

International Journal of Pharmaceutical Sciences and Drug Research

2016; 8(3): 128-133



Review Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

The Use of Viral Vectors in Gene Transfer Therapy

A. Dziaková, A. Valenčáková*, E. Hatalová, J. Kalinová

University of Veterinary Medicine and Pharmacy, Komenského 73, 04181 Košice, Slovak Republic

ABSTRACT

Gene therapy is strategy based on using genes as pharmaceuticals. Gene therapy is a treatment that involves altering the genes inside body's cells to stop disease. Genes contain DNA- the code controlling body form and function. Genes that do not work properly can cause disease. Gene therapy replaces a faulty gene or adds a new gene in an attempt to cure disease or improve the ability of the body to fight disease. Gene therapy holds promise for treating a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS. Various types of genetic material are used in gene therapy; double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), plasmid DNA and antisense oligodeoxynucleotides (ASON). The success of gene therapy depends on assuring the entrance of the therapeutic gene to targeted cells without any form of biodegradation. Commonly used vectors in gene therapy are: adenoviruses (400 clinical studies; 23.8%), retroviruses (344 clinical studies; 20.5%), unenveloped/plasmid DNA (304 clinical studies, 17.7%), adeno-associated viruses (75 clinical studies; 4.5%) and others. In this paper, we have reviewed the major gene delivery vectors and recent improvements made in their design meant to overcome the issues that commonly arise with the use of gene therapy vectors.

Keywords: *Adenoviruses, Bacteriophages, gene therapy, Retroviruses, viral vector.*

INTRODUCTION

Gene therapy is a strategy based on using genes as pharmaceuticals. It can be used effectively for curing a vast spectrum of serious acquired or congenital diseases [1], such as cancerous diseases [2-3], Acquired [4], cardiovascular diseases [5], infectious diseases, cystic muscular dystrophy [6], and X-linked severe immunodeficiency (X-linked SCID). [7] Gene therapy is a simple method based on principles of transitioning a damaged gene for

a healthy one, or by supplementing a missing gene, so expression of a required protein can be achieved. [8] Nevertheless, in practice it is a complex procedure, in which the transported gene (transgene) must overcome several obstacles for it to reach targeted nuclei of human cells, where the expression should finish accurately.

European medicine agency (EMA) defines a medicinal product in gene therapy as a biological-medicinal product meeting following descriptions: (a) it consisting of an active substance containing or composed of recombinant deoxyribonucleic acid used or to be used in human beings with respect for regulation, correction, shift, completion or excision of gene sequence; its therapeutic, prophylactic or diagnostic effect is directly related to the sequence of recombinant deoxyribonucleic acid which it comprises of, or the gene expression product of this sequence.

***Corresponding author: Prof. DVM, A. Valenčáková,**
University of Veterinary Medicine and Pharmacy;
Department of Biology, Zoology and Radiobiology;
Komenského 73, 04181 Košice, Slovak Republic;

E-mail: alexandra.valencakova@uvlf.sk

Received: 28 March, 2016; **Accepted:** 18 April, 2016

Pharmaceutics in gene therapy do not include vaccines against infectious diseases.

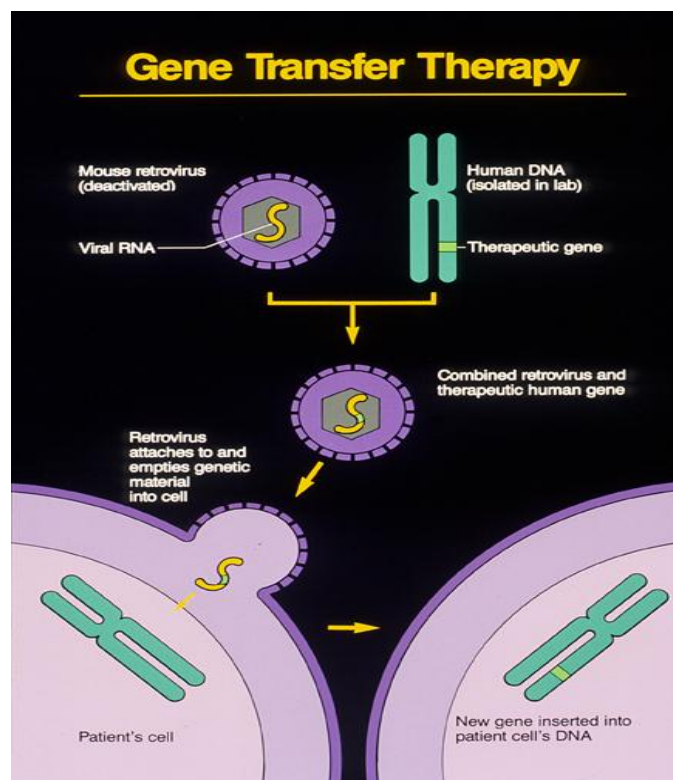


Fig. 1: The principles of gene therapy ^[9]

Food and Drug Administration (FDA) defines vectors in gene therapy as agents mediating their effects by transcription and/or translation of transmitted genetic material and/or integration to host genome, and are administered as nucleic acids, viruses, or genetically modified microorganisms. Products can be used for cell reparation *in vivo*, or can be carried to cells *ex vivo* before delivery to the recipient. ^[10]

Gene therapy is one of the fastest developing fields in medicine. ^[11] To this day, more than 1 800 approved clinical studies of gene therapy have been or are being published. The most common gene therapy is gene therapy of cancerous diseases. Worldwide, cancerous diseases represent over 60% of clinical studies concerning gene therapy, followed by monogenous and cardiovascular diseases. ^[10]

CLASSIFICATION OF GENE THERAPY

In general, gene therapy can be used as germline or as somatic gene therapy. Gene therapy of germlines depends on inserting a functional gene to germinative cells (reproductive cells) such as sperms or zygotes. This gene will be integrated to individual genomes, which will cause hereditary modification in the genetic makeup of the patient. Therapeutic genes in somatic gene therapy carried to somatic cells of the patient (non-reproductive cells) will manifest only in one generation, therefore changes and effects are limited to an individual. The results in a patient's gene characteristic are not hereditary. ^[12] The difference between these two methods is, that in somatic gene

therapy, the genetic material is inserted to certain targeted cells, where the change is not passed on to next generations, meanwhile in the gene therapy of germlines, the therapeutic or modified gene passes on to the next generation. This difference is important, because current legal regulations allow only gene therapy on somatic cells. ^[10]

GENE TRANSFER THERAPY

Gene therapy vectors

Gene therapy is a recently discovered technique for curing serious disorders (acquired or congenital) by repairing the genetic causes of these disorders, either by replacement of deformed genes by healthy ones or by supplementing missing genes. ^[13] Various types of genetic material are used in gene therapy; double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), plasmid DNA and antisense oligodeoxynucleotides (ASON). ^[14] The success of gene therapy depends on assuring the entrance of the therapeutic gene to targeted cells without any form of biodegradation. ^[15] Passive penetration through cellular membrane is prevented by the size of the DNA molecule ^[16], the sensitivity of DNA to nucleases in a biological medium and the hydrophilous polyanionic character of the DNA macromolecule. ^[15] For these reasons, the DNA must be attached to a distribution system or a vector carrying the therapeutic gene to targeted cells, protecting it from degradation caused by nucleases and ensuring transcription in the cells.

Ideal genome transfer system, called "vector", should satisfy several criteria: ^[17]

1. It cannot invoke a strong immune reaction,
2. It must be capable of transporting nucleic acids regardless of their size,
3. It must ensure permanent and regular expression of the genetic material,
4. The vector must deliver the gene only to certain types of cells, especially when targeted cells are localized in various places in the organism, or when they are a part of heterogeneous population,
5. It must be capable of penetrating into dividing and non-dividing cells,
6. It needs to be easily prepared, be low priced and available for vast usage in high concentrations,
7. It must maintain episomal position, or integrate itself to a specific location in the genome, but not integrate randomly.

Vectors for therapeutic sequence transfer to targeted cells can be divided to three basic groups: viral, non-viral (unenveloped DNA) and bacterial. Each of these groups has its own research, therapeutic indications, characteristics, pros and cons. ^[1, 18] Commonly used vectors in gene therapy are: adenoviruses (400 clinical studies; 23.8%), retroviruses (344 clinical studies; 20.5%), unenveloped/plasmid DNA (304 clinical studies, 17.7%), adeno-associated viruses (75 clinical studies; 4.5%) and others. ^[19]

VIRAL VECTORS

A virus is a biological object capable of penetration to the host cell nucleus and using the cellular mechanism for the replication and expression of its own genetic material, and subsequent spread to other cells. [20] Viruses were the first vectors used for distribution and protection of therapeutic genes, where their life cycle can be used as an advantage. [21] This type of a vector, known as "viral vector", is one of the most commonly used vectors in gene therapy, due to its capability to effectively transport genes and secure long-term expression. [22] Scientists use various viruses for transporting therapeutic genes to cell nuclei, utilizing the virus life cycle. The virus must be modified by genetic engineering for the purpose of its use as a vector for gene transfer. The pathogenic part is removed and replaced with a therapeutic gene. [23] Simultaneously, the virus keeps its non-pathogenic structures, which enable it to enter the cell. [20] The resultant non-pathogenic virus carrying the therapeutic gene is called "viral vector". Nowadays, viral vectors are commonly used for gene transfer, due to their high effectiveness *in vivo*, despite their disadvantages [24], which can be summarized into following points:

1. Acute immune reaction stimulated by viral vectors can lead to death [24-25],
2. The production of viral vectors in great quantities is very complex and costly [25],
3. The size of the genes supplied by the virus is limited.

Most commonly used viruses used as vectors are: adenoviruses, retroviruses, adeno-associated viruses (AAV) and herpes simplex viruses. [26]

ADENOVIRUSES

Adenoviruses are common DNA viruses, causing upper respiratory tract infections in humans. The genome length of double-stranded DNA in adenoviruses is 36 kilobases (kb), and can be manipulated by standard recombinant technology. [27] Adenoviruses are most commonly used vectors for gene distribution, because of their gene transfer effectiveness *in vivo* and their capability to deliver double-stranded DNA to the nucleus effectively. Their genome length enables extensive modifications and incorporation of therapeutic genes. Most of these adenoviral vectors were modified to replication-deficient forms by excision of essential genes E1A and E1B. [28-29]

Adenoviral vectors can be replication-deficient when certain genes are excised and replaced by a cassette expressing foreign therapeutic genes. These vectors are used as vaccines and in gene therapy of cancerous diseases.

Oncolytic vectors are designed to replicate primarily in cancer cells and destroy them via natural lytic viral replication process. Many clinical studies show that replication defective and replication competent

adenoviral vectors are safe and have therapeutic activity. [30]

Three generations of adenoviruses were developed. The first generation is highly immunogenic, and only one adenoviral sequence is missing – E1. The therapeutic gene is distributed to targeted cells with the rest of the viral genes. Remaining adenovirus sequences induce a strong humoral and cellular immune response targeted against modified cells. [31] Some adenovirus genes were excised in the second generation, but transduced cells still expressed a few products coded by adenovirus sequences. [32] The third generation of adenoviral vectors was developed in Frank Graham laboratory, where all viral genes were removed and an extremely low-immunogenic vector capable of distributing a large number of therapeutic genes (30 kb) was created. These vectors present very promising means for gene therapy applications, despite the production being more complex than of first generation vectors. [33]

Adenoviral vectors have several advantages: a high titer (10¹² – 10¹³ viral particles per ml) of recombinant viruses; the ability to infect post-osmotic cells; the ability to infect a vast scale of cellular types; a capability to accept foreign DNA to the extent of 8 kb, including expression cassettes or other regulative sequences; and previous experiences exist when vaccines on the basis of adenoviruses were used on humans without any side effects.

However, there are some limiting features of adenoviral vectors, and those are: insufficient permanent expression, when viral DNA is not integrated in the host genome; antigenicity against viral proteins by humoral and cytotoxic T-lymphocytes; and a possible toxicity in higher dosage. The lack of permanent expression probably does not present a problem in acute applications, as cancer, re-stenosis after angioplasty or therapy of angiogenesis. [34]

RETROVIRUSES/LENTIVIRUSES

γ-retroviruses and lentiviruses belong to the family *Retroviridae*, and they are characterized by their ability to transcribe RNA genome to a cDNA copy, which is subsequently integrated in the genome of the host cell. Retroviruses are commonly divided into simple (sometimes called oncogenic γ-retroviruses, such as mice leukemia virus) and complex (for example lentiviruses) [34]. Simple and complex retroviruses contain two copies of linear, non-segmented single-stranded RNA 7-12 kb in length, coding genes gag, pol and env. [35]

Recombinant retrovirus vectors have been used in the first gene therapy study by Rosenberger et al. in 1990. These vectors can transduce dividing cells and integrate the viral genome to the genome of host cells. Recombinant retroviruses are the most commonly used viruses in the production of anti-cancer vaccines. They incorporate therapeutic genes into cancer cells *ex vivo*

and create stable genetically modified lines of cancer cells. [36]

Almost all retroviral vectors are based on C-type mice leukemic viruses, for example Moloney virus, and more than 70% of clinical studies in cancer gene therapy uses retroviral vectors. The advantages of C-type retrovirus vectors in malignant disease gene therapy are that these were extensively studied and capable of infecting only actively dividing tissue. [37] For example, in a liver containing colorectal metastases, normal hepatocytes will be in a steady G_0 phase, meanwhile division will occur in the group of metastatic cells, although this group can present only 10% of cancer cell population. This can lead to a certain degree of tumor specificity.

There are numbers of reasons why retroviral vectors are not the best instruments for cancer cell *in vivo* modification. Recombinant retroviruses cannot reach high titer volumes; they can transduce only dividing cells and are not effective by *in vivo* delivery, because they are subjected to intense destruction by complement in the serum. [38]

Retroviral vectors are particularly preferable for cell "gene corrections", for the reason of long-lasting and stable expression of the transported transgenes and also for low efforts used for their cloning and production. Despite a few unsuccessful attempts of using retroviruses as a means of therapy, there is a new generation available with improved genome integration and safe characteristics, which makes retroviral vectors useful tools for several gene therapy applications. [39]

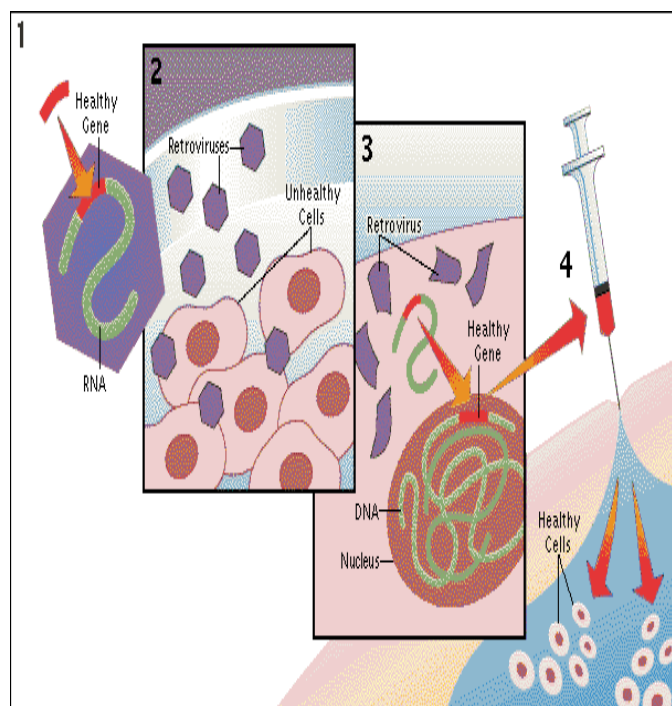


Fig. 2: Gene therapy using retrovirus vector [40]

Lentiviral vectors are effective and stable means of gene transfer to numerous cell types, such as stem cells. The safety of lentiviral vectors rose by minimizing the chance of recombination between various gene

elements, which was accomplished by keeping less than 5% of viral genome and by creating "self-inactivating" vectors. [41] Lentiviruses can be produced in high titer volumes, can transduce and integrate with a genome of non-dividing cells and effectively distribute therapeutic genes to CD34⁺ cells. Lentiviral vectors are derived from HIV, which belongs to the group of complex retroviruses. [36] The main difference between simple retroviruses (such as Moloney mice leukemic virus) and lentiviruses is, that simple retroviruses hold only three structural genes, while lentiviruses contain nine (three structural – gag, pol, env, and six regulatory – Vif, Vpr, Vpu, Tat, Rev and Nef) genes. The production of the most advanced (third generation) viral vectors on the basis of HIV requires the four-time transfecting of 293 cells (three envelope plasmids and one transfer vector). [42] Despite certain safety risks connected with the fact of HIV being a deadly virus to humans, the latest generation of lentiviral vectors will be undoubtedly soon applied in gene therapy clinical studies. [36, 43]

ADENO-ASOCIATED VIRUSES

Adeno-associated viruses (AAV) belong to the genus *Dependovirus*, part of the family *Parvoviridae*, AAV is small (20 nm), replication-deficient non-enveloped virus. The genome composes of positive or negative polarity single-stranded DNA (ssDNA), and is 4.7 kb in length. Type 2 Adeno-associated virus is a non-pathogenic DNA virus used as a vector for transport of eukaryotic genes *in vitro* and *in vivo*. AAV hold a unique set of characteristics, which can make them useful in human gene therapy. AAV infections do not require proliferation of host cells, although expression from AAV vectors can show relative preference to actively dividing cells. The wild types AAV, as well as AAV vectors, have the tendency to persist in infected cells for an extended period of time without adverse effects for the host. The wild type AAV frequently integrates in one specific region of chromosome 19, while replication-deprived AAV vectors integrate in less specific regions of the host cell genome and can persist in episomal state. AAV vectors were used for transduction of a vast scale of cell types *in vitro*, including epithelial cells of the respiratory tract, as well as bone marrow and lymphocyte-divided cells. [44]

AAV are characterized by low frequency of random incorporations to the genome and moderate immune response. As in all vector systems, the tropism of AAV limits their use in systemic application. Yet the tropism can be modified by aimed modification of the capsid and by using different serotypes, and this is how the AAV become a distribution system aimed for the right types of cells. [45]

BACTERIOPHAGES

Bacteriophages (phages) are viruses composed of a DNA or RNA genome covered by a protein capsule. They infect bacteria, either by incorporating viral DNA

to the host genome and replicating as a part of the host (lysogenes), or by simply replicating in the host cell. Afterwards they release phage particles by budding from the cell or by active lysis of the cell. [46]

Waclaw Szybalski began a new type of research on a group of lambda-phages. The goal of his experiments was to determine gene transfer, modification and regulation. [10] Szybalski knew that cells are capable of accepting foreign DNA, however, at this time; no one could successfully prove the hereditary transformation of biochemical traits until the year 1962, when Szybalski published the study "DNA-mediated heritable transformation of a biochemical trait". [47] Szybalski proved that a genetic defect can be corrected by transferring functional DNA from a different (foreign) source. Other than that, he proved that a therapeutic gene can be inherited when daughter cells express the same phenotype as the transformed parental cells. [10]

In recent years, it was proved that bacteriophages have several potential applications in modern biotechnological industry; they were designed as protein and DNA vaccine transport systems; as distribution systems in gene therapy; as an alternative to antibiotics; for the purpose of detecting pathogenic bacteria and as tools for protein, peptide or antibody "libraries" screening. This diversity, manipulation and production simplicity gives an estimate for their potential use in research, therapy and production in biotechnological and medicinal area. [46]

The capsid of bacteriophages protects DNA from degradation after injection, gives phages the capability to display foreign molecules on the phage capsid, and enables targeting of specific cell types, anticipating the success of gene therapy. Artificial covalent conjugation of molecules on the surface of the bacteriophages and phage marking were used to target and to utilize molecules on the phage surface. [48] Target sequence marking of, for example, fibroblast growth factor, was used for the purpose of phage distribution to cells with specific receptors; in the meantime, proteins sequences, such as adenoviral Penton base, mediating adhesion of the virus, entrance and endosomal release are used for amplifying phage uptake into cells and endosomal release. [49]

For the purposes of testing phage capability to target specific types of tissue, phage display libraries were studied on mice models several times, and isolated from specific tissues in every experiment. [50] For example, to isolate phages targeting the liver, mice were inoculated with a phage display library and after a liver extraction phages were isolated from this organ. This process should be repeated several times, until phages exhibit a relatively high degree of tissue-specific tropism. [46] A similar testing strategy *in vitro* was used for isolating phages displaying peptides and has shown elevated cytoplasmic uptake to mammal cells. [50] Again, this shows the universality of phage usage and

proves targeting on specific tissues can be achieved either by rational designing of phages or testing random phage displaying libraries.

For many years now, virus expression vectors have been explored as a mechanism for gene delivery, therapy and vaccine development. More recently, the next generation of virus vectors possess greater attributes such as tissue specificity and improved expression levels, while at the same time have many shortcomings of their predecessors, such as issues regarding immunogenicity and safety. Despite the scientific and technological advances there are still many uncertainties about the side effects of treatment. Furthermore, the less known effects, such as long-term expression of the introduced genes, the lack of controlling the expression of these genes and genetic modification of germ cells, are now ignored. There is no doubt that currently the main problem is the high immunogenicity of viral vectors introduced into the patient, as well as problems related to efficacy, toxicity and inflammatory response.

REFERENCES

- Gardlík R, Pálffy R, Hodosy J, Lukács J, Turna J, Celec P. Vectors and delivery systems in gene therapy. *Med Sci Monit.* 2005; 11(4):RA110-21.
- Valenčáková A, Dziaková A, Hatalová E. Gene therapy of cancerous diseases. *Int. J. Pharm. Sci. Drug Res.* 2015; 7(6):444-450.
- Rochlitz CF. Gene therapy of cancer. *Swiss Med Wkly.* 2001; 131:1-2, 4-9.
- Yu M, Poeschla E, Wong-Staal F. Progress towards gene therapy for HIV infection, *Gene Ther.* 1994; 1(1):13-26.
- Dishart KL, Work LM, Denby L, Baker AH. Gene Therapy for Cardiovascular Disease. *J Biomed Biotechnol.* 2003; 2:138-148.
- Bunnell BA, Morgan RA. Gene therapy for infectious diseases. *Clin Microbiol Rev.* 1998; 11(1):42-56.
- Kohn DB, Sadelain M, Glorioso JC. Occurrence of leukaemia following gene therapy of X-linked SCID. *Nat Rev Cancer* 2003; 3(7):477-88.
- Zhang J, Frolov I, Russell SJ. Gene therapy for malignant glioma using Sindbis vectors expressing a fusogenic membrane glycoprotein. *J Gene Med.* 2004; 6(10):1082-91.
- Bazeley R., <http://www.phgfoundation.org/news/11189/> (10.Oct.2015)
- Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. *Gene.* 2013; 525(2):162-9.
- Wysocki PJ, Grabarczyk P, Mackiewicz-Wysocka M, Kowalczyk DW, Mackiewicz A. Genetically modified dendritic cells--a new, promising cancer treatment strategy? *Expert Opin Biol Ther.* 2002; 2(8):835-45.
- Stribley JM, Rehman KS, Niu H, Christman GM. Gene therapy and reproductive medicine, *Fertil Steril.* 2002; 77(4):645-57.
- Sandhu JS, Keating A, Hozumi N. Human gene therapy. *Crit Rev Biotechnol.* 1997; 17(4):307-26. Review.
- Knipe JM, Peters JT, Peppas NA. Theranostic agents for intracellular gene delivery with spatiotemporal imaging. *Nano Today* 2013; 8(1):21-38.
- Nam HY, Nam K, Hahn HJ, Kim BH, Lim HJ, Kim HJ, Choi JS, Park JS. Biodegradable PAMAM ester for enhanced transfection efficiency with low cytotoxicity. *Biomaterials* 2009; 30(4):665-73.
- Ropert C. Liposomes as a gene delivery system, *Braz J Med Biol Res.* 1999; 32(2):163-9.
- Somia N, Verma IM. Gene therapy: trials and tribulations. *Nat Rev Genet.* 2000; 1(2):91-9.

18. Chailertvanitkul VA, Pouton CW. Adenovirus: a blueprint for non-viral gene delivery. *Curr Opin Biotechnol.* 2010; 21(5):627-32.
19. Wiley John, Sons I. Gene therapy clinical trials worldwide, In: *The Journal of Gene Medicine*, March 17th 2010, Available from: <<http://www.wiley.com/legacy/wileychi/genmed/clinical/>>
20. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat Med.* 2001; 7(1):33-40.
21. Ibraheem D, Elaissari A, Fessi H. Gene therapy and DNA delivery systems. *Int J Pharm.* 2014; 459(1-2):70-83.
22. Boulaiz H, Prados J, Marchal JA, Melguizo C, Concha A, Carrillo E, Vélez C, Martínez A, Aránega A. Modulation of Ki-67 expression and morphological changes induced by gef gene in MCF-7 human breast cancer cells. *Cell Mol Biol (Noisy-le-grand)* 2005; 51(1):87-92.
23. Bouard D, Alazard-Dany D, Cosset FL. Viral vectors: from virology to transgene expression, *Br J Pharmacol.* 2009; 157(2):153-65.
24. Munier S, Delcroix-Genête D, Carthagéna L, Gumez A, Hazan U. Characterization of two candidate genes, NCoA3 and IRF8, potentially involved in the control of HIV-1 latency. *Retrovirology* 2005; 2:73.
25. Templeton NS. Cationic liposome-mediated gene delivery in vivo. *Biosci Rep.* 2002; 22(2):283-95.
26. Walther W, Stein U. Viral vectors for gene transfer: a review of their use in the treatment of human diseases. *Drugs* 2000; 60(2):249-71.
27. Kämmer C, Doerfler W. Genomic sequencing reveals absence of DNA methylation in the major late promoter of adenovirus type 2 DNA in the virion and in productively infected cells. *FEBS Lett.* 1995; 362(3):301-5.
28. Sprengel J, Schmitz B, Heuss-Neitzel D, Doerfler W. The complete nucleotide sequence of the DNA of human adenovirus type 12. *Curr Top Microbiol Immunol.* 1995; 199(2):189-274.
29. Gardlik R, Celec P, Bernadic M. Targeting angiogenesis for cancer (gene) therapy. *Bratisl Lek Listy.* 2011; 112(8):428-34.
30. Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr Gene Ther.* 2013; 13(6):421-33.
31. Christ M, Lusky M, Stoeckel F, Dreyer D, Dieterlé A, Michou AI, Pavirani A, Mehtali M. Gene therapy with recombinant adenovirus vectors: evaluation of the host immune response, *Immunol Lett.* 1997; 57(1-3):19-25.
32. Gorziglia MI, Kadan MJ, Yei S, Lim J, Lee GM, Luthra R, Trapnell BC. Elimination of both E1 and E2 from adenovirus vectors further improves prospects for *in vivo* human gene therapy. *J Virol.* 1996; 70(6):4173-8.
33. Morsy MA, Gu M, Motzel S, Zhao J, Lin J, Su Q, Allen H, Franklin L, Parks RJ, Graham FL, Kochanek S, Bett AJ, Caskey CT. An adenoviral vector deleted for all viral coding sequences results in enhanced safety and extended expression of a leptin transgene. *Proc Natl Acad Sci U S A* 1998; 95(14):7866-71.
34. Kovesdi I, Brough DE, Bruder JT, Wickham TJ. Adenoviral vectors for gene transfer. *Curr Opin Biotechnol.* 1997; 8(5):583-9.
35. O'Keefe EP. Nucleic Acid Delivery: Lentiviral and Retroviral Vectors. In *Mather Methods* 2013; 3:174.
36. Wysocki PJ, Mackiewicz-Wysocka M, Mackiewicz A. Cancer gene therapy state of the art. *Reports of practical oncology and radiotherapy* 2002; 7(4):149-155.
37. Miller RJ Jr, Bahnson RR, Banner B, Ernstoff MS, O'Donnell WF. Neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin for locally advanced transitional cell carcinoma of the bladder. *Cancer* 1990; 65(2):207-10.
38. <http://www.wiley.com/genetherapy/clinical> (10.Oct.2015)
39. Maier P, von Kalle C, Laufs S. Retroviral vectors for gene therapy. *Future Microbiol.* 2010; 5(10):1507-23.
40. http://library.thinkquest.org/28000/load_image.php3?id=51 (10. Oct. 2015)
41. Yang S, Delgado R, King SR, Woffendin C, Barker CS, Yang ZY, *et al.* Generation of retroviral vector for clinical studies using transient transfection. *Hum Gene Ther.* 1999; 10(1):123-32.
42. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, *et al.* Gene transfer into humans-immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med.* 1990; 323(9):570-8.
43. Vigna E, Naldini L. Lentiviral vectors: excellent tools for experimental gene transfer and promising candidates for gene therapy. *J Gene Med* 2000; 2(5):308-16.
44. Flotte TR, Carter BJ. Adeno-associated virus vectors for gene therapy. *Gene Ther.* 1995; 2(6):357-62.
45. Michelfelder S, Trepel M. Adeno-associated viral vectors and their redirection to cell-type specific receptors. *Adv Genet.* 2009; 67:29-60.
46. Clark JR, March JB. Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *Trends Biotechnol.* 2006; 24(5):212-8.
47. Szybalska EH, Szybalski W. Genetics of human cell line. IV. DNA-mediated heritable transformation of a biochemical trait. *Proc Natl Acad Sci U S A* 1962; 48:2026-34.
48. Larocca D, Kassner PD, Witte A, Ladner RC, Pierce GF, Baird A. Gene transfer to mammalian cells using genetically targeted filamentous bacteriophage. *FASEB J.* 1999; 13(6):727-34.
49. Piersanti S, Martina Y, Cherubini G, Avitabile D, Saggio I. Use of DNA microarrays to monitor host response to virus and virus-derived gene therapy vectors. *Am J Pharmacogenomics* 2004; 4(6):345-56.
50. Rajotte D, Arap W, Hagedorn M, Koivunen E, Pasqualini R, Ruoslahti E. Molecular heterogeneity of the vascular endothelium revealed by *in vivo* phage display. *J Clin Invest.* 1998; 102(2):430-7.

Source of Support: Nil, Conflict of Interest: None declared.