HSV-mediated transfer of glutamic acid decarboxylase for painful diabetic neuropathy

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Inflammatory pain begins with tissue damage

Scholz & Woolf 2002
Pain-causing molecules in the periphery activate specialized primary sensory afferents whose cell bodies lie in the dorsal root ganglia.
“Second order” neurons with cell bodies in the dorsal horn of the spinal cord project rostrally to the brain.
Third order neurons transmit pain-related information to sensory and limbic cortical structures in the cerebral hemispheres.
Continued activation of nociceptors results in alterations in function and changes in gene expression in DRG and spinal cord.

**Electrophysiology**

(i) Windup

(ii) Activity-dependent CS

(iii) LTP

**Gene expression**

**DRG:**
- BDNF, substance P, bradykinin receptor...

**Dorsal horn neurons**
- c-Fos, Cox-2
- prodynorphin, NK1, TrkB...

**Spinal microglia & astrocytes**
- ERK, IL-1β, TNFα...
Premise:

Effective pain control using conventional analgesic agents is limited by the widespread distribution of the drug targets in both pain and non-pain pathways within (and outside) the nervous system.

Rationale:

Gene transfer can be used to achieve focal release of an analgesic gene product to selectively interrupt nociceptive neurotransmission.
## Gene Transfer Vectors

<table>
<thead>
<tr>
<th>VECTOR</th>
<th>SIZE (nm)</th>
<th>GENOME (kb)</th>
<th>TYPE</th>
<th>INSERT (kb)</th>
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<tr>
<td>liposome</td>
<td>100</td>
<td>8</td>
<td>DNA</td>
<td></td>
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<td>retrovirus</td>
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<td>8</td>
<td>ss-RNA</td>
<td>8</td>
</tr>
<tr>
<td>lentivirus</td>
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<td>AAV</td>
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<td>5</td>
<td>ss-DNA</td>
<td>5</td>
</tr>
<tr>
<td>adenovirus</td>
<td>100</td>
<td>36</td>
<td>ds-DNA</td>
<td>8, 30</td>
</tr>
<tr>
<td>HSV</td>
<td>200</td>
<td>152</td>
<td>ds-DNA</td>
<td>50</td>
</tr>
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</table>
heparan sulfate

GB, GC

nectin (HveC)

GD

gH, gL

dynein
A non-replicating HSV vector is created from the wild type virus by selective deletion of essential Immediate Early (IE) HSV genes. The replication incompetent vector is propagated to high titer on a complementing cell line that provides the missing gene product \textit{in trans}.
An HSV vector containing the preproenkephalin gene produces enkephalin in DRG neurons \textit{in vitro}...

...and in DRG \textit{in vivo} following subcutaneous inoculation in the foot
HSV-mediated enkephalin provides an analgesic effect in delayed phase of the formalin model of inflammatory pain
HSV-mediated enkephalin reduces cancer-related pain in the osteogenic sarcoma model in the mouse
NP2 protocol

RAC review (June 2002)

- Insertion of transgene into human-grade vector backbone
- GLP production of toxicology lot and GMP manufacturing optimization
- cGMP production and certification of Master Cell Bank
- cGMP production and certification of Master Viral Bank
- FDA-approved GLP toxicology studies
- FDA-approved GLP biodistribution studies

Drafting and submission of IND application to FDA

University of Michigan institutional approvals

- Cancer Center Protocol Review Committee (PRC)
- Institutional Biosafety Committee (IBC)
- General Clinical Research Center (MCRU) Review
- Institutional Review Board (IRB)

Certificate of Analysis

First patients enrolled (December 2008)
Human grade enkephalin-expressing HSV vector (NP2)

Completely deleted for:
ICP4
ICP27

Defective in promoter function for:
ICP22
ICP47
HSV PE gene transfer for cancer pain: Phase I trial

**Inclusion criteria**

- **Patients with terminal malignancy**
  Focal pain consistently ≥ 4 on a 10 point scale despite morphine > 200 mg/d or equivalent

- **Dose escalation design**

<table>
<thead>
<tr>
<th></th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
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<tbody>
<tr>
<td>Cohort 1</td>
<td>1x10^7 pfu</td>
<td></td>
<td></td>
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<tr>
<td>Cohort 2</td>
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<td>1x10^8 pfu</td>
<td></td>
<td></td>
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<tr>
<td>Cohort 3</td>
<td></td>
<td></td>
<td>1x10^8 pfu</td>
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<tr>
<td>Cohort 4</td>
<td></td>
<td></td>
<td></td>
<td>MTD</td>
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</tbody>
</table>
HSV PE gene transfer for cancer pain: Phase I trial

Protocol

- Clinical grade vector, inoculated in ten 100 microliter intradermal injections in the dermatome(s) corresponding to the radicular distribution of pain

- Physical examination at days 1, 3, 7, 10, 14, 21, 28 at monthly intervals up to 4 months and yearly thereafter

- **Primary Outcome**: Adverse Events

- **Secondary Outcome**: Pain Measures
  - Numeric rating scale
  - McGill Short-Form Pain Questionnaire
  - Diary of concurrent analgesic use
Neuropathic pain is pain that occurs in the setting of injury to elements of the nervous system without peripheral tissue damage.

Neuropathic pain treatment options:
- Tricyclic antidepressants
- Anticonvulsants
- Sodium channel blockers
- Opioid drugs

NNT greater than 2 to achieve a 50% reduction in pain intensity.
Pain after peripheral nerve injury is characterized by activation of microglia in gray matter of spinal cord.

HSV vectors expressing anti-inflammatory peptides IL-4 or the p55 soluble TNFα receptor produce analgesic effects that are statistically significant.
Pain after peripheral nerve injury is characterized by a loss of GABAergic tone in the spinal cord.
The GAD-expressing HSV vector produces GAD in DRG *in vivo*

GAD protein is transported to afferent terminals in the spinal cord...
...resulting in the release of GABA...

DRG neurons *in vitro*

dorsal horn *in vivo* (microdialysis)

...by a constitutively active mechanism
GABA release by the GAD-expressing vector produces a robust antiallodynic effect in the spinal nerve ligation model of neuropathic pain.
HSV-mediated GABA reduces below-level central neuropathic pain in the lateral hemisection model spinal cord injury pain

The GABA-mediated effect is blocked in part by both bicuculline and by phaclofen
**GABA release by the GAD-expressing vector reverses pain-related behaviors in the STZ model of painful diabetic neuropathy**

Thermal hyperalgesia

![Thermal hyperalgesia graph](image_url)

Cold allodynia

![Cold allodynia graph](image_url)

Mechanical hyperalgesia

![Mechanical hyperalgesia graph](image_url)
HSV GAD gene transfer for PDN: Phase I/II trial

Inclusion criteria

- Patients with painful diabetic neuropathy
  Present for at least 6 months
  Pain consistently ≥ 4 on a 10 point scale

- Stable glycemic control
  Glycosylated hemoglobin ≥ 12%

- Intraepidermal nerve fibers present
  on punch biopsy of the skin in the distal lower extremity
HSV GAD gene transfer for PDN: Phase I/II trial

Protocol

- **Clinical grade GAD-expressing vector, inoculated in ten 100 microliter intradermal injections in the L4/L5 dermatomes in the distal leg**

- 60 patients randomly assigned to receive vector or placebo in a 2:1 ratio

- **Primary Outcome Phase I: Safety**

- **Primary Outcome Phase II: Efficacy**
  determined by a 30% reduction in pain measured by the 24 hour average pain intensity at 2, 4 and 6 weeks after vector inoculation
Q1. What is the rationale for using the HCMV IEp to drive transgene expression?

A1. Self-limited expression provides safety in case of an unanticipated adverse event. Should the treatment prove effective and prolonged therapy preferable, we will proceed construct a similar vector with the LAP2 promoter driving GAD expression.
Q2. Will pre-existing anti-HSV immunity compromise the therapeutic benefit?

A2. Our data, demonstrating that re-inoculation re-establishes the therapeutic effect in several different models and with several different transgenes, suggests that this will not be a problem.

Post-hoc comparison of the therapeutic effect in patients with and without pre-existing anti-HSV titers will ultimately provide a definitive answer to this question.
Q3. Will the treatment result in the production of anti-GAD antibodies resulting in a disease phenotype?

A3. We don’t anticipate a problem, because:

(1) Single injections of the vector do not appear to cause a significant immune response.

(2) While antibodies to GAD65 are found in autoimmune disease, antibodies against GAD67 are not.

(3) Intentional immunization with GAD65:Alhydrogel is currently in Phase III trial as an immune modulating therapy for diabetes mellitus. There has been no evidence of adverse immune consequences in toxicology studies or in the trial to date.
Q4. Is there data regarding spread of vector genomes beyond the injection site?

A4. FDA-mandated biodistribution studies of 320 mice from 1 to 90 days following subcutaneous inoculation of the preproenkephalin-expressing HSV vector showed quantifiable genomes only at the injection site (early) and in DRG.
Q5. In the absence of safety data, please provide additional rationale for the phase 1/2 design.

A5. Safety and dose-finding data for HSV-mediated gene transfer will be provided by the phase 1 study of the preproenkephalin-expressing vector in patients with terminal cancer, that we anticipate will be completed by the end of this calendar year. The results of that study will inform us regarding ultimate design of the GAD PDN study.
HSV GAD protocol

RAC review (March 2009)

Insertion of transgene into human-grade vector backbone

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First patients (?)
Q6. HSV delivered subcutaneously has been shown to present antigens and...elicit an immune response. Please comment on the immunological issues.

A6. (1) The data from the re-inoculation experiments suggest this should not be a problem.
   (2) Drs. Federoff and Bowers have demonstrated that two subcutaneous inoculations of an HSV amplicon expressing $\beta_1-42$ does not lead to a detectable immune response.
   (3) There is extensive data from clinical trials in which replication competent but compromised “oncolytic” genomic HSV recombinants have been injected into tumors with no reports of systemic immunologic problems.
Q7. Provide additional rationale for the anatomical compartments that are planned for HSV gene transfer.

A7. (1) The vector will be injected intradermally to provide access to sensory nerve terminals.

   (2) The injections will be targeted to the skin of distal leg dermatomes innervated by L4 and L5 roots, in order to achieve transgene-mediated release of GABA in the dorsal horn of spinal cord at the relevant rostro-caudal level.

A8. These issues will be addressed and corrected when we draft the final consent form in consultation with our Institutional Review Board.
University of Michigan

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Jun Liu
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Munmun Chattopadhyay
Jian Hu
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Diamyd Medical

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David Krisky

University of Michigan

Kevin Weatherwax
Susan Urba
Srinivas Chiravuri
Frank Worden
Suzette Walker
Heidi L’Esperance
Mary Orr
Gayle Estep
Peptide neurotrophic factors can be used to treat polyneuropathy in animal models but have proven ineffective in human trials.

### Research Report

**Nerve growth factor administration protects against experimental diabetic sensory neuropathy**

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*Departments of a Neurology, b Medicine, Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

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<table>
<thead>
<tr>
<th>NGF</th>
<th>Neuropathy</th>
<th>Phase duration</th>
<th>Patients</th>
<th>Dosage notes</th>
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</thead>
<tbody>
<tr>
<td>NGF</td>
<td>Diabetic neuropathy</td>
<td>Phase 1–2, placebo controlled, 6 months</td>
<td>250 patients</td>
<td>Subcutaneous, 0.3 µg/kg, 3 times a week</td>
</tr>
<tr>
<td>NGF</td>
<td>Diabetic neuropathy</td>
<td>Phase 3, placebo controlled, double blind, 48 weeks</td>
<td>505 patients/NGF treated, 515 patients in placebo group</td>
<td>Subcutaneous 0.1 µg/kg, 3 times a week</td>
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<tr>
<td>NGF</td>
<td>HIV neuropathy</td>
<td>Phase 2, placebo controlled, 18 weeks</td>
<td>270 patients</td>
<td>Subcutaneous, 0.1–0.3 µg/kg, twice a week</td>
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<tr>
<td>NGF</td>
<td>HIV neuropathy</td>
<td>Phase 2, open-label follow-up study, 48 weeks</td>
<td>200 patients</td>
<td>Subcutaneous, 0.1–0.3 µg/kg, twice a week</td>
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</tbody>
</table>
HSV-mediated expression of neurotrophin-3 prevents neuropathy caused by subacute overdose of pyridoxine (400 mg/kg bid)
LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation - PDX neuropathy model.
LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation - PDX neuropathy model
LAP2-driven expression provides biologically active transgene expression at 6 months after inoculation – PDX neuropathy model
LAP2-driven expression provides biologically active transgene expression for 6 months after inoculation in diabetic neuropathy.
Subcutaneous inoculation of an NT-3 expressing HSV vector protects against the development of diabetic neuropathy over 6 months.

**sensory amplitude**

1 month 6 months 6 months

**conduction velocity**