Tampere University

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ADENOVIRUSES AS A VIRAL VACCINE PLATFORM

Faculty of Medicine and Health Technology
Bachelor's Thesis
October 2022

ABSTRACT

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Biotechnology and Biomedical Engineering
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Since the discovery of first adenoviruses almost seven decades ago, their use as vectors has been extensively studied. Today, they are the most common way to deliver transgenes in laboratories and are a widely used platform in novel vaccine development. Due to broad host tropism of adenovirus vectors and their ability to induce both strong humoral and cellular immune responses while carrying the transgene antigens, they are optimal candidates for inducing immunity against infectious viral diseases. Because adenoviral vector vaccines can be developed and produced rapidly with pre-existing manufacturing facilities and large-scale production possibilities, they offer a highly potential weapon against newly arising worldwide pandemics such as COVID-19.

In this bachelor's thesis, the characteristics of adenoviruses such as genome, structure and infection method are summarized. The means for developing adenoviral vectors and manufacturing adenoviral vector vaccines are explained and the challenges these are facing are discussed. Also, the latest advances and features of adenovirus vector vaccines against COVID-19 and Ebola are summarised. Lastly, the future perspectives of AdV vector vaccines are discussed.

Keywords: adenovirus, adenovirus vector, vaccine, vaccine development, COVID-19, Ebola

TIIVISTELMÄ

Laura Mäkelä: Adenovirusvektorit rokotekehityksessä
Kandidaatintutkielma
Tampereen yliopisto
Bioteknologia ja biolääketieteen tekniikka
Lokakuu 2022

Adenovirusten hyödyntämistä geenivektoreina on tutkittu siitä saakka, kun ne ensimmäisen kerran löydettiin melkein seitsemänkymmentä vuotta sitten. Nykyään ne ovat yleisin tapa siirtogeenien kuljetukseen laboratorioissa ja ovat laajalti käytetty tekniikka rokotekehityksessä. Adenovirukset sopivat vektoreiksi hyvin siksi, että ne pystyvät infektoimaan monia eri kudoksia ja solutyyppejä sekä pystyvät tuottamaan vahvan solu- ja vasta-ainevälitteisen immuunipuolustusreaktion. Koska adenovirusvektorit pystyvät kuljettamaan niiden genomiin lisätyn rokoteantigeenin geenisekvenssin soluun samalla matkien luonnollista virusinfektiota, ne ovat optimaalinen tapa parantaa vastustuskykyä erilaisia virusperäisiä infektiotauteja vastaan.

Tällä hetkellä käynnissä on monia prekliinisiä ja kliinisiä tutkimuksia, joissa tutkitaan adenovirusvektorirokotteiden toimivuutta erilaisia viruksia vastaan. Adenovirusvektorirokotteita voidaan kehittää suhteellisen lyhyessä ajassa ja niitä voidaan tuottaa nopeasti isoja määriä, sillä adenovirusvektorien kehitykseen vaadittavat protokollat ja metodit sekä suurimittaiseen tuotantoon tarvittavat tuotantolaitokset on kehitetty jo aikaisemmin. Kuten nähtiin COVID-19 -pandemiassa, rokotteet, joita pystytään kehittämään ja tuottamaan nopeasti, ovat ensisijaisen tärkeitä taisteltaessa nopeasti leviäviä viruksia vastaan.

Aluksi tämä tutkielma esittelee adenoviruksen erilaisia ominaisuuksia, kuten sen rakennetta, genomia ja infektiotapaa. Tarkastelussa ovat myös adenovirusvektorirokotteiden hyvät puolet sekä kehitys- ja tuotantomahdollisuudet. Lisäksi pohditaan kehitykseen liittyviä ongelmia ja haasteita. Tämän jälkeen kerrotaan adenovirusvektorirokotteiden hyödyntämisestä COVID-19 -pandemiassa sekä niiden käytöstä ebolaa vastaan. Kyseisten rokotteiden ominaisuudet esitellään viimeaikaisten tutkimusten valossa. Viimeisenä pohditaan adenovirusvektorirokotteiden tulevaisuutta.

Avainsanat: adenovirus, adenovirusvektori, rokote, rokotekehitys, COVID-19, ebola

PREFACE

I would like to thank my supervisor Amirbabak Sioofy-Khojine and the whole Virology research group for introducing me to the world of viruses. I am grateful for the guidance and encouragement throughout the writing of this thesis.

I also want to thank my friends and family who have been indispensable support during this process.

Tampere, 23rd of October

Laura Mäkelä

CONTENTS

1. INTRODUCTION

Vaccines are one of the most successful inventions of modern medicine against infectious diseases preventing 4-5 million deaths yearly and reducing healthcare costs with over 450 billion euros. (Gebre et al., 2021; https://www.who.int/news-room/facts-in-pictures/detail/immunization, viewed 11.6.2022) To this date, there are available vaccines against 25 different diseases caused by viruses, bacteria, and parasites (https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases, viewed 11.6.2022). Despite these achievements, there are still many other deathly infectious diseases for which there isn't any functional vaccines. This has led the field of vaccine development to search for novel vaccine technologies besides traditional vaccines to be able to face this problem.

One of these novel methods is using viruses as vaccine vectors. The natural function of a virus is to enter the cell and replicate the genome it is carrying by using the replication machinery of the host cell. Ideally, by utilizing these mechanisms transgenes inserted into the viral genome could be transferred into the host cell where the transgenes would be expressed. This idea has been pursued for some time now in novel vaccine development and also in the fields of gene therapy, oncology, and immunotherapy with varying results. Thus far for example retroviruses (RV), adenoviruses (AdV), adeno-associated viruses (AAV), lentiviruses (LV) and herpes viruses (HSV) have been genetically modified into vectors (Gebre et al., 2021; Zhang & Zhou, 2016). From these, especially AdV vectors have been studied extensively and used successfully in designing novel vaccines. For this reason, this thesis focuses on AdV vector based viral vaccine platforms.

AdVs were used as vectors for the first time in 1992 for gene therapy purposes. The ability of AdV to infect multiple cell types was thought to be the key for persistent transgene expression for people who were suffering from genetic disorders. However, the high immunogenicity of AdV vectors that makes them suitable platform in vaccine development today, made them a challenging method for gene therapy. (Gebre et al., 2021)

Due to the potential of AdV vectors for vaccine use, many strategies and protocols have been developed for AdV vector design and production. To ensure the safety of the vectors the

genome of AdV is modified so that the virus is non-pathogenic, and it can't replicate by deleting either only early 1 (E1) or both E1 and early 3 (E3) region from the viral genome. (Gebre et al., 2021) Easily modifiable genome and many other advantageous features such as already existing large-scale production possibilities and safety make AdV vectors today highly desirable and pursued vaccine platforms.

AdV vectors have been used successfully so far in novel vaccines against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that caused the worldwide COVID-19 pandemic. (AstraZeneca, 2022) Also, AdV vector vaccines against Ebola have been used. (Gebre et al., 2021) In addition to this, there are many studies, pre-clinical and clinical, where AdV vector-based vaccines are tested against viruses such as human immunodeficiency virus (HIV) and influenza viruses. Success in these ongoing studies could potentially save hundreds of thousands of deaths caused by viral infections each year. (Zhang & Zhou, 2016; https://ourworldindata.org/hiv-aids, viewed 5.7.2022)

2. ADENOVIRUSES

AdVs are non-enveloped double stranded (ds) DNA viruses enclosed with icosahedral capsid. They belong to *Adenoviridae* family that is divided into six generas: *Atadenovirus, Aviadenovirus, Ichtadenovirus, Siadenovirus, Testadenovirus* and *Mastadenovirus. Mastadenoviruses* include all human adenoviruses. (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=10508, viewed 9.6.2022) Adenoviruses were discovered in 1953 when Rowe et al. published an article describing an agent that causes human adenoid degeneration (Rowe et al., 1953). Later this agent was named as human adenovirus (HAdV) after the human adenoid tissues from which it was isolated for the first time.

To this date there have been reported to be over 120 AdV serotypes. These are encountered in different species of mammalians, fish, birds, and reptiles (Kulanayake and Tikoo, 2021). More than 70 of these serotypes are HAdVs and can be divided into seven groups from A to G based on various characteristics. Serotypes that belong to groups B, C, and E infect both upper and lower respiratory tracks, and the serotypes belonging to groups B, D and E infect conjunctiva. Serotypes of groups F and G cause infections in gastrointestinal tract. Usually, these infections

are mild for those with intact immune system. (Radke & Cook, 2018) However, HAdV infections can be severe for immunologically compromised people (Kulanayake and Tikoo, 2021).

2.1 GENOME

Genome of AdV consists of non-segmented, usually 26 to 48 kb long, double stranded DNA (dsDNA). (Kulanayake and Tikoo, 2021). As seen in *Figure 1* there are internal terminal repeats (ITRs) in both ends of the DNA strand from where the replication starts. Next to the 5' ITR there is a viral packaging sequence essential for the correct packaging of the viral progeny. (Lee et al., 2017; Saha et al., 2014) Coding regions of the genome can be classified into early (E) and late (L) regions based on the time point they are transcribed post-infection, prior to or following the DNA replication. Transcripts and proteins encoded by the early genes such as E1A, E1B, E2, E3 and E4 activate transcription of other viral genome regions and modify the cellular environment suitable for the synthesis of the viral DNA and production of viral progeny. For example, the E1A products activate viral gene expression whereas proteins of the E3 region possess counter-immune system functions. Late regions, L1-L5, encode mainly the alternatively spliced structural proteins of AdV that are transcribed only after the major latent promoter (MLP) is activated by synthesis of viral DNA. (Saha et al., 2014)

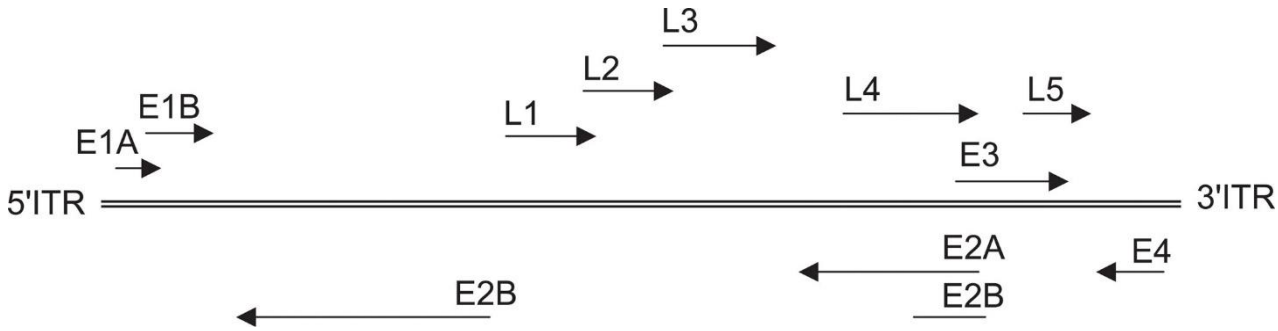

Figure 1: Illustrative picture of AdV's genome. *(modified from Tatsis & Ertl, 2004)*

2.2 STRUCTURE

AdVs' icosahedral capsid consists of three major and four minor capsid proteins (*Figure 2)*. The protein compositions vary between different genus of *Adenoviridae* family. (Kulanayake and Tikoo, 2021) The major capsid proteins hexon, penton and fibre are essential not only for forming a protective shell around the core and viral DNA but also for the host cell entry. The fibre contains a knob structure on its C-terminal end that is the key structure for the attachment to the host cells attachment receptors. On the other hand, the penton mediates the interaction between the virus and host cell integrins as a part of the cell entry. (Stasiak & Stehle, 2020)

The minor capsid proteins, protein (p) IX, pIIIa, pVI, and pVIII stabilize the structure of the virion through protein-protein interactions (Kulanayake and Tikoo, 2021).

The mastadenovirus (mAdV) capsid proteins surround the core containing the viral DNA and core proteins pV, pVII, pVIVa2, Mu, terminal protein (TP) and adenovirus protease (AVP). The core proteins mediate different protein-protein and protein-DNA interactions inside the virion condensing the DNA to fit inside the capsid. Also, they are essential throughout the infection starting from the disassembly of the viral capsid upon entry, ending with the assembly of viral progeny. (Kulanayake and Tikoo, 2021)

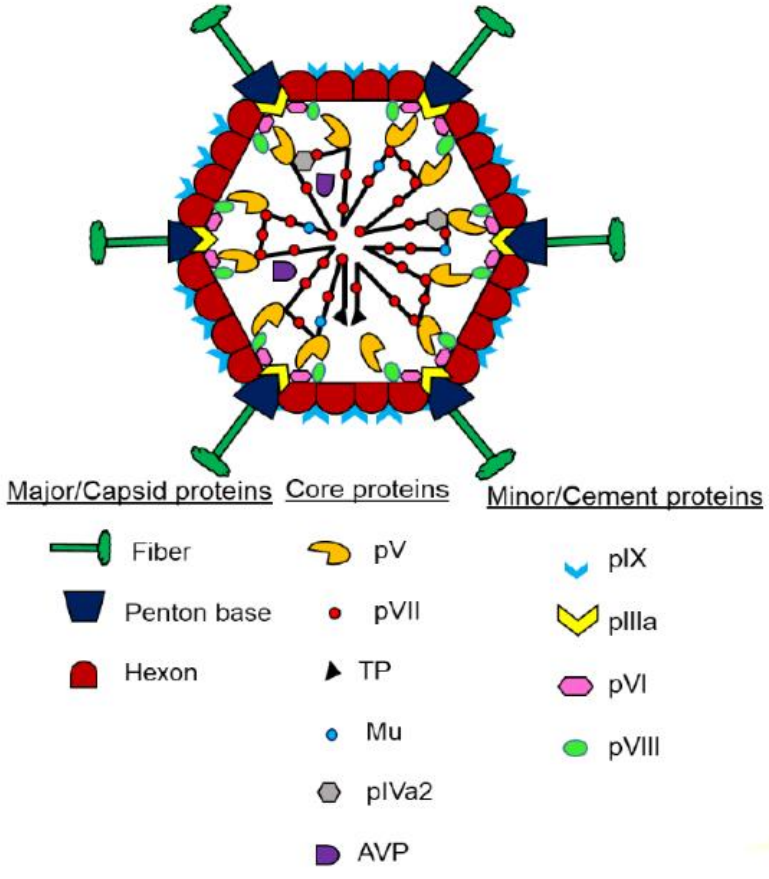

Figure 2: Illustrative picture of the structure and protein composition of AdV. Picture is not proportional. (Kulanayake and Tikoo, 2021)

2.3 AN OVERVIEW OF THE INFECTION AND REPLICATION

As AdVs have broad host tropism they can infect different types of cells and tissues. This is partly due to AdVs ability to utilize plenty of different host cell receptors for cell entry. Two types

of receptors are needed for AdV to gain access into the host cell: the attachment receptor and the integrin entry receptor. Five best known and structurally studied attachment receptors are Coxackie and Adenovirus Receptor (CAR), CD46, desmoglein-2 and the glycans GD1a and polysialic acid. (Stasiak & Stehle, 2020) The receptors used for cell entry are serotype specific.

At the beginning of the infection the knob domain of the fibre protein binds to an attachment receptor. This is followed closely by the binding of the viral penton to the integrin entry receptor. The integrin-penton interaction stimulates integrin clustering, which leads to downstream signaling for the cell to take the virus in via endocytosis. (Stasiak & Stehle, 2020) The hexon protein also interacts with scavenger receptors assisting the cell entry. Endocytic intake induces partial uncoating of the virus and release of lytic pVI that breaks the membrane of the endosome releasing AdV to the cytoplasm. (Flatt & Butcher, 2019; Kulanayake and Tikoo, 2021)

Transition of the virus from cytoplasm to the nucleus occurs by utilizing cells natural intracellular traffic machinery and activation of nuclear localization signal (NLS) containing viral proteins such as pVII. (Flatt & Butcher, 2019) Partially uncoated AdV binds to dynein-dynactin motor that transports it along microtubules to the direction of its minus end near the centrosome and the nucleus. How AdV is able to do this is not well understood. Once AdV is near the nucleus, it binds to nuclear pore complex (NPC) and undergoes the final uncoating stage, releasing the viral DNA into the nucleus. Several NLS containing pVIIs bind to viral dsDNA enabling the use of nuclear transportation proteins importins to get into the nucleus. (Flatt & Butcher, 2019) After DNA replication pVII also translocates the newly synthesized structural proteins from the cytoplasm to the nucleus prior to formation of viral progeny (Lee et al., 2017).

To replicate viral genome and to produce needed viral proteins, AdV exploits replication machinery of the cell without integrating its genome into host genome. The ITRs act as origin of replication in both sides of the linear viral dsDNA. Before the replication of viral DNA, early genes are transcribed since the transcripts are needed for regulatory purposes both in DNA replication and expression of the L region, and for packaging the viral progeny later. When replicated DNA and core proteins have been packaged into newly formed capsids, viral progeny is released from the cell via lysis killing the host cell. (Lee et al., 2017)

3. ADENOVIRUS VECTORS

Nowadays AdV vectors are one of the most common ways of delivering transgenes into mammalian cells in laboratories and clinical trials (Saha et al., 2014; Lee et al., 2017). One of the reasons for this is that adenoviruses are reasonably safe to handle in the laboratory and use as they do not cause severe infections for immune competent people (Radke & Cook, 2018). This facilitates the development and production of AdV vectors when high level biosafety class premises are not required. Also, because AdV vectors have been widely studied and they have been tested in many clinical trials, safe dosage and administration routes are well known (Lee et al., 2017). Furthermore, the fact that AdV doesn't integrate its viral genome into the host cell's also increases the safety of using AdV vectors as random insertion mutagenesis is more unlikely to happen compared to when lentiviruses or retroviruses are used as vectors. (Zhang & Zhou, 2016)

Another advantageous feature especially as a vaccine is that AdV vectors have high immunogenicity and trigger both innate and adaptive immunity. The main trigger for innate immunity is the recognition of viral genome of AdV vector by pattern recognition receptors (PRRs). This in turn, activates the downstream adaptive immunity and antibody production. As the vector as such inflicts immune response enough, there is no need for adjuvants that are usually crucial in the case of traditional vaccines. This eases the development of the AdV vector-based vaccines when the composition of the vaccine can be simpler. (Gebre et al., 2021)

What adds to the utility of AdV vectors from vaccine perspective is that AdVs can infect wide range of different cell types and both dividing and non-dividing cell populations (Lee et al., 2017). Broad host tropism leads to increased probability of successful transgene expression and consequently increased yield of antigen *in situ*. Simultaneous transgene antigen expression with activation of innate immunity is the key for activation of adaptive immunity to induce vaccine immunity including antibody production and forming the immune memory. (Gebre et al., 2021)

3.1 VECTOR DESIGN

Adenoviruses are genetically modified in order to make them suitable for being used as vectors. The most common modifications for vaccine purposes are deletion of E1 coding cassette with or without additional deletion of E3. (Gebre et al., 2021; Zhang & Zhou, 2016). The coding

cassettes can be seen in Figure 3. Vectors with these deletions are called the first-generation vectors. For gene and cell-based therapy purposes a second generation AdV vectors have been developed from which other early regions E2 and E4 have been also deleted. AdV vectors from which the whole viral genome, excluding the ITRs and the packaging signal, has been deleted are the third-generation vectors. These, so called high-capacity adenovirus vectors (HC-AdVs) or gutless AdVs, were especially developed to be as minimally immunogenic as possible and thus are not optimal for vaccine use. (Lee et al., 2017)

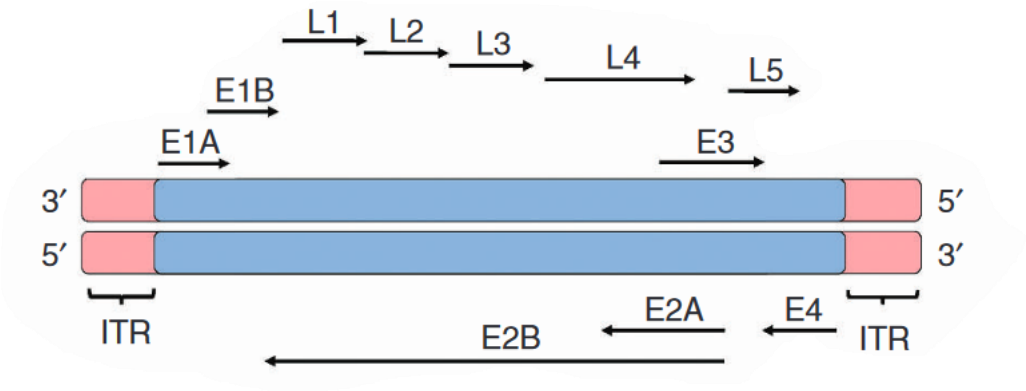

Figure 3: Modified schematical picture of coding cassettes of AdV. E1A, E1B and E3 are normally deleted from vaccine vectors. (Afkhami et al., 2016)

AdV vectors can be replication competent or replication incompetent and this depends on the deletion of E1 coding cassette. E1 genes affect the expression of other viral genes that are needed in the end of replication cycle. Deletion of the whole E1 or part of it makes the virus non-replicating. (Zhang & Zhou, 2016) Thus, deletion of E1 increases the safety of AdV vectors to be used in vaccines while also freeing space for the insertion of vaccine antigen transgene. (Gebre et al., 2021)

The deletion of E3 is done mainly to free more space for transgene insertion. However, as E3 genes are known to help the infected cell to hide from and decrease the activation of innate immunity, this deletion is optimal for vaccine vectors. (Gebre et al., 2021) Together with deletion of E1 and E3 AdV vectors can carry relatively large transgene cargo up to about 8 kb in size allowing the generation of multivalent vectors against complex pathogens (Lee et al., 2017; Saha et al., 2014; Afkhami et al., 2016). Deleting the viral genome in this magnitude also highly reduces the produced viral antigens resulting in decreased harmful immune response against the vector. (Afkhami et al., 2016) Activated immune system against the vector would complicate the cell entry of the vector deteriorating the wanted expression of antigen transgene and later production of antibodies.

The genome size in whole must be considered while designing novel vectors as the size affects the stability of the vector. It has been shown that adenoviruses modified genome below 75 % or above 105% of the wild type's size is not stable and tends to rearrange while stable virions, and in this case stable vectors, must have genome size of 90% of the wild type's size. (Saha et al., 2014) Therefore, if the vectors used in given vaccine have deletions in both E1 and E3 the inserted gene must cover the size of deleted coding cassettes. In case of AAV vectors it has been suggested that non-coding so called stuffer DNA could be used to achieve the genome size close to wild type's size if the inserted transgene can't cover the deletions. (Saha et al., 2014) This could be possible solution for AdV vectors also if needed.

Another factor that must be considered during the vector design process is the serotype of AdV. It has been shown that there is a difference in immune response with different serotypes. (Zhang & Zhou, 2016) The different immunogenicity profiles mean that certain highly immunogenic serotypes such as human adenovirus 5 (HAdV5), HAdV26, HAdV35 and HAdV11 can be more successful in vaccine use than others. (Mendonça et al., 2021) In addition, as AdVs do not usually cause severe infections in people there is a broad pre-existing immunity against the common serotypes, HAdV5 being one of them. (Zhao et al., 2018) This limits the use of these serotypes and can hinder the efficiency of them as most people have neutralizing antibodies (NAbs) against them. This obstacle is avoided by using less seroprevelant HAdVs such as HAdV26 or HAdV35, or AdVs of different species such as chimpanzee (ChAdV) Another option is to modify the vector capsid so that the vectors are able avoid the pre-existing NAbs. (Gebre et al., 2021) Also, serotype specific NAbs can be problematic in cases when a full protection against a disease requires more than one dose of vaccine. After the first dose of AdV vector vaccine NAbs will also form against the virus which can affect the efficiency of the second dose when the same serotype is used as vector in the booster injection. This, however, can be avoided by using different serotypes at each dose. (Mendonça et al, 2021)

3.2 VECTOR PRODUCTION

Currently there are two production strategies that are mainly used for insertion of transgene antigen into AdV vector. (Vemula & Mittal, 2010; Afkhami et al., 2016) These are homologous recombination in mammalian cells and direct cloning *in vitro* which will be covered in detail below. Also, alternative methods including use of *E. coli* and yeast artificial chromosome (YAC) in yeasts for homologous recombination as well as harnessing bacteriophage P1 Cre/LoxP recombination system for the same purpose have been reported. (Afkhami et al., 2016)

3.2.1 HOMOLOGOUS RECOMBINATION

The most widely used method, the homologous recombination, is carried out by using two different plasmids in mammalian cell (*Figure 4)*. The first so called shuttle plasmid contains a 5' end of the adenoviral genome and an inserted transgene replacing the E1 coding cassette of the virus genome. The E3 region can also be replaced with a transgene in this plasmid. The other plasmid contains adenoviral genome slightly overlapping the 3' end of the shuttle plasmid genome and without the coding cassettes that have been replaced with transgenes in the shuttle plasmid. These are transfected into mammalian cells where homologous recombination happens between the plasmids producing recombinant adenoviral genome with inserted transgenes. (Afkhami et al., 2016; Vemula & Mittal, 2010)

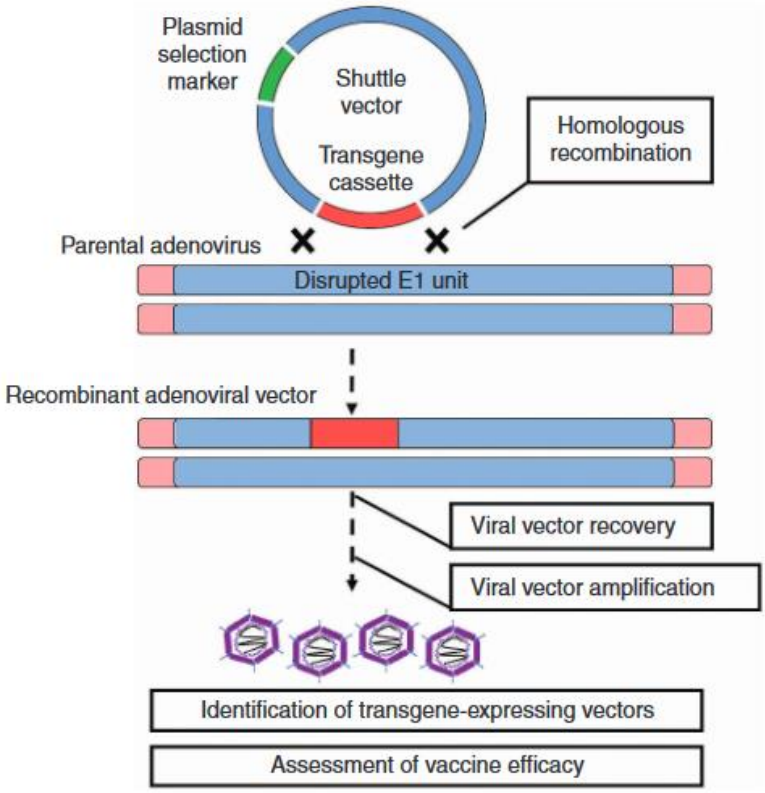

Figure 4: Homologous recombination technique for adenoviral vector production. Homologous recombination occurs in E1 providing mammalian cell line. (Afkhami et al., 2016)

The cells used for homologous recombination are the same that are used for viral vector packaging. As the E1 cassette is vital for the replication of adenovirus, recombinant vectors that lack it must be propagated in mammalian cells such as human embryonic kidney (HEK-293) cells that provide the functions of E1. HEK-293 line was developed in 1977 by making an insertion of E1 from HAdV5 to produce the first generation E1 deleted AdV vectors. (Kovesdi &

Hedley, 2010) It has been and is still widely used cell line for producing AdV vectors for both preclinical and clinical applications. (Afkhami et al., 2016)

Even though homologous recombination is the most common way of producing adenoviral vectors it has its own disadvantages. Firstly, homologous recombination of two plasmids doesn't occur efficiently in mammalian cells. This can lead to production of vectors that don't have the desired transgene cassette. (Afkhami et al., 2016) Due to this, recovering large enough stock of vectors that efficiently express the transgene may require several rounds of viral propagation and purification slowing down the vaccine production process. Furthermore, the production of replication-incompetent vectors in E1 providing cells such as HEK-293 can lead to trace amount of replication-competent adenoviral (RCA) vectors which is against the safety rules in vaccine production. (Afkhami et al., 2016; Vemula & Mittal, 2010) This is because of a massive homology between most E1 deleted plasmids produced via homologous recombination and the E1 sequence from HAdV5 in HEK-293 cells, that sometimes leads to unwanted homologous recombination between the plasmids and the cellular genome. RCA contamination is highly undesirable and thus highly monitored when producing vector stocks for clinical use. (https://www.genengnews.com/sponsored/overview-manufacturing-adenoviral-vectors-at-large-scale/, viewed 13.7.2022)

3.2.2 DIRECT CLONING

The other way of producing recombinant adenoviral vectors is the direct cloning (*Figure 5)* in which two plasmids are used. The first one works as a shuttle vector with the inserted transgene and the other one consists of adenoviral genome with the E1 cassette replaced by a linker region. Both the linker region and the transgene insert are constructed so that they are flanked by the same rare restriction sites such as PI-Sce I and I-Ceu I that can't be found from the adenoviral genome. By using standard molecular cloning techniques, the transgene can then be ligated to adenoviral backbone. Produced recombinant plasmids can be amplified in suitable bacterial cell line after which they also are transfected into packaging cells such as HEK-293. (Afkhami et al., 2016)

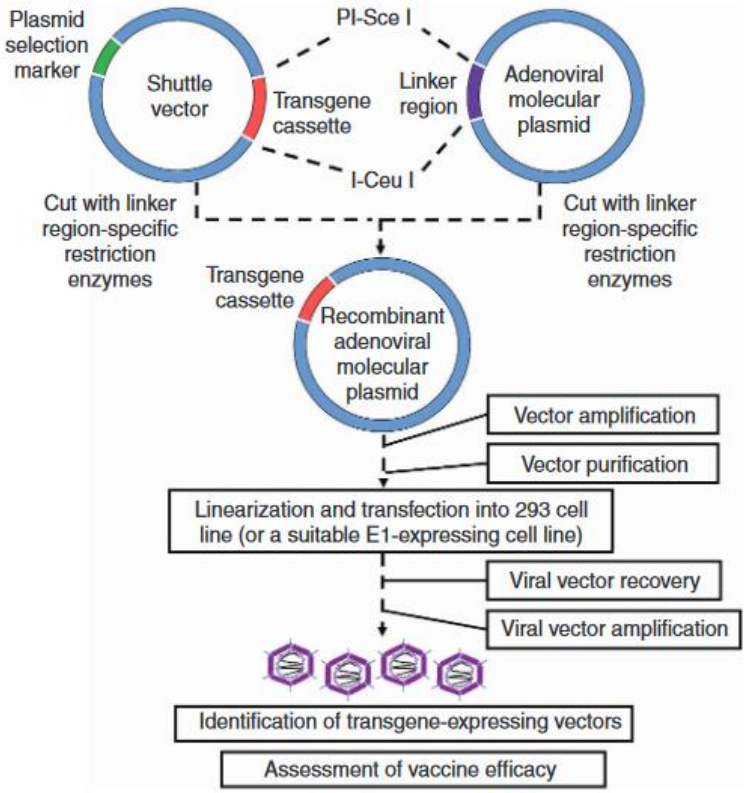

Figure 5: Direct cloning technique for adenoviral vector production. (Afkhami et al., 2016)

While direct cloning can be more challenging method from these two due to the requirement of precise sequence map of the whole adenoviral genome and wider range of different reagents and molecular biology techniques it has the means of overcoming the problems homologous recombination has. Because the recombinant adenoviral plasmids are produced *in vitro* and only after that transfected to packaging cell line there is no concern of RCA contamination. (Afkhami et al., 2016) This is a highly desirable feature for clinical applications. Direct cloning also evades the need of several rounds of viral purification since there is no risk of missing the transgene in the product as described for homologous recombination method earlier. However, both methods of homologous recombination and direct cloning share a common defect that the origin of the E1 coding cassette of HEK-293 cells is HAdV5 and is not fully compatible with some AdV serotypes used as a vector. Consequently, using HEK-293 cells can provide lower yields of viral vectors with these serotypes and slow down the vaccine production. This, however, can be overcome by using a different cell line such as human embryonic retinal (PRE.C6) cells or transfecting the packaging cells with a third plasmid expressing the proteins of E1 instead. (Kovesdi & Hedley, 2010; Akhami et al., 2016)

3.3 MANUFACTURING

Due to the long history of using AdV vectors in preclinical and clinical applications the means for large-scale production of AdV vector vaccines do already exist. Produced AdV vectors can be easily purified from the packaging cell lysate by caesium chloride (CsCl) gradient ultracentrifugation. (Zhang & Zhou, 2016) Other methods such as affinity purification chromatography and ion change chromatography have also been used with recombinant viral vectors and could be implemented for purification of AdV vectors. (Vemula & Mittal., 2010)

The vectors can be expanded in large-scale in both wave and stirred tank suspension culture bioreactors using PER.C6 cells or HEK-293 cells that have been adapted to grow in suspension. For example, Janssen has been able to produce millions of doses of adenovirus vector-based Ebola vaccines by using PER.C6 cells. (Gebre et al., 2021) Suspension bioreactors also provide an opportunity to scale up to even 10 000 litres capacity enabling rapid vaccine production in cases of emerging epidemics and pandemics (Vemula & Mittal, 2010)

4. ADENOVIRUS VECTOR BASED VACCINES

Even though AdV vector vaccines have been a long-pursued idea, only relatively few vaccines have actually reached the needed approvals for human use applying this technology. The biggest successes with AdV vector viral vaccine platforms have been achieved so far with COVID-19 pandemic with millions of AdV vector vaccine doses given to humans across the world. (Mendonça et al., 2021) This, however, has to be interpreted with caution since the licence for the vaccine was for emergency use in the beginning and the use in different age groups was also limited. In addition to COVID-19 vaccines, an Ebola vaccine using AdV vector platform, Zabdeno by Janssen-Cilag International N.V., has gained a marketing authorization from the European Medicines Agency (EMA) to be used in Europe. (https://www.ema.europa.eu/en/medicines/human/EPAR/zabdeno#authorisation-details-section, viewed 4.8.202)

Due to the suitability of AdV vectors for vaccines, they have also been studied against numerous other viral pathogens (*Table 1)*. Studies aiming to design AdV vector-based vaccines against bacteria and protozoa have also been done for example for human Tuberculosis and Malaria. (Gebre et al., 2021; Zhang & Zhou, 2016) However, as AdV vectors mimic natural viral

infection, thus inducing immune responses especially against viruses, their use as a vaccine has mainly been focused on preventing viral diseases. Many different AdV serotypes have been utilized in vaccine studies, including some rarer serotypes such as canine AdV (CaAdV). However, most of them are modified from HAdV or ChAdV.

Table 1: List of AdV vector-based viral vaccines under the development or in use. All mentioned serotypes are not used in one vaccine but in some cases two or more serotypes are utilized in different vaccine doses.

Pathogen	*Serotype of the AdV vector used*
SARS-CoV-2	HAdV5, HAdV26, ChAdVY25
Ebola virus	HAdV5, HAdV26, HAdV35, ChAdV3
HIV/SIV	HAdV5, HAdV26, HAdV35
Influenza virus	HAdV4, HAdV5
Hepatitis C virus	ChAdV3, ChAdV63
Rabies virus	HAdV5, ChAdV68, CaAdV type 2
Dangue virus	HAdV5
MERS	HAdV5, HAdV41

4.1 ADENOVIRUS VECTOR VACCINES IN COVID-19 PANDEMIC

The outbreak of SARS-CoV-2 in Wuhan 2019 resulted in worldwide COVID-19 pandemic leading to rapid vaccine development against the virus. Both traditional and novel vaccine platforms were pursued to develop a vaccine. The urgency caused by quickly spreading pandemic, however, favoured the novel vaccines relying on messenger RNA (mRNA) or AdV vector technologies owing to the fast development and pre-existing production possibilities. (Mendonça et al., 2021) The traditional vaccine production relies on producing the viral pathogen in cell culture followed by purification and formulation or producing the subunit vaccines using protein expression technology. Each case relies on generating vast amounts of virus or subunit and subsequent purification and formulation slowing down the development process. This has been solved in mRNA vaccines but still counts as one limiting factor in AdV based vaccines.

The many advantageous features of AdV vectors for vaccine use have resulted in five nationally or originally World Health Organization (WHO) Emergency Use Listing (EUL) approved AdV vector-based vaccines against SARS-CoV-2. (Mohamed et al., 2022) Today, two of these have been fully licenced in European Union (EU). (https://www.vaccination-info.eu/en/covid-19/covid-19-vaccines, viewed 17.10.2022) The first AdV vector-based vaccine to be EUL approved in 2021 was Vaxzevria designed by Astra Zeneca and University of Oxford. Quickly after this also Covishield™ by Serum Institute of India Pvt. Ltd and Johnson & Johnson vaccines were approved. Vaxzevria and Johnson & Johnson vaccines remain the only AdV vaccines that have reached a full licence from EU later. Year after the first EUL approvals in 2022 also Convidecia by CanSino Biologics Inc. achieved EUL approval. (https://extranet.who.int/pqweb/vaccines/vaccinescovid-19-vaccine-eul-issued, viewed 3.8.2022) The fifth AdV based-vaccine, Sputnik V by Gamaleya Research Institute of Epidemiology and Microbiology, has been used since 2021 in Russia and many other countries where it has been approved for emergency use. (Mendonça et al., 2021)

Common features in all of these vaccines are the use of full-length or pre-fusion stabilized form of the spike (S) protein of SARS-CoV-2 as an antigen. Pre-fusion stabilized protein has been stabilized to its trimeric prefusion form through a mutation to achieve a higher immunogenicity and improved protein expression. Also, they all use replication incompetent first-generation E1/E3 deleted AdV vectors. (Mendonça et al., 2021) The serotype of the AdV vector and the number of needed doses, however, vary between the vaccines. In Vaxzevria and Covishield™ the used vector is ChAdOX1 that was originally generated by modifying ChAdVY25 serotype in 2012 when studying a vaccine candidate for Middle East respiratory syndrome (MERS).

(Mendonça et al., 2021) The use of chimpanzee adenovirus rather than humans is to evade the pre-existing seroprevelance that is the limiting factor for many HAdV serotypes. Also, because the guidelines for designing and manufacturing that specific vector did already exist it was an optimal candidate to meet the need of rapid COVID-19 vaccine production. The efficacy of Vaxzevria was shown to be 75% in preventing symptomatic COVID-19 disease using prime-boost regimen before the EUL approval. (Mendonça et al, 2021)

Convidecia uses HAdv5 as vector despite the broad pre-existing immunity that reaches up to 90% in some countries. (Travieso et al., 2022) In early human trials it was reported that seroprevelance limited the efficiency of the first dose of the vaccine administered by intramuscular (IM) injection. However, in 2021 an interim analysis showed that Convidecia was able to prevent 90% of the severe COVID-19 after one dose leading to the first national approvals of the vaccine in China and a few other countries. (Mendonça et al., 2021)

Johnson & Johnson vaccine on the other hand is HAdV26 vector-based vaccine. In pre-clinical phase the vaccine was able to elicit strong humoral and cellular immune responses despite the usage of the less immunogenic serotype HAdV26. In January 2021, an interim data with over 44 000 vaccinees showed that the vaccine was safe and 85% effective at prevention of the severe COVID-19 disease leading to the emergency use approvals in over 40 countries. (Mendonça et al., 2021) However, efficacy and safety study done in March 2022 showed the efficacy of Johnson & Johnson vaccine to be only 41.7% against all emerged SARS-CoV-2 variants. (Sadoff et al., 2022)

Sputnik V vaccine consist of two dose heterologous prime-boost regimen utilizing both HAdv5 and HAdV26. (Travieso et al., 2022) This is to avoid the immune response against the second dose of vaccine and also because heterologous prime-boost regimen has been shown to be more immunogenic than homologous prime-boost regimen. (Mendonça et al., 2021) The vaccine was emergency use approved nationally in Russia and over 50 other countries 2021 after interim data with over 21 000 vaccinees showed the vaccine to be 91,5% efficient against COVID-19. (Mendonça et al., 2021; https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker, viewed 15.8.2022)

A rare prothrombotic blood clotting syndrome, today known as Vaccine-induced immune thrombotic thrombocytopenia (VITT), emerged as possible side effect of COVID-19 AdV vector vaccines in March 2021. (Mendonça et al., 2021) It was soon discovered to be linked especially with Vaxzevria, Covishield™ and Johnson & Johnson vaccines which administration was paused to review the data. The use was restarted after in all countries, except Denmark, since

the positive factors for public health outweighed the possibility of the rare side effect. Because VITT cases have only been reported with AdV vector vaccines and study has shown that the used S-protein antigen does not cause or stimulate thrombosis, AdV vectors have been thought to be the problem. (Mendonça et al., 2021) The mechanism is not completely understood but studies suggest that some adenoviral components form complexes with platelet factor 4 (PF4) and with proinflammatory reactions caused by the vaccine these complexes stimulate production of anti-PF4 antibodies that subsequently cause thrombosis in VITT. (Greinacher et al., 2021) However, as there hasn't been any or only few cases of VITT reported with other AdV vector vaccines such as Sputnik V, Convidecia or Ad26.ZEBOV-GP Ebola vaccine, the cause of VITT is still unclear. It is unknown if this is because of a difference in reporting or safety of the vaccines. (https://www.uptodate.com/contents/covid-19-vaccine-induced-immune-thrombotic-thrombocytopenia-vitt, viewed 17.10.2022)

4.2 ADENOVIRUS VECTOR VACCINE AGAINST EBOLA

Ebola virus was discovered in 1976. The worst Ebola outbreaks to this date have been seen in Guinea, Sierra Leone and Liberia in 2014 where over 10 000 people died, and in 2018 in Democratic Republic of the Congo where Ebola caused over 2000 deaths. (Barry et al, 2021) Before the first major outbreak in 2014 the Ebola vaccine development was already focused especially on viral vector platforms, but the development was slow because of the rare occurrence and severity of Ebola outbreaks.

The first clinical trial of adenovirus vectors was done in 2010 with two dose HAdV5 vector vaccine encoding the GP protein from two different strains of Ebola virus. (Zhang & Zhou, 2016) The vaccine was well tolerated and induced both humoral and cellular immune responses, but the vaccine composition was proven to be poorly balanced. In 2014, clinical trials were done for one dose ChAdV3 based vaccine with 111 vaccinees. Some participants received also MVA-BN-Phyllo boost dose that consisted of GP protein of Ebola virus and antigens from filovirus. (Zhang & Zhou, 2016) The results were enough for moving to efficacy trials and proved that boost regimen could potentially induce longer protection against the virus.

Recently in 2021, the first heterologous two dose regimen AdV vector-based Ad26.ZEBOV with MVA-BN-Filo Ebola vaccine was approved by the European Commission under exceptional circumstances. Ad26.ZEBOV uses replication incompetent HAdV26 vector encoding a one variant of GP protein. MVA-BN-Filo uses replication incompetent vaccinia Ankara vector that encodes GP proteins from Marburg virus and different strains of Ebola virus and nucleoproteins from Taï Forest virus. The vaccine has also received EMA marketing authorization in Europe

and is now recommended by the WHO Strategic Advisory Group of Experts on Immunization (SAGE) to be used in lower risk populations preventively. (Barry et al, 2021)

After the recommendation of SAGE further studies have been done to estimate the effectiveness and safety of the vaccine. In one Phase II study the two-dose vaccine was tested with different vaccination frequencies in healthy African adults, children and adolescents as well as HIV-positive adults. Also, possible effects of booster dose of Ad26.ZEBOV after one year was tested. (Barry et al, 2021) The study showed that the optimal vaccination frequency between the two doses is 56 days and confirmed that the vaccine is overall well tolerated in healthy and HIV-positive population. They also showed that the vaccine was able to induce long term immune memory and the booster dose of Ad26.ZEBOV increased the amount of binding antibodies 55-fold. Thus, they showed the vaccine to be a highly potential prophylactic candidate against Ebola. More studies are being done to test the safety and tolerability in groups such as infants and pregnant women.

5. CONCLUSION

The successful use of AdV vector-based vaccines against COVID-19 and Ebola has highlighted the attractiveness of AdV vectors, especially in vaccines. AdV vectors are easy to design due to the ease of manipulation of the genome and their specific features such as immunogenicity, stability and broad host tropism that make them an optimal platform for vaccines. As AdV vectors have been used so long and tested widely in many fields of medicine, standardized and scalable amplification and purification methods as well as large-scale commercial production facilities with required biosafety classifications are already available. As a result, the quick development of AdV vector vaccines is possible thus making them highly desirable tool for fighting new rapidly spreading pandemics in future. It has been estimated that due to climate change associated increased emergence rates of zoonotic diseases, the probability of occurrence of extreme epidemics and probability of observing pandemics like COVID-19 during one’s lifetime can increase up to threefold in following decades. (Marani et al., 2021) These predictions emphasize the need for vaccine platforms such as AdV vectors that are highly adaptable for emerging pathogens of great variety, and which can also be produced rapidly as were the vaccines against COVID-19.

AdV vectors have a strong track record of usage in pre-clinical and clinical studies which have shown the safety of AdV vectors. After COVID-19, AdV vector vaccines have been used by many with different ages, genders and nationalities all over the world proving the suitability of AdV vector vaccines for wider distribution with relatively few side effects at population level. The most common symptoms of COVID-19 vaccines, including AdV vector vaccines, have been irritation at the injection site, headache, fever and nausea, and all in all, the level of adverse events has been mild to moderate. (https://www.cdc.gov/coronavirus/2019-ncov/vaccines/expect/after.html, viewed 10.10.2022; Mendonça et al., 2021) However, the severe VITT that emerged only after wider distribution of certain AdV vector vaccines has raised the question of the safety of AdV vector vaccines again. As the mechanism of the VITT is not completely understood and only common feature with the VITT cases have been the vaccines utilizing AdV vector platform there is a likely possibility that some AdV vector vaccine components, including virus proteins, HEK-293 cell line proteins or free DNA, have been the cause. (https://www.uptodate.com/contents/covid-19-vaccine-induced-immune-thrombotic-thrombocytopenia-vitt, viewed 23.10.2022) The question of what in AdV vector vaccines causes the VITT must be answered in studies to come to be able to avoid the VITT with AdV vector vaccines developed in future.

Also, the issue of seroprevelance to the serotypes commonly used as vectors remains relevant in the future. A good example of this is HAdV5 that has all desirable features, including a good immunogenicity profile, for vaccines but has moderate to high seroprevelance levels around the world. Utilizing other rare serotypes must be further investigated to achieve effective vaccines which can avoid the pre-existing NAbs. Also, the development of new AdV vectors from rare serotypes provide wider range of novel vectors available which can foster quicker development of the new AdV vector-based vaccines in future. This also provides a possibility of developing vaccines that require frequent booster doses.

In general, the full potential of AdV vector vaccines is yet to be explored. Despite the availability of many pre-clinical and clinical studies AdV vector vaccine development against viruses such as HIV and influenza has had only little progress. (Zhang & Zhou, 2016) To overcome the difficulties in developing new AdV vector-based vaccines against the already existing diseases and newly arising pandemics, the current problems with AdV vector vaccines must be resolved. For this, expanding knowledge of AdV vector vaccines is needed and bigger financial support towards AdV vector vaccine development is required. AdV vector vaccines, however, provide a great tool for fighting infectious diseases and thus are worthy investment for the future.

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