

# An Introduction to Virotherapy



Siddhi Mathur

*THE HERPES SIMPLEX VIRUS AND ADENOVIRUS ARE AT THE FOREFRONT OF VIROTHERAPY – AN APPROACH THAT USES VIRUSES TO DELIVER SPECIFIC GENES OR SELECTIVELY LYSE TUMOUR CELLS. THE PROMISING RESULTS FROM THIS FIELD OF RESEARCH HAVE MANY IMPLICATIONS WHICH INCLUDE A NOVEL WAY OF TARGETING SPECIFIC CANCER CELLS. THIS ARTICLE GIVES A DETAILED BACKGROUND OF THE ABOVEMENTIONED VIRUSES, THE MECHANISMS BY WHICH THEY OPERATE, AND HOW THEY HAVE EMERGED AS PRIME CANDIDATES FOR USE IN VIROTHERAPY.*

**V**irotherapy is a recent strategy that could potentially be used to cure cancer by infecting tumor cells with a virus. Modern virotherapy is now being tested in humans after showing promising results when tested in mice. This paper explores the use of the herpes simplex virus (HSV), and its potential as a cure for breast cancer. However, it is not the only virus undergoing research. Another commonly known virus, the adenovirus, is being explored for its use in the killing of cancerous cells by inducing apoptosis or by the remission of genes that increase cancerous cells' susceptibility to chemotherapy. This paper will discuss two strategies: using viruses to express therapeutic genes to replace ineffective or absent genes, and the other, involving the use of oncolytic viruses to replicate only in tumor cells, and destroy them. The

workings and uses of the adenovirus and the HSV as vectors for gene therapy and oncolytic viral therapy in cancer patients will also be explored.

## HSV

HSVs are widespread amongst different species, and are host-adapted pathogens. There are two types of HSV: HSV-1 and HSV-2 (Dupont et al., 2008). These two viruses are identical in terms of physical properties; however, they differ in epidemiological characteristics. The HSV virion itself contains a linear double-stranded DNA genome, found inside a protein shell. This shell, also known as the HSV nucleocapsid, is surrounded by a layer of viral protein called the tegument. This layer is

## Genome size of potential virus vectors for the nervous system

Adeno-associated virus	8,500	base pairs
Adenovirus	35,000	base pairs
Herpes simplex virus	150,000	base pairs
Lentivirus	10,000	base pairs

**Table 1:** This table compares the size of the HSV to other common viruses in terms of base pairs (Latchman, 2001).

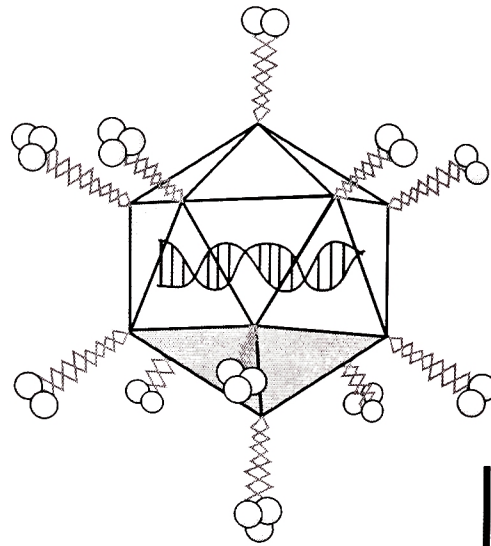
enclosed within a lipoprotein envelope containing glycol proteins, which are responsible for the attachment of the HSV to host cells. The most important biological characteristic of HSVs is their ability to permanently infect a host. Despite the host's immune system, the virus can be reactivated periodically, either spontaneously or by stimuli (Dupont et al., 2008).

*HSV as a Vector*

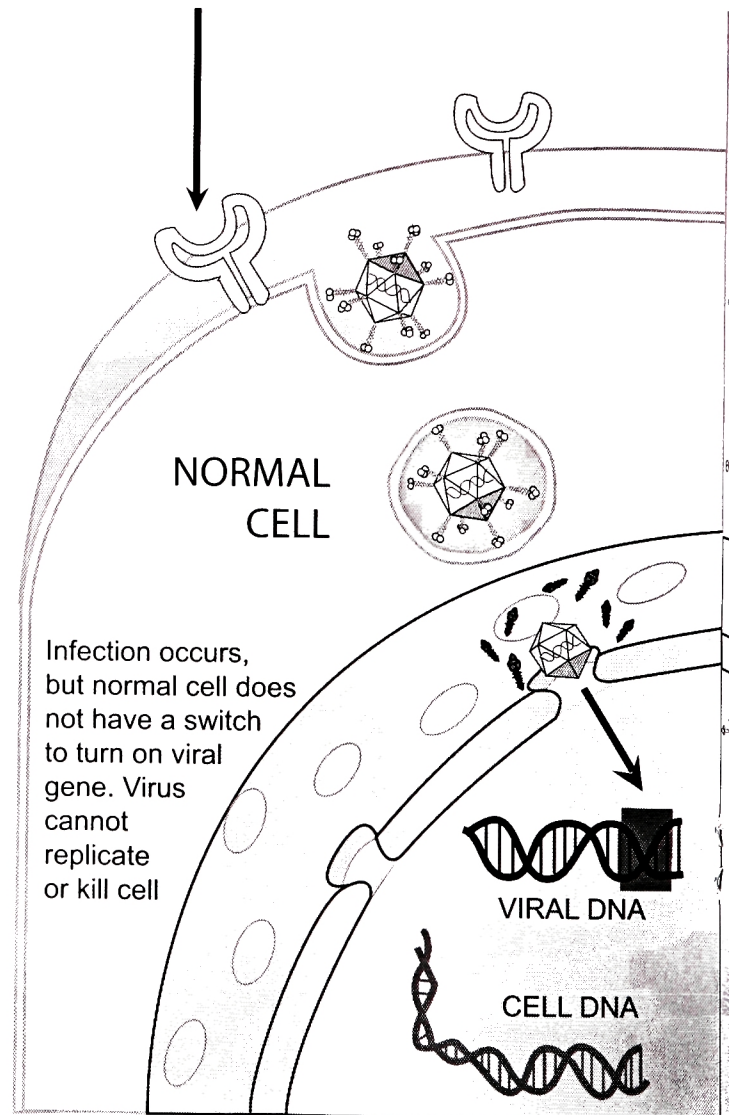
HSV-1 is one of the viruses most studied as a potential gene therapy vector (Latchman, 2001). Its genome consists of 152 kilobase pairs of linear DNA and 84 conjugate genes (Beutler et al., 2006). A useful property of HSV is that almost half of the virus' genome can be deleted, yet it can still replicate with efficiency and carry out all necessary processes (Beutler et al., 2006). However, if the HSV needs to be engineered with an amplicon (a small segment of DNA), more than 50% of the virus needs to be left intact. The HSV can either encode the therapeutic gene into its genome, or it can become part of an amplicon delivery system.

In the amplicon delivery system, the therapeutic gene is cloned into a eukaryotic plasmid that contains an HSV origin and a packaging signal (Beutler et al., 2006). This process allows the plasmid to replicate as the HSV reproduces with a HSV helper vector (Beutler et al., 2006). Moreover, the employment of the plasmid vector simplifies the manufacturing process and reduces the severity of the effects that live HSV could have (Beutler et al., 2006).

**VIROTHERAPY WITH TRANSCRIPTIONAL TARGETING**

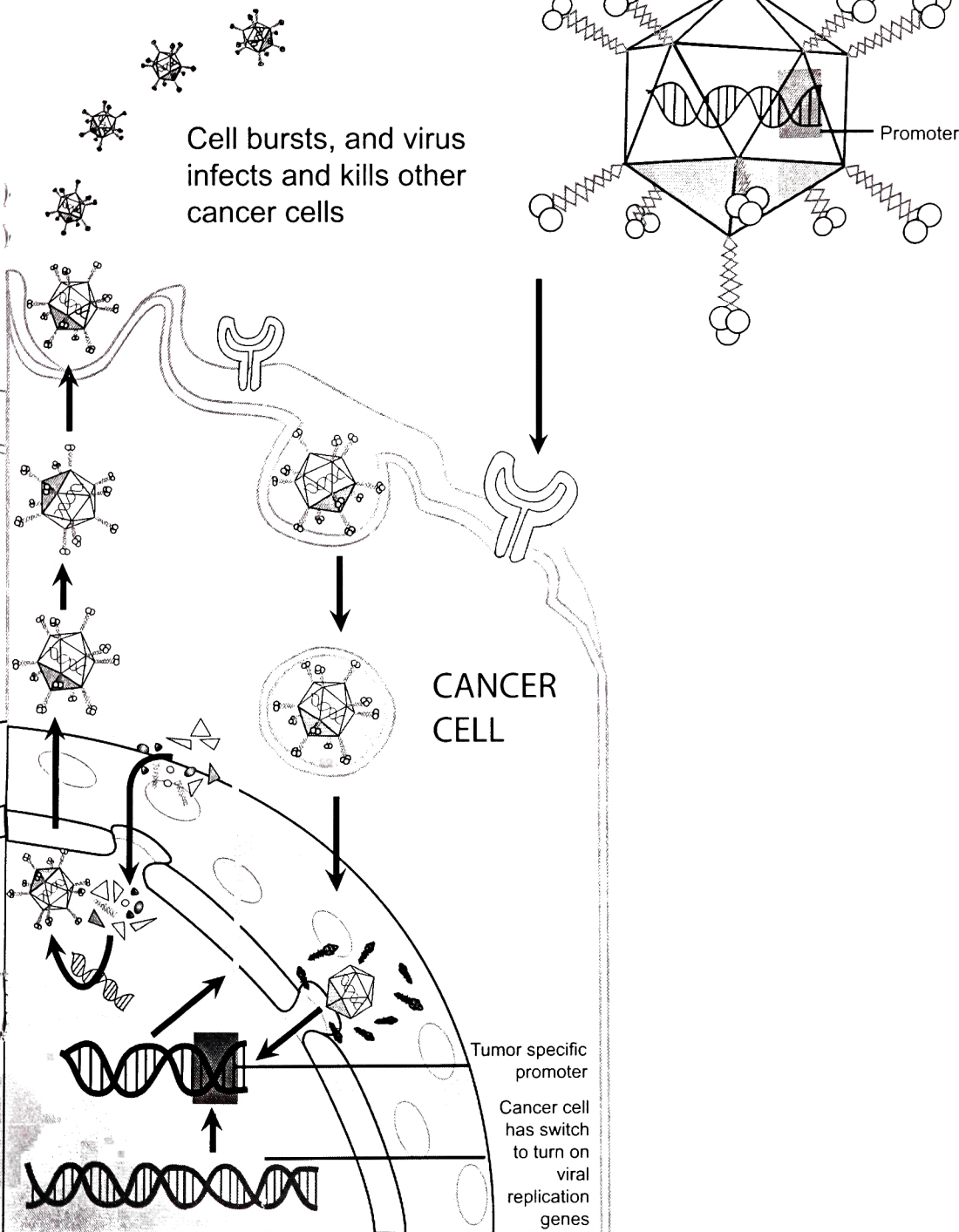


Engineered adenovirus with tumor-specific promoter links to essential virus gene



This image describes transcriptional targeting. A tumor-specific gene is juxtaposed beside one of the essential virus genes. In the above illustration by a darkly shaded area on the viral DNA.

# TRANSCRIPTIONAL TARGETING



In this process the snippet of the promoter-virus' essential genes. This is shown in the virus' DNA (Curiel & Nettleback, 2003).

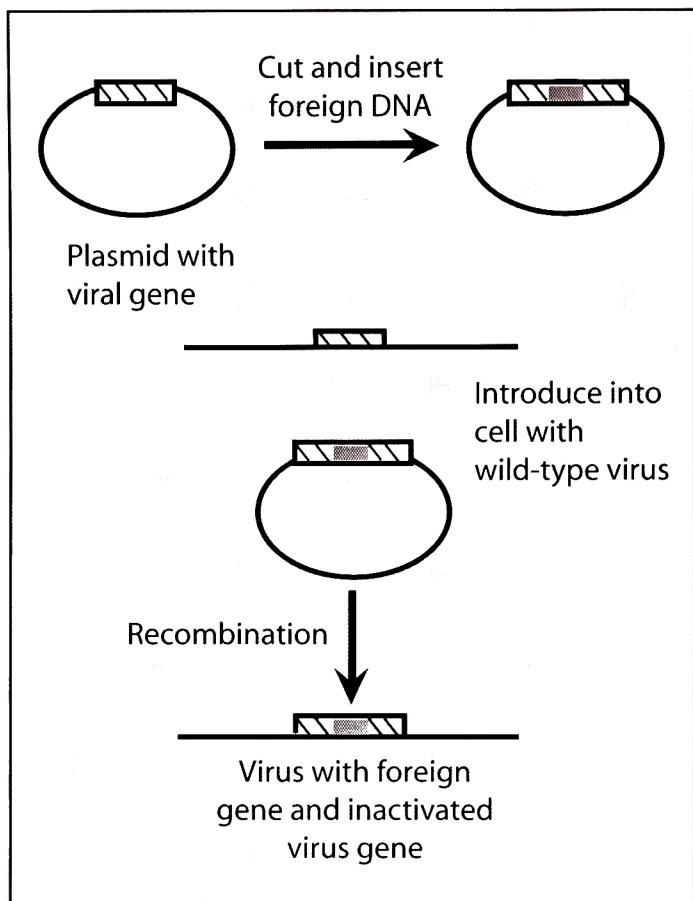
The second method [Figure 1], involves inserting the therapeutic gene directly into the HSV genome, which can be accomplished by cloning the gene into the plasmid vector using a particular technique that allows the gene to be 'flanked' by specific HSV viral sequences (Latchman, 2001). Further, when the plasmid with the gene is transfected into cells along with HSV DNA, recombination occurs between the viral sequences in the plasmid vector and the corresponding sequences in the virus genome (Latchman, 2001). This results in the introduction of the new gene into the HSV genome.

As mentioned earlier, one of the greatest advantages of using HSV as a vector is its large genome compared to other viruses being researched for gene therapy, and the large number of unessential genes. These genes, once removed, make room for therapeutic genes.

Furthermore, such a characteristic is particularly important because most viruses cannot package large genes (Latchman, 2001). However, HSV can accommodate 30 to 40 kilobase pairs of genetic material after removing its own genetic material, especially since the removal of its own genetic material does not affect the growth of the virus (Latchman, 2001). HSV will be useful in the future as many cancer therapies require large genes (Latchman, 2001).

Human breast cancer cells are particularly sensitive to HSV-1 cytotoxicity (Liu et

**Figure 1:** Introduction of foreign DNA into the herpes simplex virus (HSV)-1 genome by recombination (Latchman, 2001).



al., 2001). The HSV oncolytic vectors are suitable for breast cancer tumor therapy because the mutant viruses replicate in the cells with uncontrollable cell division only, and not in the normal cell tissue (Liu et al., 2001). For example, cancer killing vectors like G207 are created by inserting foreign genes and mutations into the virion's original genome (Harvard Medical School, 2005). The G207 vector is also based on the removal of the genes that allow it to withhold replication in non-dividing cells. Since these genes are not essential for replication in rapidly dividing cells, the virus replicates only in cancer cells. The G207 vector, originally meant for brain tumors, has proved useful in the treatment of multiple human tumors including breast cancer (Liu et al., 2001). Further research is looking into the use of the G207 HSV vectors to remove breast cancer tumor cells from bone marrow (Liu et al., 2001). Moreover, the G207 vector is very sensitive to antiviral drugs like acyclovir and gangcyclovir, which means that if the vector were to replicate uncontrollably, it could easily be controlled with the help of these drugs (Liu et al., 2001). This feature, along with the ability of HSV to replicate only in tumor cells, adds to the safety of this treatment option.

## ADENOVIRUS

The adenovirus, a cause for the common cold, is under intense research and has been deemed useful in the development of virotherapy for the treatment of malignant tumors (Curiel & Nettleback, 2003). Further, the adenovirus is particularly helpful because it has been studied for years by scientists trying to find a cure for the common cold. A setback that researchers face is that many people have been exposed to adenoviruses and carry the antibodies against them. Therefore, injecting shots of the adenovirus as cancer therapy can cause severe flu-like symptoms prompting the body to annihilate the dose given, thereby inhibiting the therapy (Curiel & Nettleback, 2003). Nonetheless, the body's response to the virus ensures that its replication remains controlled (Curiel & Nettleback, 2003).

### *Adenovirus as a vector*

The adenovirus is comprised of a 20-sided protein case or capsid, containing DNA and 12-protein arms. The adenovirus is different from retroviruses used in gene therapy since it does not integrate its DNA into infected cells. To ensure the accuracy of adenoviruses, researchers are working on two mechanisms: transductional targeting and transcriptional targeting (Curiel & Nettleback, 2003).

Transductional targeting incorporates the ability of the virus to identify and enter tumor cells. This is an important strategy as the adenovirus generally penetrates normal tissue cells instead of tumor cells. This can be changed by attaching special antibodies onto the arms of the virus like a socket wrench and these antibodies attach to only tumor-specific proteins. Once attached, the virus is engulfed into the cell, where the viral capsid enters the nucleus through a pore and injects its own DNA (Curiel & Nettleback, 2003). Once copies of the viral DNA have been made, proteins are synthesized to form many more adenoviruses. The virus then causes the cell to burst, allowing all the adenoviruses to leak out (Curiel & Nettleback, 2003).

Transcriptional targeting involves placing a snippet of DNA called a tumor-specific promoter next to one of the adenovirus' essential genes (Curiel & Nettleback, 2003). This promoter acts as an 'on' switch that permits the genes to function only in cancer cells. These engineered viruses can enter any cell in the body, but they only reproduce in cancer cells (Curiel & Nettleback, 2003). However, when

they enter a tumor cell, the tumor-specific promoter allows them to be copied and thousands of copies result as the new virions break out of the tumor cells and spread (Curiel & Nettleback, 2003).

The adenovirus infects cells and then induces them to enter the 'S' phase of the cell cycle (Bischoff et al., 1996). The human gene E1B codes for a protein that inactivates the tumor-suppressing cellular protein p53 (Bischoff et al., 1996). The E1B-deleted adenovirus replicates only in p53 deficient cells (Bischoff et al., 1996). Injections of the E1B-deleted adenovirus significantly reduced the size of the tumors in mice being tested (Bischoff et al., 1996). Further, in mice co-infected with adenovirus and HSV, improved results were observed in the treatment of colon cancer (Blaese, Morris & Wildner., 1999). The rationale behind this treatment is that the adenovirus comprises the suicide genes while HSV provides a safety mechanism that will abort the infection if uncontrolled growth of the virus occurs (Blaese et al., 1999).

## CONCLUSION

Individuals previously infected with adenovirus or HSV will have acquired an immunological response to either virus. This leaves very little room for researchers to develop a vaccine that uses these viruses, as they cannot predict how the patient's immune system will react. More research is required before utilizing it as a cure. Thus far, this technology has been tested on mice, while other animal models have been created. Phase I and II human trials have also been conducted. Progress has been made in these models, tumor regression has been observed, and translation of the finalized treatment to humans may be expected in the near future. **M**

## REFERENCES

- Curiel, D. Nettleback, D. (October 2003). Tumor-Busting. *Scientific American*, 68-75
- Dupont, Herbert, Humes, Gardner, Laurence et al. (Ed.). (2008). *Kelley's Textbook of Internal Medicine*. Philadelphia, PA: Lippincott Williams and Wilkins.
- Beutler, Ernest, Kaushansky, Kenneth, Kipps et al. (2006). *Williams Hematology*. United States of America: McGraw Hill.
- Latchman, D (2001). *Gene Therapy with HSV Vectors: Progress and Prospects for Neuroscience*. *The Neuroscientist*. 7, 528-537.
- Liu X., Martuza R., Mazumder A., Meehan K., Rabkin S., et al. (2001). Biological Purging of Breast Cancer Cells Using an Attenuated Replication-competent HSV in Human Hematopoietic Stem Cell Transplantation. *Cancer Research*. 61, 3009-3015.
- Bischoff J. R., Kirn D. H., Williams A., Heise C., Horn S., et al. (1996). An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*, 274,373-376.
- Blaese, M. Morris, J. Wildner, O. (1999). Therapy of Colon Cancer with Oncolytic Adenovirus Is Enhanced by the Addition of HSV-thymidine kinase. *Cancer Research*. 59, 410-413.
- Molecular Neurosurgery Laboratory - Gene Therapy. (2005). Retrieved January 28, 2007, from Harvard Medical School: Richard B. Simches Research Centre Web site: <http://btrc.mgh.harvard.edu/MNL.htm>