Replication Defective Herpes Simplex Virus Type 1 Vectors

Applications to Treatment of Sensory Nerve Disease

Nurel Therapeutics Inc
RD-HSV Vectors

Advantages: neuronal tissues

- Ease of construction
- Large or multi-transgene capacity
- Targeted delivery sensory ganglion by retrograde transport
- Stable persistence in neurons in non-integrated state
- No toxicity for neurons
- Capable of short or long-term transgene expression
- Successful for treatment of animal models of disease
- Scalable manufacture with no wild type type recombinants
- tk gene as a safety feature

Limitations: non-neuronal tissues

- Difficult to target non-neuronal tissues
- Toxicity and transgene expression are linked to a toxic gene
Best Gene Therapy Targets

- elimination of IE genes
- targeted delivery to sensory neurons

Treatment of sensory neuron diseases: pain and neuropathy

Scalable manufacture of RD-HSV vectors
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HSV Targeted Delivery

Skin Surface

Sensory Nerve Endings
HSV Transport in Axon

Spinal Cord

Skin

QuickTime™ and a Sorenson Video decompressor are needed to see this picture.
Expression of HSV vectors in neurons

*In vitro* and *in vivo*
Short Term Treatments (2-4 wks)
Applications to Pain and Neuropathy
Using the HCMV Promoter
Chronic Pain

An Unmet Medical Need that Affects 60 Million Americans At Sometime During Their Lives And Costs Billions of Dollars Annually In Health Care Expenses And Job-Related Absenteeism
Design and Application of HSV Vectors To the Treatment of Chronic Pain

Preclinical Studies that Support A Phase 1/2 Randomized Dose Escalation Study Design to Evaluate Safety and Provide Early Evidence for Clinical Efficacy
Neurophysiology of Nociception

Cortex → Thalamus → Midbrain → Pons → Medulla → Spinal Cord

Free nerve endings detect noxious stimuli

Dorsal root ganglia (DRG)

C fibers
Aδ fibers

Primary afferents

Synapse in Spinal SDH

2nd order neuron

Synapse in Thalamus

3rd order neuron

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Julius and Basbaum, Nature, 2001
• Formalin
• CFA
• Osteolytic Sarcoma
• Nerve Ligation
• Spinal Cord Injury

Inflammatory Pain

Neuropathic Pain
control SHPE-inoculated

SV40
pA
HCMV
IEp
PPE
loxp
loxp

SHPE

tk

ICP4

ICP4
Spinal nerve ligation model of neuropathic pain
Mechanical Allodynia

- Use calibrated von Frey Filaments.
- Press against plantar surface of paw.
- Determine 50% g threshold using the up-down method.
Neuropathic pain. L5 spinal nerve ligation causes mechanical allodynia.

![Graph showing response rate vs stimulus (gms).](image-url)

Response rate (pre-operative)
Neuropathic pain. L5 spinal nerve ligation causes mechanical allodynia.
Neuropathic pain: Transduction of lumbar DRG with the PE-HSV vector reverses nerve injury-induced mechanical allodynia (3.5 wks post-op)
Neuropathic pain: Transduction of lumbar DRG with the PE-HSV vector reverses nerve injury-induced mechanical allodynia (3.5 wks post-op)
SHPE continues to provide an antinociceptive effect after 2 weeks of morphine treatment (tolerance)
Conclusions

1. Enk gene therapy produced an anti-allodynic effect
2. Effect did not induce tolerance and was additive with morphine
3. Pursuing GAD67 for spinal cord injury pain and endomorphin for herpes zoster pain
Reversal of Diabetic Neuropathy
Using HSV NGF or NT3 Gene Transfer
Can Neurotrophic Gene Transfer Reverse Diabetic Neuropathy?

Neuropathy:
• Electrophysiology
• Thermal hypoalgesia

Vectors:
• NGF expressing (SHN)
• NT-3 expressing (QHL2NT3)
Can Neurotrophic Gene Transfer Reverse Diabetic Neuropathy?

Days 1 and 3
100 mg/kg STZ

Days 45-47
Confirm Neuropathy

Days 93-95
Evaluate Neuropathy

Day 60
Inject Vectors

Day 100
Euthanize, ICC

Day 100

Blood Glucose (mg/dL)

(5) (20)

FSA (μV)

Foot WL (sec)

CTL  STZ

CTL  STZ

CTL  STZ
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Day 60
Inject Vectors

Day 100
Euthanize, ICC
Treatment of pyridoxine-induced peripheral sensory neuropathy using NGF or NT3 gene transfer:

Long-term latency (LAP2) gene expression and therapeutic efficacy using HSV Vectors in a mouse model
Electrophysiology of Protected Sensory Neurons

A  

foot sensory amplitude (µV)

SHN  SLN  QLNT3  SHZ

* * *  

B  

H-wave (mV)

SHN  SLN  QLNT3  SHZ

* *  

C  

slips

SHN  SLN  QLNT3  SHZ

* * *  

Pyridoxine
Conclusions

1. The LAP2 promoter is effective for long-term therapeutic gene expression
2. NT3 is an effective treatment of peripheral sensory neuropathy
Harvest
- decant liquids
- no cell scraping
- serum free

Salt release
- reduce binding of contaminants
- increase titer

0.65u TFF
- removal of macromolecule contaminants

0.10u TFF
- size limited concentration

Ion exchange
- chromatography

0.10u TFF
- re-concentration of final product

HSV Vector Manufacture Process
(1) Harvest (2) post clarification (3) post concentration (4) post column (5) final
Particle Analysis

Particle/PFU ratio = 2.7 to 3.3
HSV vectors are ready for human trials because they are (i) safe, (ii) effective in the treatment of preclinical models of chronic pain and neuropathy, (iii) amenable to targeted gene delivery to their natural site, sensory ganglia, by simple skin inoculation and (iv) scalable manufacturing processes are now available.
## Acknowledgements

### Co-Investigators

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Bill Goins, Darren Wolfe, Dave Krisky

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- Karina Soares  
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- Jim Wechuck

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- Han Li  
- Ying Jiang

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