

Research at the

BARROW

**Neurological
Institute**

“unlocking the mysteries...”

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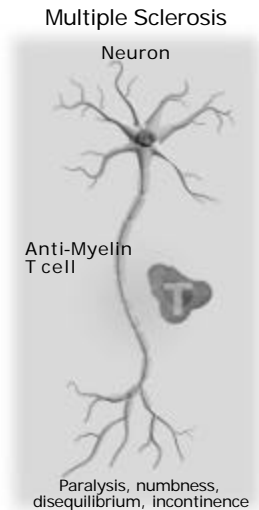
The Team

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Multiple Sclerosis (MS)

- ☞ **Autoimmune disease of nervous system**
- ☞ **Immunological attack on the myelin sheath**
- ☞ **400,000 patients in US**
- ☞ **Women > men**
- ☞ **Age < 40 years**
- ☞ **Clinical course**
 - ☞ **Relapsing remitting**
 - ☞ **Secondary progressive**
 - ☞ **Primary progressive**
 - ☞ **Progressive relapsing**



MS Target Antigens

- ☞ **Known and well characterized self antigens**
 - ☞ **Human immunology**
 - ☞ **Established animal models**
- ☞ **Myelin basic protein (MBP)**
- ☞ **Proteolipid protein (PLP)**
- ☞ **Myelin oligodendrocyte glycoprotein (MOG)**
- ☞ **Myelin associated glycoprotein (MAG)**

Trials of MBP-Specific Immunotherapy of MS

- ☞ **MBP-directed immunotherapy decreases antigen-specific immune responses**
- ☞ **Appears to be a safe and effective approach**
- ☞ **Glatiramir Acetate (Copaxone)**
 - ☞ Random polymers of 4 amino acids derived from immunodominant epitope of myelin basic protein (MBP)
 - ☞ Decreased antigen-specific T cell response, Th2 deviation
 - ☞ Approved for treatment of MS
- ☞ **MBP8289 (17 AA fragment of MBP)**
 - ☞ Tolerization and decreased antibody response to MBP
 - ☞ Appears effective in phase II trial
- ☞ **APL (Altered MBP83-99)**
 - ☞ Decreased antigen-specific T cell response
 - ☞ Appears effective in phase II trial

Understanding Th1 and Th2 Immunity: CD4 Helper T cells

Th1 cell	Th2 cell
Pro-inflammatory	Anti-inflammatory
IL2	IL4
IL12	IL5
IFN-g	IL10
TNF-a	IL13
activate macrophages	regulatory
promote cell mediated immunity	activate B cells-promotes humoral immunity

Th1 Cells mediate disease in animal model

- ☞ In MS lesion, staining for the T Cell Receptor (TCR) reveals T cells infiltration perivascular (inflammation) [Trapp 1998]
- ☞ Th1 cells directed against myelin antigens (ie MBP) are key mediators in the CNS damage seen in MS
- ☞ Th1 cytokines predominate in MS relapse
- ☞ Th1 cells mediate disease in EAE mice, via MBP-reactive T cells

Mechanism of Action of Glatiramir Acetate: understanding “Bystander Suppression”

- ☞ GA has high affinity to MHC groove of TCR displacing other antigens
- ☞ or GA is engulfed by APC and presented to Tcell
- ☞ Either way the result is clonal expansion and the generation of “an army” of Th2 biased cells in the periphery
- ☞ These GA-specific Th2 cells injected into mice prevented EAE when animals were subsequently immunized with spinal cord homogenates
- ☞ Why are these GA-specific T cells Th2 biased??

GA does not prevent immune cells from entering CNS

- ☞ **Why then do we see resolution of Gad enhancing lesions in the long run with GA?**
- ☞ **Because GA specific Th2 cells in the periphery do themselves enter the CNS early in the treatment**
- ☞ **They initiate and orchestrate a repair process from within the CNS....by the following proposed mechanisms....**

The mechanism of “bystander suppression”

- ☞ **GA-Th2 cells in CNS get reactivated by APCs in CNS (likely microglia or macrophages that infiltrated into CNS)**
- ☞ **These educated GA-Th2 cells can recognize a variety of different antigens**
- ☞ **This is consistent with GA’s original design to mimic the structure of MBP**
- ☞ **Thus you have expansion of these GA “educated” Th2 cells in the CNS**

GA Specific Th2 cells

- ☞ **Release anti-inflammatory cytokines**
- ☞ **Which then impairs the expansion of the actual myelin reactive T cells...thus as a bystander the GA specific Th2 cells reprograms or repopulates the milieu**
- ☞ **Following this logic, GA should be effective against other autoimmune diseases**

GA Specific Th2 cells may have neuroprotective function

- ☞ **T cells , B cells and macrophages produce neurotrophic factors (such as brain derived neurotrophic factor)**
- ☞ **These neurotrophic factors may attenuate injury and have a neuroprotective role**
- ☞ **Further some evidence suggest that autoreactive T cells themselves serve a neuroprotective role in SCI (Hauben 2000)**
- ☞ **GA T cells improved the survival of retinal ganglion neurons in two different animal models (Kipnis 2000; Schori 2001)**
- ☞ **This may explain reduced proportion of black holes in MS patients (Filippi 2001)**

Why not combine the effects of IFN and GA?

- ☞ **IFN works by blocking the inflammatory cells from the CNS**
- ☞ **IFN may inhibit the expansion of GA specific Th2 cells and block their entrance into the CNS where they would act through bystander suppression**
- ☞ **Possibly antimetabolic compounds PRIOR to GA may be helpful in eradicating autoreactive Th1 cells, thus making the expansion of Th2 cell milieu even more pronounced**

Core Functions of BNI Clinical Neuroimmunology Laboratory

- ☞ **Our focus is on immune mediated disorders of nervous system or neuromuscular junction**
- ☞ **Monitor how immune responses are altered by a given immunoregulatory drug**
- ☞ **Determination of mechanism of actions of immune modulator agents in human and animal models with the ultimate goals of optimization clinical protocols, enhance potency of a given drug and test combinational therapy.**
- ☞ **We aim to understand the nature of autoimmune disorders, e.g. factors controlling initiation and progression of autoimmunity.**
- ☞ **In the education arena:
Training of post doc, clinical fellow and PhD student to prepare their future careers in Neuroimmunology.**

*BNI Clinical
Neuroimmunology Laboratory*

- ☞ **MOG-induced EAE in C57BL6 mice and AChR-induced EAMG in Lewis rats and C57BL6 mice have been established.**
- ☞ **Established immune assays: T cell proliferation, cytokine release, autoantibody assay, immunohistochemistry, multi-color FACS analysis, RNA isolation, etc.**

Specific questions

- ☞ If GA-reactive Th2-like regulatory cells mediate local bystander suppression, then the effect is not MBP or MS specific, GA should have beneficial effects in other Th1 mediated disease like myasthenia gravis.

Protocol: Therapeutic effects of GA in experimental myasthenia gravis, a disease induced mainly by immune attack on acetylcholine receptor (AChR) at the neuromuscular junctions.

Implications? other autoimmune disease where Th1 cell is dominant.

Specific questions

- ☞ How long does GA-induced immune effects last?

Protocol : A Study to Evaluate the Sustained Clinical and Immunological Effects of Glatiramer Acetate in Patients with Relapsing Remitting Multiple Sclerosis

Implications? Optimal frequency and dosage of GA to reduce side effects and patients cost

Specific questions

- ☞ How can we boost beneficial efficacy of GA?

Protocol: Effect of co-immunization of incomplete Freund's adjuvant (IFA) and GA in MOG-induced EAE (experimental autoimmune encephalomyelitis).

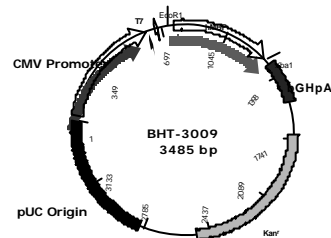
Implications: make GA more effective in MS.

BHT-3009 Treatment of Multiple Sclerosis

- ☞ **Antigen-specific immunosuppression - Well established**
 - ☞ Pre-clinical models
 - ☞ Approved product - Copaxone
 - ☞ Multiple products in clinical development
- ☞ **Plasmids**
 - ☞ Typically used to enhance immunity
 - ☞ Immunosuppressive in correct context
- ☞ **BHT-3009**
 - ☞ Strong pre-clinical rationale
 - ☞ Good safety profile
 - ☞ Phase I trial designed for careful safety monitoring

BHT-3009: Designed for Immunosuppression

- Encodes DNA for full length hMBP
- No adjuvant
- e coli is where it is initially developed but then e coli is lysed and gone
- What is around the hMBP are sequences involved in the processes of getting the e coli to grow
- Immunostimulatory sequences removed , just the hMBP DNA



EAE Model of MS

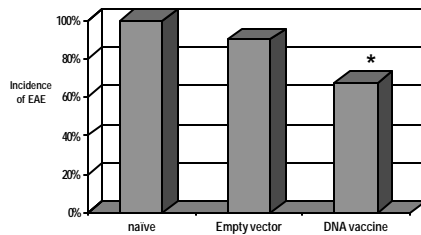


0	Healthy
2	Hind Limb paraparesis
3	Hind Limb paralysis
4	Complete paralysis
5	Death

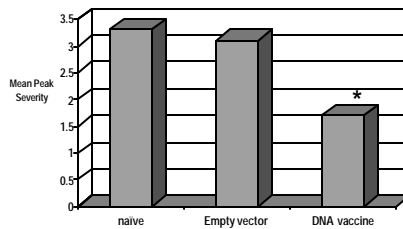


BHT-DNA Prevents EAE

(published in Journal of Immunology 1999)



* $p < 0.0022$
compared to
empty vector



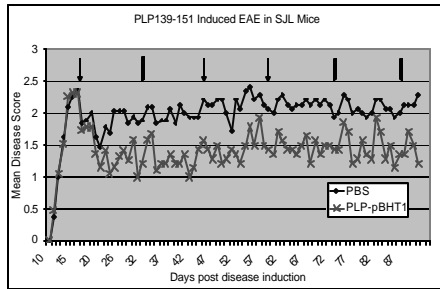
* $p < 0.005$
compared to
empty vector

• Reduction in disease incidence and severity with a single antigen DNA in a prevention model

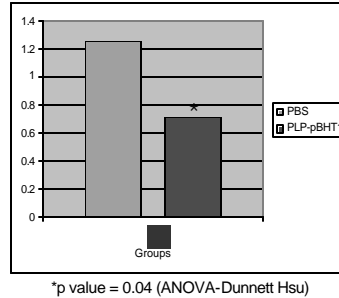
• MOA demonstrated to be anergy of autoreactive T cells

BHT-DNA Treats Established EAE

Mean Disease Score



Relapse Rate



Non-clinical Safety Studies of Repeat Dose IM Administration

- ☞ **Biodistribution and expression (mice):**
 - ☞ Detectable at site of injection (SOA) and lymph nodes (LN) for 2-4 weeks
- ☞ **Toxicity (monkeys):**
 - ☞ No significant toxicity
 - ☞ Minor changes in body temperature and blood pressure
- ☞ **Safety (mice):**
 - ☞ No toxicity observed
 - ☞ Does not cause or worsen EAE

Atorvastatin

- ☞ **Mild anti-inflammatory effects**
- ☞ **Shifts immune response from harmful Th1 to non-harmful Th2**
- ☞ **Early evidence for efficacy treating MS**
- ☞ **May improve efficacy of BHT -DNA in pre-clinical models**

Summary of Pre-clinical Data

- ☞ **EAE/efficacy data support a DNA dose frequency of biweekly or monthly.**
- ☞ **EAE/efficacy data supports efficacy over a wide range of DNA dose levels.**
- ☞ **No concerning safety or toxicology issues.**

Protocol BHT-3009-01

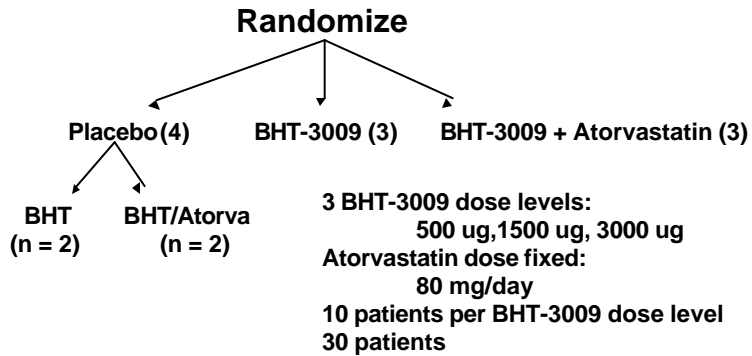
- **Placebo-controlled trial of BHT-3009 alone or combined with Atorvastatin in patients with multiple sclerosis**

- **Centers:**
 - **Barrow Neurological Institute – Tim Vollmer, M.D.**
 - **Montreal Neurologic Institute – Jack Antel, M.D.**
 - **University of Southern California – Les Weiner, M.D.**
 - **University of British Columbia – Joel Oger, M.D.**

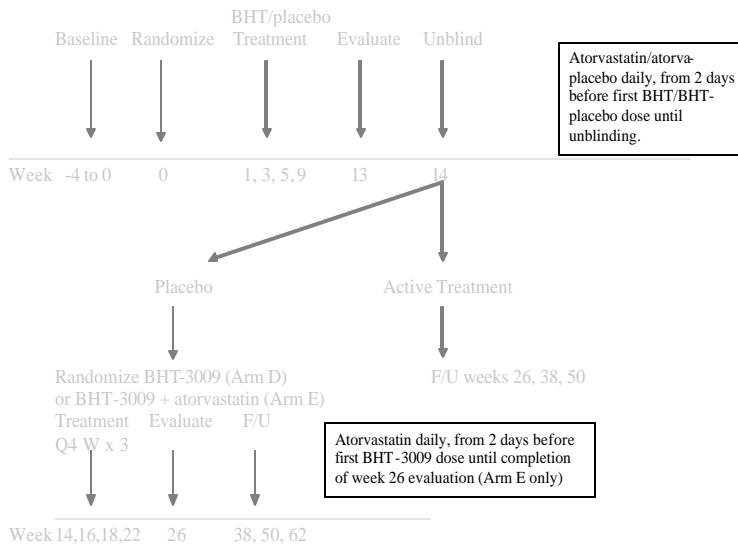
Study Design

- ☞ **Three arms**
- ☞ **Randomized**
- ☞ **Double-blind**
- ☞ **Placebo-controlled**
- ☞ **Cross-over from placebo to active**

BHT-3009-01 SCHEMA



Treatment Schedule



Objectives

- ☞ **Safety**
- ☞ **Determine phase II dose**
- ☞ **Immune response**
- ☞ **Describe clinical course**
- ☞ **Explore biomarkers of MS activity**

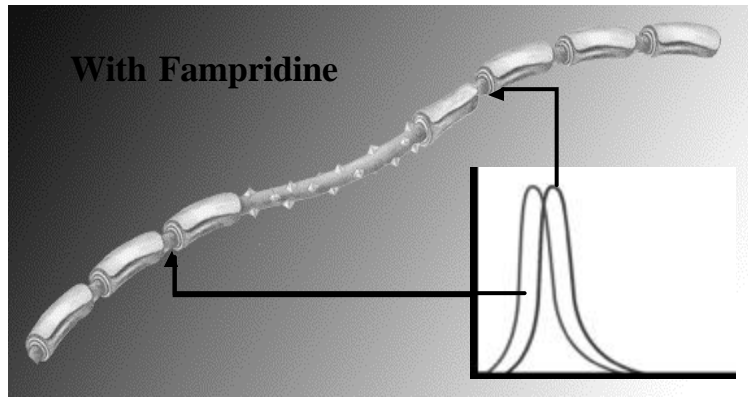
Fampridine (4-Aminopyridine; 4-AP)

History of Fampridine-
SR

- ☞ **Freely crosses BBB**
- ☞ **Blocks fast-activating voltage-gated K^+ channels**
- ☞ **Prolongation of action potential**
- ☞ **Increased safety factor for firing action potential**
- ☞ **Possible enhancement of synaptic transmission**

Mechanism of Fampridine

History of Fampridine-
SR



Highlights of Clinical Research in MS

IV and Oral IR Formulations

History of Fampridine-
SR

- ☞ **1983: First clinical study in MS (Jones, et al)**
 - ☞ effects on visual function
- ☞ **1987-91: Series of studies in MS (Stefoski and Davis)**
 - ☞ effects on motor function
- ☞ **1993: First large study (70 pts) in MS (van Diemen, et al)**
 - ☞ broad effects on disability (EDSS)
 - ☞ dose and serum level related to efficacy and safety
 - ☞ plasma levels variable and difficult to control with IR form

Delivery and Formulations of Fampridine

History of Fampridine-SR

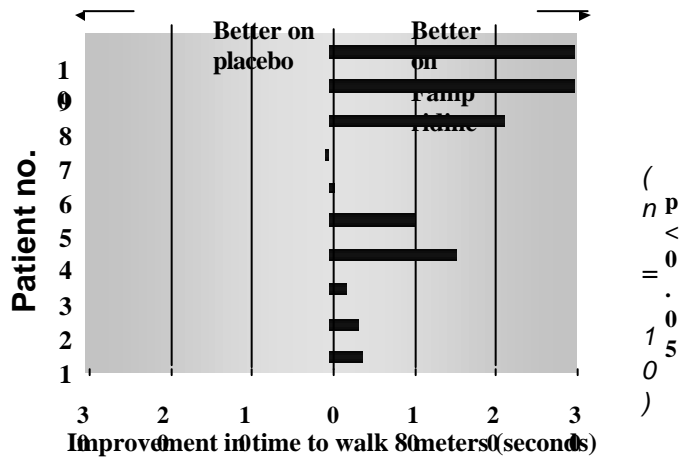
- ☞ **Intravenous administration (6 to 15 mg/hr)^{1,2}**
- ☞ **Immediate release tablets or capsules (<80 mg/day)³⁻⁵**
 - ☞ **enteric coated**
 - ☞ **non-enteric coated**
- ☞ **Sustained release tablet (Elan/Acorda)⁶**
 - ☞ **well tolerated at doses up to 50 mg/day**
 - ☞ **achieves peak concentration gradually**
 - ☞ **longer, well-maintained drug effect with twice-a-day dosing**

Fampridine-SR Facts

History of Fampridine-SR

- ☞ **Proprietary, oral, sustained-release formulation developed by Elan Pharmaceuticals**
- ☞ **Designed for twice-a-day dosing**
- ☞ **Overcomes rapid peaks of plasma level and food effect**
- ☞ **Acorda Therapeutics licensed the formulation from Elan**
- ☞ **Advanced clinical development in SCI and MS**

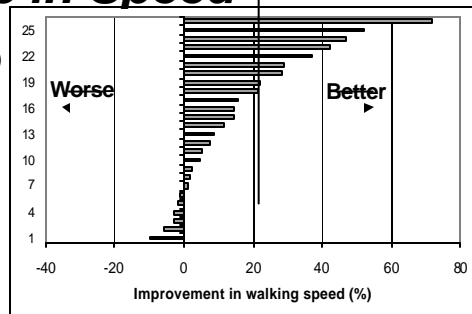
**Phase 2 MS Study ELN 0995-001US *:
Time to Walk 8 Meters (Schwid et al. 1997)**



*Double-blind, placebo-controlled, crossover design

**MS-F201 Study: 25-Foot Walk –
Change in Speed**

Fampridine-SR
(20-50 mg/day)



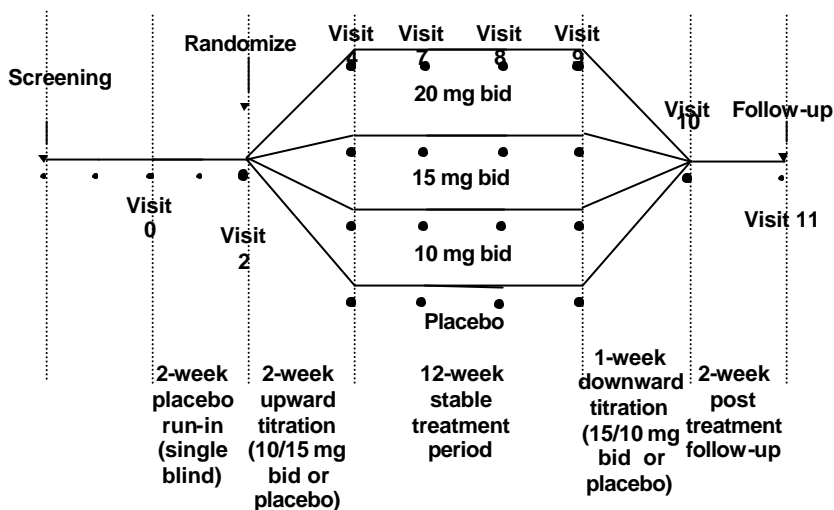
Placebo



MS-F202 Summary

- Clinically definite Multiple Sclerosis (MS)
- Between the ages of 18 and 70
- Able to complete 2 trials of the Timed 25-Foot Walk at the Screening Visit
- Average time : between 8 and 60 seconds
- Primary endpoint: % improvement in average walking speed during the stable dosing period, relative to the baseline dosing period (placebo run-in), using the timed 25-foot walk
- Safety and efficacy

MS-F202 Study Design



MS-F202 Conclusions

- ☞ **Consistent trends on 25-foot walk in planned analysis**
- ☞ **Statistical significance on walking speed for two-group analysis of 10 and 15 mg b.i.d. vs. placebo**
- ☞ **Statistically significant improvement in LEMMT at 10 and 15 mg b.i.d.**
- ☞ **Confirmed favorable AE profile**
- ☞ **Confirmed that 10 and 15 mg b.i.d. are both candidates for further development**
- ☞ **Additional analyses, including drug levels, ongoing**

