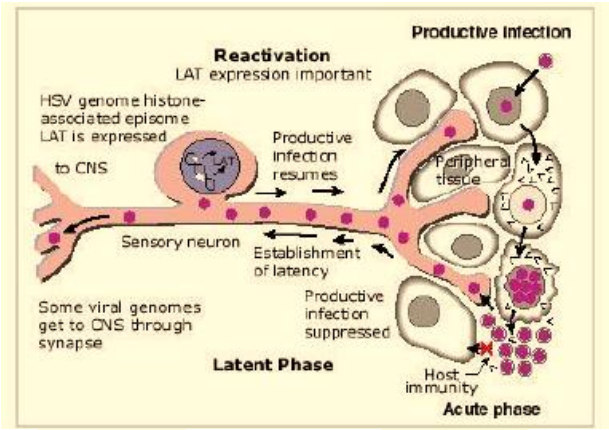
Vektor Herpes virus

Herpes Virus

Herpes simplex virus (HSV): a complex human neurotrophic virus

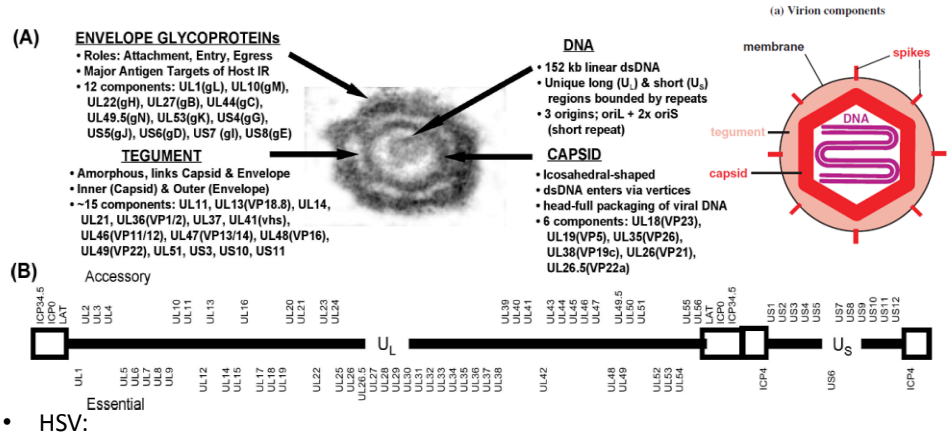


Body periphery (oral / genital epithelial cells)
- Infection
- Lytic multiplication

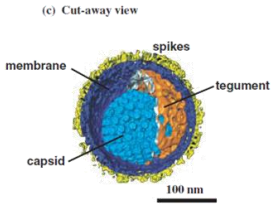
Sensory nerve endings innervating site of multiplication
- Virus enter sensory nerve endings

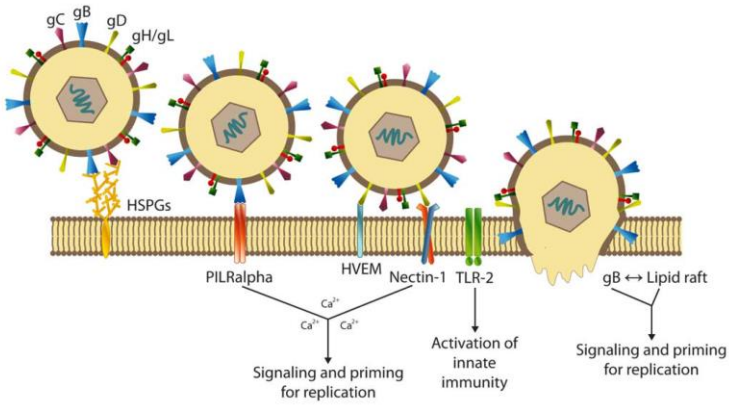
retrograde transported

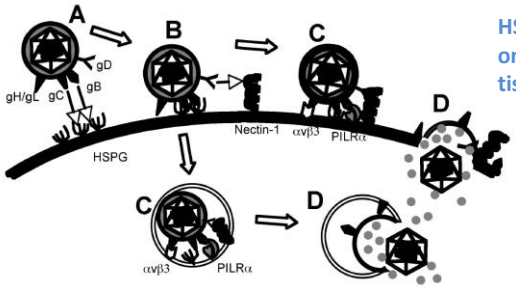
Nucleus of sensory neurons
- virus Latent



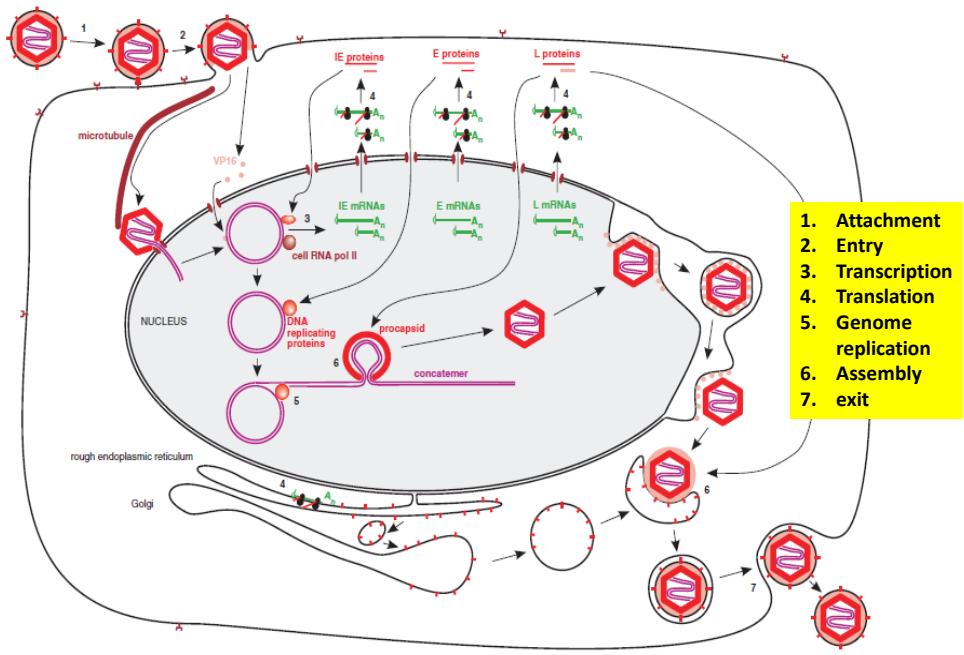
- HSV:
  - icosahedral capsid→ viral DNA genome in association with core proteins.
  - linear double stranded DNA of 152 kb encoding at least 80 gene products
  - Around the capsid → an amorphous layer : tegument + 20 different proteins with structural and regulatory roles, surrounded by an external envelope containing different glycoproteins involved in different functions, among which the first steps of binding and entry into the host cell.



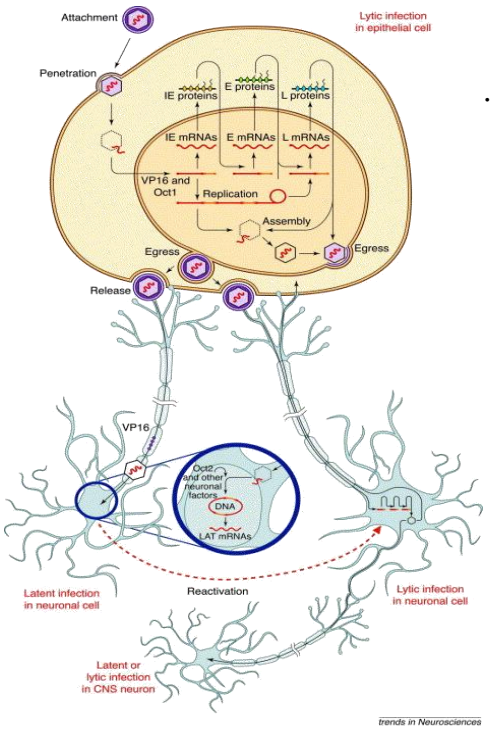




HSV receptors are ubiquitous on almost all cell types and tissues



HSV-1 replication cycle. Stages in virion assembly include procapsid construction, packaging a copy of the genome in the procapsid and acquisition of the envelope by budding into a vesicle within the cytoplasm



- **The HSV-1 life cycle.** The herpes simplex virus type 1 (HSV-1) enters epithelial cells by fusion of the virus envelope with the plasma membrane and the virus capsid is transported to the nuclear membrane. Viral DNA (represented in red and orange) is released into the nucleus where, upon interaction with a multiprotein complex formed by the VP16 tegument protein, the cellular factor Oct1 and other cellular factors, it is transcribed in a sequential cascade with immediate–early (IE) first, early (E) next and, finally, late (L) genes. Viral proteins are translated in the cytoplasm, and viral DNA is replicated and packaged into capsids in the nucleus. Capsids bud out through the nuclear membrane, acquiring the envelope during transit. Enveloped virions are then released at the cell membrane. The resulting virus particles can encounter and fuse with the cell membrane of peripheral nerve termini that innervate the site of primary infection. The viral nucleocapsid then travels via retrograde axonal transport to the neuronal cell body, where the virus can either proceed through the highly regulated cascade of lytic gene expression or enter latency, during which the lytic gene program is interrupted. As VP16 and the viral capsid reach the nucleus separately, it has been hypothesized that, if the protein is lost during axonic transport, entrance in latency is favoured. The virus possesses a natural promoter system (LAP1 and LAP2) that remains active during latency, producing a family of non-polyadenylated latency-associated transcripts (LATs). Neuronal transcription factors, such as Oct2 and Pou4, have been reported to regulate gene expression negatively in latently infected neurones[64]. The virus is capable of reactivating itself from the latent state in response to a wide variety of stimuli that allow it to enter the lytic portion of the HSV life cycle. At this stage, progeny virions can be transported back to the site of the primary infection or the virus can enter the CNS.

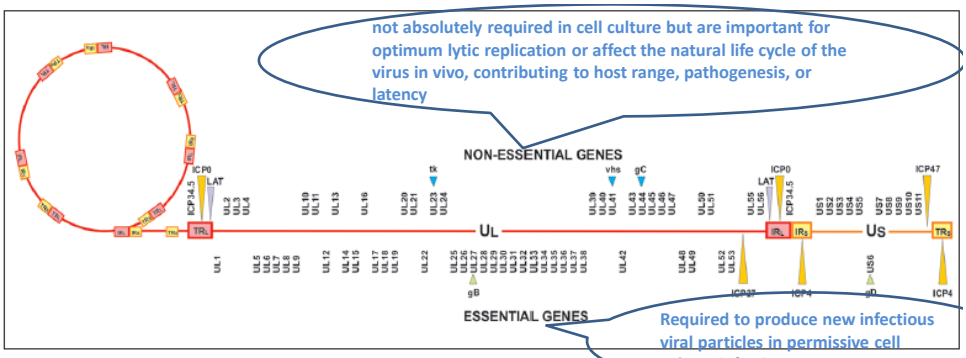
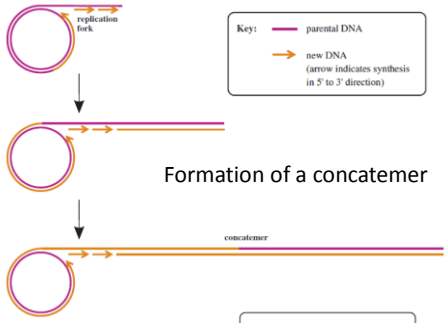


Figure 1. Herpes simplex virus Type 1 genome. The rolling circle replication mechanisms, essential and nonessential accessory genes are depicted.

- The genome is a linear double stranded DNA of 152 kb encoding ~ 80 gene products. The genome is replicating by a rolling circle mechanism forming head-to-tail concatamers.
- During the replication, the presence of inverted repeats sequences flanking the two unique segments of the genome (unique long UL and unique short US) are causing the formation of four isomers equally infectious (Fig. 1).



Formation of a concatemer

- In the viral genome there are approximately 80 gene products :
  - immediate early (IE or a),
  - early (E or b) and
  - late (L or g)
- The viral DNA contains ~37 essential genes.
- The US region of the genome→ 1 essential gene : glycoprotein D → replacement opportunity → 40-50 kb of exogenous sequences→ reduce pathogenicity and loss of viral activities → not efficient for gene delivery

HSV for gene therapy vectors

- Attractive aspects from HSV
  - HSV displays a broad host cell range and its cellular receptors, HS, PILRα, HVEM, and nectin-1 and 2, are widely expressed on the cell surface of numerous cell types.
  - HSV is highly infectious.
  - Non-dividing cells may be efficiently infected and transduced by HSV.
  - Almost half of the about 90 known viral genes are nonessential for growth in tissue culture and then may be deleted to create genomic space for exogenous transgenes and to delete functions essential for viral virulence and toxicity *in vivo*.
  - Recombinant HSV vectors can be easily produced to high titer and purity without wild type (wt) contaminants.
  - The latent behaviour of the virus may be exploited for stable long-term expression of therapeutic transgenes in neurons.
  - HSV possesses the interesting features to be transported retrogradely in neurons and transferred across synapses and it is possible to take advantage of this virus characteristic to trace neuronal pathways

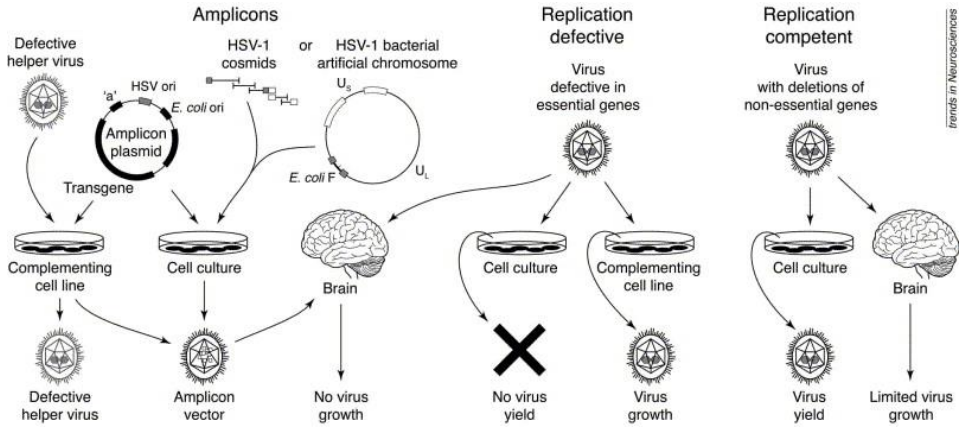
HSV-1 genome and h**SV-derived** v**ectors**

- HSV as a vector for several potential applications in human health.
  i. delivery and expression of human genes to the nervous system cells
  ii. destruction of cancer cells
  iii. prophylaxis and immunotherapy against tumors
  iv. prophylaxis against infections with HSV and other infectious diseases.

Hespes Virus Vector

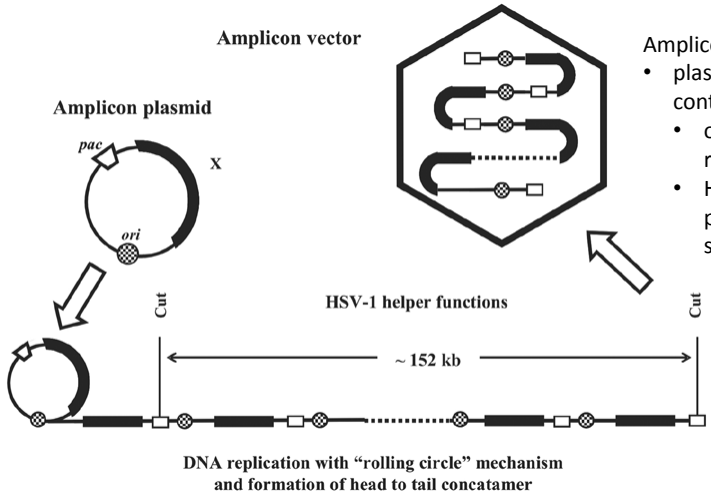
Vectors based on HSV Type 1 :

a) amplicon vectors,
b) replication-defective viruses
c) replication competent vectors
d) bacterial artificial chromosome (BAC) vectors
e) genetically engineered replication-competent viruses with restricted host range.



- **HSV-1 vector strategies for gene transfer to the CNS.** The replication and packaging of amplicons, which uses a defective helper virus, requires a cell line that provides the complementing functions. The progeny will be a mixture of defective and amplicon-containing particles. Alternatively, the helper functions can be provided by a library of overlapping cosmids or by a bacterial artificial chromosome in a standard cell culture. In these cases, the progeny will be formed exclusively by amplicon vectors. Replication-defective HSV-1 vectors that contain deletions of essential functions cannot replicate in standard cell culture, and require complementing cell lines that provide the respective missing function. Replication-competent vectors with deletions of non-essential genes do not require specialized cell lines, and can be propagated in standard cell cultures. Abbreviations: 'a', HSV packaging recognition sequence; *E. coli*F, *E. coli* F-plasmid backbone; *E. coli* ori, *E. coli* origin of replication; HSV ori, HSV origin of replication; U_L,unique long segment; U_S, unique short segment.

Amplicon vector



Amplicon vector:

- plasmid-derived vectors contain :
  - origin of HSV DNA replication (*ori*)
  - HSV cleavage–packaging recognition sequences (*pac*)

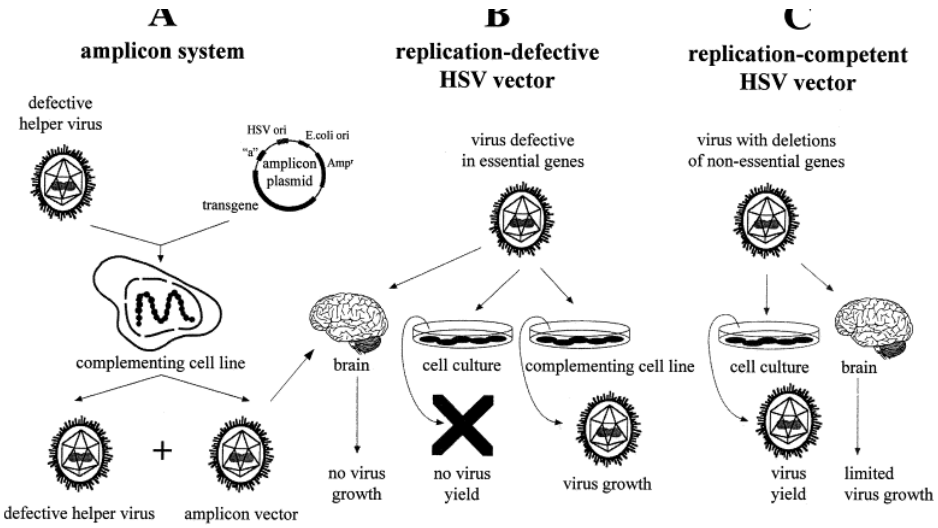


Fig. 2. HSV-1 vector strategies for gene transfer to the central nervous system. (A) Amplicons require the use of defective HSV-1 recombinants to package concatamers of an amplicon-plasmid DNA molecule containing the transgene in a specific cell line capable of complementing the defective mutant by providing the gene product in trans. In order for the plasmid to be packaged into HSV-1 particles, the plasmid must contain an HSV-1 origin of replication (HSV ori) and the packaging signal (`a' sequence). A mixed population of particles, primarily consisting of defective virus with a small percentage of packaged amplicon, can be inoculated into the brain. (B) Defective HSV-1 vectors containing deletions of essential functions cannot replicate in standard cell culture, and require specific cell lines which provide the respective gene product in trans for propagation. (C) HSV-1 recombinant vectors with deletions of non-essential genes do not require specialized cell lines, and can be propagated to high titers in standard cell culture

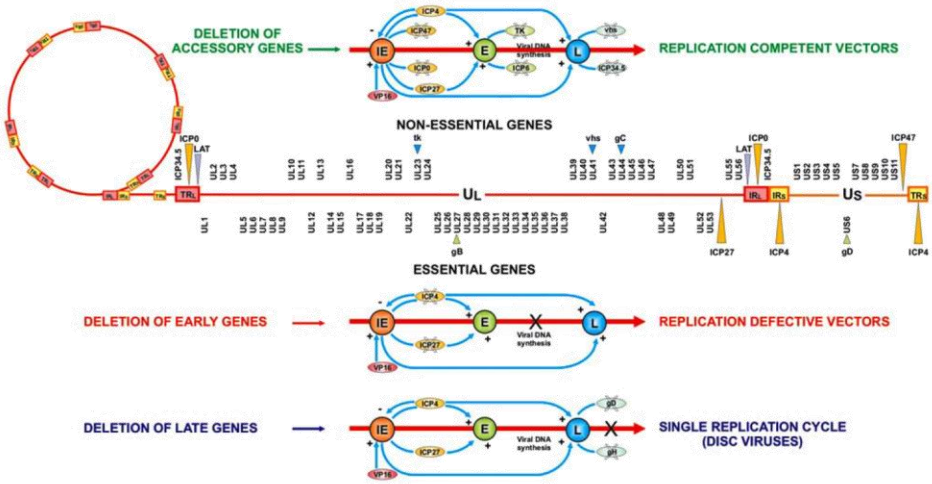
HSV Amplicon vectors

- HSV Amplicon vectors →
  - identical to wild type HSV-1 structurally, immunologically and host-range points of view
  - carry a concatemeric form of a plasmid DNA, named the amplicon plasmid, instead of the viral genome.
- several advantages as gene delivery vehicles:
  - a large transgene capacity (150 kb);
  - the repetitive character of the genome carried by the amplicon particle ensures the introduction of multiple copies of the transgene per infected cell;
  - the ability to infect a wide variety of cell types, including dendritic cells;
  - the ease of vector construction;
  - the limited toxicity due to the lack of viral coding sequences.

- contaminant helper particles, even if defective, induced significant cytotoxicity and inflammatory responses
- The last generation of helper system:
  - consists of the entire HSV-1 genome, without pac signals,
  - bacterial artificial chromosome (BAC) in *E. coli* supplying the full set of transacting HSV-1 functions.

Replication-defective vectors

- viral vectors → mutated or deleted "essential" genes
  - several replication-defective vectors have been constructed in which the IE genes, expressing infected cell proteins (ICP) 0, 4, 22, 27 and 47, have been deleted in various combinations
- For in vitro replication → complemented in trans



Replication-competent vectors
Attenuated HSV vectors

- Deletion of some non essential viral genes results in viruses that retain the ability to replicate in vitro, but are compromised in vivo, in a context dependent
- Toxic and/or pathogenic properties of the virus → disabled prior its use as a gene delivery vector.

HSV-1 based v**ectors** a**pplications**

- **HSV-1 based** v**ectors for** nervous system gene therapy
- HSV-1 vector design
  - elimination of the lytic viral gene expression and of the innate and immune responses (toxicity);
  - engineering of promoter systems to achieve appropriate, lasting transgene expression;
  - identification of strategies to target heterologous gene expression to specific neurons; and
  - simultaneous expression of multiple genes.

Table 1. Experimental Gene Therapy of Neurological Disorders Using HSV Vectors

Pathological Disturbances/Clinical Indications	Therapeutic Transgenes	Stage	Ref.
Replication-Defective Vectors			
Epilepsy	FGF-2, BDNF	Preclinical	[35]
Multiple sclerosis	IL-4, IL-1ra	Preclinical	[34, 48, 49]
Alzheimer's disease	shRNA, neprilysin	Preclinical	[36]
Parkinson's disease	GDNF bcl-2 Erithropoietin	Preclinical	[51, 52] [50, 51] [37]
Diabetes	Neurotrophic factors	Preclinical	[56-60]
Chronic pain	Preproenkephalin	Phase I	[38, 39, 88]
Lysosomal storage diseases: Tay-Sachs	HexA α subunit	Preclinical	[42]
Replication-Competent Vectors			
Lysosomal disorders: MPS VII	β-glucoronidase	Preclinical	[41]
Multiple sclerosis	IL-4, IL-10	Preclinical	[76]
Ischemic brain injury	HSV-2 ICP0PK	Preclinical	[77]
Chronic pain	Preproenkephalin	Preclinical	[69, 70]

HSV-1 based vectors applications

- **HSV-1 based vectors for vaccination**
- several advantages of using HSV-1 as vaccine vectors for the delivery of foreign antigens :
  - to elicit strong and durable immune responses by various routes of inoculation
  - the viral DNA persists inside the host's cell nucleus as an episomal element, thus eliminating the safety concerns deriving from the random integration of the viral genome into the host's DNA;
  - They carry the *tk* gene, encoding the viral thymidine kinase, that, in case of undesired effects, can be used, in combination with specific antiviral drugs, to kill the virus-harbouring cells.

HSV-1 BASED VECTORS FOR Vaccination

HSV Strain	Genetic Modification	Therapeutic Transgenes	Clinical Indications	Ref.
Replication-Competent Vectors				
NV1020 (HSV-1)	Deletion in one copy of ICP34.5 + *tk* under ICP4 promoter control + deletion in UL24, 55 and 56	none	Anti HSV vaccine	[121, 122]
RAV9395 (HSV-2)	Deletion in both copy of ICP34.5 + deletion in UL55 and 56 + deletion in both copy of ORF P	none	Anti HSV vaccine	[118]
AD-472 (HSV-2)	Deletion in both copy of ICP34.5 + deletion in UL55 and 56 + deletion in both copy of ORF P + deletion of UL43.5 + deletion of US10-12	none	Anti HSV vaccine	[126, 127]
NS-gEnull (HSV-1)	Deletion of US8	none	Anti HSV vaccine	[128]
ImmunoVEX^{HSV2}	Deletion of genes involved in immune evasion	none	Anti HSV vaccine	www.biovex.com
Replication-Defective Vectors				
dl5-29 (HSV-2)	Deletion of UL5 and 29	none	Anti HSV vaccine	[99, 103, 105]
dl5-29-41L (HSV-2)	Deletion of UL5 and 29 + deletion of UL41	none	Anti HSV vaccine	[99, 104, 105]
DISC-gH (HSV-1)	Deletion of gH	none	Anti HSV vaccine	[101, 106]
DISC gH (HSV-2)	Deletion of gH	mGM-CSF or hIL-12	Anti HSV vaccine and anti tumour vaccine	[108, 109]
CJ9gD (HSV-1)	Deletion of UL9	Over-expression of gD	Anti HSV vaccine	[110-112]
TOH-OVA (HSV-1)	Deletion of ICP4, 22, 27, and 47	Ovalbumin	Anti bacterial infections	[94]
d106 (HSV-1)	Deletion of ICP4, 22, and 27	HIV-1 Tat	Anti HIV vaccine	[114]
d81 (HSV-1)	Deletion of ICP27	SIV Env and Nef	Anti SIV vaccine	[115]
HSV-SIV d106 (HSV-1)	Deletion of ICP4, 22, 27, and 47	SIV Gag, Env, and Tat-Rev-Nef fusion protein	Anti SIV vaccine	[95, 116]
d106S (HSV-1)	Deletion of ICP4, 22, 27, and 47 + increased acyclovir resistance	HIV-1 Tat	Anti HIV vaccine	[117]

HSV-1 BASED VECTORS FOR CANCER GENE THERAPY

- HSV vectors have
  - wide-range natural hosts
  - efficiently infect numerous human tumour cell lines *in vitro*.
-

Table 3. Summary of Oncolytic Vectors and Clinical Trials

HSV Strain	Genetic Modification	Therapeutic Transgenes	Stage	Clinical Indications	Ref.
dlsptk	*tk* deletion	none	Preclinical		[148, 240]
hrR3	UL39 disruption (large RR subunit)	none	Preclinical		[155, 241, 242]
HSV1716	Deletion in both copies of ICP34.5	none	Human clinical trials Phase I (recruitment not yet open)	Glioma, melanoma, head-and-neck cancer. Non-CNS solid tumours	[167, 169, 170, 243, 244]
R3616	Deletion in both copies of ICP34.5	none	Preclinical		[151, 157]
R4009	Stop codon in both copies of ICP34.5	none	Preclinical		[151, 157]
G207	Deletion in both copies of ICP34.5 + disruption of UL39	none	Human clinical trials phase I, IB, and II	Recurrent brain cancer (glioma, astrocytoma, glioblastoma	[161-165, 245, 246]
MGH-1	Deletion in both copies of ICP34.5 + disruption of UL39	none	Preclinical		[149, 166]
MGH-2	Deletion in both copies of ICP34.5 + disruption of UL39	CYP2B1 and shiCE	Preclinical		[190]
R7020 (NV1020)	Deletion in one copy of ICP34.5 + *tk* under ICP4 promoter control + deletion in UL24, 55 and 56	none	Human clinical trials phase I and II	Liver, metastases derived from colorectal cancer.	[121, 122, 176, 177]
G47Δ	Deletion in both copies of ICP34.5 + disruption of UL39	none	Preclinical		[144]
Myb34.5	Deletion in both copies of ICP34.5 + disruption of UL39 + insertion of an ICP34.5 gene under the control of the B-myc promoter	none	Preclinical		[247, 248]
DF3γ34.5	Deletion in both copies of ICP34.5 + insertion of an ICP34.5 gene under the control of the DF3/MUC1 promoter	none	Preclinical		[139, 249]
HF10	Spontaneous generation of HSC-1 variant	none	Human clinical trials		[215, 216]
NV1042	HSV-1/HSV-2 intertypic recombinant + contains only one ICP34.5 copy	Murine IL-12	Preclinical		[192-195]
OncoVex^{GM-CSF}	Deletion in both copies of ICP34.5 + deletion of ICP47	GM-CSF	Phase II/III	Breast cancer, head-and-neck cancer, melanoma	[160, 250]
RAMBO	Deletion in both copies of ICP34.5 + UL39 disruption	IE4/5 prom-Vstat120	Preclinical		[214]
NP2 enkephalin expressing vector	Deletions in ICP4, 27, 22 and 47	human prepro-enkephalin	Phase I	Chronic pain from terminal cancer	[38, 39]

FACTORS THAT MAY AFFECT HSV VECTORS EFFICACY for tumor gene therapy

- the immune response to viral vectors, and
  - Immune Response to HSV Infection
  - Effect of Innate and Adaptive Immunity on Vector Efficacy
- the complexity of the tumour mass
  - ***Enzyme Treatment or Expression***
  - ***Viral-Induced Apoptosis***
  - ***How to Investigate Viral Spread Inside Tumour ECM***.

TARGETING REPLICATION-COMPETENT VECTORS

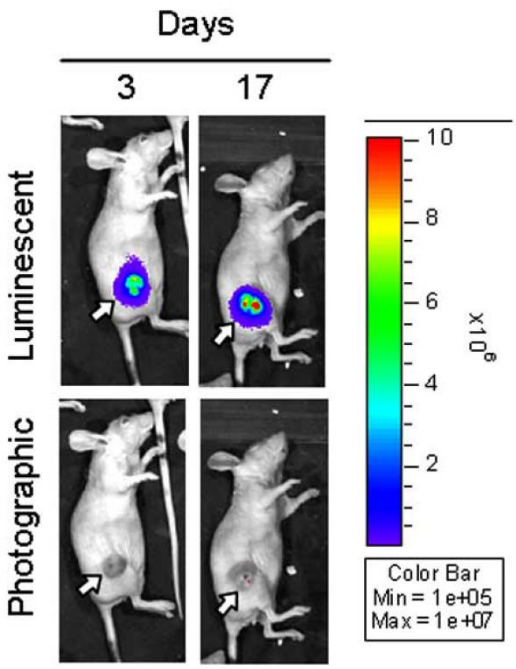
- Aim:
  - To prevent damage of healthy tissues,
  - to decrease the risk of germ line transduction,
  - to design vectors that can be administered intravenously

Table 4. Summary of HSV-1 Replication-Competent Vectors Targeting Strategies

HSV Strain	Genetic Modification	Targeting Molecule	Targeting Tissue/Organ	Ref.
Targeting of Entry				
KgBpK$^-$gC$^-$	Deletion in pK region of gB + deletion of gC		General	[251]
KgBpK$^-$gC-EPO	Deletion in pK region of gB + deletion of gC	Insertion of gC-EPO fusion protein	EPO-receptor positive cells	[252]
KgBpK$^-$gC:preS1ap	Deletion in pK region of gB + deletion of gC	Insertion of gC:preS1ap fusion protein	Hepatocytes HBV receptor expressing cells	[253]
R5111	Deletion in pK region of gB + deletion of HS binding regions of gC and gD	Insertion of gC-IL-13 and gD-IL-13 fusion molecules	IL-13Rα2 receptor expressing cells	[255]
KgBpK$^-$gC$^-$gD$^-$	Deletion in pK region of gB + deletion of gC + deletion of gD	Pseudotyping with VSV-G	VSV susceptible cells	[254]
R-LM113	Modification of gD coding sequence	Insertion of gD-anti-HER2 single-chain antibody fusion molecule	HER-2-expressing tumour cells	[257, 258]
HSV1716	Modification of gD coding sequence	Insertion of gD-scFv anti-CD55or anti CD38 or anti-EGFR fusion molecules	Tumour cells	[259]
Targeting of Replication/Expression				
CEAICP4;	Deletion of either ICP4 promoter	Substitution with CEA promoter	Tumour cells	[139]
CEAγ34.5	Deletion of ICP34.5 promoter	Substitution with CEA promoter	Tumour cells	[139]
DF3γ34.5	Deletion of ICP34.5 promoter	Substitution with DF3/MUC1 promoter/enhancer sequences promoter	DF3/MUC1 expressing tumour cells	[139, 249]
KeM34.5	G207 backbone	Insertion of Musashi1 promoter driving ICP34.5	Malignant glioma	[265, 266]
rQNestin34.5	G207 backbone	Insertion of rQNestin promoter driving ICP34.5	Brain tumours	[234]

Imaging gene therapy

- Radionuclide Imaging
- Bioluminescence Imaging (BLI)



NEW PERSPECTIVES IN TUMOUR ONCOLYTIC VIROTHERAPY

- Oncolytic Virotherapy for the Cancer Stem Cells
- MicroRNA in Oncolytic Virotherapy