IL-13 longitudinally and classified individuals based on the absence of lines producing a particular cytokine (complete suppression). As previously reported,8 the frequency of IFN-$\gamma$–producing lines decreased during glatiramer acetate treatment. In the combined population (glatiramer acetate plus albuterol group and glatiramer acetate plus placebo group), there was a significant increase ($P=0.02$) in the odds of complete suppression at 6 months and 12 months at the glatiramer acetate dosage of 100 µg/mL. Although the glatiramer acetate plus albuterol group generally had greater odds of complete suppression of IFN-$\gamma$ compared with the glatiramer acetate plus placebo group (Table 3 and eFigure 3), only a trend was observed at 12 months for the stimulation at 100 µg/mL (odds ratio, 4.18; 95% CI, 0.88-19.90; $P=0.07$).

Similarly, the odds of complete suppression for IL-13–producing lines increased in the combined treatment groups at the stimulations of 50 µg/mL and 100 µg/mL at the 6-month and 12-month time points ($P<0.03$).

### Table 2. Clinical and Magnetic Resonance Imaging End Points (Change From Baseline)$^a$

<table>
<thead>
<tr>
<th>Time Point, mo</th>
<th>Glatiramer Acetate Plus Albuterol Sulfate Group, Raw Change</th>
<th>Glatiramer Acetate Plus Placebo Group, Raw Change</th>
<th>Difference Between Groups, Model Based</th>
<th>Robust P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.08 (0.33)</td>
<td>−0.13 (0.26)</td>
<td>0.26 (0.09)</td>
<td>.005</td>
</tr>
<tr>
<td>12</td>
<td>0.06 (0.32)</td>
<td>−0.14 (0.39)</td>
<td>0.21 (0.10)</td>
<td>.047</td>
</tr>
<tr>
<td>18</td>
<td>0.18 (0.41)</td>
<td>0.03 (0.42)</td>
<td>0.09 (0.14)</td>
<td>.51</td>
</tr>
<tr>
<td>24</td>
<td>0.16 (0.32)</td>
<td>0.04 (0.45)</td>
<td>0.09 (0.13)</td>
<td>.51</td>
</tr>
<tr>
<td></td>
<td>Multiple Sclerosis Functional Composite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.13 (0.71)</td>
<td>0.01 (0.73)</td>
<td>0.21 (0.20)</td>
<td>.31</td>
</tr>
<tr>
<td>12</td>
<td>0.28 (0.54)</td>
<td>0.007 (0.740)</td>
<td>0.30 (0.19)</td>
<td>.12</td>
</tr>
<tr>
<td>18</td>
<td>0.30 (0.71)</td>
<td>0.14 (0.49)</td>
<td>0.07 (0.18)</td>
<td>.71</td>
</tr>
<tr>
<td>24</td>
<td>0.28 (0.52)</td>
<td>0.30 (0.71)</td>
<td>−0.09 (0.19)</td>
<td>.64</td>
</tr>
<tr>
<td></td>
<td>Paced Auditory Serial Addition Test, z Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.18 (0.56)</td>
<td>−0.29 (0.48)</td>
<td>0.48 (0.15)</td>
<td>.002</td>
</tr>
<tr>
<td>12</td>
<td>−0.15 (0.47)</td>
<td>−0.42 (0.61)</td>
<td>0.32 (0.17)</td>
<td>.07</td>
</tr>
<tr>
<td>18</td>
<td>0.10 (0.50)</td>
<td>−0.24 (0.67)</td>
<td>0.41 (0.19)</td>
<td>.03</td>
</tr>
<tr>
<td>24</td>
<td>−0.005 (0.330)</td>
<td>−0.34 (0.80)</td>
<td>0.38 (0.21)</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Timed 25-Foot Walk, z Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.13 (0.23)</td>
<td>−0.06 (0.69)</td>
<td>0.21 (0.38)</td>
<td>.71</td>
</tr>
<tr>
<td>12</td>
<td>−0.15 (0.47)</td>
<td>−0.24 (0.67)</td>
<td>0.41 (0.19)</td>
<td>.03</td>
</tr>
<tr>
<td>18</td>
<td>0.10 (0.50)</td>
<td>−0.24 (0.67)</td>
<td>0.41 (0.19)</td>
<td>.03</td>
</tr>
<tr>
<td>24</td>
<td>−0.005 (0.330)</td>
<td>−0.34 (0.80)</td>
<td>0.38 (0.21)</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>9-Hole Peg Test, z Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>−0.06 (0.98)</td>
<td>−0.11 (0.60)</td>
<td>0.18 (0.25)</td>
<td>.47</td>
</tr>
<tr>
<td>12</td>
<td>0.04 (0.81)</td>
<td>−0.01 (0.63)</td>
<td>0.08 (0.23)</td>
<td>.74</td>
</tr>
<tr>
<td>18</td>
<td>0.13 (1.02)</td>
<td>0.19 (0.73)</td>
<td>−0.15 (0.30)</td>
<td>.63</td>
</tr>
<tr>
<td>24</td>
<td>0.21 (0.66)</td>
<td>0.16 (0.81)</td>
<td>0.02 (0.26)</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Brain Parenchymal Fraction</td>
<td></td>
<td></td>
<td>.10</td>
</tr>
<tr>
<td>12</td>
<td>−0.002 (1.140)</td>
<td>−0.60 (0.66)</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gadolinium-Enhancing Lesions</td>
<td></td>
<td></td>
<td>.87</td>
</tr>
<tr>
<td>12</td>
<td>−0.69 (1.40)</td>
<td>−1.71 (3.74)</td>
<td>. . .</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: Ellipses, not applicable.

$^a$The intent-to-treat results are presented. The Multiple Sclerosis Functional Composite P values are from a mixed-effects model with a subject effect and robust standard errors; the brain parenchymal fraction $P$ value is from an analysis of covariance model; and the gadolinium-enhancing lesions $P$ value is from a Wilcoxon rank sum test on the change from baseline.
for all comparisons). Although the glatiramer acetate plus albuterol group had a greater increase at 6 months and 12 months for both stimulations (Table 3 and eFigure 3), the only significant difference was at 12 months for the 100 µg/mL stimulation (odds ratio, 4.61; 95% CI, 1.02-20.90; \( P = .048 \)).

Analysis of the secondary immunologic end points showed that IL-5 production did not change significantly over time in either group or when the groups were combined (data not shown). Conversely, analysis showed decreased proliferation in both groups at the 6-month and 12-month time points at the 50 µg/mL stimulation, but no significant difference was observed between the 2 groups.

Although treatment with glatiramer acetate plus albuterol led to slower brain atrophy over the first year (Table 2), the difference between groups was not statistically significant (\( P = .10 \), analysis of covariance; and \( P = .06 \), Wilcoxon rank sum test). Both groups showed a decrease in the number of gadolinium-enhancing lesions at month 12, but there was no significant difference between the 2 groups. The maximum number of gadolinium-enhancing lesions at 12 months in each group was 2.

**SAFETY**

Many subjects experienced adverse events, but most were mild (Table 4). One subject in each treatment group had a serious adverse event. Of the moderate or severe adverse events, 3 were considered possibly related to the study treatment, including reaction at the glatiramer acetate injection site, leg weakness, and chest tightness. The remaining events were unlikely or unrelated to the study treatment (eTable). Mild tremor was reported by 2 subjects in the glatiramer acetate plus placebo group and by 11 subjects in the glatiramer acetate plus albuterol group.

---

**Table 3. Estimated Probability of Complete Suppression and Odds Ratios From Repeated-Measures Logistic Regression Analysis**

<table>
<thead>
<tr>
<th>Immunologic End Point</th>
<th>Glatiramer Acetate Plus Albuterol Sulfate Group</th>
<th>Glatiramer Acetate Plus Placebo Group</th>
<th>Difference Between Groups</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interferon ( \gamma ), mean (SE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.13 (0.05-0.27)</td>
<td>0.16 (0.05-0.40)</td>
<td>1.57 (0.31-7.97)</td>
<td>.59</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.23 (0.10-0.47)</td>
<td>0.32 (0.15-0.55)</td>
<td>1.70 (0.47-6.16)</td>
<td>.42</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.45 (0.25-0.66)</td>
<td>0.20 (0.07-0.47)</td>
<td>4.18 (0.88-19.90)</td>
<td>.07</td>
</tr>
<tr>
<td><strong>Interleukin 13, mean (SE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.15 (0.07-0.30)</td>
<td>0.26 (0.11-0.50)</td>
<td>0.82 (0.18-3.68)</td>
<td>.80</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.23 (0.09-0.46)</td>
<td>0.37 (0.18-0.60)</td>
<td>1.94 (0.52-7.27)</td>
<td>.33</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.53 (0.31-0.74)</td>
<td>0.37 (0.17-0.62)</td>
<td>4.61 (1.02-20.90)</td>
<td>.048</td>
</tr>
</tbody>
</table>

Abbreviation: Ellipses, not applicable.

*The intent-to-treat results are presented for glatiramer acetate stimulation (100 µg/mL). Estimates were obtained from the generalized estimating equation model.*

---

**Table 4. Adverse Events (AEs)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glatiramer Acetate Plus Placebo Group (n=345)</th>
<th>Glatiramer Acetate Plus Albuterol Sulfate Group (n=399)</th>
<th>Combined Groups (n=744)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of serious AEs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Subjects with serious AEs</td>
<td>1/20 (5.0)</td>
<td>1/23 (4.3)</td>
<td>2/43 (4.7)</td>
</tr>
<tr>
<td>No. of AEs by severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>328 (95.1)</td>
<td>374 (93.7)</td>
<td>702 (94.4)</td>
</tr>
<tr>
<td>Moderate</td>
<td>17 (4.9)</td>
<td>24 (6.0)</td>
<td>41 (5.5)</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>1 (0.3)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Life threatening</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects with AEs by severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>(n=20)</td>
<td>(n=23)</td>
<td>(n=43)</td>
</tr>
<tr>
<td>Moderate</td>
<td>12 (60.0)</td>
<td>13 (56.5)</td>
<td>25 (58.1)</td>
</tr>
<tr>
<td>Severe</td>
<td>8 (40.0)</td>
<td>9 (39.1)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Life threatening</td>
<td>0</td>
<td>1 (4.3)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Fatal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serious AE related to study drug</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No. of deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Due to rounding, percentages do not total 100.*
Modulation of immune responses has been demonstrated in subjects receiving glatiramer acetate treatment. Induction of glatiramer acetate–reactive T<sub>H2</sub>-like regulatory suppressor cells is thought to be the primary mechanism in the therapeutic action of this drug. In addition, it was suggested in 2005 that glatiramer acetate affects properties of antigen-presenting cells. Glatiramer acetate–reactive T cells may also produce neurotrophic factors (eg, brain-derived neurotrophic factor) that protect neurons and axons in the area of injury. As previously reported in 2000, we found that treatment with glatiramer acetate led to a gradual decrease in proliferation and cytokine production by glatiramer acetate–specific cells that was evident by 6 months after initiation of therapy (eFigure 3); it was found that administration of glatiramer acetate plus albuterol led to an earlier decrease in glatiramer acetate–specific T-cell responses that became significant at 12 months, at which time IL-13 production was significantly lower in the subjects treated with glatiramer acetate plus albuterol. The fact that IL-13–producing lines tended to decrease during glatiramer acetate treatment was surprising, especially given previous reports of increased T<sub>H2</sub> cytokines. However, it was noted at least in 1 study that the observed T<sub>H1</sub>1 to T<sub>H2</sub> shift is a statistical phenomenon, as individual patients showed a T<sub>H1</sub>1-biased rather than T<sub>H2</sub>2-biased cytokine profile, despite prolonged treatment with glatiramer acetate. The discrepancy could be related to the longer follow-up in that study compared with the previous observations. However, the observed decrease in proliferation, as well as the decrease in cytokine secretion, is compatible with the hypothesis of energy development.

Adrenergic mechanisms have a major role in the modulation of the immune response, and experiments in the experimental allergy encephalomyelitis model showed worsening of disease by chemical sympathectomy and suppressed disease by β-adrenergic agonists. Indeed, earlier work showed that decreased IL-12 may be a mechanism by which β-adrenergic agonists may have a positive effect on T<sub>H1</sub>–mediated autoimmune disease. More recently, adrenergic and dopaminergic pathways were reported to have a role in regulatory T-cell function in humans. In that study, the clinical response occurred earlier than the observed immunologic effects, suggesting that albuterol may have beneficial immunologic effects other than those we monitored during the present trial.

Disease-modifying therapies could decelerate brain atrophy by reducing inflammation and possibly by promoting remyelination and repair. Brain atrophy measurements can provide an estimate of the amount of tissue destruction due to the pathologic processes in MS. Glatiramer acetate treatment was reported to have no significant effect on the rate of brain volume change during short-term therapy, while long-term therapy with glatiramer acetate was reported to prevent the loss of brain parenchyma in subjects with relapsing-remitting MS. We found a smaller change in BPF at 12 months in the glatiramer acetate plus albuterol group compared with the glatiramer acetate plus placebo group. Although the difference was not statistically significant, our study was not powered to detect a difference in this outcome. The slower rate of BPF change in the glatiramer acetate plus albuterol group agrees with our finding of earlier clinical stabilization in this group. Our reported change in BPF among the glatiramer acetate plus placebo group (mean, −0.68% per year) is comparable to the reported BPF change in subjects treated with glatiramer acetate.

The MSFC includes quantitative functional measures of leg, hand, and arm, and cognitive function. The observed early improvement in the MSFC among subjects treated with glatiramer acetate plus albuterol suggests that albuterol-mediated immune modulation is associated with clinical benefit, although we could not detect statistically significant differences in immune responses at the early time points. Notably, the effect on the MSFC was mostly driven by improvement in the 25-foot walk. Improvement in lung function by albuterol therapy is a potential explanation for the improved walking time, but we believe that this is unlikely. The time of appearance of both clinical and MR imaging–monitored therapeutic effects to glatiramer acetate was reported to be around 6 months after the start of treatment; therefore, the addition of albuterol to glatiramer acetate therapy at the time of treatment initiation may accelerate clinical response. Albuterol is generally a safe medication but may not be appropriate for patients taking β-blockers or monoamine oxidase inhibitors or for patients with anxiety disorders.

We conclude that treatment with glatiramer acetate plus albuterol is well tolerated and improves clinical outcomes in patients with multiple sclerosis. The combined regimen seems to enhance clinical response during the first year of therapy.

Financial Disclosure: Dr Khoury has received consulting or lecture fees from EpiVax, LifeCycle Pharmaceutical, PDL, BioPharma, Repligen, and Wyeth Pharmaceuticals. Dr Hafler has received consulting or lecture fees from Allozyne, Inc, ElsAI Research Institute, Xceed Molecular Corporation, and Actelion. Dr Weiner has received consulting or lecture fees from AutolImmune, Biogen Idec, EMD Serono, Enzo, Gentech, Teva Neuroscience, and Vascular Biogenics and has received grant support from Millennium Pharmaceuticals. Dr Buckle has received consulting or lecture fees from Bayer, Biogen Idec, EMD Serono, Pfizer, and Teva Neuroscience.

Funding/Support: This study was supported in part by Autoimmunity Center of Excellence Study grant U19A1046130 from the National Institute of Allergy and Infectious Diseases (Dr Khoury).

Online-Only Material: The eAppendix, eTable, and eFigures are available at http://www.archneurol.com.

REFERENCES


