OBA Protocol #1007-1053
Phase 1 /2 Randomized, Blinded, Placebo-Controlled, Sequential Dose Escalation Study of the Safety and Pharmacodynamics of BHT-3034, an Acetylcholine Receptor Tolerizing Plasmid

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Outline

- Background
  - BHT-DNA plasmids
- Proposed mechanism of action of BHT-3034
- Review of animal studies
  - Expression of transgene
  - Early vs late disease
  - Lack of evidence for exacerbation
  - Selection of dose range
  - Interim toxicology study results
- MG and unmet medical need
  - Alternative therapies
- Proposed patient population
  - Ocular and mild generalized MG
  - Corticosteroid use during study
Rationale

- **Pathogenesis of MG**
  - Autoimmune process directed against extracellular domain of \( \alpha \) chain of human nicotinic acetylcholine receptor (AChR), leading to muscular weakness

- **BHT-3034** is a DNA plasmid expression vector encoding the extracellular domain of the \( \alpha \) chain of AChR

- **Goal:** Antigen-specific tolerance to AChR

- **Potential advantage:** leave intact other immune responses and immune surveillance
Other BHT-DNA Plasmids

- **BHT-3009 for multiple sclerosis**
  - OBA Protocol #0403-633: RAC Meeting June 8, 2004
  - Completed Phase 1 and Phase 2 studies
  - No safety signals seen in >200 MS patients dosed for up to one year

- **BHT-3021 for type 1 diabetes**
  - OBA Protocol #0604-769: RAC Meeting June 21, 2006
  - Ongoing Phase 1 study (T1D)
  - No safety signals seen in >50 T1D patients dosed weekly for 12 weeks
BHT-3034 Plasmid

Figure 1 – Structural Diagram of BHT-3034

BHT-3034
3696 bp

<table>
<thead>
<tr>
<th>Features</th>
<th>Position (bp)</th>
</tr>
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<tbody>
<tr>
<td>Promoter</td>
<td>32-621</td>
</tr>
<tr>
<td>Intron</td>
<td>702-834</td>
</tr>
<tr>
<td>Chrna1</td>
<td>845-1483</td>
</tr>
<tr>
<td>Poly A signal</td>
<td>1527-1751</td>
</tr>
<tr>
<td>Antibiotic Res</td>
<td>1924-2718</td>
</tr>
<tr>
<td>Origin</td>
<td>3018-3691</td>
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</table>
Proposed mechanism of BHT-DNA Induced Tolerance

**Immunizing vaccine (adjuvant)**

**Tolerizing DNA Vaccine**

**Activation:**
- ↑ proliferation
- ↑ IFN-γ
- ↑ autoantibodies

**Tolerance:**
- ↓ proliferation
- ↓ IFN-γ T cells
- ↓ autoantibodies
BHT-3034 Therapy Reduces B cell and T cell Responses

Antigen Encoding DNA

Antigen

APC (e.g. Dendritic cell)

T Cell

Tolerance:

↓ proliferation
↓ IFN-γ T cells
↓ autoantibodies

Antibody Reduction

Decreased T cell Number

RIA data

<table>
<thead>
<tr>
<th>% change</th>
<th>PBS</th>
<th>rBHT-3034</th>
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<tbody>
<tr>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.1</td>
<td></td>
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<tr>
<td></td>
<td>-0.2</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>-0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.5</td>
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</table>

p=0.016

No. of IFN-γ spots/million SP cells

<table>
<thead>
<tr>
<th>PBS</th>
<th>mBHT-3034</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>20</td>
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</table>
Animal Models and Species

- Pharmacology studies performed in mice (C57Bl/6) and rats (Lewis)
  - Gold standard for studies of MG
  - Genetically susceptible animals
  - Antigen specific induced disease model
  - Similar to human disease
    - Animals develop anti-AChR antibodies, complement deposition at NMJ, T cell involvement, progressive muscle weakness
  - Two species differ in dominant epitopes

- Toxicology study performed in Lewis rats used in efficacy EAMG studies.
BHT-3034 Expression Peaks Within One Week

Weekly Injections intended to maintain constant levels of peak expression
BHT-3034 Suppresses Disease in EAMG Studies

Mouse “Prevention” Study

Weekly IM injections
50µg Dose
n=7-8

Rat “Treatment” Study

Weekly IM injections
250µg Dose
n=17
BHT-3034 is More Effective Treating Early/Mild Disease in Rat EAMG Study M20

**Rats ≥2 at Start of Therapy (n=12)**

**Rats ≤1 at Start of Therapy (n=5)**
BHT-3034 Does not Induce anti-AChR Antibodies

EAMG Study M20 (Rat)

Mouse Immunogenicity Study (healthy C57Bl/6 mice)

Weekly dosing for 5 months in C57Bl/6 mice (n=5). No anti-AChR Antibodies induced
Toxicology Study Design

13 week GLP repeat dose (QW) toxicology study in Lewis Rat

**Dose levels:** 0.25mg, 1.0mg

**Dose regimen:** Weekly IM dosing

**Endpoints:** Clinical observations; body weights; food consumption; full necropsy with organ weights; complete tissue panel histopathology; clinical chemistry; immune cell phenotyping; hematology and clotting analysis.

**10 Week Interim data:**
- No deaths on study
- No adverse clinical symptoms associated with drug
- No differences in average body weight or food consumption
- Necropsy performed Sept 1
Phase 1/2 Dosing is Supported by Efficacy and Safety Study Dose Regimens

Rationale for Weekly Dosing:
• Effective in rat and mouse EAMG studies
• Effective in pre-clinical studies for other autoimmune diseases
• Effective in BHT-3021 Trial in T1D
• Consistent with expression profile

Rationale for Clinical Dose Range:
• MRSD is 1.0-4.0 mgs (based on tox NOAEL with 10X safety margin)
• Anticipated starting dose of 0.2 mgs (50-200X safety margin)
• Active doses in BHT-3009 and BHT-3021 Trials are in 1.0mg range
• Highest clinical dose proposed (10 mg) approaches practical limit based on concentration limits of DNA and the maximum IM dose volume.
• No safety signals in 3-6mg doses (12x weekly) in T1D Trial
Myasthenia Gravis Summary

- **Most common NMJ disorder**
  - Prevalence 1/10-20K
  - AChR-Ab discovered in 1973
- **Clinical features**
  - Ptosis, diplopia in 90%
  - Only 15% remain purely ocular; most generalize in 2-3 yrs
  - Bulbar, truncal, proximal > distal limb involvement
  - Respiratory weakness in 30%, crisis in 20%
- **Diagnosis**
  - Edrophonium test, Rep stim/SFEMG, AChR-Ab, MuSK-Ab
  - Chest CT/MRI for thymoma
- **Treatment**
  - Pyridostigmine, corticosteroids, azathioprine, cyclosporine, mycophenolate, tacrolimus
  - Crisis: Plasma exchange, IVIG
Corticosteroids

- Marked improvement in >80% of patients
  - 28% remission
  - 53% normal ADLs, minor symptoms

Favorable response in approximately 80%

- 15% improved with functional limitations
- Remainder refractory

- Maximal benefit in 5-6 mo
## Immunosuppressive agents

<table>
<thead>
<tr>
<th>Medication</th>
<th>Controlled studies</th>
</tr>
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<tbody>
<tr>
<td>Azathioprine</td>
<td>+</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>+</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>+</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>+</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>+</td>
</tr>
<tr>
<td>IVIG</td>
<td>+</td>
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**Mycophenolate mofetil**

Randomized, double-blinded, controlled studies in generalized AChRAb+ MG

<table>
<thead>
<tr>
<th></th>
<th>MSG-Roche</th>
<th>Aspreva</th>
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</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>80</td>
<td>136</td>
</tr>
<tr>
<td>Duration</td>
<td>3 mo/6 mo open label</td>
<td>9 mo</td>
</tr>
<tr>
<td>MM dose</td>
<td>1250 mg bid vs. placebo</td>
<td>1000 mg bid vs. placebo</td>
</tr>
<tr>
<td>Prednisone at entry</td>
<td>None</td>
<td>≥ 20 mg qd or qod equivalent</td>
</tr>
<tr>
<td>Prednisone during study</td>
<td>20 mg qd</td>
<td>Tapered to 7.5 mg qd or 15 mg qod</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Δ QMG score</td>
<td>Reaching MMS or PR from wks 32-36</td>
</tr>
</tbody>
</table>
Mycophenolate mofetil

- MSG-Roche study
  - n=39 on pred/placebo; 41 on pred/MM
- No significant difference in ΔQMG at 3 mo
  - -4.4 on MM vs. -3.6 on placebo (p=0.71)
- No significant difference in 2° outcomes
  - MG-ADL, MMT, SF-36, AChRAb levels
- MM was well tolerated
  - Diarrhea in 16%, infection in 13% in blinded phase on MM

Mycophenolate mofetil

- Aspreva study
  - n=88 on pred/placebo; 88 on pred/MM
  - n=144 completed study

- No significant different in reaching treatment response of MMS/PR
  - 44.3% on MM vs. 38.6% on placebo (p=0.541)

- No significant difference in 2° outcomes
  - QMG, MG-ADL, SF-36, global assessments
  - Trend for greater prednisone dose reduction, decline in AChRAb, hospitalizations if on MM, but not significant

- MM overall well tolerated
  - Headache (12%), nausea (9%) most common side effects
  - One death related to study drug (pneumonia in MM group)

- Sanders et al. Neurology 2008;71:400
The “muddle” of the MM trials

- Greater than expected response to prednisone alone
  - MSG study did not select prednisone-resistant patients
- Potential confounding factors
  - Rigorous definition of response in Aspreva study for which there was no preliminary data
  - Short duration of studies: 12, 36 weeks
  - Older patients, more men
  - Duration of disease (up to 3 years)
# Immunosuppressive agents

<table>
<thead>
<tr>
<th>Medication</th>
<th>Major adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>HTN, DM, weight gain, bone loss, cataracts, ulcers, psychologic disorders</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Fever, abdominal pain, hepatotoxicity, n/v, anorexia, leukopenia, skin rash</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Hirsutism, tremor, gum hyperplasia, HTN, hepatotoxicity, nephrotoxicity</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Alopecia, leukopenia, n/v, skin discoloration, anorexia, hemmorhagic cystitis, malignancy</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Hyperglycemia, HTN, headache, hyperkalemia, nephrotoxicity, diarrhea, n/v</td>
</tr>
</tbody>
</table>
Transternal thymectomy effect on remission

- **1940-57**
  - Significant effect (20% vs. 10%)

- **1958-1965**
  - Similar remission and improvement rates

- **1966-2000**
  - Slightly higher mortality and lower remission rates in thymectomy group

P values vs. 1940-57

Lifetime Course of MG
David Grob, MD, 1919-2008
Unmet Need: Ocular and Mild Generalized MG

- Ocular MG
  - ~60% of patients symptomatic on pyridostigmine alone
  - Many patients unwilling to initiate treatment with corticosteroids or other immunosuppressives
  - 50-60% of patients → generalized disease within 1-2 years

- Mild Generalized MG
  - Chronic disease → chronic therapy
    - Spontaneous remission rare; remission with aggressive Rx ~20%
    - Adverse effects of chronic therapy with corticosteroids or other immunosuppressives

- BHT-3034 has the potential to
  - ↓ symptoms of MG
  - ↓ need for corticosteroids or other immunosuppressives
  - ↓ progression of ocular to generalized disease
Proposed Study Population

- Adults, ages 18-75, inclusive
- MG by standard diagnostic criteria
- Ocular or mild generalized disease
- Within 18 months of symptom onset
- AChR antibody +
- Symptomatic (not well controlled on current Rx)
- Stable acetylcholinesterase inhibitor and corticosteroid use (if applicable)
- No other immunosuppressives, cell-depleting Rx
Corticosteroid Use During Study

- Inclusion
  - Corticosteroid use up to 20 mg/day prednisone (or equiv.) allowed
- Concomitant medication
  - For those on steroids, “effort should be made to keep the dose of corticosteroid constant throughout the study, and in particular throughout the Evaluation Period.”
  - For those not on steroids, effort should be made to optimize symptom control without use of corticosteroids, in particular throughout the Evaluation Period.”
  - If steroid dose ≥ 60mg/day prednisone for ≥ 7 consecutive days, then study drug should be discontinued

- Reflects variation in clinical practice with respect to steroid use (dose, adjustment, schedule)
  - Strict algorithm considered impractical for Phase 1/2 study designed for safety and pharmacodynamic endpoints
- Provides strict boundary to ensure subject safety
  - Safety of low-moderate dose steroids supported by animal study
- Acknowledge standardizing steroid use will be important in later studies