SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 5.
Issue: 3.
Page N. 264-272
Year: 2021

Review Article www.wjahr.com

# THE VICTORIOUS WEAPON OF MOLECULAR BIOLOGY: THE VIRAL VECTOR

**[1]Arunava Chandra Chandra, [1]Kushal Nandi, [1]Amrita Chakraborty, *[1]Dr. Dhrubo Jyoti Sen and [2]Dr. Dhananjoy Saha**

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, Techno India University, Salt Lake City, Sector-V, EM-4, Kolkata-700091, West Bengal, India.
[2]Deputy Director, Directorate of Technical Education, Bikash Bhavan, Salt Lake City, Kolkata–700091, West Bengal, India.

**Received date:** 21 March 2021 **Revised date:** 11 April 2021 **Accepted date:** 01 May 2021

***Corresponding author: Dr. Dhrubo Jyoti Sen**
Department of Pharmaceutical Chemistry, School of Pharmacy, Techno India University, Salt Lake City, Sector-V, EM-4, Kolkata-700091, West Bengal, India.

**ABSTRACT**

*Since 1960 our medical science is contributing many things in our advanced healthcare system. Vector is nothing it is a vehicle used to transfer the genetic material into target Cell. In this article we are discussing about vectors, different type of vectors and their application in molecular biology at the Pandemic situation of Covid-19. Viruses have evolved elegant strategies to reach and enter specific target cells where they seize the cellular machinery to express viral genes and assemble progeny particles. In the same way, viral vectors represent the most effective means of gene transfer to modify specific cell type or tissue, and can be manipulated to express therapeutic genes. Viral vectors are usually derived from parental wild type viruses whose viral genes (essential for replication and virulence) have been replaced with the heterologous genes intended for cell manipulation. They can be used as in-vitro tools for biomolecular and gene functional studies, but also to accomplish more demanding tasks such as treat genetic disorders, fight cancer, drive tissue regeneration and monitor cell function. Nowadays victors are played common role in advanced therapies like gene therapies, cell therapies, Viral victor purification and also in vaccine production. Viral victors are now currently involving all across the world to development of effective Covid-19 Vaccine in the pandemic situation of Coronavirus.*

**KEYWORDS:** *Vectors, Genetic material, Target Cell, Molecular biology, Heterogenous genes, Biomolecular, Vaccine Production.*

## INTRODUCTION

Considering that the Latin word “vector” comes from the word “vehere”, which means "to carry”. Viral vectors are the commonly used tool used by molecular biologists to deliver genetic material into cells. Delivery of genes by a virus is termed as transduction and the infected cells are described as transduced. Viral Vectors are widely used in gene therapy. The COVID-19 pandemic has underlined the importance of vaccine development for the global population. According to the World Health Organization (WHO), around 151 vaccine candidates have been subjected to preclinical studies and 42 vaccines have reached clinical trials. The importance of viral vector manufacturing is highlighted by the fact that there are at least two adenovirus-based vaccine candidates that are currently in phase III development while another one has been approved in Russia.

Due to the relative ease of manufacturing viral vector vaccines, effective packaging cell line systems have been developed for many viral vector systems such as Ad (adenoviruses), AAV (Adeno-associated viruses), flaviviruses, and lentiviruses. This has also facilitated the rapid and efficient large-scale production of vaccine candidates that are eligible for clinical applications against COVID-19. In July 2020, an article was published in the 'Nature' journal where the chimpanzee Ad vector ChAdOx1 nCoV-19 was engineered to express the SARS-CoV-2 S protein. When this protein was subjected to immunization of mice and rhesus macaques, it induced strong humoral and cellular immune responses and prevented COVID-19 pneumonia in macaques. More such research involving viral vectors is expected to continue at least for the next one or two years. This is expected to boost market growth. Viruses can usually infect more than one type of cell. Thus, when viral vectors are used to carry genes into the body, they might infect healthy cells as well as cancer cells. Viral vectors are complex—significantly more complex than traditional recombinant protein therapeutics. Besides consisting of both proteins and nucleic acids, viral vectors present daunting production challenges.

**Figure-1: Viral vector analysis.**

**Figure-2: Viral Vector Separations.**

They are distinguished by large flow-through channels that are designed for working with large proteins, plasmid DNA, virus-like particles, and viruses—including adeno-associated viruses. The columns rely on convection to provide rapid and low-shear mass transfer.

**Types of Viral Vectors**

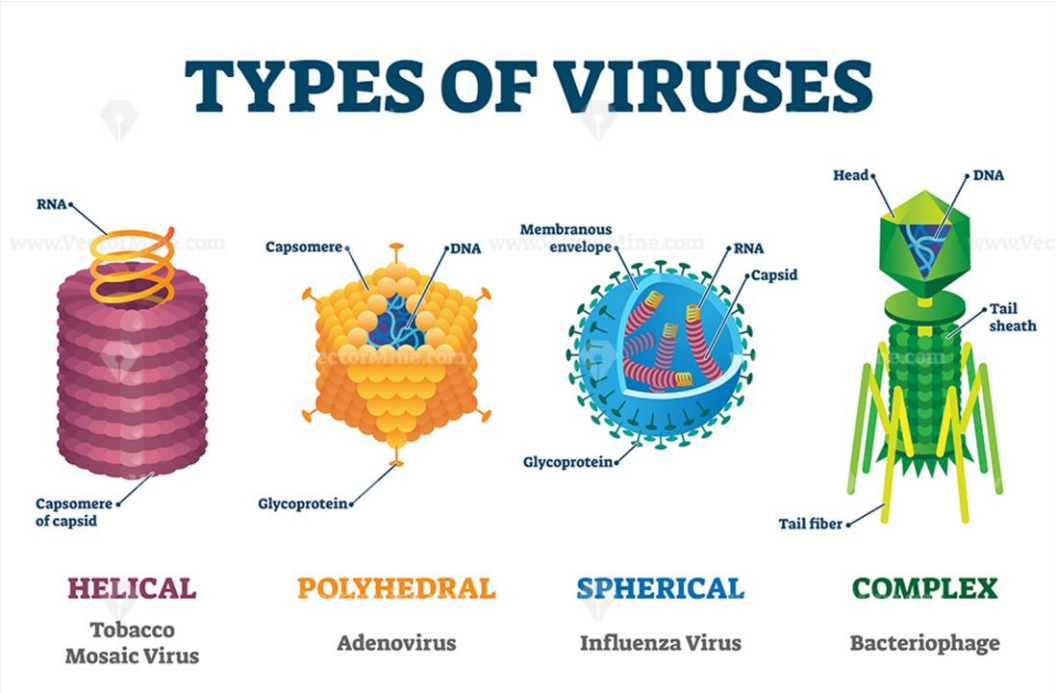



**Figure-3: Different types Of Viral Vectors.**

**Adenoviral Vectors:** Adenovirus vectors are non-toxic, non-integrating, non-enveloped viruses with a linear double-stranded DNA. Adenoviral vectors are based on Ad5 serotype. Adenoviral vectors have a wide range of action and are able to deliver nucleic acids to both dividing and non-dividing cells. This can make their use in basic research difficult, but they are sometimes used in vitro. When utilized *in-vivo*, adenoviral vectors often precipitate immune elimination of the cells, which also limits their functionality. Adenoviruses are often responsible for respiratory, gastrointestinal and eye infection that affect humans. As a result, research is currently being conducted to investigate the use of adenoviral vectors in applications of gene therapy and vaccination. To enter the cells Ad5-based vectors use the Coxsackie-Adenovirus Receptor (CAR). Adenovirus vectors provide strong, transient gene expression or - knockdown are used for gene therapy, vaccine, and cancer therapy development[1,2]

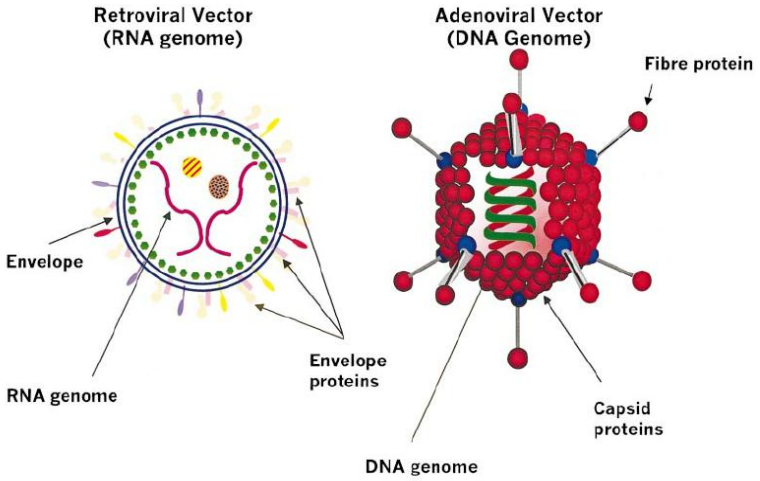



**Figure-4: DNA & RNA Virus Morphology.**

**Adeno-associated viral vectors (AAV):** AVV become a tool of choice for many preclinical *in-vivo* and *in-vitro* experiments and clinical studies in the field of cell and gene therapy. Ability to generate recombinant virus lacking viral genes but containing transgene, very low immunogenicity and long-term transgene expression makes adeno-associated viruses one of the safest and most common tools for clinical applications. Adeno-associated viruses (AVV) have linear single-stranded DNA (ssDNA) genome of approximately 4.5 kb, flanked by two inverted terminal repeats (ITR) and two open frames – rep and cap. Rep gene encodes regulatory proteins involved in genome replication and packaging. Cap gene encodes 3 capsid proteins: VP1, VP2 and VP3 in a ratio of 10:1:1. Cap defines cell/tissue tropism. AVV are presented in various serotypes which express different tissue tropism. Based on this feature, certain serotypes are recommended when a specific tissue is planned to be targeted. Modifications of promoter or capsid allow to generate AAV which targets specific tissues or cells.[3,4]

**Herpes Simplex Virus Vectors:** This type of viral vector has the ability to deliver large-scale quantities of exogenous DNA. The primary concerns with the use of herpes simplex virus to deliver genetic material are cytotoxicity and the maintenance of transgene expression.

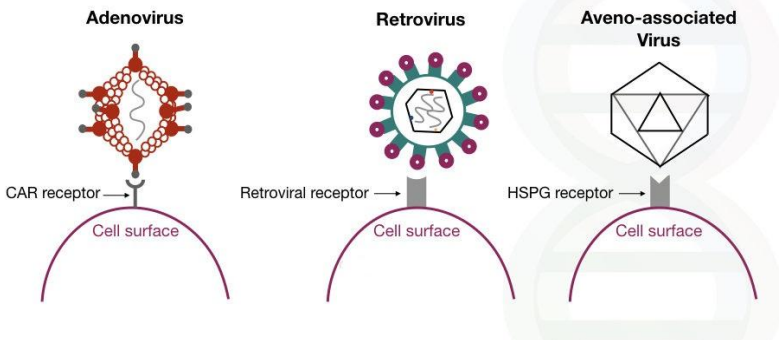



**Figure-5: CAR [Chimeric antigen receptor T cells] receptor, Retroviral receptor, HSPG [Heparan sulfate proteoglycans] receptor.**

**Retroviral Vectors:** Retroviral vectors are commonly used and known to integrate into the genome of the infected cell in a stable and permanent fashion. Reverse transcriptase in the virus allows integration into the host genome. There are two types of retroviral vectors: replication-competent and replication defective. Usually replication-defective vectors are preferred in practice as they allow for several rounds of replication due to their coding regions. However, for transduction to occur retrovirus cells require mitotic cell division to be in process, which puts a significant limit on their utility. Because of this, some cells such as neurons are resistant to the effect of retroviral vectors.[5,6]

**Lentiviral Vectors:** Lentiviruses are a type of retrovirus that are able to integrate into non-dividing cells and do not require mitotic cell division in order to function. Instead, the genome enters the cell DNA via reverse transcription and is incorporated in a random position of the cell genome. The unpredictability of the integration site is a significant inhibitory factor of this type of vector. It may lead to a disturbance in cellular function and has the potential to lead to cancer development. Despite this, research is supportive of the use of lentivirus as a vector and has demonstrated that the risk of negative consequences is low.[7,8]

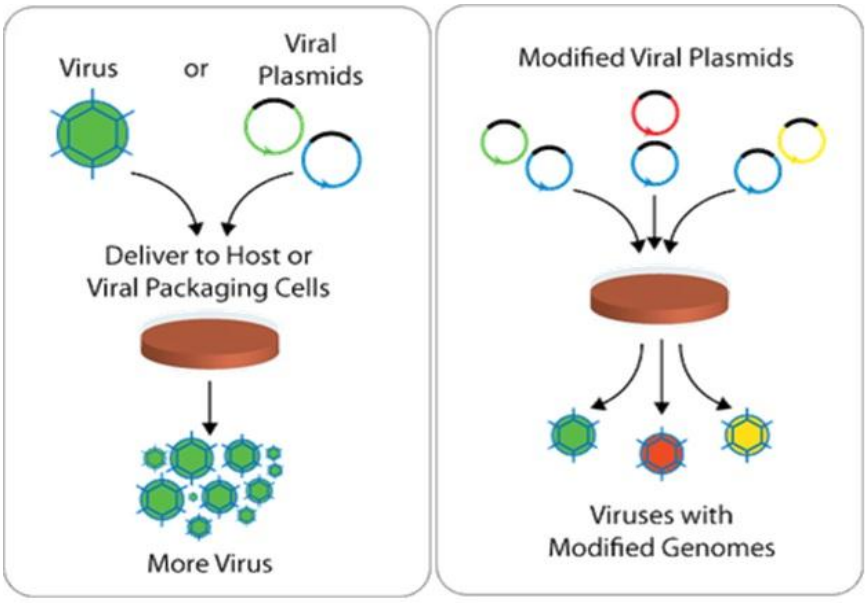
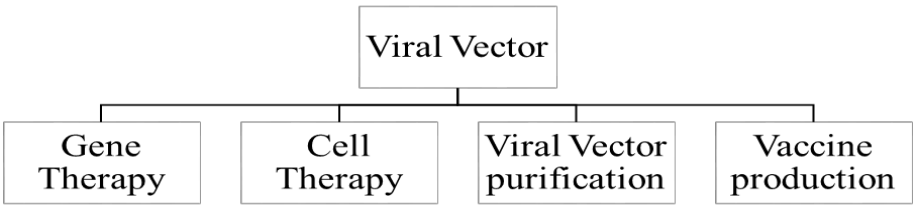



**Figure-6: Viral cell packaging & modification of viral genomes by changes in Viral genomes.**

**Manufacturing of viral vectors:** The promising results from clinical studies of the use of viral vectors to address important medical needs, have surged the interest in developing scalable and cost-efficient manufacturing processes. For gene therapy alone, the global market value is estimated to exceed 10 billion USD by 2025. For regulatory compliance, products intended for therapeutic use should be well characterized and manufactured to high purity, efficacy, and safety, and high levels of GMP compliance should be met. The continuous development of recombinant viral vectors expands the commercial product pipeline, prompting the use of virus vector manufacturing platforms.

**Applications of Viral Vectors**




**Gene Therapy:** All viruses attack their hosts and introduce their genetic material into the host cell as part of their replication cycle. This genetic material contains basic 'instructions' of how to produce more copies of these viruses, hijacking the body's normal production machinery to serve the needs of the virus. The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells becoming infected. Some types of viruses actually physically insert their genes into the host's genome. This incorporates the genes of that virus among the genes of the host cell for the life span of that cell. Viruses like this could be used as vehicles to carry 'good' genes into a human cell. First, a scientist would remove the genes in

the virus that cause disease. Then they would replace those genes with genes encoding the desired effect (for instance, insulin production in the case of diabetics). This procedure must be done in such a way that the genes which allow the virus to insert its genome into its host's genome are left intact.

**Viral vectors based on DNA for gene delivery systems:** Viral vectors based on DNA for gene delivery are usually longer lasting and integrating into the genomes. DNA-based viral vectors are lentivirus, poxvirus, adenovirus, adeno-associated virus, retrovirus, human foamy virus (HFV) and herpes virus.[9] DNA-based viral vectors for gene delivery systems utilize the viral vectors to deliver genetic materials to the host cells. The viral vectors are efficient for delivering the genetic materials to the host cells.[10] The DNA-based viral vectors for the gene delivery systems include plasmids containing transgenes for gene therapy. Although the DNA-based viral vectors are in early stages of clinical trials, the class of materials has been emerged to yield extremely promising candidate of gene delivery systems for gene therapy in the wide range of diseases including cancer, AIDS, neurological disorders such as Parkinson's and Alzheimer's diseases, and cardiovascular disorders.[11,12]


**Figure-7: Viral Vector based COVID-19 Vaccine.**

**Viral vectors based on RNA for gene delivery systems:** Viral vectors based on RNA for gene delivery have developed for the ability to transcribe directly for infectious RNA transcripts. RNA-based gene delivery is not permanent and usually transient. In the gene delivery systems based on RNA, human foamy virus, oncoretro-viral vectors, and lenti-viral vectors are included in the gene therapy. The sophisticated system provides the RNA-dependent polymerase complexes coupled with negative-strand RNA templates. RNA-based gene delivery systems have carried out for HIV with lenti-viral vectors-modified CD 34(+) cells in patients undergoing transplantation for AIDS-related lymphoma.[13,14]

**Oncolytic viral vectors for gene delivery systems:** Oncolytic viruses (OVs) are an emerging treatment option for cancer type diseases. They have recently focused on cancer research aiming to develop their therapeutic treatment potential.[15] The positive and negative effects of the multitude for their modification have discussed to increase their infectivity, anti-tumor immunity and treatment safety for the interaction of OVs and tumor cells. Oncolytic adenovirus co-expressing IL-12 and IL-18 improves tumor-specific immunity via differentiation of T cells expressing IL-12Rβ2 or IL-18Rα.[16] The ultimate aim is to design a virus that is able to replicate effectively within the host, special target and lyse tumor cells. Adenovirus-mediated decorin expression induces cancer cell death through activation of p53 and mitochondrial apoptosis.[17] Oncolytic adenovirus (Ad) vectors present a promising modality to treat cancer by using the gene therapy.[18] Oncolytic adenovirus expressing IL-23 and p35 elicits IFN-ɤ-and TNF-α-Co-producing T cell-mediated antitumor immunity. It has reported that cytokine immune-gene therapy is one of promising strategies for cancer treatments.[19-22]

**Viral Vectors in Vaccine Production:** Recombinant vectors can be used to deliver antigens and to stimulate immune responses in humans. Viral vectors possess various intrinsic properties which may lend to advantages and disadvantages for usage for a given therapeutic application. The safety and flexibility of recombinant viral vectors have lead to their usage in gene therapy, virotherapy, and vaccine applications. In this chapter, we will discuss the utility and importance of recombinant vectors as vaccine agents. This chapter will highlight some of the uses of recombinant viral vectors for therapeutic vaccines; and will mostly focus on the application of a range of recombinant viral vectors for prophylactic vaccines against infectious agents.

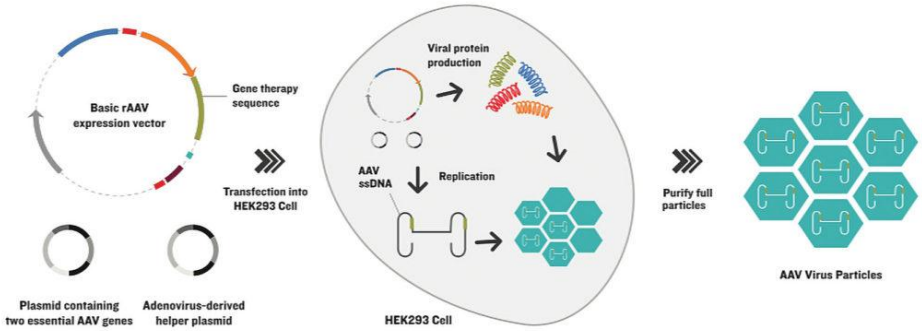



**Figure-8: To produce recombinant adeno-associated virus (rAAV).**

Vigene Biosciences uses a triple plasmid transient transfection method. Outlined here, the method eliminates the need for a helper adenovirus by introducing a basic rAAV expression vector in one plasmid (top left), and additional DNA in two other plasmids (bottom left). The plasmids are transfected into the production cell line (middle), which produces mature rAAV capsids containing the transgene of interest. Once purified (right), these viral particles can infect patient cells to deliver their DNA payload but cannot replicate further. Viral vector-based vaccines differ from most conventional vaccines in that they don't actually contain antigens, but rather use the body's own cells to produce them. They do this by using a modified virus (the vector) to deliver genetic code for antigen, in the case of COVID-19 spike proteins found on the surface of the virus, into human cells. By infecting cells and instructing them to make large amounts of antigen, which then trigger an immune response, the vaccine mimics what happens during natural infection with certain pathogens - especially viruses. This has the advantage of triggering a strong cellular immune response by T cells as well the production of antibodies by B cells. An example of a viral vector vaccine is the rVSV-ZEBOV vaccine against Ebola.


**Figure-9: COVAXIN &COVISHIELD COVID-19 Vaccine.**

**How Do They Works? (Example COVID-19 Viral Vector Vaccine):** Viral vector vaccines use a modified version of a different virus (the vector) to deliver important instructions to our cells.

**First,** the vector (not the virus that causes COVID-19, but a different, harmless virus) will enter a cell in our body and then use the cell's machinery to produce a harmless piece of the virus that causes COVID-19. This piece is known as a spike protein and it is only found on the surface of the virus that causes COVID-19.

**Next,** the cell displays the spike protein on its surface, and our immune system recognizes it doesn't belong there. This triggers our immune system to begin producing antibodies and activating other immune cells to fight off what it thinks is an infection.

**At the end of the process,** our bodies have learned how to protect us against future infection with the virus that causes COVID-19. The benefit is that we get this protection from a vaccine, without ever having to risk the serious consequences of getting sick with COVID-19. Any temporary discomfort experienced after getting the vaccine is a natural part of the process and an indication that the vaccine is working.

| Virus | Advantages | Disadvantages |
|---|---|---|
| **Retrovirus** | Long-term gene expression | Generation of replication-competent virus potential for tumorigenesis Infects dividing cells only |
| **Lentivirus** | Long-term gene expression<br>Infects non-dividing and dividing cells | Generation of replication-competent virus potential for tumorigenesis |
| **Vaccinia virus** | High immunogenicity<br>Safety: used as a smallpox vaccine<br>High titer production | Pre-existing immunity |
| **Adenovirus** | High immunogenicity<br>Safety: used in many clinic trials<br>High titer production | Pre-existing immunity |
| **Adeno-associated virus** | Long-term gene expression<br>Non-pathogenic virus | Low titer production |
| **Cytomegalovirus** | Induces a unique CTL response<br>Protects against SIV infection in an animal model | Pre-existing immunity<br>Risk of pathogenesis in specific individuals |
| **Sendai virus** | High immunogenicity | Pre-existing immunity |

**Figure-10: Viruses based Viral Vectors advantages & disadvantages.**

**Advantages**

- ❖ High efficiency gene transduction
- ❖ Highly specific delivery of genes to target cells.
- ❖ Induction of both humoral and cell-mediated immune responses
- ❖ Improved efficacy and safety
- ❖ Reduced administration dose
- ❖ Enable large-scale manufacturing

Potential targets ranging from cancers to a vast number of infectious diseases

**Disadvantages**

- ✓ Risk of integrate into the host genome and lead to other diseases
- ✓ The presence of pre-existing immunity against the vector caused by previous exposure to the virus and the production of neutralizing antibodies can reduce vaccine efficacy

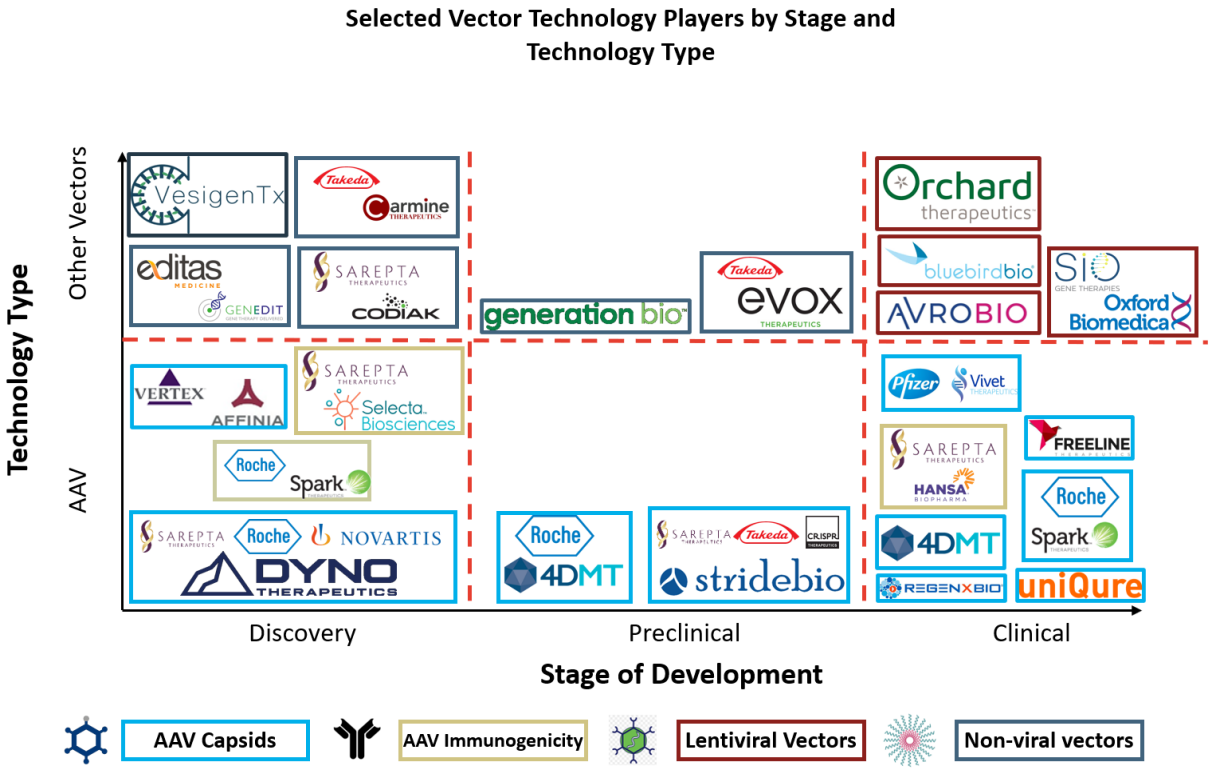



**Figure-11: Global companies race & involve in viral vectors invitation, Purification, Manufacturing.**

**Conclusion:** Three major considerations exist for the use of viral vectored vaccines in humans, the first of which is safety. Some platforms, such as Ad vectors, have been tested extensively in human clinical trials for many years and are accepted as being safe for human use. Other platforms, such as VSV, are still in their infancy and human safety remains an unknown factor. As more clinical trials take place, we will gain a better understanding of these vectors and their performance in humans. The second consideration is pre-existing vector immunity, which varies in significance depending on the vector of choice. For Ad or vaccinia vectors, this may have a serious impact on vaccine vector efficacy in humans; for the zoonotic virus vectors, such as avipox viruses or NDV, pre-existing immunity is not likely to play as significant a role in developmental and clinical applications. Finally, the third consideration for vaccine vector development is the vector's genomic capacity for a transgene insert. Depending on the vector, these exogenous DNA sequences can range in length from less than 1 kb to 35 kb. However, with increased transgene size often comes genetic instability and decreased virus yield in production. These must all be factored together to determine the ideal virus vaccine vector that is suitable for use in humans, but also maintaining a high level of efficacy.

## REFERENCES


1. Schalk, J. A., Mooi, F. R., Berbers, G. A., van Aerts, L. A., Ovelgonne, H., and Kimman, T. G. Preclinical and clinical safety studies on DNA vaccines, Hum. Vaccin, 2006; 2: 45–53.
2. Thomas, C. E., Ehrhardt, A., and Kay, M. A. Progress and problems with the use of viral vectors for gene therapy, Nat. Rev. Genet, 2003; 4: 346–358.
3. Mairhofer, J., and Grabherr, R. Rational vector design for efficient non-viral gene delivery: challenges facing the use of plasmid DNA, Mol. Biotechnol, 2008; 39: 97–104.
4. Bower, D. M., and Prather, K. L. Engineering of bacterial strains and vectors for the production of plasmid DNA, Appl. Microbiol. Biotechnol, 2009; 82: 805–813.
5. Navarro, J., Oudrhiri, N., Fabrega, S., and Lehn, P. Gene delivery systems: Bridging the gap between recombinant viruses and artificial vectors, Adv. Drug Deliv. Rev., 1998; 30: 5–11.
6. Bergelson, J. M., Cunningham, J. A., Droguett, G., Kurt-Jones, E. A., Krithivas, A., Hong, J. S., et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5, Science, 1997l 275: 1320–1323.
7. Tomko, R. P., Xu, R., and Philipson, L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses, Proc. Natl. Acad. Sci. USA, 1997; 94: 3352–3356.
8. Berkner, K. L. Development of adenovirus vectors for the expression of heterologous genes, Biotechniques, 1988; 6: 616–629.
9. Roth, J. A. Adenovirus p53 gene therapy, Expert Opin. Biol. Ther, 2006; 6: 55–61.
10. Mata-Espinosa, D. A., Mendoza-Rodriguez, V., Aguilar-Leon, D., Rosales, R., Lopez-Casillas, F., and Hernandez-Pando, R. Therapeutic Effect of Recombinant Adenovirus Encoding Interferon-[gamma] in a Murine Model of Progressive Pulmonary Tuberculosis, Mol. Ther, 2008; 16: 1065–1072.
11. Happel, K. I., Lockhart, E. A., Mason, C. M., Porretta, E., Keoshkerian, E., Odden, et al. Pulmonary Interleukin-23 Gene Delivery Increases Local T-Cell Immunity and Controls Growth of Mycobacterium tuberculosis in the Lungs, Infect. Immun, 2005; 73: 5782–5788.
12. Goncalves, M. A. Adeno-associated virus: from defective virus to effective vector, Virol. J., 2005; 2: 43.
13. Mogler, MA, Kamrud, KI, RNA-based viral vectors. Expert Rev Vaccines, 2015; 14(2): 283–312. doi: https://doi.org/10.1586/14760584.2015.979798. PMID: 25382613.
14. DiGiusto DL, Krishnan A, Li H, Li S, Rao A, Mi S, Yam P, Stinson S, Kalos M, Simon J, Lacey F, Yee JK, Li M, Couture L, Hsu D, Forman SJ, Rossi JJ, Zaia JA. RNA-based gene therapy for HIV with lentiviral vector-modified CD34(+) cells in patients undergoing transplantation for AIDS-related lymphoma. Sci Transl Med, 2010; 2(36): 36–43.
15. Howells A, Marelli G, Lemoine NR, Wang Y, Oncolytic virus-interaction of virus and tumor cells in the battle to eliminate cancer. Front Oncol, 2017. http://doi.org/https://doi.org/10.3389/fonc.2017.00195.
16. Choi I-K, Lee J-S, Zhang S-N, Park J, Lee K-M, Sonn CH, Yun C-O. Oncolytic adenovirus co-expressing IL-12 and IL-18 improves tumor-specific immunity via differentiation of T cells expressing IL-12Rß2 or IL-18Rα. Gene Ther, 2011; 18(9): 942–7.
17. Yoon A-R, Hong JW, Yun C-O. Adenovirus-mediated decorin expression induces cancer cell death through activation of p53 and mitochondrial apoptosis. Oncotarget, 2017; 8(44): 1–20.
18. Choi JW, Lee YS, Yun C-O, Kim SW. Polymeric oncolytic adenovirus for cancer gene therapy. J Control Release, 2015; 10(219): 181–91. https://doi.org/10.1016/jconrel.2015.10.009 Epub 2015 Oct 23.
19. Choi I-K, Li Y, Oh E, Kim J, Yun C-O, Oncolytic adenovirus expressing IL-23 and p35 elicits IFN-ɤ- and TNF-α-co-producing T cell-mediated antitumor immunity, PLoS One, 2013; 8(7): e67512. https://doi.org/https://doi.org/10.1371/journal.pone.0067512.
20. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the

pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology, 2013; 144: 512–27.
21. El-Aneed A. Current strategies in cancer gene therapy. Europ J Pharmacol, 2004; 498: 1–8.
22. Baban CK, Cronin M, O'Hanlon D, O'SullivanG C, Tangney M. Bacteria as vectors for gene therapy of cancer. Bioengineered Bugs, 2010; 1(6): 385–94.