Bedside to Bench to Bedside: MS to EAE to MS

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Disclosures

Dr. Voskuhl is an inventor on a UCLA use patent for estriol in MS. She also formerly did consulting for Synthetic Biologics.
**MS & EAE**

Most widely used for MS model
Standard Preclinical Study MS drug development
If works in EAE, may not work in MS
If does not work in EAE, even less likely to work in MS

**Necessary, but Not Sufficient**

Prerequisite to advance to next stage of drug development

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**MS & EAE**

Autoimmunity model – Neurodegenerative model

Peripheral immune activation
WM: immune cell infiltration and glial activation
  WM: demyelination
  WM: axonal loss
GM: synaptic loss
GM: neuronal loss
GM atrophy on in vivo MRI
WM & GM: Electrophysiology
MS & EAE

Which EAE model: Depends on the Question:

Monophasic – Relapsing – Chronic progressive

Pregnancy

Sex Differences

EAE as a model for MS
Bench to Bedside: Does It Work?

Based on:

novel molecule
cutting edge technique

Problem:

Redundancy
heterogeneity
not physiologically important
A Different Approach

Bench to Bedside
(molecule or technique based)

Bedside to Bench to Bedside
(clinical observation based)

1. Start with a clinical observation
2. Unravel mechanism at lab bench
3. Target mechanism in a clinical trial

Clinical Observations

Pregnancy: Decreased relapses
Sex Differences: F > M susceptibility
               M > F progression
Clinical Observation Late Pregnancy: Relapses by 75%

†Sex Hormones: Estiol, Estradiol, Progesterone, other


Estrogens

Estradiol –  
"gold standard"  
binds high affinity to ERα & ERβ  
ERα toxicity breast and uterus

Estriol –  
"weak"  
ERβ > ERα  
safest of the estrogens since 1980s

Spence & Voskuhl, Frontiers in Neuroendocrinology 2012
**Preclinical: Estriol Treatment in EAE**

Four strains, both sexes, pre and post tx
spinal cord: ↓ inflammation, ↑ axons, ↑ myelin
Holmdahl, Voskuhl, Offner, Whitacre

ERα and ERβ: Direct Neuroprotective Effects in EAE


**Direct Neuroprotective Effects:**
**Preclinical Data**

![Diagram](image)

Treatment
- IFN Beta
- Copaxone
- Mitoxantrone
- Tysabri
- Fingolimod
- Teriflunomide
- BG12
- Estriol?

Direct Neuroprotective Effects:
- Decreased inflammation

J. Neuroscience 2006, PNAS 2007, Brain 2010,
Pilot Estriol Clinical Trial in MS

Clinical Data:

Oral estriol 8 mg/day for 6 months: mid-pregnancy level
10 women, single arm, crossover, monthly MRIs
Compared to 6 months Pre-Tx:
— MRI gado lesions ↓ by 70-80%
--↓ TNFα, ↑IL5, ↑IL10 in PBMCs
--↓MMP9


Double Blinded, Placebo Controlled Multicenter Trial

Primary Outcome: Relapse Rate
Phase II (Clinical, not Surrogate)
Powered at p = 0.1
Treatment Duration = 24 months
“Add on” study in RRMS (no placebo only)
Copaxone (Glatiramer Acetate – GA)
n = 158
16 sites across U.S.
Site Neurologists / # randomized

University of California Los Angeles: Barbara Giesser / 27
Ohio State University: Michael Racke / 19
Washington University: Anne Cross / 12
Johns Hopkins University: Peter Calabresi - John Ratchford / 12
University of Pennsylvania: Dina Jacobs / 11
University of Texas, Southwestern: Elliot Frohman - Angela Bates Flores / 11
University of Colorado: John Corboy / 10
University of New Mexico: Corey Ford / 10
University of Chicago: Anthony Reder - Jacqueline Bernard / 10
UMDNJ / Rutgers: Suhayl Dhib-Jalbut / 9
University of Utah: John Rose / 8
University of Minnesota: Gareth Parry - Gary Beaver / 8
University of Kansas: Sharon Lynch / 7
Mayo Clinic Arizona: Dean Wingerchuk / 6
Dartmouth University: Lloyd Kasper - Andrew Pachner / 3
Columbia University: Mark Tullman / 1

Estriol Trial, NIH sponsored, 16 sites, 164 patients:
Primary Outcome Measure: Relapse Rates

Phase II powered:
Reduce relapses by 1/3
24 month duration
p = 0.1

Result:
Hit Primary (24 months)
Rapid Onset (12 months)
“Floor Effect” Estriol + GA
Clinical Observation: Estrogens and Cognition

Healthy women:
Cognitive decline in E removal (ovariectomy)
Cognitive improvement with estradiol treatment

Healthy rats and mice:
Cognitive decline in E removal (ovariectomy)
Cognitive improvement with estradiol treatment (ERβ)

Estriol Treatment In EAE: Hippocampus

Hippocampus: preserved CA1 volume
↓ microglia activation, ↑ synapses

Lab Invest, 2010, 2012
Estriol Treatment in EAE: Synapses & Function

Estriol Trial in MS
Effect on Cognition

** P < 0.05, *P < 0.10

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Clinical Observation: F > M (3:1)

Bench: Female > Male: EAE in SJL Mice

Sex Hormones
Sex Chromosomes

Lower testosterone in MS (normal = 300-900 ng/dL; MS < 500 ng/dL)

Voskuhl, Annals of Neurology 1996

F > M (3:1) - Testosterone

Bench EAE:
Castration worse / Testosterone Tx better:

LN, spleen – immunomodulation
WM: less inflammation, sparing myelin & axons

Cua & Stohlman, Bebo & Bourdette; Cuprizone: Ghandour & Schumacher, Brain, 2013
MS Pilot Testosterone Clinical Trial:

Single arm, crossover design, 10 MS men
Testosterone Levels: low to high normal

Arch. Neurol., 2007

MS Pilot Testosterone Clinical Trial:
Whole Brain Atrophy

Arch. Neurol., 2007
MS Pilot Testosterone Clinical Trial: Gray Matter Atrophy

Clinical Observation:
Testosterone Tx improves cognition in normals andropause +/- dependence on conversion to E

Testosterone Tx in EAE: Hippocampal GM

△ synapses, ▲ cognitive function
Recent Further Clinical Observations
Endogenous Testosterone in MS men

Brigham & Women’s MS male cohort:

Cross- sectional:
Low T – higher EDSS

Longitudinal:
Low T - performed worse on cog testing next 2 years

Bove, Chitnis, MS 2014

A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED, MULTICENTER PHASE II
TRIAL TO INVESTIGATE THE EFFECTIVENESS OF TESTOSTERONE TREATMENT IN
MEN WITH MULTIPLE SCLEROSIS

PPI: Rhonda Voskuhl (UCLA)

Co-Investigators:
Ronald Swerdloff (UCLA)
Tanuja Chitnis (Brigham & Women’s)
Riley Bove (Brigham & Women’s)
Douglas Arnold (NeuroRx, McGill)
Allan MacKenzie-Graham (UCLA)

25 Clinical Sites across the United States
Bedside to Bench to Bedside: Clinical Observations

1. Pregnancy is protective in MS
2. Males are less likely to get MS: F>M (3:1)
3. Males: worse disability progression

Clinical Observation
Males: worse disability progression

Why is there more neurodegeneration in men?
Testosterone neuroprotective.

Sex chromosomes XX vs XY?
Use XX vs XY (chromosome vs. hormone)
Use bone marrow chimeras (CNS vs immune)
4 Core Genotypes

Female gonads

XX  |  XY-

Male gonads

XX_{Sry}  |  XY_{Sry}

Genotype

Determine whether there are sex chromosome effects in the CNS during EAE

Day -56: Ovariectomy

XX_{Donor} Recipient 4 weeks old

Day -49: Inject \(-1.5 \times 10^7\) cells via tail vein

Day -49: Harvest bone marrow cells

Chimera

Bone marrow chimeras
Group 1) XX immune, XY CNS (XX\rightarrow XX)
Group 2) XX immune, XX CNS (XX\rightarrow XY)

PNAS 2013
Determine whether there are sex chromosome effects in the CNS during EAE

Day -56: Ovariectomy

Day -49: Harvest bone marrow cells

Days -50 & -49: 425 RADS

Day -49: Inject ~1.5x10^7 cells via tail vein

Bone marrow chimeras

Group 3) XY+ immune, XY- CNS (XY→XX)
Group 4) XY+ immune, XX CNS (XY→XY)

Clinical Observation: M > F Progression

XY- CNS, compared with XX, confers greater EAE disease severity

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PNAS 2013
Clinical Observation: M > F Progression

XY- CNS, as compared to XX, have less myelin and fewer axons in spinal cord

Clinical Observation: M > F Progression

XY- CNS, as compared to XX, have less myelin and fewer Purkinje cells in cerebellum
Clinical Observation: M > F Progression
XY- CNS, as compared to XX, have less synapses in cerebral cortex

First to show sex chromosome complement effect on neurodegeneration

PNAS 2013
Bedside to Bench to Bedside:
MS to EAE to MS

1. Pregnancy decreases MS relapses
   - Estriol Tx for females
2. Males are less likely to get MS: F>M (3:1)
   - Testosterone Tx for males
3. Males worse disability progression
   - XY deleterious in CNS

Research in Sex Differences

Using EAE
1. Sex hormone effects
2. Sex chromosome effects
MS to EAE to MS

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(molecule or technique based)

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