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A Review of Virus-Vectored Vaccines: Current Production Methods, Uses, Issues, and Future Perspectives

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Abstract

Viral vector vaccines have recently risen to the forefront of vaccinology, but there are multiple factors that must be addressed before they achieve widespread approval and use. The concept of using viruses as vectors is not new, and numerous attempts to create vaccines that utilize virus vectors have been made in the past 25 years. Despite this, only one viral vector vaccine has gained full approval by the FDA. The goal of this review is to provide a comprehensive overview of the types of viral vector vaccines, production and purification methods, current and future viral vector vaccines, the issues and limitations of viral vector vaccines, and how they can be improved for future use.

One Sentence Summary

A review of current and future viral vector vaccines, production methods, and improvements needed.

Introduction

Currently, most Food and Drug Administration (FDA)-approved vaccines are live attenuated, inactivated, or subunit vaccines and provide humanity with immunizations to a wide range of pathogens (*2*). Since Edward Jenner's development of the first vaccine in the late 1700s, humanity has made great strides in the field of vaccinology. Despite these advances, the rate of vaccine development over the past 40 years has slowed drastically and there are still many infectious diseases that do not have FDA-approved vaccines, even after decades of rigorous research and development (*3, 4*). Some of the major factors for this decline in development include the individual properties of various viruses, deficiencies in animal models to assess protection and safety in some populations, and prohibitive costs that make the field unattractive for pharmaceutical companies to fund (*3*). Additionally, traditional vaccination methods may simply just be unable to produce results with some of these more difficult pathogens (*4*), and thus, new methods must be developed. One such method is viral vectored vaccines. Although viral vector vaccine approaches have not yet become commonplace in human vaccines, the idea of using viruses as vectors is not new. The first successful demonstration of using a virus as a vector to transfer genetic material into a mammalian cell was published in 1972 using the SV40 virus (*5*). 11 years later the first proof-of-concept for a viral vectored vaccine was published (*6*), and a year later research was published showing that a recombinant vaccinia virus expressing hepatitis B surface antigen (Ag) effectively immunized chimpanzees against hepatitis B (*7*). Since then, several viral vector vaccines have been developed for animal use, however, use in humans has not caught on so quickly, in part due to higher regulatory standards and safety concerns (*8*). Currently, six viral vector vaccines have been approved for use by the FDA, with four of them being against SARS-CoV-2 and gaining approval in the last year in response to the

COVID-19 pandemic and the other two being for Ebola. The first viral vector vaccine to gain approval was rVSV-ZEBOV (sold under the brand name Ervebo), which is the only approved replication-competent viral vector vaccine to this day. The five other viral vectored vaccines are Zabdeno/Mvabea for Ebola, Oxford-AstraZeneca for SARS-CoV-2, Sputnik V for SARS-CoV-2, Janssen for SARS-CoV-2, and Convidecia for SARS-CoV-2, all of which are nonreplicating.

The primary applications for viral vector vaccines are in emerging infectious diseases and those that traditional vaccination strategies have, thus far, been unable to prevent reliably. These diseases/pathogens with currently limited/no vaccine availability include HIV/AIDS, hepatitis C (HCV), malaria, tuberculosis, respiratory syncytial virus (RSV), cytomegalovirus (CMV), and non-Ebola viruses that cause hemorrhagic fever like Nipah and Lassa viruses.

The goal of this review is to provide a comprehensive overview of the history, production and purification methods, and future directions for improvement of viral vectored vaccines. While this review is intended to look at viral vector vaccines as a whole, particular focus will be placed on adenovirus-based vectors, due to these vectors being the most commonly used and widely researched for vaccine purposes.

Types of Viral Vector Vaccines

Viral vector vaccines could be classified in a variety of different ways, such as the family or species of virus used, cell types targeted, classes of pathogens they are designed to protect against, so on and so forth. Viral vectors are commonly divided into two broad classes, replication-deficient (RD) and replication-competent (RC). In both cases, vectors will act as genetic vaccines since they are able to either infect and undergo viral replication or introduce genetic material into a host cell through transduction. Even though viruses are not living

organisms, both types are generally considered “live” vaccines due to the principles behind viral vector vaccines acting as live viruses to deliver genetic material to cells using the same mechanisms a wildtype virus would (*9*). Additionally, even though some would consider them viral vector approaches, virus-like particles (VLPs) will not be discussed in detail since they are essentially a viral capsid featuring a desired protein Ag without any genetic material and, as such, are more in line with traditional inactivated or subunit vaccines than they are with viral vector vaccines.

Common Viruses Used as Vectors

Many viruses have the ability to be used as vectors and each family has its own advantages and drawbacks. Some of the most commonly used viruses belong to the retrovirus, lentivirus, adenovirus (Ad), poxvirus, and rhabdovirus families. Retroviruses and lentiviruses have large packing capacities, good integration abilities, can transduce non-dividing and dividing cells, and can be pseudotyped fairly easily, however, there are concerns surrounding insertional mutagenesis with these vectors (*4*). Ad vectors have a broad tropism, show strong gene expression, and are generally quite safe due to low pathogenicity, but the seroprevalence of existing antibodies (Abs) to human Ad strains is quite high (*4, 8*). All current viral vectored SARS-CoV-2 vaccines and Zabdeno, the first dose of the Zabdeno/Mvabea Ebola vaccine, are based on Ad platforms. Poxvirus vectors show high immunogenicity and are able to be produced in cultures reliably, but seroprevalence of preexisting Abs is high. Mvabea, the second dose of the Zabdeno/Mvabea Ebola vaccine uses a modified vaccinia Ankara vector, which is a type of poxvirus. Rhabdovirus based vectors induce a strong humoral immune response, are readily pseudotyped, and have a low seroprevalence of existing antibodies, but include the possibility of neuro-virulence with some virus species, specifically rabies virus (*4*). rVSV-EBOV is a

recombinant, replication-competent modified vesicular stomatitis virus (VSV), a member of the rhabdovirus family, which expresses a Zaire Ebolavirus surface glycoprotein (*4*).

Replication-Deficient

RD vectors are those that are missing one or several genes required for genome replication and thus cannot propagate outside of specific cells that have been modified to stably express the missing genes (*10*). Production of RD vectors often involves the usage of a specific cell lines such as HEK-293 or Vero cells, which are collectively known as helper or production cells. These cells express genes necessary for viral replication that aren't normally present in human cells, and thus allow propagation of the viruses until they reach suitable titers (*9*). These production cell lines will be discussed in more detail later. Since the cells of the vaccinated host lack the genes that the modified viruses need for propagation, these vectors are unable to propagate in host cells. Cell cultures can be made suitable for vector replication via transient transfection or stable integration of viral replication genes into the cell line genome. Some vectors, such as the recombinant modified vaccinia virus Ankara (MVA), are not totally replication deficient, as they can replicate normally in embryonic fibroblasts of chickens and are only RD in mammalian cells (*11*). Since these vectors are unable to replicate and produce infectious progeny in human cells, the safety profile for them is generally high (*4*). Because of this high safety profile, a majority of viral vector vaccine candidates use RD vectors (*9*). Currently, there are 5 RD viral vectored vaccines that have gained approval for use in at least one country: Oxford-AstraZeneca (ChAdOx1 nCoV-19), Janssen (Ad26.COV2.5), Convidecia (AD5-nCOV), and Sputnik V (Gam-COVID-Vac) vaccines for SARS-CoV-2 and the Zabdeno (Ad26.ZEBOV)/Mvabea (MVA-BN-Filo) prime-boost regimen vaccine for Ebola.

Replication-Competent

RC vectors function similarly to RD vectors in the sense that they are both genetic vaccines, meaning they contain genetic material that will lead to expression of a desired protein Ag, and require specific cell substrates for production. However, RC vectors do not rely on production cell lines or transient transfection with support plasmids to propagate. RC vectors are cultured in Good Manufacturing Practice (GMP) eukaryotic cell lines, similar to currently available live-attenuated vaccines (*9*). With all viral vector vaccines, the primary concern with their usage is safety, specifically when RC vectors are used due to risk of recombination, which could reintroduce a level of pathogenicity to the vector (*9*). RC vectors are typically more immunogenic than RD vectors due to RIG-I-like receptor activation being a strong pro-inflammatory signal that is effective in inducing T cell responses to the virus (*12*). Additionally, since the vector will undergo multiple replication cycles, the presence of Ag is sustained for a longer duration in the body, leading to a prolonged immune response and increased Ab production (*9*). Currently, there is one RC vector approved for usage for the prevention of Ebola, Ervebo (rVSV-ZEBOV).

Production and Purification Methods

Engineering Viruses to be Used for Delivery of Desired Antigens

Production of viral vector vaccines begins with creation of the vector that will express the desired Ag. Production methods for vectors will vary based on the type of virus used, this review will focus specifically on Ad vectors. Ad vectors can be divided into 3 subdivisions based on how much of the genome is changed: first-generation (FGAd), second-generation (SGAd), and high capacity (HCAd) (also known as third-generation, gutless, gutted, or helper-dependent) (*13, 14*) (Figure 1). Adenoviruses can be classified into over 50 different serotypes that vary in

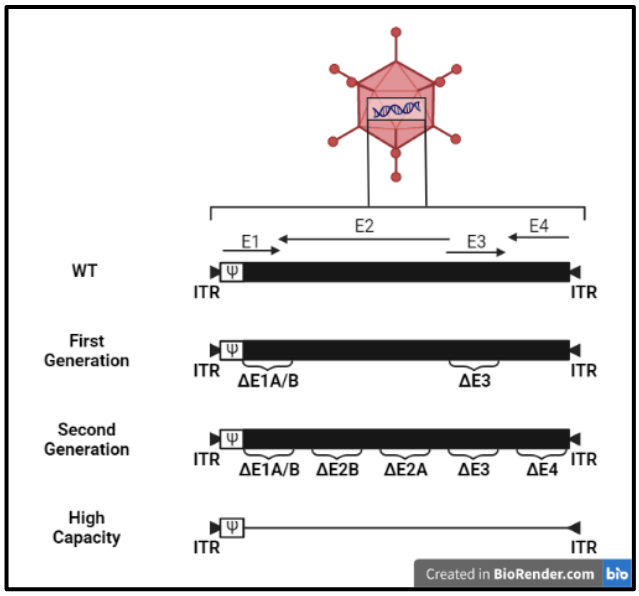

Figure 1: Basic genome schematic of wildtype, first generation, second generation, and HCAd. The early transcription regions are indicated by arrows above WT, adenovirus genome indicated by black bars, and packaging signal (Ψ) is indicated with an open box.

prevalence based on region (*15*). Ad serotype 5 (Ad5) is a commonly used and extensively studied Ad serotype with a genome size of 36 kb (*16*). Within the Ad genome, regions are classified as early or late depending on whether their expression is before or after DNA replication. The early regions are E1A, E1B, E2, E3, and E4. E1A and E1B products are critical for viral replication (*17*) and regulation of p53 and p73 (*18*). Additionally, E1A promotes cell proliferation via repression of retinoblastoma protein activity (*18*). E2 proteins mediate DNA replication, while E3 proteins alter host immune responses and E4 proteins alter host cell signaling (*16*). FGAd vectors are created by substituting either E1A/B or E1A/B and E3 with a transgene(s) of interest of up to 8.2 kb (*13, 14, 16, 17*). Since E1A and E1B are necessary for virus replication, culturing FGAd vectors requires a cell line that can provide their gene products. It just so happens that human embryonic kidney 293 (HEK-293) cells were originally immortalized by integrating E1A and E1B genes into chromosome 19 of the cells, making them an ideal cell line for production (*19*). SGAd vectors were designed to decrease Ad-specific Ags and contain deletions in the E2 or E4 regions to complement the deletions in E1 and E3, enabling up to 14 kb of transgene(s) to be inserted (*13, 14, 17, 18, 20*). Since SGAd vectors are also lacking the E1 region, a complementing cell line must once again be used. HCAd vectors feature deletions of all viral genes aside from the Ψ and two ITR regions, allowing for a significant transgene packing

capacity of up to nearly 38 kb (*13, 14, 17, 18, 20*). In addition to the HCAd plasmid lacking the entire Ad genome minus Ψ and ITR regions, a helper virus (HV) featuring a deleted E1 region is required to provide the other viral proteins needed to rescue the HCAd vectors. The Ψ region of the HV is flanked by *loxP* inserts which, in a Cre recombinase-expressing cell line such as 293Cre, excises the packaging signal for the HV (*18, 20, 21*). Removal of Ψ from the HV genome helps ensure that the only the sequence with the transgene(s) of interest are packaged into the HCAd vector products (*14, 17, 20, 21*) (Figure 2). One issue with HCAd vectors is that when the genome size is less than ~27 kb, the vectors tend to spontaneously rearrange their DNA to return the genome to a size similar to the wild type (*22*). To combat this, HCAd vectors featuring transgenes smaller than 27 kb will also feature non-coding "stuffer" DNA (*22*).

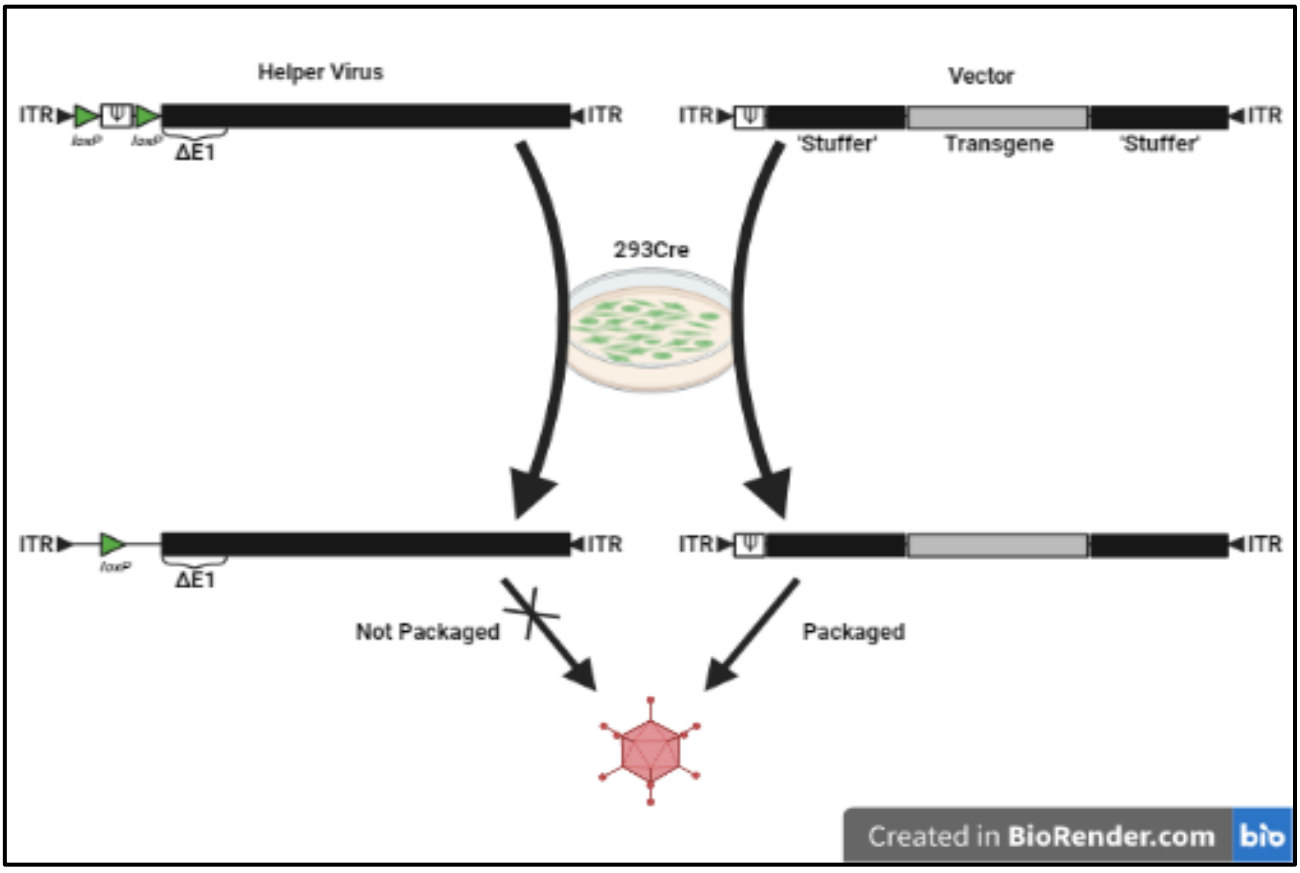

Figure 2: Schematic representing the creation of HCAd vectors with assistance from a helper virus.

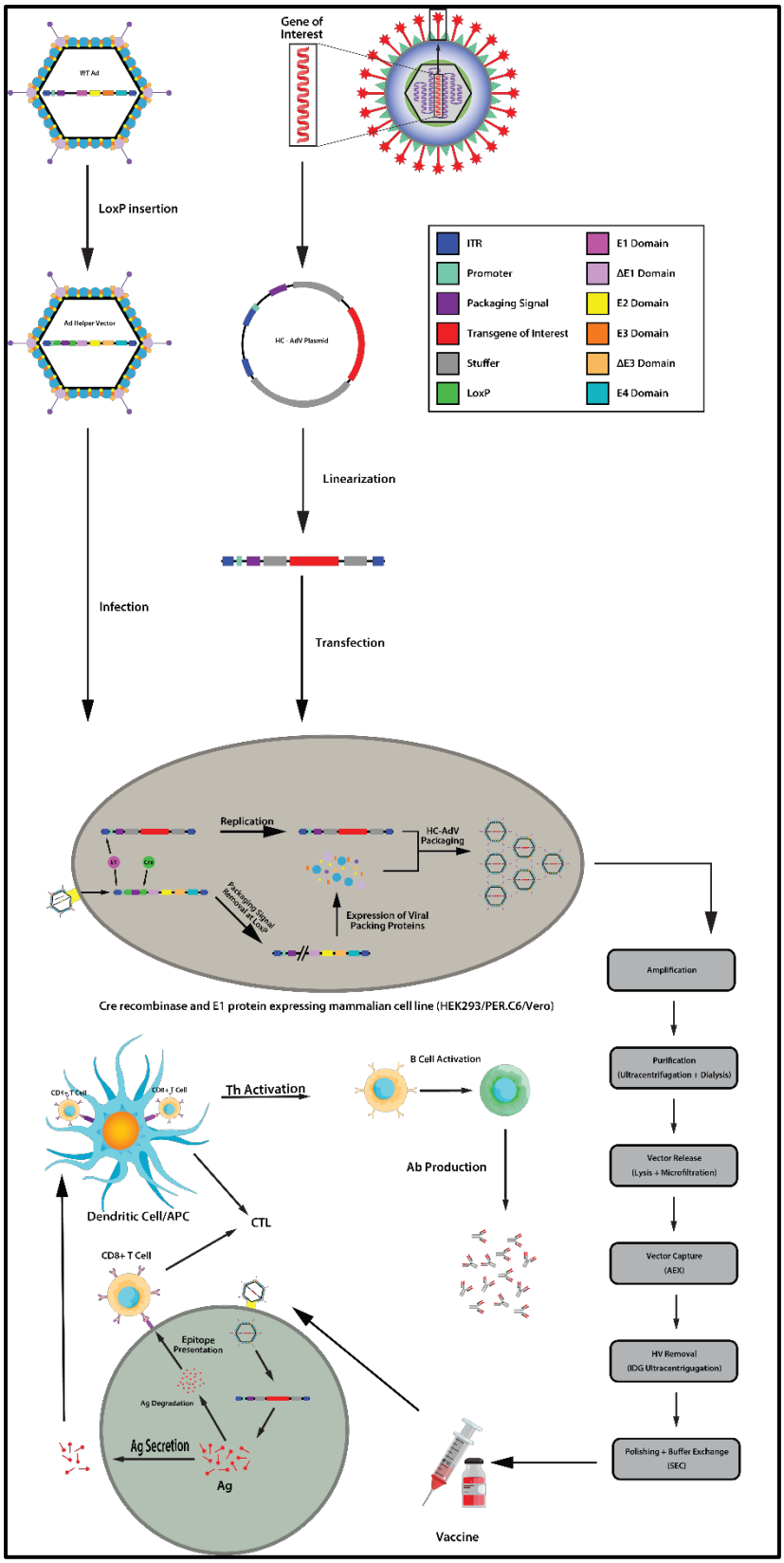

Figure 3. Overview of viral vector vaccine production process using HC-Ad vector as an example. This process will vary based on the type of virus used as a vector.

Inclusion of stuffer sequences has shown the additional benefit of increasing transgene expression, both *in vivo* and *in vitro* (*23*). A schematic showing the general process of HC-Ad vector creation can be found in Figure 3.

Cell Lines Used for Production

The most commonly used cell line for production of viral vector vaccines is human embryonic kidney (HEK) 293 cells. HEK293 cells are convenient due to the ease of culturing, rapid reproduction, tolerance for a variety of transfection methods, and efficient protein production (*24*). There are multiple variants of HEK293 cells that can be used for biopharmaceutical production, including HEK-293H, HEK-293T, and HEK-293EBNA1. For production of viruses and viral vectors, HEK-293T cells are typically used. HEK-293T cells express the simian virus 40 large T Ag and produce high titers of viral gene vectors (*25*). Another commonly used cell line are Vero cells. The Vero cell line was established from the kidney of an African green monkey in 1962, and since then multiple derivatives have been established (*26*). The extensive experience with production of viral vaccines, as well as enhanced viral production rates for a variety of viruses, make Vero cells an attractive choice for production cultures (*26*). Vero cells are used for production of the Ervebo vaccine (*26*). The cell line used for production of viral vector vaccines will depend on the vector platform used, as some viruses will reach sufficient titers more quickly in one cell line than another. Despite having multiple suitable choices, vector production in cell cultures remains a major limiting factor in the cost and rates of production for viral vector vaccines and further optimizations are critical for these vaccines to become commonplace.

While production of vectors in cultures is a well-characterized process, there is great difficulty to meet large demands for vaccine doses, such as the case of a pandemic. A common

way to amplify production speeds is through the use of bioreactors. A bioreactor is an apparatus used for growing an organism or virus at an industrial scale under controlled conditions. Currently, there are two primary options for bioreactors suitable for production of lentivirus and adenovirus vectors, iCELLis Nano and Univercells scale-X hydro, both of which show similar production efficiency (*27*). A brief overview of some common bioreactor systems and the vectors produced in them can be found in Table 1.

Table 1. Overview of common bioreactor systems used for vector production. LV-lentivirus, AAV-adeno-associated virus, Ad-adenovirus, HSV-1-herpes simplex virus type 1. Adapted from (*1*).

System	Vector(s)
HYPER*Flask* (Corning)	LV
Roller Bottles	AAV γ-Retroviral
Cell Factories (Nunc) or CellSTACKS (Corning)	LV γ-Retroviral AAV Foamy Virus
iCELLis™(Pall)	Ad AAV γ-Retroviral LV
Spinner Flask, Stirred Bioreactor	Ad γ-Retroviral Rabiesvirus Baculovirus AAV LV
CellCube (Costar)	HSV-1 γ-Retroviral
Wave Bioreactor (GE Healthcare)	LV AAV γ-Retroviral
Chemap CF-2000 bioreactor (Mannedorf)	LV AAV
Spinferm Bioreactor (Braun Biotechnology)	AAV
Celligen Plus (NewBrunswick Scientific)	Ad
scale-X™ hydro (Univercells)	Ad AAV LV

Purification Standards and Processes

Production of clinical grade viruses in production cells requires manufacturing processes both upstream and downstream that are expensive, time consuming, and at times lack scalability. In the case of Ad vectors specifically, one limiting factor is the necessity to grow vector cultures on mammalian cell lines (*28*). Prior to 2020 and the COVID-19 pandemic, Ad vectors had not been manufactured at a scale suitable for an effective response to a pandemic (*28*). Exact production processes will vary based on the type of vector used, however they should remain relatively consistent in facilities that specialize in a specific vector platform.

Production of viral vector vaccines, similar to other investigational drugs, must comply with current Good Manufacturing Practice (cGMP) regulations as required by the Food, Drug, and Cosmetic Act. As such, extensive quality control and characterization of recombinant vectors is a requirement for production of viral vector vaccines that are safe, pure, and effective.

Current Viral Vector Vaccines

Currently, only one viral vector vaccine has proven clinical efficacy and gained full FDA approval, Ervebo (*29*). rVSV-ZEBOV (Ervebo) is a replication competent live-attenuated Ebolavirus vaccine based on a recombinant vesicular stomatitis virus (rVSV) platform (*29*). Work on this vaccine originally started in 2004 with the creation of plasmids expressing the positive strand complement of the VSV genome with a transgene site coding for Zaire Ebolavirus glycoprotein (*30*). The viability of rVSV-ZEBOV as a vaccine was soon demonstrated in nonhuman primates, showing better results compared to a similar Ad-based vaccine candidate (*31*). Despite the early success, clinical development proceeded slowly, with phase I/II trials only beginning in 2014 (*32*). In 2019, during an outbreak in the Democratic Republic of the Congo, rVSV-ZEBOV was used and demonstrated an efficacy of 97.5% (*29, 33*). These results lead to European Medicines Agency (EMA) conditional marketing approval, WHO approval, and finally FDA approval in late 2019 (*29*).

Zabdeno/Mvabea is a two-dose, prime-boost regimen Ebola virus vaccine that utilizes AdVac (Janssen) and MVA-BN (Bavarian Nordic) technologies (*29*). The first dose administered is Zabdeno (Ad.26.ZEBOV) with Mvabea (MVA-BN-Filo) administered 8 weeks later (*29, 32*). Due to this vaccine following a prime-booster immunization strategy with 8 weeks between doses, Zabdeno/Mvabea is not suitable for outbreak response. Zabdeno is a FGAd vector based on the Ad serotype 26 (Ad26) platform featuring deletions in the E1 and E3 regions (*34*). PER.C6 cells were co-transfected with plasmids for the Ad.26.ZEBOV vector as well as a plasmid encoding the E1 and E3 regions of the Ad genome to allow production of vector progeny (*34*). The Mvabea dose is constructed from a modified Vaccinia Ankara virus (MVA) that encodes the Zaire Ebola virus Mayinga strain glycoprotein, Sudan Ebola virus Gulu strain

glycoprotein, and Ebola virus Taï Forest strain nucleoprotein, as well as the Marburg virus Musoke strain glycoprotein (*29, 35*). Preclinical studies featuring Zabdeno/Mvabea showed full protection against Ebola virus challenge in nonhuman primates, and phase I, II, and III clinical trials have thus far shown strong neutralizing antibody responses with CD4+ and CD8+ T cell responses in vaccinated individuals (*35-37*). Due to the relative rarity and danger associated with Ebola virus, efficacy data is not currently available. However, the EMA granted approval for Zabdeno/Mvabea based on predictive efficacy from animal studies in July of 2020 (*38*).

Benefits and Limitations of Viral Vector Vaccines

Traditional vaccines have a long history of successful clinical applications for globally problematic diseases such as polio, HBV, measles, and smallpox. However, they have been unable to match that same success with more difficult diseases like HIV/AIDS, HCV, malaria, tuberculosis, and RSV. Viral vector vaccines may provide a path towards our goals of creating vaccinations for these problematic diseases through a variety of beneficial properties. Traditional vaccines are typically paired with another substance, called an adjuvant, to prime the body's immune response when exposed to the vaccine Ag and induce production of neutralizing Abs against the desired pathogen. Viral vector vaccines are self-adjuvanted, as our immune systems have adapted over time to recognize viral intruders (*9*) without the requirement of additional immunostimulatory substances. Viral nucleic acids are recognized by Toll-like receptors 3, 7, 8, and 9, ultimately inducing secretion of pro-inflammatory cytokines and chemokines through MAPK and NF-κB pathways (*9*).

Safety is a topic of great concern when discussing the possibility of using a pathogen as a prevention method. This is especially the case with RC vectors, as there is a risk of recombination during each replication cycle. If the proper mutations occur, these vectors have

potential to regain some pathological functions that were lost in the process of attenuation. Depending on the type of virus used as a vector, the consequences of this could be quite severe.

Another issue with viral vector vaccines is production. Even in the case of vectors that are widely used, such as Ad vectors, generation of recombinant vectors remains time-consuming and relatively labor-intense due to the complexity of production (*39*). This issue proves to be a limiting factor for viral vector vaccines for two reasons. The first reason is that initially slow production speeds decrease the ability of these vaccines for outbreak responses. The second reason is that due to the complexity of downstream processes, it can be quite difficult for these vaccines to be produced where they are needed. Improvements are needed to standardize production processes to allow efficient and economically viable production of these vaccines (*39*).

Improving Viral Vector Vaccines

One way viral vector vaccine production can be improved is by increasing production efficiency. This can be done through improving yields, development of standardized manufacturing and purification processes, and developing methods to upscale production. The general process of vector production begins with developing the desired vector, amplification of a seed stock of that vector, followed by harvesting the vectors for downstream processing (*40*). Downstream processing is multistep process where collected vectors are purified, most commonly via depth filter clarification, tangential flow filtration (TFF), anion exchange (AEX) chromatography, and another TFF step (*40*). The complexity of this process is typically considered disadvantageous for the response to emerging pathogens (*28*). To optimize production yield, 3 primary factors need to be considered: multiplicity of infection at the time of infection, time that virus particles spend in the bioreactor, and the concentration and metabolic

state of the cells in the culture media. In other words, the process must optimize the ratio of viable cells to infectious particles, the harvest time that yields the maximum titer of vectors, and the health and nutritional supply to cell cultures used (*41*).

One potential way to improve vector yield is by developing new cell lines that produce vectors at higher titers more quickly. Recently, Wei et al. experimented with ways HEK-293 cells could be modified to make Ad vector production more efficient (*39*). To accomplish this, the researchers modified HEK-293T cells to create a new cell line, rapid adenovirus production and amplification (RAPA), that stably expresses pTP and E1A genes that are found in the E1 region of the Ad genome and are deleted in Ad-based vectors (*39*). The effect of these genes on vector production was measured by transfecting the cells with an Ad vector encoding red fluorescent protein (RFP) and gaussia luciferase (GLuc), AdR-GLuc (*39*). Parental cells that co-expressed pTP and E1A produced higher numbers of RFP+ cells in culture and GLuc activity increased 259% compared to normal 293T cells (*39*). Three days following transfection, the viral titers produced in RAPA cells were 6-fold higher than the titers produced in standard 293T cells (*39*). Additionally, functionality of produced vectors was tested by transducing mesenchymal stem cells with Ad vectors containing a transgene encoding BMP9 (*39*). Increased BMP9 in mesenchymal stem cells leads to differentiation to osteogenic precursor cells (*39*). AdBMP9 vectors produced in RAPA cells induced expression of alkaline phosphatase, a marker of early osteogenic differentiation, at levels nearly 4-fold higher than vectors produced in standard 293T cells (*39*). Integration of RAPA cells, or another cell line that enhances production of vectors, into existing bioreactor systems could greatly enhance production efficiency of Ad vectors to be used as vaccines. Development of additional cell lines to be used for viral vector platforms

utilizing different viruses could improve the viability of large-scale production of viral vector vaccines to meet global needs.

Expense of production is another important factor in improving viral vector vaccines efficacy at a global scale. Since resource-poor regions show disproportionately high burdens of disease, ensuring low cost of production and the ability to use standard equipment present at most sites is of utmost importance (*28, 42*). Recently, Joe et. al. set out to create a large-scale manufacturing strategy for Ad vectors that was cost efficient, quick to produce large volumes with global availability, and consistently high quality (*28*). The developed model achieved low costs by utilizing equipment and materials that are already widely available (*28*). Additionally, they simplified downstream processing by removing a step of TFF and demonstrating efficient recovery of purified vectors through direct loading of clarified lysate onto AEX (*28*). Combining these improvements, Joe et. al. were able to nearly double the productivity of previous published batch or fed-batch processes while keeping a resulting price per dose under 1 EUR (*28*).

Another area that viral vector vaccines could be improved is evasion of preexisting neutralizing Abs against the vectors. This is most significant when considering Ad and AAV based vector platforms, which can carry a relatively high seroprevalence based on serotype of virus and population demographics. One way of doing this is by making modifications to the vector capsid via genetic or chemical approaches (*43*). Some genetic approaches for Ad vectors specifically could include insertion of peptides into the fiber, hexon, or pIX capsid proteins; pseudotyping of fiber proteins with fibers of a different serotype; introduction of point mutations in fiber or hexon proteins; introduction of cysteine-rich moieties in the hexon or fiber proteins; and exchange of hypervariable loops (HVRs) of hexon proteins with HVRs of a different serotype (*43*). This type of modification may find additional usage to enhance targeting of

vectors for specific cell types through use of adapter molecules that bind to capsid fiber proteins (*43*).

There are two primary applications for genetic modifications to the viral coat: evasion of immune defenses and enhanced tropism towards a specific cell type or tissue. The most obvious genetic modification to increase host immune evasion is through the use of HC-Ads. By removing the vast majority of the viral genome, viral factors that trigger an immune response are also reduced. However, since efficient packaging requires a genome size of 27 kb and the total capacity of HCAd vectors is around 36kb, stuffer DNA must often be used to reach the minimum packaging threshold (*44*). By using sequences of intronic human genes, such as hypoxanthine-guanine phosphoribosyltransferase (HPRT) as stuffer DNA, vectors can achieve more prolonged transgene expression while minimizing immune response via CTLs (*23*). Another way to avoid immune detection is by directly mutation the highly variable regions (HVR) of the Ad hexon protein. By deleting a stretch of negative amino acids in HVR1 while also including a mutation to FX, binding of vectors by IgM antibodies and complement components can be ablated (*45*). To summarize the work on this topic by Stasheva et al., Ad vectors with mutated HVR1s resist inactivation through liver cell sequestration and blood factors while not inducing hepatotoxicity (*45*). To my knowledge, this is the most complete example of genetically modifying Ad vector capsids to evade immune detection to this day. That being said, this is an area of active research and requires further development before seeing use in clinical trials.

Another way to modify vectors to evade immune responses is through chemical shielding. This method effectively “caps” the vector in a chemical coat to prevent unwanted recognition by both innate and adaptive immune cells. Two compounds commonly used for this

purpose are polyethylene glycol (PEG) and poly-N-(2-hydroxypropyl)methacrylamide (pHPMA) (*43*).

A recent study by N. N. Francini et al. explored how modifications to pHPMA coating could increase resistance of Ad vectors to neutralizing Abs without diminishing effects *in vivo (40)*. By incorporating diazonium salts onto a pHPMA backbone, the researchers optimized Ad capsid shielding against neutralizing Abs (*40*). The result was Ads that retained biological activity in vitro and in vivo, while avoiding neutralization by Abs (*40*). Additionally, chain length of the modification to pHPMA was found to modulate both the protection from neutralizing Abs and transfection efficiency, suggesting that fine tuning modifications to the polymer coat may allow better control over preservation of viral activity, circulation time, and internalization at target cells (*40*).

Conclusion

Viral vector vaccines are a promising method of modern immunization that are becoming important tools in the development of vaccines against emerging and problematic pathogens. However, some aspects of viral vector vaccines are distinctly limited at this time. Limiting factors include seroprevalence of neutralizing Abs against commonly used vectors, manufacturing process limitations, and safety concerns. The potential improvements presented in this review are just a few possible directions to take to increase the viability of viral vector vaccine usage at a large scale. Further development and employment of these and other novel techniques and improvements will fortify the prospect of viral vector vaccine usage for emerging pathogens and those where traditional vaccine approaches have failed.

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