Risk considerations of the growing biomedical use of herpevirus vectors
Summary

- Herpesvirus Vectors
- Use in Humans
- Biosafety Risks
Herpesvirus vectors in Humans

Vaccines:
9 Human Herpesviruses have been identified

**Lysis / Latency switch**

1. Herpes Simplex Virus – 1 (HSV-1)
2. Herpes Simplex Virus – 2 (HSV-2)
3. Varicella Zoster Virus (VZV)
4. Epstein Barr Virus (EBV)
5. Cytomegalovirus (CMV)
6A. Human B Lymphotrophic Virus
6B. Human B Lymphotrophic Virus
7. Roseolovirus
8. Karposi’s sarcoma herpesvirus (KSHV)
HSV-1 ds DNA genome

encodes ~80 genes (152 kb!)

Essential Genes

Non Essential Genes

- Immediate Early gene
- Early Gene
- Strict Late gene
- Expression stage not known
HSV-1 genes are expressed in a hierarchical cascade.
Why herpesvirus vectors?

- Preferential replication in tumor cells
- Large genome size for big or multiple transgenes
- Latency – proof of possibility to silence genome
- High infectivity in many cell types
- Neuronal transport – gene delivery to deep tissues
- Gene transfer in postmitotic cells, episomal persistence
HSV-1 vector types

Replicative (Conditional):
- ONCOLYTICS, VACCINES

Replication-incompetent:
- CANCER IMMUNOTHERAPY,
  NEUROLOGICAL GENE
  THERAPY, VACCINES

Amplicons (Gutless):
- PRECLINICAL

productive viral genome

NON-productive viral genome

NON-viral genome
HSV-1 vector types

1 REPLICATIVE
HSV-1 vector types

2 REPLICATION-INCOMPETENT

- Transgene expression
- Latency-associated transcripts
- Lytic cycle expression
- Quiescent viral genes
- Non-productive viral genome
HSV-1 vector types
3 AMPLICONS
Use in Humans
# Viral vaccines against HHVs

<table>
<thead>
<tr>
<th>Virus</th>
<th>Status and Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epstein Barr Virus</td>
<td>Modified vaccinia and adenovirus</td>
<td>Cohen JI (2015) Epstein–barr virus vaccines <em>Clinical &amp; Translational Immunology</em> 4: e32 doi 10.1038/cti.2014.27</td>
</tr>
</tbody>
</table>
IMLYGIC, 1st of a kind

US approval for drug that turns herpes virus against cancer

Amgen wins EU green light for first virus-based cancer drug

IMLYGIC, 1st of a kind

- Phases I - III
- Safe, no deaths, no serious AEs
- Priming dose 10^6 particles → 10^8 particles x ~15
- Detectable in blood, urine (1/5), not in tears, nasal mucosa, feces
- Little at injection site

# Oncolytic Herpesvirus Clinical Trials

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Company</th>
<th>Mutations/Disruptions</th>
<th>Tumor Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMLYGIC (T-VEC, Oncovex, Talimogene Laherparevee)</td>
<td>Amgen</td>
<td>ΔICP34.5, ΔICP47, expresses GM-CSF</td>
<td>melanoma, liver tumor, head &amp; neck carcinoma</td>
</tr>
<tr>
<td>OrienX010</td>
<td>OrienGene Biotechnology</td>
<td>ΔICP34.5, ΔICP47, expresses GM-CSF</td>
<td>glioblastoma</td>
</tr>
<tr>
<td>SEPREHVIR (1716)</td>
<td>Virttu Biologics</td>
<td>ΔICP34.5</td>
<td>Hepatocellular carcinoma, glioblastoma, mesothelioma, neuroblastoma</td>
</tr>
<tr>
<td>G207</td>
<td>Medigene</td>
<td>ΔICP34.5, disrupted ICP6</td>
<td>glioblastoma</td>
</tr>
<tr>
<td>HF10</td>
<td>Takara Bio</td>
<td>Inactivated UL43, UL49.5, UL55, UL56</td>
<td>breast cancer, melanoma, pancreatic cancer</td>
</tr>
<tr>
<td>G47Δ</td>
<td>Medigene</td>
<td>ΔICP34.5, ΔICP47, disrupted ICP6</td>
<td>prostate cancer</td>
</tr>
<tr>
<td>NV1020</td>
<td>Medigene</td>
<td>Haploid ICP4, ICP0, ICP34.5, ΔTK, inactive UL24</td>
<td>Liver metastatic colorectal cancer</td>
</tr>
</tbody>
</table>


## Nonreplicative Herpesvector Clinical Trials

<table>
<thead>
<tr>
<th>Vector</th>
<th>Source</th>
<th>Characteristics</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISC-GMCSF</td>
<td>Xenova</td>
<td>ΔgH (HSV-2), expresses GM-CSF</td>
<td>melanoma</td>
</tr>
<tr>
<td>NUREL-C2</td>
<td>Uni Pittsburgh</td>
<td>Inactive ICP4, ICP27, ICP22, expresses TNF &amp; Connexin43</td>
<td>glioblastoma</td>
</tr>
<tr>
<td>NP2</td>
<td>Periphagen</td>
<td>ΔICP4, ΔICP27, expresses proenkephalin</td>
<td>intractable pain in cancer</td>
</tr>
</tbody>
</table>

First clinical trial of gene therapy for pain shows substantial pain relief for patients

Date: April 12, 2011
Source: University of Michigan Health System

Joseph Glorioso, PhD, Receives Pioneer Award for Engineering Herpes Simplex Virus Gene Delivery Systems

New Rochelle, NY, February 19, 2014—Joseph C. Glorioso, III, PhD (University of Pittsburgh School of Medicine, PA) devoted much of his research career to developing new gene delivery systems for treating a number of genetic and acquired diseases, including cancer, neurodegenerative disease, and autoimmune disease. Glorioso, who received the 2014 Pioneer Award for Engineering Herpes Simplex Virus Gene Delivery Systems, is the first recipient of the award, which recognizes the achievement of a single investigator in the field of gene delivery.

Dr. David Fink receives national VA research award from top officials in surprise ceremony

Posted on December 16, 2014

Gene therapy pioneer, U-M Medical School Department of Neurology chair and longtime Veterans Affairs researcher David Fink, M.D., received the 2014 Paul B. Magnuson Award from VA in a surprise ceremony at the VA Ann Arbor Healthcare System on Monday.

Dr. Fink is a staff neurologist, and an investigator with the Geriatric Research, Education and Clinical Center, at the Ann Arbor VA. He is also the Robert Brear Professor of Neurology at U-M. He has been a neurologist at VA Ann Arbor for 19 years and has also served asVA Associate Director for Research. Fink’s research has been recognized with awards and grants from the VA, the American Heart Association and the U.S. military.

As Pot represents the most imp opportunity to study com acquired diseases, includ
HSV-1 vectors are transported from skin to spinal cord

Minimally invasive delivery into poorly accesible tissues
Recessive: mutations in FXN reduce expression of frataxin, an essential protein which is especially vital in certain neurons of the CNS:

PROGRESSIVE DEGENERATION OF:
- Dorsal Root Ganglion neurons
- Sensory tracts of spinal cord
- Dentate nuclei of the cerebellum

Other neurological applications: Friedreich’s Ataxia
HSV-FXN vector injected into footpad: $8.75 \times 10^5$ PFU/mouse footpad

5 days after injection: 50-400 genomes per DRG detected and one animal that had 270,000 genomes

SPECIFIC transgene targeting into DRGs
Other neurological applications: enhancing HSV-1 to silence sensory afferents in the bladder

Emergence of a spinal micturition reflex after SCI: abolition by silencing of hyperexcited C-fiber bladder afferents by gene therapy to restore continence and micturition, (ELPIS)
Biosafety Risks
Shedding

- **Replication competence**: ability to multiply and amplify in human host affects dissemination in the body and may increase shedding. Possibility of recombination with wildtype virus.

- **Immunogenicity**: vectors that elicit a strong immune response are cleared from circulation more rapidly than weakly immunogenic vectors → shorter duration shedding. In multiple administrations, shedding may be for a shorter duration in the later dose cycles than early doses (immune-priming).

- **Persistence and latency**: duration may be longer due to persistence or latency (eg neurons, leukocytes) followed by reactivation. Shedding may be intermittent and unpredictable (reactivation stimuli)

- **Tropism**: may affect what samples should be collected to assess shedding. Modifications of tropism may alter shedding profile because of retargeting of the product to different tissues or organs.
Shedding measurements

Viremia and virus shedding
No viral shedding was observed in any patient on this trial as all HSV-1 cultures including blood, buccal swab, and urine at all study visits through day 28 were negative. PCR for HSV-1 genomes were also negative in all buccal swab and urine samples. Blood PCR for HSV-1 genomes were negative at baseline, day 0, and day +1 following virus injection. In contrast, blood PCR for HSV-1 genomes at day +4 turned positive in 1 patient at dose level 1, 2 patients at dose level 2, and all 3 patients at dose level 3 (6 of 9 patients total). In 2 patients, PCR remained positive at day +7, and in one of those patients (HSV04), it remained positive through day 28. Unfortunately, this patient's disease rapidly progressed leading to hospice care so we were unable to confirm viral clearance at a later time point.

Detection of Multiple Strains of Latent Herpes Simplex Virus Type 1 Within Individual Human Hosts

MARCIA E. LEWIS,†† WAI-CHOI LEUNG, VERONA M. JEFFREY, AND KENNETH G. WARREN
Neurovirology Research Unit, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

Received 22 March 1984/Accepted 11 June 1984

One hundred and fifteen isolates of herpes simplex virus were recovered from parallel explant cultures of trigeminal and vagus ganglia and trigeminal nerve roots derived from 20 unselected human cadavers. Restriction enzyme patterns of strains recovered from 18 of 20 individuals could be differentiated from individual to individual, although all isolates from a single host were identical. Isolates from two individuals differed among themselves in the number and location of certain restriction enzyme sites.
Superinfection Prevents Recombination of the Alphaherpesvirus Bovine Herpesvirus 1

François Meurens,¹ Frédéric Schynts,² Günther M. Keil,³ Benoît Muylkens,¹ Alain Vanderplasschen,¹ Pierre Gallego,⁴ and Etienne Thiry¹*

In vivo, the rise of recombinant viruses can be modulated by different factors, such as the dose of the inoculated viruses, the distance between inoculation sites, the time interval between inoculation of the first and the second virus, and the genes in which the mutations are located.

The dramatic effect of the time interval on the rise of recombinant viruses is particularly important for the risk assessment of recombination.
Intracerebral recombinant HSV-1 vector does not reactivate latent HSV-1

Q Wang¹, J Guo¹ and W Jia¹,²
¹Departments of Surgery and Ophthalmology, University of British Columbia; and ²British Columbia Cancer Agency, Vancouver, Canada
Pharmacovigilance

- Route of administration: should be considered in the selection of sample types to collect in a shedding study. e.g., in addition to routine samples (urine, feces, saliva): skin swabs at injection site for intradermal administration; nasopharyngeal washes for inhalation or intranasal delivery.

- Tests for adventitious agents introduced during manufacture (e.g., mycoplasma)

- Tests to distinguish between wildtype and vector

- Herpesvirus PCR and plaque assays offered by CROs
HSV vectors have an excellent safety profile

*Preferential replication in tumor cells*

*Immune tolerance: prevalence >90% human population*

*Natural OFF state in neurons: potential for neurological therapy*

*Clinical Record: no deaths or serious adverse events*
Future regulatory challenges...

More complex vectors:

1. Multiple, and/or really big transgenes
2. Cell-type retargeting - cell entry
   - innate defences
   - transcription factors
   - miRNA
3. Chimeras with other viruses

Lim F (2013) HSV-1 as a Model for Emerging Gene Delivery Vehicles
ISRN Virology Article ID 397243, 12 pages doi 10.5402/2013/397243
Megavirales

The third biggest virus is *Phycodnavirus*.
*Infecting algae*
Double stranded DNA
Size = 250-560 kbp
(Mimivirus = 1181 kbp)

*Pandoravirus salinus*
- Base pairs: 2.5 million
- Length: 1,000 nm
- Diameter: 500 nm

*Megavirus chilensis*
- Base pairs: 1.26 million
- Diameter: 500 nm

*Influenza type A*
- Base pairs: 13,500
- Diameter: 100 nm

A phycodnavirus at cell surface
Plant genomes enclose footprints of past infections by giant virus relatives

Florian Maumus\textsuperscript{1,*}, Aline Epert\textsuperscript{2}, Fabien Nogué\textsuperscript{2} & Guillaume Blanc\textsuperscript{3,*}

Chlorovirus ATCV-1 is part of the human oropharyngeal virome and is associated with changes in cognitive functions in humans and mice

Robert H. Yolken\textsuperscript{a,1}, Lorraine Jones-Brando\textsuperscript{a}, David D. Dunigan\textsuperscript{b}, Geetha Kannan\textsuperscript{c}, Faith Dickerson\textsuperscript{d}, Emily Severance\textsuperscript{a}, Sarven Sabunciyan\textsuperscript{a}, C. Conover Talbot Jr.\textsuperscript{1,*}, Emese Prandovszky\textsuperscript{a}, James R. Gurnon\textsuperscript{b}, Irina V. Agarkova\textsuperscript{b}, Flora Leister\textsuperscript{a}, Kristin L. Gressitt\textsuperscript{a}, Ou Chen\textsuperscript{a}, Bryan Deuber\textsuperscript{a}, Fangrui Ma\textsuperscript{b}, Mikhail V. Pletnikov\textsuperscript{c}, and James L. Van Etten\textsuperscript{b,1}
Thank you for your attention