Induction of Antigen-Specific Tolerance in Multiple Sclerosis After Immunization With DNA Encoding Myelin Basic Protein in a Randomized, Placebo-Controlled Phase 1/2 Trial

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Objective: To assess safety and immune modulation by BHT-3009, a tolerizing DNA vaccine encoding full-length human myelin basic protein, in patients with multiple sclerosis (MS).

Design: The study was a randomized, double-blind, placebo-controlled trial. Subjects receiving placebo were crossed over into an active arm after treatment unblinding.

Setting: The trial was conducted at 4 academic institutions within North America.

Patients: Thirty patients with relapsing-remitting or secondary progressive MS who were not taking any other disease-modifying drugs were enrolled in the trial. Further, the patients were required to have either 1 to 5 gadolinium-enhancing lesions on screening brain magnetic resonance imaging (MRI), a relapse in the previous 2 years, or disease worsening in the previous 2 years.

Interventions: BHT-3009 was administered as intramuscular injections at weeks 1, 3, 5, and 9 after randomization into the trial, with or without 80 mg of daily oral atorvastatin calcium in combination. Three dose levels of BHT-3009 were tested (0.5 mg, 1.5 mg, and 3 mg).

Main Outcome Measures: The primary outcome measures were safety and tolerability of BHT-3009. Secondary outcome measures included the number and volume of gadolinium-enhanced lesions on MRI, relapses, and analysis of antigen-specific immune responses.

Results: BHT-3009 was safe and well tolerated, provided favorable trends on brain MRI, and produced beneficial antigen-specific immune changes. These immune changes consisted of a marked decrease in proliferation of interferon-γ-producing, myelin-reactive CD4+ T cells from peripheral blood and a reduction in titers of myelin-specific autoantibodies from cerebral spinal fluid as assessed by protein microarrays. We did not observe a substantial benefit of the atorvastatin combination compared with BHT-3009 alone.

Conclusion: In patients with MS, BHT-3009 is safe and induces antigen-specific immune tolerance with concordant reduction of inflammatory lesions on brain MRI.

Trial Registration: clinicaltrials.gov Identifier: NCT00103974.

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Multiple sclerosis (MS) is a chronic demyelinating disease characterized by a coordinated immune attack on the myelin sheath in the central nervous system (CNS), leading to eventual damage of the underlying axon. There are elements of an antigen-specific adaptive immune response in MS involving T cells and antibodies, as well as many aspects of an innate immune response that includes the presence in brain lesions of mediators such as complement and microglia, which process myelin-derived autoantigens.¹ The cause of MS is unknown, but substantial evidence exists that autoreactive immune cells and antibodies that recognize specific myelin antigens, such as myelin basic protein (MBP), as well as a subset of inflammatory cyto-
...kines, such as interferon-γ or more recently IL-17 (interleukin 17), play a fundamental role in its pathogenesis. There are no approved MS therapies aimed specifically toward down-regulating antigen-specific autoreactive immune cells. Development of a therapy that could down-regulate or tolerize the pathogenic antigen-specific auto-immune cells in MS provides the propitious opportunity of maximizing efficacy while minimizing potential adverse effects.

One such approach involves the use of plasmid DNA vaccines encoding 1 or more myelin antigens. Initially, intramuscularly injected plasmid DNA was demonstrated to be a relatively efficient method of gene transfer without the potential adverse consequences associated with viral-mediated gene transfer. It was then quickly discovered that plasmid DNA could also be used as a vaccine because immune responses were generated against the encoded protein product of the DNA plasmid. Recent work by ourselves and others has demonstrated that DNA vaccines can be used instead to down-regulate or alter an ongoing immune response in various animal models of autoimmunity, providing the framework for the human studies we describe herein.

We present herein the results of the first DNA vaccine, to our knowledge, used to treat autoimmune disease in humans. We have engineered a DNA vaccine called BHT-3009 encoding full-length human MBP and have treated 30 patients with relapsing-remitting (RRMS) or secondary progressive MS (SPMS) in a randomized, multicenter, double-blind, placebo-controlled, dose-escalation phase 1/2 trial. The results reported herein demonstrate that BHT-3009 is safe and well tolerated and produced favorable brain magnetic resonance imaging (MRI) and immune response changes.

METHODS

SUBJECTS

After providing informed consent, patients were enrolled at 4 clinical sites in North America. The protocol was reviewed and approved by both the Canadian and US regulatory agencies as well as ethics committees at each site. An independent data and safety monitoring board oversaw the study. This study was performed according to the Declaration of Helsinki guidelines. Eligible patients were 18 years or older and had a definite diagnosis of MS by the McDonald criteria, RRMS or SPMS, and a screening disability score on the Kurtzke Expanded Disability Status Scale (EDSS) between 2.5 and 6.5, inclusive. Further, eligible patients had either (1) 1 to 5 gadolinium-positive lesions on screening MRI, (2) a clinical relapse within 2 years prior to screening, or (3) disease worsening in the previous 2 years. Patients were excluded if treated with high-dose corticosteroids in the month prior to screening, glatiramer acetate within 12 months prior to screening, or interferon-β within 1 month prior to screening.

STUDY DESIGN

A total of 30 patients with RRMS or SPMS were enrolled in 1 of 3 arms: placebo, BHT-3009 alone, or BHT-3009 plus atorvastatin calcium (Lipitor, Pfizer Inc, New York, New York). Patients were divided into 3 dose cohorts of BHT-3009: 0.5 mg, 1.5 mg, or 3 mg per dose. After a subject passed the screen, randomization was performed by a third-party organization (Pacific Data Designs, San Francisco, California). Both the sponsor and all personnel at the clinical sites were blinded to this randomization process.

BHT-3009 was administered intramuscularly at weeks 1, 3, 5, and 9 after randomization. Oral atorvastatin calcium was administered as an immunomodulatory agent at a dose of 80 mg once daily, beginning 2 days before the first BHT-3009 dose and continuing until the treatment blind was broken (at week 13). The BHT-3009 and atorvastatin combination arm was tested because of the theoretical benefit of adding a helper T cell subtype 2 promoting adjuvant. Preclinical studies have shown that atorvastatin by itself can treat animal models of MS by promoting helper T cell subtype 2 deviation of the autoimmune response. Plas- cebo consisted of either saline injections or placebo tablets, as appropriate. Magnetic resonance imaging and other safety evaluations were performed twice at baseline and at weeks 5, 9, 13, 26, 38, and 50. Patients initially randomized to placebo were rerandomized into one of the active treatment arms at week 13 ( crossover patients). These patients were dosed and evaluated in an analogous way to those originally randomized to one of the active treatment arms, except in an open-label manner.

At each safety evaluation visit, assessment of the patient included history and physical examination (including injection-site examination after receiving dose), vital signs, adverse-event monitoring, hemolologic testing, blood chemistries, and urinalysis. In addition, EDSS assessment, an electrocardiogram, and blood tests for measurement of antinuclear antibody titers and anti–double-stranded DNA levels were performed at screening and at week 13. The National Cancer Institute Common Terminology Criteria for Adverse Events were used for scoring the severity of adverse events. Patients who were experiencing a relapse were evaluated and received standard of care using the best judgment of the treating physician.

END POINTS

The primary outcome measure was safety as determined by the number of adverse events, neurologic assessments of relapses and disability, MRI measurements of gadolinium-enhancing and T2 lesions, and standard laboratory evaluation of hematological, renal, and liver function. A relapse was defined as the appearance or reappearance of 1 or more neurologic abnormalities persisting for at least 48 hours and immediately preceded by a period of relatively stable or improving disease for at least 30 days. Other outcome measures included the assessment of the impact on antigen-specific immune response as measured by T-cell activity assays on peripheral blood and autoantibody measurements on cerebral spinal fluid (CSF), when available.

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging of the brain with injection of gadolinium was performed according to a standardized protocol. A central MRI reading unit (NeuroRx Research, Montreal, Quebec, Canada) qualified each participating site’s MRI departments before the study opened at the site. The MRI reading unit evaluated MRIs for quality and measured the study end points according to standardized protocols without knowledge of the patients’ treatment assignments.

T-CELL ASSAYS

Fresh peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll gradient techniques at baseline, week 9, and week 50 in a subset of patients in the trial. On the day...
of collection and immediately on purification of the PBMCs, they were incubated with a variety of myelin antigens and 5,6-carboxyfluorescein diacetate succinimidyl ester, a vital dye used to measure proliferation. Three positive controls were used: anti-CD3, tetanus toxoid, and glatiramer acetate. The PBMCs were cultured for 10 days, then stimulated with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin A, after which the cells were stained with anti-CD3, anti-CD4, and an intracellular cytokine stain for IFN-γ or IL-4. Cells were acquired on a fluorescent-activated cell sorter and the data were analyzed by gating on lymphocytes and CD4+ cells.

PROTEIN MICROARRAY ASSAYS

Custom myelin peptide arrays, each containing 127 myelin and control peptides printed in duplicate, were produced by JPT Peptide Technologies GmbH (Berlin, Germany). As previously described, arrays were blocked with phosphate-buffered saline containing 3% fetal calf serum and 0.05% Tween 20, probed with 1:10 dilutions of CSF, and autoantibody binding detected using Cy-3–labeled goat-antihuman IgG/M antisera samples (Jackson Immunoresearch, West Grove, Pennsylvania). Arrays were scanned with the GenePix 4000B scanner (Molecular Devices Corporation, Sunnyvale, California), and GenePix Pro 5.0 software (Molecular Devices Corporation) was used to quantitate net median pixel intensities for each peptide. Two arrays were probed with each CSF sample, and the heatmap presents reactivities for all peptides for which there was more than a 2-fold and more than a 25–fluorescence unit difference between the pretreatment and posttreatment median values.

RESULTS

DNA VACCINE CONSTRUCTION

We isolated the full-length complementary DNA encoding the 18.5-kDa isofrom of human MBP from a human brain complementary DNA library. This was cloned into a modified expression plasmid driven by a eukaryotic promoter. The expression plasmid was created as a derivative of the pVAX1 plasmid, where certain immunostimulatory CpG motifs were removed and immunoinhibitory GpG motifs were included. We have previously shown that oligonucleotides containing these GpG motifs either alone or combined with DNA vaccine plasmids were effective in treating animal models of several prototypic autoimmune diseases. The plasmid thus constructed, BHT-3009, was then manufactured under good manufacturing practice conditions and formulated in a phosphate-buffered saline solution containing calcium.

DEMOGRAPHICS AND TRIAL DESIGN

The flow of subjects through the study is illustrated in Figure 2. The first subject was screened in June 2004, and the last was screened in December 2005. The first patient completed dosing (ie, week 13) in October 2004, and the last patient completed dosing in July 2006. The
The demographic composition of the 3 treatment arms was balanced among the groups. A total of 11 patients with RRMS, 13 patients with SPMS without relapses, and 6 patients with SPMS with relapses were enrolled in the study. Patients were randomized without regard to their clinical subtypes, and thus, the final distribution of patients by subtype of MS is shown in Table 1. The median age of 50.1 years and the mean EDSS score of 4.6 indicated the patient population had moderately advanced disease.

**ADVERSE EVENTS AND CLINICAL RELAPSES**

Adverse events that were considered to be treatment related or possibly treatment related are tabulated in Table 2. All adverse events were considered to be mild to moderate in severity and brief. No adverse events grade higher than 3 was considered to be treatment related. Furthermore, the percentage of patients with adverse events in the BHT-3009 treatment arm was no greater than that with placebo. Routine clinical laboratory testing did not

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**Table 1. Disposition of Patients**

<table>
<thead>
<tr>
<th></th>
<th>0.5-mg Dose</th>
<th>1.5-mg Dose</th>
<th>3-mg Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>BHT-3009</td>
<td>BHT-3009 + atv</td>
</tr>
<tr>
<td>RRMS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Patients</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SPMS w/ relapse</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SPMS w/o relapse</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 2. Treatment-Related AE**

<table>
<thead>
<tr>
<th></th>
<th>BHT-3009</th>
<th>BHT-3009 + atv</th>
<th>Total BHT-3009</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>14</td>
<td>14</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Patients with AE</td>
<td>8 (57.1)</td>
<td>6 (42.9)</td>
<td>14 (50.0)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Total AE</td>
<td>16</td>
<td>13</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Patients who experienced indicated AE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site</td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>5 (17.9)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Gas/abdominal pain, discomfort</td>
<td>0</td>
<td>1 (7.1)</td>
<td>1 (3.6)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Upset stomach</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Sour taste</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>0</td>
</tr>
<tr>
<td>Constitutional</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (7.1)</td>
<td>1 (7.1)</td>
<td>2 (7.1)</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Dizziness/imbalance</td>
<td>0</td>
<td>1 (7.1)</td>
<td>1 (3.6)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Sweats</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>0</td>
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<tr>
<td>Weakness</td>
<td>0</td>
<td>1 (7.1)</td>
<td>1 (3.6)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gynecologic</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Urinary urgency</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Pain</td>
<td>0</td>
<td>2 (14.3)</td>
<td>2 (7.1)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Contusion</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Palpitations</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (3.6)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Abbreviations**:
- AE: adverse events
- atv: atorvastatin calcium
- NA: not applicable
- RRMS: relapsing-remitting multiple sclerosis
- SPMS: secondary progressive multiple sclerosis
- w/, with
- w/o, without

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Table 1: **Disposition of Patients**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>BHT-3009</th>
<th>BHT-3009 + atv</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRMS</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SPMS w/ relapse</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SPMS w/o relapse</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2: **Treatment-Related AE**

<table>
<thead>
<tr>
<th></th>
<th>BHT-3009</th>
<th>BHT-3009 + atv</th>
<th>Total BHT-3009</th>
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</thead>
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At 1 clinical site, the peripheral immune response of myelin antigen–specific CD4+ T cells was measured through the course of the study (Figure 3 and Figure 4). This was measured with a flow cytometer–based proliferation assay as described in the “Methods” section.

Using this assay, data at baseline and after treatment with BHT-3009 were obtained from a total of 6 patients. Of these, 5 patients were found to have significant proliferation of IFN-γ–producing CD4+ T cells reactive to myelin antigens at baseline, and all had a decline in proliferation with BHT-3009 (Figure 4). No such decrease was observed in their response to tetanus toxoid or to global T-cell responses, such as with anti-CD3 or glatiramer acetate, indicating that antigen-specific tolerance had occurred in these 5 patients. This antigen-specific decrease in T-cell activity persisted up to 50 weeks after randomization in several patients.

Further, in none of the patients, including the single patient who had no activity at baseline, was there a significant induction of myelin antigen–specific T-cell proliferation at any point after BHT-3009 treatment. Taken together, these data thus demonstrate that BHT-3009 did not induce a cellular immune response to MBP or any of the other myelin antigens tested and suggest that it is an effective agent for the induction of antigen-specific tolerance to myelin proteins in patients with MS.

### MRI DATA

Contrast-enhanced brain MRI was obtained at baseline and during treatment as described in the “Methods” section. No statistically significant increases in the number of gadolinium-enhancing lesions, volume of gadolinium-enhancing lesions, and volume of T2 lesions were observed with either BHT-3009 alone or in combination with atorvastatin compared with placebo. Rather, there was a trend toward a reduction in lesion activity after treatment relative to the placebo group (Table 3). The gadolinium lesion number and volume increased in the placebo arm relative to baseline, whereas the gadolinium lesion number decreased with BHT-3009 alone and the gadolinium lesion volume decreased with both BHT-3009 alone and with atorvastatin (Table 3). These lesion changes were favorable with BHT-3009 but did not reach statistical significance.

### ANTIGEN-SPECIFIC IMMUNE RESPONSE WITHIN THE CNS

To measure the antigen-specific immune response in the CNS as opposed to peripheral blood, patients were given the option of volunteering to undergo lumbar punctures for CSF collection. Five patients volunteered during the screening period and 3 of these patients had a follow-up lumbar puncture after completing dosing with BHT-3009. The CSF was analyzed for autoantibody titers using a protein microarray as described in the “Methods” section.

The results shown in Figure 5 demonstrate that treatment with BHT-3009 significantly decreased autoantibody titers in CSF to multiple myelin antigens in all 3

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### Table 3. MRI Gd-Positive Lesion Number and Volume

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample Size</th>
<th>Baseline</th>
<th>During Treatment</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-positive lesion&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>12</td>
<td>0.63 (15/24)</td>
<td>1.12 (38/34)</td>
<td>+0.49 (+79)</td>
</tr>
<tr>
<td>BHT-3009</td>
<td>9</td>
<td>0.88 (15/17)</td>
<td>0.32 (8/25)</td>
<td>−0.56 (−64)</td>
</tr>
<tr>
<td>BHT-3009 + atvb</td>
<td>9</td>
<td>0.61 (13/16)</td>
<td>0.81 (22/27)</td>
<td>No change</td>
</tr>
<tr>
<td>BHT-3009 + atvb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14</td>
<td>0.95 (20/21)</td>
<td>0.78 (29/37)</td>
<td>−0.17 (−18)</td>
</tr>
<tr>
<td>BHT-3009 + atvb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14</td>
<td>0.62 (13/21)</td>
<td>0.67 (28/42)</td>
<td>+0.05 (+8)</td>
</tr>
<tr>
<td>Gd-positive lesion, cm&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>12</td>
<td>0.0393</td>
<td>0.0874</td>
<td>+0.0481 (+122)</td>
</tr>
<tr>
<td>BHT-3009</td>
<td>9</td>
<td>0.1545</td>
<td>0.0270</td>
<td>−0.1275 (−83)</td>
</tr>
<tr>
<td>BHT-3009 + atvb</td>
<td>9</td>
<td>0.0916</td>
<td>0.0626</td>
<td>−0.0289 (−32)</td>
</tr>
<tr>
<td>BHT-3009 + atvb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14</td>
<td>0.1410</td>
<td>0.0873</td>
<td>−0.0537 (−38)</td>
</tr>
<tr>
<td>BHT-3009 + atvb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14</td>
<td>0.0698</td>
<td>0.0472</td>
<td>−0.0225 (−32)</td>
</tr>
</tbody>
</table>

Abbreviations: atv, atorvastatin calcium; Gd, gadolinium; MRI, magnetic resonance imaging.

<sup>a</sup>Values in parentheses indicate total number of Gd-positive lesions over the total number of MRIs, which in most cases included 2 per patient at baseline and 3 per patient during treatment. Values outside the parentheses indicate the total number of lesions divided by the total number of MRIs.

<sup>b</sup>Includes data for crossover patients who were initially randomized to placebo. The last single scan before crossover was used as the baseline scan on these patients.

<sup>c</sup>Values indicate the total volume of lesions divided by the total number of MRIs.
patients who had follow-up lumbar punctures. Importantly, this reduction in autoantibody titers was not restricted to MBP but was also observed with other antigens, such as proteolipid protein (PLP), suggesting that BHT-3009 can cross-tolerize to other antigenic components of the myelin sheath. Although these specific results were not repeated with conventional enzyme-linked immunosorbent assay, we have previously shown that this assay correlates well with and is more sensitive than enzyme-linked immunosorbent assay.

The results reported herein demonstrate that BHT-3009 is safe and well tolerated, produced favorable trends in MRI brain lesions, and was associated with antigen-specific down-regulation of autoimmune activity in both blood and CSF. The combined delivery of self-antigens along with backbone modifications of the CpG content in the DNA plasmid may be sufficient to cause the
down-regulation of the autoimmune process, leading to antigen-specific tolerance and less disease activity. The BHT-3009 plasmid encodes MBP, one of the major auto-antigens putatively involved in MS pathogenesis. Further, the plasmid backbone has been modified in such a way as to reduce the number of immunostimulatory Cpg motifs and increase the number of immunoinhibitory GpG motifs. Preclinical experiments indicated that this combination of full-length self-antigen delivery and an immunoinhibitory DNA backbone could

Figure 4. Reduction in antigen-specific T-cell activity with BHT-3009. Shown are the percentages of specific interferon (IFN)–γ–positive CD4+ T-cell responses (y-axis) to various antigens (x-axis) at several points for 6 patients: patient 003 (A), patient 002 (B), patient 028 (C), patient 011 (D), patient 027 (E), and patient 020 (F). For each patient, the right panel represents T-cell responses to the positive controls (anti-CD3, tetanus toxoid [TT], and glatiramer acetate [GA]). The left panel shows the responses to various myelin antigen peptides or whole myelin basic protein (w-MBP). The peptides used in the assay are the following: MBP83-99, a mixture of MBP14-33 and MBP114-126 (MBPa); a mixture of MBP111-129 and MBP145-164 (MBPb); a mixture of proteolipid protein (PLP) 191-210, PLP184-199, and PLP178-197 (PLPa); a mixture of PLP189-154, PLP89-106, and PLP42-59 (PLPb); a mixture of myelin-oligodendrocyte glycoproteins MOG35-55, MOG145-160, and MOG1-20 (MOG); and no peptide (nil). Samples were drawn at 3 different points: baseline, week 9, or week 50. Week 9 data for the patient in part D was not analyzable because of poor assay conditions. Patients in panels A, B, and C were in the BHT-3009 plus atorvastatin calcium arm, and patients in panels D, E, and F were in the BHT-3009 arm. The only patient among these who experienced a relapse was patient 028 (C), who had a relapse 7 weeks and 29 weeks after the last dose of BHT-3009. Positive responses are indicated with asterisks (except in the case of the positive controls where this is not indicated because the majority of data points are positive). For a particular response to be considered positive, 2 criteria had to be met: an absolute proliferation greater than 2% and a stimulation index greater than 2.0 (stimulation index is defined as the specific proliferation with a given antigen divided by that in the nil). For part E, all of the myelin antigen–specific proliferation at week 50 is judged to be negative because although many samples have a greater than 2% specific proliferation, the stimulation indexes are less than 2.0 because of the relatively high proliferation in the nil sample. Sample F is negative for all myelin antigens. During the study, the responses to the myelin antigens generally decreased in each case, whereas the positive control responses remained relatively stable, reinforcing the antigen-specific nature of the action of BHT-3009.
oligodendrocytic basic protein; OSP, osteopontin; PLP, proteolipid protein.
myelin-oligodendrocyte glycoprotein; MOBP, myelin-associated

the reactivity of myelin-reactive autoantibodies. All 3 of these patients were
predosing and postdosing CSF samples were analyzed, there is a decrease in
posttreatment sample in any patient are shown. In all 3 patients where both
25–fluorescence unit difference in reactivity between a pretreatment and
represented by the heatmap, with red being the highest reactivity and blue,
myelin peptides (far right column) was measured by protein array and
after the first dose with BHT-3009 (post). Autoantibody reactivity to various

discussed in animal models of autoimmunity and has been
observed in patients undergoing allergen desensitization. There are 2 mechanisms by which bystander suppression
and thus tissue-specific tolerance may have occurred with
BHT-3009 treatment: induction of regulatory T cells or via
dendritic cell–mediated cross-tolerance. Regulatory T cells
generated against MBP could traffic to a lesion within the
CNS and have beneficial effects either directly on other
myelin-reactive T cells or on resident myelin antigen–
representing within dendritic cells of cervical lymph nodes.
In all 3 of these patients were randomized to the BHT-3009–only arm (ie, without atorvastatin calcium).
αBCrys indicates α-B-crystallin; MBP, myelin basic protein; MOG,
myelin-oligodendrocyte glycoprotein; MOP, myelin-associated
oligodendrocytic basic protein; DSP, osteopontin; PLP, proteolipid protein.

lead to favorable immunological changes in patients
with MS.

Statins, a class of lipid-lowering drugs that includes
atorvastatin, have proven efficacious in some animal models
of autoimmunity and in early-stage human trials. We
tested whether high-dose, daily oral atorvastatin could
potentiate the efficacy of BHT-3009. The clinical, MRI,
and immunological data indicate that combining oral ator-
vasatin with our BHT-3009 vaccine provided little if any
additional benefit to what was observed with BHT-3009
alone.

In this trial, we demonstrated based on every clinical
and paraclinical parameter that BHT-3009 is safe and well
tolerated. There were no increases in clinical relapses,
disability, drug-associated laboratory abnormalities,
adverse events, or the number and volume of contrast-
enhancing lesions on brain MRI with BHT-3009 treat-
ment compared with placebo. In fact, there was a trend
oward a decrease in the number and volume of contrast-
enhancing lesions in the brain in patients treated with
BHT-3009 compared with placebo. One consideration in
interpreting the MRI data is that a relatively higher num-
ber of patients with SPMS without relapses were ran-
domized to placebo, and thus, conclusions solely based
on the MRI data should be avoided.

Because of the unique antigen-specific nature of the
mechanism of action of BHT-3009, we also carried out
immunological assays in several patients, analyzing T-
cell and autoantibody responses in blood and CSF, re-
spectively. In all 5 patients who had MBP- or PLP-
specific activity at baseline, the antigen-specific autoreactive T-cell response diminished with BHT-
3009 by week 9, as measured by the proliferation of IFN-
γ–producing CD4+ T cells. In no case was MBP- or PLP-
specific T-cell activity induced with BHT-3009, providing
further evidence that our DNA vaccine approach leads
to tolerance rather than active immunization. Using pro-
tein microarrays, we demonstrated that compared with the
screening samples, there was a relative decrease in the
myelin-reactive autoantibody titers in the CSF of 3
individuals. Further, the data demonstrate that this de-
crease in myelin-reactive autoantibody titers extends be-
yond MBP to other myelin antigens, such as PLP, myelin-
oligodendrocyte glycoprotein, and αB-crystallin.

This expansion of the tolerogenic response beyond the
antigen encoded by BHT-3009 is a demonstration of a phe-
nomenon known as “bystander suppression” or “infectious
tolerance.” Bystander suppression is well
described in animal models of autoimmunity and has been
observed in patients undergoing allergen desensitization.

There are 2 mechanisms by which bystander suppression
and thus tissue-specific tolerance may have occurred with
BHT-3009 treatment: induction of regulatory T cells or via
dendritic cell–mediated cross-tolerance. Regulatory T cells
generated against MBP could traffic to a lesion within the
CNS and have beneficial effects either directly on other
myelin-reactive T cells or on resident myelin antigen–
presenting microglia, rendering them tolerance promot-
ing. An exhaustive search for regulatory T cells was not
conducted in the trial, and thus, we can neither exclude
nor conclude that regulatory T cells are induced by BHT-
3009. Animal model studies are being conducted in ad-
inution to assays in humans as part of an ongoing, larger
phase 2b trial to examine this possibility.

The other mechanism by which bystander suppression
can be incurred is via dendritic cells. It has recently
been demonstrated that a variety of CNS myelin anti-
gens, including MBP and PLP, can be found within cer-

cral draining lymph nodes of both primates with ex-
perimental autoimmune encephalomyelitis and humans
with MS. It has been proposed that dendritic cells within
cervical lymph nodes can present multiple myelin anti-
gens and thus are central to the observed phenomenon
of epitope spreading. Further, it is argued that these mul-
tiple myelin antigen–presenting dendritic cells should play
an essential role in any approach designed to reverse epi-

tope spreading (ie, bystander suppression) within the pe-

dipheral immune system because they can potentially
cross-tolerize to multiple antigens simultaneously. BHT-
3009 is administered within the deltoid muscle of the arm,
and because we know from preclinical studies that ex-
pression of BHT-3009 can be detected in draining lymph
nodes, BHT-3009 is likely to be distributed to and ex-
pressed within dendritic cells of cervical lymph nodes.

Figure 5. Decrease in cerebral spinal fluid (CSF) autoantibodies. The CSF
was obtained from 5 patients at baseline (pre) and in 3 patients at 13 weeks
after the first dose with BHT-3009 (post). Autoantibody reactivity to various
myelin peptides (far right column) was measured by protein array and

-1000
2500
10 000
5000
2500
1000
250
100
25
10

MBP51-70
MBP31-50
MOBP31-50
MOG21-40
MOG91-110
GSP131-150
PLP131-150
PLP178-197
PLP181-200
PLP201-220
Thus, BHT-3009 may tolerate to MBP-reactive T cells circulating through these lymph nodes, but by virtue of these multiple myelin antigen–presenting dendritic cells now rendered tolerance promoting by BHT-3009, other myelin-reactive T cells (eg, PLP-reactive T cells) may also become tolerated.

We have demonstrated in this first, to our knowledge, in-human trial of a DNA vaccine for autoimmune disease that the approach is safe and well tolerated. We describe evidence for induction of favorable trends on brain MRI, indicating a reduction in the inflammatory response in the CNS. BHT-3009 reduced antigen-specific immune responses both in the peripheral immune system and the CNS, including reductions in proliferation of IFN-γ-producing, myelin-reactive CD4+ T cells and levels of CSF antibodies directed against myelin. Based on these encouraging results, a 1-year, double-blind, randomized, placebo-controlled phase 2b trial of BHT-3009 in approximately 290 patients with RRMS has commenced. If successful in MS, antigen-specific DNA vaccines can be developed for prevention or treatment of related diseases, such as type 1 diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis, and myasthenia gravis.

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