

Baseline plasma anti-MBP antibody levels correlate with subject response to BHT-3009, a novel, antigen-specific tolerizing DNA vaccine therapy for MS patients

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Abstract

Background: BHT-3009 is the first antigen-specific tolerizing DNA vaccine being tested for the treatment of patients with Multiple Sclerosis (MS). BHT-3009 encodes for full-length human myelin basic protein (MBP). Within a Phase 2 study of BHT-3009 in relapsing-remitting MS subjects, plasma from 87 subjects was available at baseline for the measurement of anti-MBP antibody levels. Given BHT-3009's proposed mechanism of action, we tested the hypothesis that BHT-3009 may be most effective in subjects with strong pre-treatment anti-MBP immune responses.

Methods: BHT-3009 was injected intramuscularly in weeks 0, 2, 4, and then once every 4 weeks thereafter through week 44. The primary outcome was determined as a measure of new gadolinium (Gd) enhancing lesions on six brain MRIs performed every 4 weeks from weeks 28 through 48. Secondary endpoints included T2 lesions and annualized relapse rates (ARR). Blood from 87 subjects was collected at baseline and tested for anti-MBP IgM antibodies reactive to 23 MBP peptides in a protein microarray assay. Using a threshold value for positivity, the number of anti-MBP antibodies positive (NUM) was calculated for each subject and correlated to MRI Gd lesions, T2 lesion, and ARR.

Results: Compared to placebo-treated subjects, sub-populations of the 0.5mg BHT-3009-treated subjects with increased baseline plasma NUM had significantly fewer new Gd-enhancing and T2 lesions and a trend towards lower ARR. The magnitude of the difference between the 0.5 mg BHT-3009 and placebo sub-populations increased with increasing NUM. At a threshold plasma NUM \geq 12, a >70% decrease in new Gd-enhancing lesions ($p=0.02$) and a >65% decrease in new T2 lesions ($p=0.04$) was observed in the 0.5 mg BHT-3009 sub-population compared to the corresponding placebo sub-population. Subjects with plasma NUM \geq 12 also exhibited a similar trend in ARR. The ARR for the NUM \geq 12 sub-population was 0.7 in the placebo-treatment group compared to 0.42 in the 0.5mg BHT-3009-treatment group. For subjects with NUM \geq 14 the ARRs were 0.79 and 0.22 in the placebo- and 0.5mg BHT-3009-treatment groups, respectively.

Conclusions: Baseline blood plasma anti-MBP IgM levels can be used to identify populations of MS patients in which treatment with 0.5mg BHT-3009 is associated with a significant reduction in new Gd-enhancing lesions, T2 lesions and relapses after one year of treatment with BHT-3009. BHT-3009 has the potential to be the first personalized medicine for MS patients.

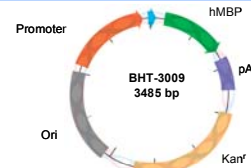
Background

Results from the main phase 2 trial of BHT-3009 have been previously reported (Garren et al. *Ann Neurol* 2008; 63:611-620). In summary, the overall results demonstrate:

- Excellent safety & tolerability
- Decrease in several brain MRI lesion parameters
- CSF auto-antibodies: antigen-specific immune tolerance

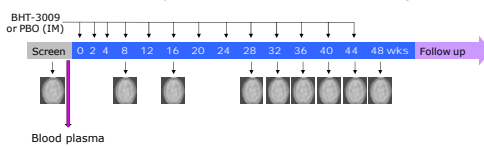
In addition to the main phase 2 trial, we performed an Immunology Study that examined immune responses of a subset of subjects who were enrolled in the phase 2 trial. A tolerizing vaccine such as BHT-3009 is likely to be most effective in the context of an ongoing, active immune response that can undergo induction of tolerance. Thus, we hypothesized that MS patients with strong immune responses to MBP peptide epitopes at baseline should benefit the most from treatment with BHT-3009. The study reported here was performed to obtain data on a possible correlation between baseline blood plasma anti-MBP antibodies and the level of benefit after treatment with BHT-3009.

Materials & Methods

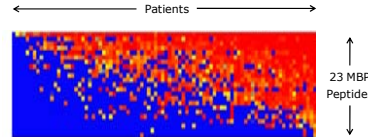


Plasmid (Fig 1). The structure of the BHT-3009 human MBP encoding DNA plasmid vaccine used in the trial is diagramed above.

Subjects: Inclusion criteria for the main phase 2 included definite diagnosis of RRMS, age of 18 to 55 years, EDSS of 0 to 3.5, at least one relapse in the past one year, and \leq 5 Gd⁺ lesions on screening brain MRI.



Design (Fig 2). A total of 289 RRMS patients were randomized 1:1:1 in one of three arms: placebo (PBO, phosphate buffered saline), 0.5 mg BHT-3009, or 1.5 mg BHT-3009. BHT-3009 was administered intramuscularly at weeks 0, 2, 4, and every 4 weeks thereafter until week 44, for a total of 13 doses (vertical arrows). One MRI with Gd was obtained at screen, eight MRIs were obtained during the treatment period, the last six of which were used for the current analysis. Blood plasma was obtained at baseline from 87 patients in the Immunology Study (purple arrow), and then were frozen and stored at -80° C.



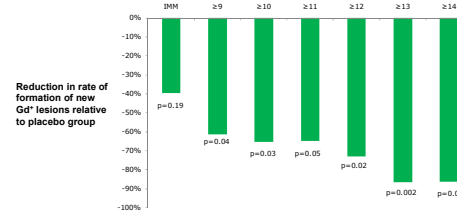
Antigen Array (Fig 3). Peptide microarrays were performed to measure the levels of anti-MBP IgM antibodies. Pre-treatment plasma anti-MBP peptide antibody measurements for all 87 subjects are displayed in a heat map format. Each box represents one subject's antibody response to one MBP peptide and the amount of antibody is shown as a heat value (blue=low and red=high). The number of human MBP peptide epitopes recognized by plasma antibodies, or NUM, was calculated by tallying the total number of myelin peptides on the array that exhibited a threshold median value for all replicate features of each peptide on the array; typically a median reactivity of \geq 10,000 digital fluorescence units. Subjects were sorted by the number of MBP peptides detected (NUM) from the highest NUM on the right to the lowest NUM on the left.

Results

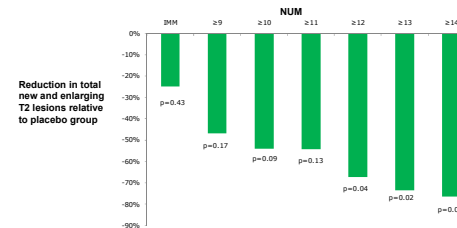
Table 1. Number of subjects for each NUM sub-population

	Placebo	0.5 mg	1.5 mg	Total
NUM \geq 14	11	10	9	30
NUM \geq 13	11	13	12	36
NUM \geq 12	14	18	15	47
NUM \geq 11	15	21	16	52
NUM \geq 10	17	26	19	62
NUM \geq 9	19	27	20	66
NUM \geq 8	20	28	20	68
All Imm	31	30	26	87

MRI Results



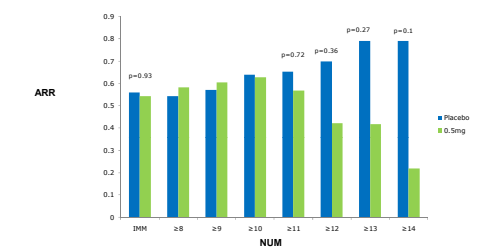
Reduction in Gd⁺ lesions with 0.5mg BHT-3009 (Fig 4). The magnitude of the difference between the 0.5mg BHT-3009 treatment group and the placebo treatment group increases with increasing NUM (73% for NUM \geq 12 and 86% reduction for NUM \geq 14). The 1.5mg dose treatment group exhibited a non-significant trend in reduction in the rate of formation of new Gd⁺ enhancing lesions compared to the placebo group (not shown). P values calculated from Generalized Linear Model with negative binomial with the number of lesions on MRI scan at baseline as a covariate.



Reduction in T2 lesions with 0.5mg BHT-3009 (Fig 5). The magnitude of the difference between the 0.5mg BHT-3009 treatment group and the placebo treatment group increases with increasing NUM (67% for NUM \geq 12 and 77% reduction for NUM \geq 14). The 1.5mg dose treatment group exhibited a non-significant trend in reduction in the rate of formation of T2 lesions compared to the placebo group (not shown). P values calculated as above.

Results

Relapse Results



Reduction in ARR with 0.5mg BHT-3009 (Fig 6). Total annualized relapse rates (ARR) in placebo-treated subjects tended to increase slightly with increasing NUM, while ARR in 0.5mg treated subjects decreased with increasing NUM. The magnitude of the difference between the 0.5mg BHT-3009 treatment group and the placebo treatment group also increased with increasing NUM (38% reduction in ARR for NUM \geq 12 and 67% reduction in ARR for NUM \geq 14). The 1.5mg dose treatment group did not exhibit any statistically significant differences between the placebo group (not shown). P values calculated from Generalized Linear Model with negative binomial with the number of relapses in the two years prior to treatment as a covariate.

Summary & Conclusions

Baseline blood plasma anti-MBP levels identifies responders to 0.5mg BHT-3009 after one year of treatment based on:

- Reduction in brain Gd-enhancing lesions (Fig 4)
- Reduction in brain T2 lesions (Fig 5)
- Reduction in clinical relapses (Fig 6)

BHT-3009 is exceptionally safe, causes antigen-specific tolerance, and improves brain lesions and clinical relapses in patients with high anti-MBP levels in their blood.

Disclosures

HG and RK are employees of and own stock and/or options in Bayhill Therapeutics
WHR, PJJ, and LS are consultants for and own stock and/or options in Bayhill Therapeutics
AR is a consultant for Bayhill Therapeutics