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Viral Vectors for Gene Therapy: A Review

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ABSTRACT

Gene therapy provides modern medicine with new perspectives that were unimaginable two decades ago. Advancement in molecular biology and, especially, molecular medicine is now changing the basics of clinical medicine. Reports of long-term side effects of the first successful human gene therapy study have slowed the penetration of DNA usage into clinical routine. However, the main safety problem lies in the secure and efficient delivery of genes into target cells and tissues. A number of older and more recently discovered techniques have been developed for therapeutic gene transfer. A variety of viral and non-viral possibilities are available for basic and clinical research. In viral vectors, virus particles are used to carry nucleic acids into cells[1]. The commonly used Viral Vectors are Adenovirus, Adeno-associated virus, Retrovirus, Lentivirus and Herpes simplex virus. The diverse nature of different vectors and the variability of different diseases mean that there will almost certainly be no "one size fits all" vector. Compared to naked DNA, virus particles provide a relatively efficient means of delivering nucleic acids into cells, for expression as recombinant genes. This review includes structure, function, advantages and disadvantages of various viral vectors for gene delivery.

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INTRODUCTION

Gene therapy offers new treatment possibilities for many common acquired and inherited human diseases where conventional clinical procedures are less effective. These diseases include monogenic disorders, such as cystic fibrosis, but also more complex disorders, such as cardiovascular diseases, diseases of nervous system, autoimmune diseases, and cancer [1].

Viruses have complex structures and life cycles and many are pathogens, but several viruses are highly efficient gene delivery vehicles. Non-viral vectors generally cannot approach the efficiency of viral vectors when considered on the basis of gene copies that must be presented to the target cell in order that one or several copies can be expressed in the cell nucleus. For viral vectors, the usual approach is to remove the unneeded or pathogenic features while retaining the efficiency of gene delivery, expression and persistence where appropriate. The goal of non-viral vector design is usually to add features to DNA to mimic those functions which viruses already perform well. The design of viral vectors addresses many common considerations for both safety and biological activity including removal of virulence genes, absence of replication competent parental virus and host responses to components of the vector. Also, particular considerations for individual classes of vectors reflect the properties of the parental virus, the target organ or disease, and the gene to be delivered. Retrovectors integrate efficiently, so insertional mutagenesis was considered an important issue [28].

Converting a Virus into a Vector

The viral life cycle can be divided into two temporally distinct phases: infection and replication. Infection results in the introduction of the viral genome into the cell. This leads to an early phase of gene expression characterized by the appearance of viral regulatory products, followed by a late phase, when structural genes are expressed and assembly of new viral particles occurs. In the case of gene therapy vectors, the viral particles encapsulate a modified genome carrying a therapeutic gene cassette in place of the viral genome. Transduction is defined as the abortive (non-replicative or dead-end) infection that introduces functional genetic information expressed from the recombinant vectors into the target cell. The viral genome comprises both genes and *cis*-acting gene regulatory sequences. Although some overlap exists, most *cis*-acting sequences map outside of the viral coding sequences. This spatial segregation of genes and *cis*-acting sequences along the viral genome is exploited in the design of recombinant viral vectors. To generate a vector, coding genes and *cis*-acting sequences are separated into distinct nucleic acid molecules to prevent their reconstitution by recombination into productive viral particles [2].

In the case of genetic diseases caused by a mutation in a specific gene or its product, gene therapy usually involves the delivery of a functional copy of this gene into a target cell or tissue to achieve a therapeutic benefit [3]. However, it can also be a tool for the treatment of non-genetic and polygenic disorders by delivering genes that stimulate immune response,

suicidal genes inducing cell death, genes modifying cellular information or developmental program, or genes producing a therapeutic protein with specific functions [4]. Recent sobering reports on the long-term outcome of the first successful clinical gene therapy study have renewed the discussion about the safety of DNA use in the treatment of diseases [5]. Therefore, current studies are aimed at developing effective and safe techniques and vectors to deliver genes into tissue as well as to improve the regulation of transgene expression.

The main aim of gene therapy is to correct a genetic defect by transferring of a functional normal copy of the gene into cells. Gene therapy applies to:

– Genetic disorder (deficiency)
– Cancer
• Genetic predisposition
• Mutation in oncogene or tumor suppressor gene
– Autoimmunity diseases: rheumatoid arthritis
• Delivery of counteracting gene
– Viral infections
• Delivery of DNA molecules that will produce RNA or proteins against the virus

The following are rationale for viral vector use. They are

• Virus is obligatory intracellular parasites
• Very efficient at transferring viral DNA into host cells
• Target specific cells: depending on the viral attachment proteins (capsid or glycoproteins)
• Gene replacement: non-essential genes of virus are deleted and exogenous genes are inserted.

The commonly used Viral Vectors are:

1.Adenovirus
2. Adeno-associated virus (AAV)
3.Retrovirus
4. Lentivirus
5. Herpes simplex virus (HSV).

Adenoviruses

Adenovirus (Ad) was first discovered in 1953 in human adipose tissue [6]. This virus has since been classified into six species (A–F) that infect humans, and these species are subdivided into over 50 infective serotypes [7]. From the variety of known Ads, researchers have concluded that viruses Ad2 and Ad5 of species C are the most effective for creating viral vectors for use in gene therapy [8]. Ad vectors, now one of the most widely studied vector

forms, are prominently used in worldwide clinical trials. As of March 2011, 402 of the total 1,703 gene-therapy clinical trials included studies with Ad vectors [9].

Structure

The Capsid

The Ad capsid is a nonenveloped, icosahedral protein shell (70–100 nm in diameter) that surrounds the inner DNA-containing core. The capsid comprises 12 identical copies of the trimeric hexon protein [10]. An entameric penton base protein is located at each vertex of the capsid, and from it extends a trimeric fiber protein that terminates in a globular knob domain [11].

The Genome

The genome of the Ad is a linear, double-stranded DNA (dsDNA) ranging from 26 to 40 kb in length [12]. This linear form is organized into a compact, nucleosome-like structure within the viral capsid and is known to have inverted terminal repeat (ITR) sequences (103 base pairs in length) on each end of the strand [11]. The viral genome comprises two major transcription regions, termed the early region and the late region [13, 14]. The early region of the genome contains four important transcription units (E1, E2, E3, and E4). Table 1 outlines the functions of each unit of the early region. Table 2 describes advantages and disadvantages of adenoviral vectors.

Table 1: Early transcription units and their functions (Ad virus)

Transcription unit	Function
E1A	Activates early-phase transcription and induces the S phase of the host cell
E1B	Codes for E1B 19K and E1B 55K, which inhibit apoptosis and allow for viral replication
E2	Codes for DNA polymerase (pol), preterminal protein (pTP), and DNA-binding protein (DBP)
E3	Codes for proteins that block natural cellular responses to viral infection
E4	Codes for a variety of proteins that perform in DNA replication, mRNA transport, and splicing

Table 2: Advantages and disadvantages of adenoviral vectors

Advantages	Disadvantages
Ability to infect both dividing and quiescent cells	Long-term correction not allowed
Stability of recombinant vectors Humoral and cellular immune	Humoral and cellular immune response from high vector doses
Large insert capacity	
Nononcogenic	
Can be produced at high titers	

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Adeno-Associated Virus (AAV)

AAV originates from the *Dependovirus* genus of the *Parvovirus* family and was first discovered in 1965 as a coinfecting agent of the Ad [15]. This small virus is naturally replication-defective and requires the assistance of either a helper virus, such as the Ad or the herpes virus, or some form of genotoxic stress to replicate within a host cell nucleus [16].

Structure

The Capsid

The AAV capsid is a nonenveloped, icosahedral protein shell, 22 nm in diameter [17]. Each serotype of this virus has its own characteristic capsid with a special affinity for certain host cell receptors, allowing it to be used to target a variety of tissue types [18, 19].

The Genome

The genome of AAV is composed of a linear, single-stranded DNA with two open reading frames flanked on each end by a 145-bp ITR sequence [17-19]. The 5¢ open reading frame contains nucleotides that code for four important replication proteins, Rep 78, Rep 68, Rep 52, and Rep 40. The 3¢ open reading frame codes for three capsid proteins, VP1, VP2, and VP3. Table 3 outlines the functions of each of these proteins. Table 4 describes advantages and disadvantages of Adeno-Associated Virus vectors.

Table 3: Functions of Rep and Cap proteins (AAV)

Protein	Function
Rep 40 Rep 52	Participate in the generation and accumulation of single-stranded viral genome from the double-stranded replicative intermediates
Rep 68 Rep 78	Interact with Rep-binding elements and ITRterminal resolution sites to assist in the DNA replication process
Cap (vp1, vp2, vp3)	All share the same V3 regions but have different N-termini – used to form the capsid structure in a ratio of 1:1:10

Table 4:Advantages and disadvantages of AAV vectors [20]

Advantages	Disadvantages
Nonpathogenic	Smaller size limits the amount of foreign genes that can be inserted
Broad host and cell type tropism range	Slow onset of gene expression
Transduce both dividing and non dividing cells	
Maintain high levels of gene expression over a long period of time (years) in vivo	

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Retroviruses

Retroviruses are known for their ability to reverse the transcription of their single-stranded RNA genome, thus creating dsDNA to replicate after infecting host cells. These viruses are most generally categorized as either simple (oncogenic retroviruses) or complex (lentiviruses and spumaviruses) [21]. This section discusses the simple oncogenic retroviruses – most commonly the murine leukemia virus – before discussing the complex retroviruses in the lentivirus section. The oncogenic retroviruses are limited by their inability to infect non-dividing cells; however, they are considered extremely useful for tissue engineering studies, particularly those concerning bone repair.

Structure

The Capsid

The retroviral capsid is an enveloped protein shell that is 80–100 nm in diameter and contains the viral genome [22]. The envelope structure surrounding the capsid is actually a lipid bilayer that originates from the host cell and contains both virus-encoded surface glycoproteins and trans membrane glycoproteins [23]. The basic retroviral structure is similar to lentiviruses (HIV-1).

The Genome

The genome of the retrovirus is a linear, nonsegmented, singlestranded RNA, 7–12 kb in length [21]. The simple class of retroviruses contains three major coding segments and one small coding domain. The major segments contain three genes – gag, pol, and env – which code for proteins important in viral integration, replication, and encapsulation [22]. The small coding domain contains the pro gene, which encodes for viral protease [23]. A more detailed description of the coding segments and their protein products may be found in Table 5.

Table 5: Functions of retroviral genes

Protein	Function
gag	Codes for the viral core
pol	Codes for reverse transcriptase and integrase
env	components of the viral envelope proteins
pro (small coding domain)	Encodes a viral protease

Retroviral vectors are widely used in studies of tissue repair and engineering. Because these vectors can be used to infect dividing cells without producing any immunogenic viral proteins while also becoming a permanent part of the host cell genome, they have proven to be an extremely useful tool in gene-therapy research. These vectors are limited only by their relatively small carrying capacity and their inability to infect nondividing cells; however, these disadvantages have not kept them from being the most widely used vectors in the research of gene and cell therapy [24].

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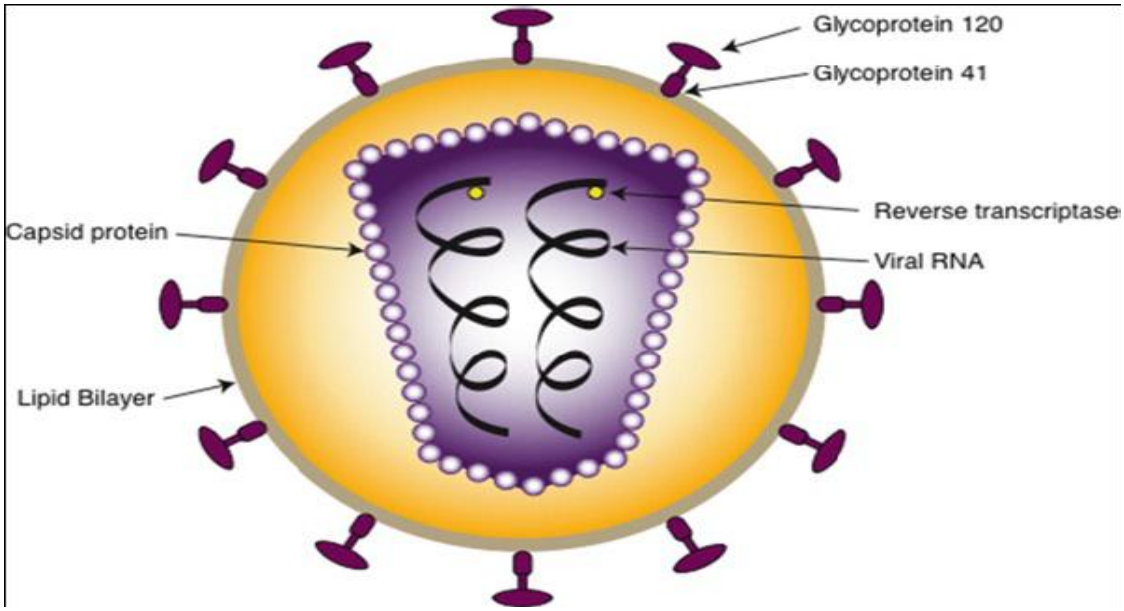

Fig 1: Schematic diagram of the basic physical structure of a retrovirus (shown here is the structure of the HIV-1 lentivirus).

Lentiviruses

Lentiviruses, a subcategory of the retrovirus family, are known as complex retroviruses based on the details of the viral genome. The most common example of a lentivirus is the human immunodeficiency virus type 1 (HIV-1).

Structure

The lentiviral capsid is the same as that of the simple retroviruses. The lentiviral genome, like that of other retroviruses, contains a single-stranded RNA, 7–12 kb in length [23].

Table 6 shows Genes expressed in HIV-1 lentivirus.

Table 6: Genes expressed in HIV-1 lentivirus.

Protein	Function
Rev	An RNA-binding protein that acts to induce the transition from the early to the late phase of HIV gene expression
Tat	An RNA-binding protein that enhances transcription 1,000-fold
Nef	Disturbs T-cell activation and stimulates HIV infectivity
Vpr	Mediates HIV to infect nondividing cells
Vpu	Enhances the release of HIV-1 from the cell surface to the cytoplasm
Vif	A polypeptide necessary for the replication of HIV-1

These genes are nonessential and absent in lentiviral vectors. The rev gene along with the simple genes gag, pol, and env are expressed on plasmids that are present in packaging cells.

Herpes Simplex Virus

Actually, many different varieties of the HSV have been discovered. The most common of these, known as HSV-1, is well known by the average person as the viral cause for cold sores. One of the most intriguing aspects of this virus is its ability to infect a host and then remain latent for a period before reappearing again [25]. Research on this virus continues in hopes that its unique characteristics will lead to a breakthrough in gene therapy.

Structure

The Capsid

The HSV has an icosohedral protein shell that is covered by a viral envelope. Embedded within the envelope are a variety of glycoproteins important for the viral attachment to host cellular receptors. Tegument is a layer of proteins and enzymes coded for by the viral genome that lies between the capsid core and the viral envelope [25].

The Genome

The HSV genome consists of a dsDNA (152 kb in length) that code for up to 90 different proteins important for viral attachment and replication[26]. This genome is further organized into what are known as unique long and unique short segments, and these segments are capped on each end by inverted repeat sequences[22]. Table 7 outlines the functions of a, b, and g genes of HSV-1.

Table 7: Functions of a, b, and g genes of HSV-1

Protein class	Function
a	Major transcriptional regulatory proteins – necessary for the synthesis of b and g proteins
b	Include DNA polymerase and transcriptional factors involved in viral replication
g	Primarily serve as structural proteins

The main advantage of the HSV is its ability to remain latent within host cells after infection. This distinctive feature, along with the fact that the virus is naturally neurotropic, allows it to infect neural cells and, therefore, to assist in treating neural diseases. Most of the animal studies performed with HSV have involved either the treatment of brain tumors or Parkinson's disease. In both cases, gene therapy using the herpes simplex viral vector has shown promising results [27].

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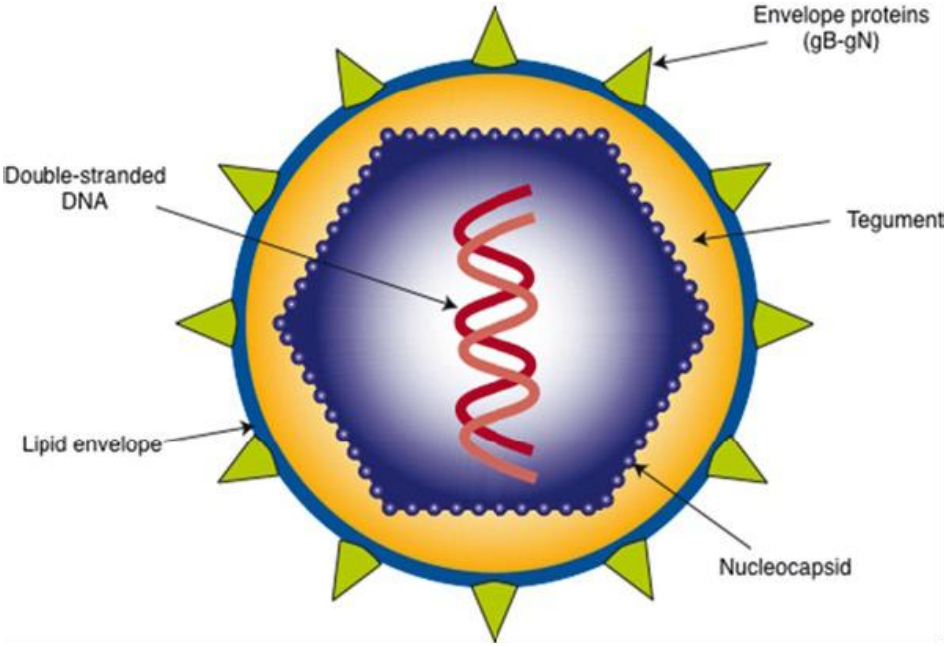

Fig 2: Schematic diagram showing the structure of the herpes simplex virus.

Comparison of different viral vectors

Viral vector	Titers	Insert size	Manipulation of tropism	Immunogenicity
Adenovirus	1011	2-38kb	good	very high
Retrovirus	107	1-7kb	good	low
Lentivirus	107	7-18 kb	good	low
AAV	107	4.5kb	not so good	low
Herpesvirus	107	50kb	not so good	low

CONCLUSION

Adenovirus vectors generate significant host cellular immune responses and require strategies to remove or prevent expression of adenovirus genes or possibly to develop approaches to transient immune modulation. For AAV vectors an important issue has been to develop efficient production systems. Development of viral vectors requires analysis of the biology and life cycle of the parental virus and the structure of the virus and its genome. Genetic analysis is also required to determine which viral elements are required in cis and which genes can be supplied in trans. This allows the design of complementation systems to produce viral vectors which use generic packaging cells to supply the trans complementing functions. Packaging cell lines can then be used as producer cells for the generation of particular vectors. The main issues to be confronted are increasing the titer of vector and decreasing or eliminating the production of replication competent or wild type virus. Purification of vectors is important for safety and product identity and because impurities may severely impact the biology of the vector. Once viral vectors can be produced, then the design rules or limitations for gene cassettes and regulatory elements can be determined [28].

Vectors can be assessed with in vitro experiments but these may not be predictive of their behavior in vivo. Many cells used in vitro culture are transformed, and even primary cells do not necessarily reflect the function of the same cells in vivo. Thus, it is essential to perform extensive in vivo experiments in animals to assess organ and cell targeting, the efficiency, specificity and persistence of gene expression and the likelihood of toxicity and host immune and inflammatory responses [28, 30].

Experiments in animals are also important because the properties of vectors may vary significantly from those that are exhibited by the parental virus. However, there is a paucity of defined, disease-specific animal models. Also, there is no well-established track record for determining which animal species or models are most useful for determining the potential biological efficiency of gene delivery and expression or the likelihood of dose-limiting toxicities.

Gene therapy in patients began with ex vivo approaches by taking advantage of the stable integration of retrovectors to modify rapidly dividing cells (notably lymphocytes) that were then returned to the patient to treat either rare monogenic diseases, e.g., adenosine deaminase deficiency, or a common acquired disease such as cancer. However, in vivo delivery systems have rapidly been adopted as is reflected in the use of adenovirus and AAV vectors. [28]

The ability of viruses to deliver foreign DNA to cells for therapeutic purposes has been exploited in numerous different contexts. The diverse nature of different vectors and the variability of different diseases mean that there will almost certainly be no “one size fits all” vector. Clinical trials have shown that certain vectors have great potential for specific diseases. For example, retroviral vectors have had great success in treating X-SCID, whereas lentiviral vectors have been used to target various neurological diseases, including Parkinson’s and ALD, and other clinical trials have employed AAV vectors to treat monogenic disorders, such as Duchenne muscular dystrophy and hemophilia B. Although no viral vector has yet received clinical approval in Europe or the USA, the encouraging results from clinical trials, coupled with continual improvements in vector design and safety, shows that this technology has immense potential [29, 30].

REFERENCES

[1] Roman Gardlík, Roland Pálffy, Július Hodosy, Jan Lukacs, Jan Turna, Peter Celec. Med Sci Monit 2005; 11(4): RA110-121.
[2] Kay MA, Glorioso JC, Naldini L. Nat Med 2001 Jan; 7(1):33-40.
[3] Orkin SH. Clin Immunol Immunopathol 1986; 40: 151–6.
[4] Rubanyi GM. Mol Aspects Med 2001; 22: 113–42.
[5] Hacein-Bey-Abina S, Von Kalle C, Schmidt M et al. Science 2003; 302: 415–19.
[6] Rowe WP, Huebner RJ, Gilmore LK, Parrott RH and Ward TG. Proc Soc Exp Biol Med 1953; 84: 570–573.

[7] Shenk T. Adenoviridae: The viruses and their replication in Fields Virology (Fields BN, Ed), Lippincott-Raven Publishers, Philadelphia PA 1996; 2111-2148.
[8] Kovesdi I, Brough DE, Bruder JT and Wickham TJ. Curr Opin Biotechnol 1997; 8: 583–589.
[9] http://www.wiley.co.uk/ genetherapy/clinical.
[10] http://www.google.com.om/search?tbo=p&tbm=bks&q=inauthor:%22Thomas+E.+Shenk %22
[11] Douglas JT. Mol Biotech 2007; 36: 71–80.
[12] Campos SK and Barry MA. Curr Gene Ther 2007; 7: 189–204.
[13] Boyer J and Ketner G. Adenovirus Late Gene Expression, in Aednovirues: Basic Biology to Gene Therapy (Seth P Ed.) RG Landes Bioscience Austin TX 1999; 69–77.
[14] Rux JJ and Burnett R. In Adenoviruses: Basic Biology to Gene Therapy, Seth P, Ed) Medical Intelligence 15. RG Landes Bioscience Austin TX 1999; 5-15.
[15] Goncalves MA. Virol J 2005; 2: 43.
[16] Büning H, Braun-Falco M and Hallek M. Cells Tissues Organs 2004; 177: 139–150.
[17] Ding W, Zhang L, Yan Z and Engelhardt JF. Gene Ther 2005; 12: 873–880.
[18] Mc Carty DM, Fu H, Monahan PE, Toulson CE, Naik P and Samulski RJ. Gene Ther 2003; 10: 2112–2118.
[19] Kotin RM, Menninger JC, Ward DC and Berns KI. Genomics 1991; 10: 831–834.
[20] Coura Rdos S and Nardi NB. Virol J 2007; 4: 99.
[21] http://www.ncbi.nlm.nih.gov/books/NBK19383/
[22] Osten P, Grinevich V and Cetin A. Handbook Exp Pharmacol 2007; 177–202.
[23] Zhang X and Godbey WT. Adv Drug Deliv Rev 2006; 58: 515–534.
[24] Robbins PD, Tahara H and Ghivizzani SC. Trends Biotechnol 1998; 16: 35–40.
[25] http://www.ncbi.nlm.nih.gov/books/NBK7934/#A3304.
[26] Kufe DW, Frei III E, Holland JF, Weichselbaum RR, Pollock RE, Bast RC et al. Cancer Medicine, BC Decker, Columbia 2006; 7: 220-227.
[27] Casper D, Engstrom SJ, Mirchandani GR, Pidel A, Palencia D, Cho PH et al. Cell Transplant 2002; 11: 331–349.
[28] http://www.freewebs.com/viralman/v05a_vir.pdf
[29] Warnock JN, Daigre C, Al-Rubeai M. Methods Mol Biol 2011; 737: 1-25.
[30] http://www.gch.ulaval.ca/agarnier/GCH2100/Cours_2/Warnock%20et%20al%202011.pdf.