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Current approaches for the development of vaccines against infectious viral diseases

Současné přístupy k vývoji vakcín proti infekčním virovým onemocněním

Bachelor´s thesis

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Declaration:

I hereby declare that this work was solely undertaken by myself and that I referenced all the information sources and literature thoroughly. This work or any part thereof has not been used to acquire another academic title.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 1.5.2022 Soňa Vargová

Abstract: Vaccination remains one of the most successful biomedical interventions for preventing viral diseases. While early vaccines were developed by attenuating the infectious agent in cell cultures or by inactivation, new delivery platforms are on the rise thanks to the advent of genetic engineering. The COVID-19 pandemic stimulated the rapid adoption and a massive deployment of these platforms. Viral vector vaccines elicit antigen expression within cells and induce a robust cytotoxic T cell response, unlike protein subunit vaccines conferring mainly humoral immunity. mRNA vaccines also deliver the antigen inside the cells while offering more manageable and faster manufacturing possibilities. Unlike DNA-based vaccines, mRNA does not enter the nucleus, and thus, the probability of disrupting gene expression in the recipient cell is diminished. This thesis aims to offer an overview of current approaches in vaccinology and discuss the various platforms in use. The thesis will also present recent advances in the development of prophylactic vaccines against infections with human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV) and also will focus on a recently proposed strategy for vaccine development based on non-cognate ligands mimicking epitopes recognised by broadly neutralising antibodies (bNAbs).

Keywords: vaccines, prophylactic vaccination, virus, viral antigen, HCV, HIV, mimotope, broadly neutralising antibody

Abstrakt: Vakcinace zůstává jednou z klíčových biomedicínských intervencí v prevenci virových nákaz. Zatímco rané vakcíny byly vyvíjené atenuací infekčního agens pasážovaním nebo inaktivací, díky pokrokům v oblasti genového inženýrství jsou na vzestupu nové vakcinační platformy. Pandemie nemoci COVID-19 způsobila rychlou adopci a celosvětové nasazení těchto platforem. Vektorové vakcíny vyvolávají expresi antigenu v buňkách a indukují robustní odpověď cytotoxických T lymfocytů, čímž dosahují lepších výsledků, než vakcíny podjednotkové, které stimulují primárně humorální imunitu. mRNA vakcíny nabízejí jednodušší a rychlejší výrobu i škálovatelnost. Na rozdíl od vakcín na bázi DNA, mRNA nevstupuje do buněčného jádra, a tím se snižuje pravděpodobnost narušení genomu buněk. Tato práce si klade za cíl nabídnout přehled současných přístupů ve vývoji protivirových vakcín a diskutovat o různych užívaných platformách. Práce také představí nedávný vývoj profylaktických vakcín proti viru lidské imunodeficience (HIV-1) a viru způsobujícího žloutenku typu C (HCV) a novou strategii založenou na proteinech mimikujících epitopy infekčních agens, a to pomocí „otisků“ paratopů široce neutralizujících protilátek.

Klíčová slova: vakcína, profylaktická vakcinace, virus, virový antigen, HCV, HIV, mimotop, široce neutralizující protilátka

List of abbreviations

COVID-19	Coronavirus disease 2019
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
HIV-1	human immunodeficiency virus type 1
HCV	hepatitis C virus
mRNA	messenger ribonucleic acid
DNA	deoxyribonucleic acid
bNAbs	broadly neutralising antibodies
NCLS	non-cognate ligand strategy
PRR	pattern-recognition receptor
APC	antigen-presenting cell
MHC	major histocompatibility complex
TCR	T cell receptor
BCR	B cell receptor
VLP	virus-like particle
HBsAg	hepatitis B surface antigen
IFN	interferon
TLR	Toll-like receptor
IL	interleukin
HPV	human papillomavirus
UTR	untranslated region
ORF	open reading frame
eIF4e	eukaryotic translation initiation factor 4E
RIG-I	retinoic acid-inducible gene I
ABD	albumin-binding domain
kb	kilobase
gp	glycoprotein

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1. Introduction

The importance of vaccination and its benefits for the human species cannot be overstated. Considered one of the most significant advances of modern medicine, they reduce mortality and morbidity and can eradicate causative infectious agents (Andre et al., 2008). It is estimated that vaccines avoid 6 million deaths per year, while also lowering the economic burden on global healthcare systems (Kennedy, 2020).

As the COVID-19 pandemic nears its end, it is essential to stress the vital role modern biotechnology has had in mitigating the crisis' impact. Humanity reached an era of unprecedented technological advancement, and the rapid deployment of vaccines targeting SARS-CoV-2 could well be considered the pinnacle of contemporary science. However, the development of new antiviral vaccines faces challenges. There remain multiple viruses against which no prophylaxis is commercially available, the most iconic example being the human immunodeficiency virus, with over a million deaths per year.

The thesis aims to elaborate on the current concepts used in developing vaccines against conventional and emerging viruses and compare various platforms in terms of immunogenicity, efficacy, and safety. The particular focus of this work will be dedicated to the issue of recent advancements in the development of prophylactic vaccines against evasive viruses that pose a global health challenge - hepatitis C virus and human immunodeficiency virus. These are also a subject of employing non-cognate ligand vaccination strategy, developed at the Laboratory of Ligand Engineering at the Institute of Biotechnology of the Czech Academy of Sciences, therefore particularly relevant.

2. Immunology

This chapter will briefly discuss the immunological phenomena connected to the development of adaptive immunity against vaccine-targeted viral components. These interactions are essential in determining the efficacy and immunogenicity of various vaccination platforms. A protein subunit vaccine example will be used for the following explanation of the related phenomena.

Most of the current vaccination strategies depend on producing humoral immunity, that is, antibody-mediated response (Lambert et al., 2005). Antibodies can bind to the viral surface, thus preventing the virion from entering permissible cells. It is widely established that neutralising antibodies correlate significantly with protection from infection (Plotkin and Plotkin, 2008). Another observation supporting this claim is the protective effect of maternal antibodies in the newborn (Zinkernagel and Hengartner, 2006). Besides preventing the virion from entering the cell, antibodies can activate the complement cascade, thus further enhancing virus neutralisation and achieving a pro-inflammatory state (Daniels et al., 1970).

After introducing the vaccine to the system, usually via an intramuscular injection, the first response is local inflammation. Adjuvant in the substance binds to the Pattern recognition receptors (PRRs) of antigen-presenting cells (APCs), resulting in their activation and uptake of the antigen. Subsequently, the antigen is processed within the APC, resulting in the loading of peptides onto the MHC II receptor. After migrating to the lymph node, the presented peptides on the APCs surface activate CD4+ helper T cells through interaction with T cell receptors (TCR), inducing the production of pro-inflammatory cytokines (e.g. IL-2). In turn, B cells can internalise the antigen directly through the B-cell receptors (BCR) and undergo clonal expansion after presenting the peptides to the CD4+ T cells and receiving a signal. This process involves maturation of the antibody response to increase the affinity of the antibody to its epitope and isotypic shift dependent on the type of cytokines produced (den Haan et al., 2014). The result is the differentiation of B cells to plasma cells, capable of secreting antibodies, and memory cells. Plasma cells produce antibodies specific to the vaccine protein, raising serum antibody concentration significantly for two weeks

following the vaccination. Long-lived plasma cells, capable of producing antibodies for decades, migrate to the bone marrow. After the subsequent encounter with the pathogen, CD8+ memory T cells can proliferate rapidly, and CD8+ effector T cells can activate apoptosis of the infected cells.

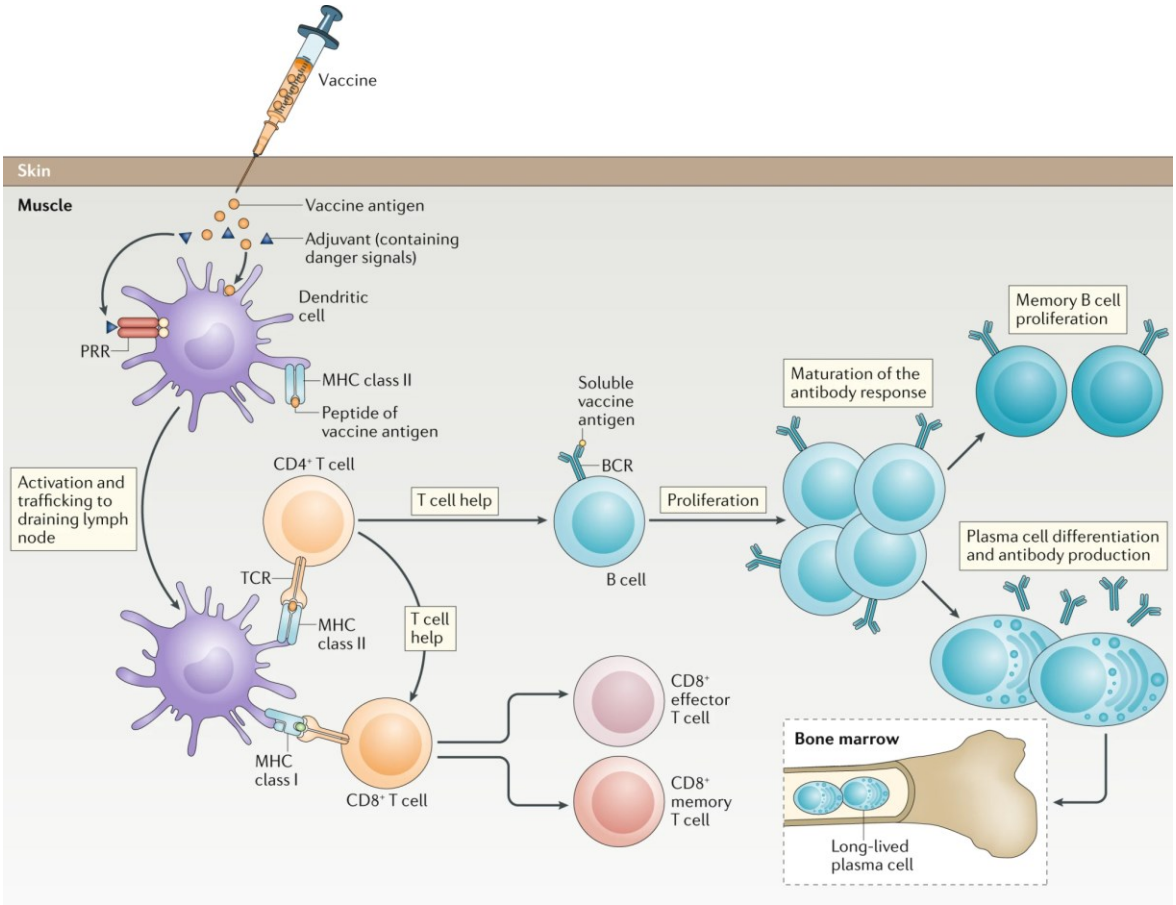

Figure 1: Interactions between adaptive and innate immune systems (Pollard and Bijker, 2021)

3. History of antiviral vaccination

Since the development of the first antiviral vaccine against variola in 1798 by E. Jenner (Jenner, 1800) and the rabies vaccine in 1885 (Pasteur, 1885), the technology has progressed substantially - nowadays, moving from using inactivated whole virus particles or live attenuated approaches to synthetic vaccines based on nucleic acids, virus-like-particles (VLPs) or vector delivered platforms.

First attempts at immunisation against viral diseases could be traced to 16th century China, where the preferred method was to inhale infectious scabs or pustules from the victims. This practice was called variolation. However, there are reports of Indian Buddhists ingesting snake venom to become immune to its effect, inducing toxoid-like immunity - suggesting the idea of immunisation dates as far back as the 7th century. Variolation found its way from Asia through Turkey to England in the 18th century. Interestingly, English farmers practised "vaccination" long before Jenner's discoveries (Pead, 2003). After publishing his work, Jenner started to inoculate people using an arm-to-arm approach, effectively passaging the virus.

Another significant advance in the field was Louis Pasteur's work on the attenuation of bacteria, subsequently applying a similar approach to protect animals against cholera, anthrax and finally, the rabies virus. These efforts culminated when Pasteur used this technique to save the lives of children bitten by a rabid dog. It should be noted that the public was challenging vaccination from its very inception.

In 1886, Daniel Elmer Salmon and Theobald Smith published the work on a "killed vaccine" aimed at hog cholera. Following their discovery, various killed vaccines were developed. Wilhelm Kolle developed a human cholera vaccine after Robert Koch discovered the bacterium *Vibrio cholerae*. At the beginning of the 20th century, five vaccines were used: two containing live viruses (variola, rabies) and three containing killed bacteria (typhoid, cholera and plague).

In 1931 E. W. Goodpasture started using the chorioallantoic membrane of a chicken egg as a medium to cultivate viruses, which enabled a safer and more cost-effective approach for subsequent development of a live-attenuated vaccine against Yellow fever by Theiler and Smith (1937). The breakthrough in the advancement of antiviral vaccines came with the advent of cell cultures and the discovery that the viruses can propagate there (Enders et al., 1949). In 1955 a formalin-inactivated polio vaccine by Salk et al. (1954) was licensed for use. Some scientists believed that a live vaccine would provide long-lasting protection from the disease, and subsequently, a live attenuated vaccine grown in monkey kidney cell culture was licensed in 1960 (Sabin et al., 1954). In the 1960s, three classical vaccines were developed: measles, mumps and rubella, all attenuated by passaging in eggs or cell culture. Using the

newly established yeast expression system, Valenzuela et al. (1982) have synthesised the hepatitis B surface antigen (HBsAg), which was consequently used for vaccination purposes.

Methods arising from genetic engineering and synthetic biology will be discussed in detail in the following chapters.

4. Approaches

4.1 Whole virus

These approaches employ the application of a whole virion into the vaccinated individual, either attenuated or inactivated (killed). Though robust in their ability to produce an immune response, these approaches are obsolete and will only be discussed briefly.

4.1.1 Live attenuated

This platform is relatively successful at stimulating both branches of immunity. The viruses can be attenuated by serial passage through a foreign host cell culture or reverse genetics. For example, it is possible to modify the NS1 gene providing the influenza virus with the ability to bind interferon (IFN) and thus hinder the antiviral immune response (Hai et al., 2008). These vaccines are replication-competent and can provide long-term, possibly lifelong immunity without boosting. A possible setback is an application to immuno-compromised individuals, as this can result in severe adverse effects. Though the concept of attenuation predates the discovery of viruses, it is still used in the prophylactic formulae against measles, mumps, rubella and polio.

4.1.1 Non-live (inactivated)

These consist of whole virus particles grown in cultures, inactivated by physical, chemical methods, or both. Formaldehyde and β-Propiolactone (BPL) are widely used to inactivate licensed human vaccines, though other approaches also exist.

Adjuvants are routinely added to non-live vaccines to enhance the immune response. As such, they allow the use of a lesser amount of antigen, thus providing an economic benefit. There are only a few adjuvants in use in licensed vaccines. This, however, is subject to a slow change, as liposome-based adjuvants or oil-in-water emulsions are now being licensed for use. Immune response developed after administering a non-live vaccine can be further enhanced by using newly developed adjuvants providing danger signals to the innate immune system, such as TLR4 stimulants. It has been recognised that cellular cytokines can enhance immune responses or direct them towards T helper 1 or T helper 2 pathways; IL-12 and granulocyte-macrophage colony-stimulating factor (GM-CSF) have featured notably in this regard.

4.2 Protein-based platforms

4.2.1 Subunit vaccines

Usually delivered in the form of a purified protein subunit, these vaccines are currently used the most due to their enhanced safety and scalability. A disadvantage of this approach is the reduced ability to stimulate the immune response and, therefore, the requirement for the use of adjuvants, repeated doses or higher dosages. (Plotkin, 2014).

There are essentially two means to acquire the proteins: disassembling the infectious virion (split-product vaccine) or expressing the proteins in genetically modified organisms, such as *Escherichia coli.* Each expression system provides its advantages and disadvantages. For example, the prokaryotic expression system cannot fold the protein correctly and carry out the posttranslational modifications

common in eukaryotes. The protein display techniques have been reviewed elsewhere and will not be discussed further.

4.2.2 Virus-like particles

Another possible approach is to use viral surface proteins with the ability to self-assemble, forming VLPs and mimicking the surface morphology of the pathogen. These empty particles do not contain the viral genome and are thus replication incompetent. Self-assembling proteins are usually manufactured in a compatible expression system by DNA plasmid transfection. Another means of displaying the antigen on the surface of a VLP is by conjugation of the antigen of interest to the VLP-forming molecules by a chemical crosslinker.

This method was pioneered by Kirnbauer et al. (1992) using baculoviral expression of the L1 major capsid protein of human papillomavirus type 16 (HPV-16). They observed that L1 particles assembled into structures that resembled the native virions. Moreover, these particles were able to induce the production of neutralising antibodies, thus blocking the virus attachment to the basement membrane of the susceptible epithelia.
Given the highly repetitive pattern of antigen display, VLPs are capable of eliciting a more robust immune response than subunit vaccines. The conformation of the epitopes is also successful at mimicking the native viral structure, thereby offering a higher titre of the conformation-dependent NAbs compared to subunit vaccines.

4.3 Viral vector-based platforms

The rationale behind vector-based vaccines consists of inserting a DNA sequence coding for the antigen of interest into an appropriate viral vector with the subsequent ability to infect the permissible cells. The cells then express the antigen on their MHC I complex, thus generating an immune response. This technology was developed in 1984 using a vaccinia virus vector expressing the hepatitis B surface antigen. Moss et al. (1984) demonstrated the production of antibodies first in rabbits, followed by the referenced study on chimpanzees.

A possible problem when employing this approach is the alternation of a host's genome; therefore, it is crucial to select vectors in favour of the most remarkable safety/efficacy ratio. One of the means to establish the safe expression of the antigen is to disrupt the genes responsible for the replication of the viral vector.

Adenoviral vectors are the most widely used for this purpose, as they can infect multiple types of cells, are efficient in expressing the transgenic antigen, and rarely integrate into the hosts' genome (Robert-Guroff, 2007). The disadvantage of using such vectors is the possibility of pre-existing immunity, which could hinder the proper development of immune response, targeting the vector instead. Various studies report high titer levels of neutralising antibodies targeting Adenovirus type 5, with Mast et al. (2010) finding only 14.8% of participants negative for said antibodies. To address this issue, it is of paramount importance to study the epidemiology of used vectors and either choose a prime-boost strategy using different serotypes (Thorner et al., 2006) or develop alternative vectors as vaccine vehicles, such as non-human Ads of simian origin (Tatsis et al., 2006).

4.4 Nucleic acid-based platforms

4.4.1 DNA-based platforms

The DNA-based platform employs the transfection of an antigen-coding DNA plasmid into the cells to provide an immune response. This concept was first pioneered in 1993 by the biotechnological company Vical observing that cDNA can be used to produce foreign protein in mice when applied intramuscularly. Mice injected by plasmid DNA coding for influenza A nucleoprotein were able to generate cytotoxic T lymphocytes specific for the said protein. On top of that, Ulmer et al. (1993) demonstrated protection from a heterologous strain of influenza A virus.

Donnelly et al. (1997) postulated that the antigens mimic natural infection, eliciting MHC class I and class II T-cell responses and antibody responses. However, the performance of DNA vaccines was found to be suboptimal in clinical trials. (Kutzler and Weiner, 2008) The possible reason for this is the predominantly

intramuscular administration of the agent, yielding a primarily cell-mediated immune response.

Various techniques could improve DNA vaccine efficacy, one being electroporation. Electroporation is an application of a small electric field to destabilise the membrane and increase the uptake of DNA into the cells (Tieleman, 2004). This can increase the uptake of nucleic acids, generating a more robust immune response, with Hirao et al. (2008) finding higher levels of cellular immunity, as well as humoral. This study also demonstrates the potential of using novel adjuvants, such as IL-12 to improve seroconversion. Among other possible improvements in the delivery of DNA vaccines is an intradermal application, which results in direct transfection of APCs and the display of the antigen on MHC I surface glycoprotein (see chapter 2 - Immunology).

Some safety concerns were a long-lasting expression of the antigen and possible genome integration causing mutations or deregulating gene expression. Wolff et al. (1992) demonstrated that a vector was detectable as long as 19 months after the application. There was, however, no chromosomal integration.

DNA immunisation is not prevalent in human medicine, with no vaccine being fully approved for human use. There are, however, some preparations approved for veterinary use, for example, targeting the West Nile virus and various clinical trials in progress (Ledgerwood et al., 2011).

4.4.2 RNA-based platforms

This approach relies on administering mRNA coding for the antigen of interest, subsequently being translated within the target cells. When employing this approach, there are two possible options: conventional or non-replicating mRNA and self-amplifying or replicating mRNA.

The structure of mRNA vaccines mimics the structure of eukaryotic mRNA, with a 5′ 7-methylguanosine triphosphate (m7G) Cap and a poly(A) tail at the 3’ end. An open reading frame (ORF) is also present and enclosed by the untranslated regions

(UTR). The cap is crucial to initialise the translation in a eukaryotic cell as it binds to the eukaryotic translation initiation factor (eIF4E) (Sonenberg and Gingras, 1998). The capping strategy generally employs either an enzymatic reaction, such as the vaccinia capping system (Yisraeli and Melton, 1989), or a synthetic cap analogue. 3' poly(A) tail enhances the delivered vaccine's stability and protects it from the exonucleases present in the interior of the cell.

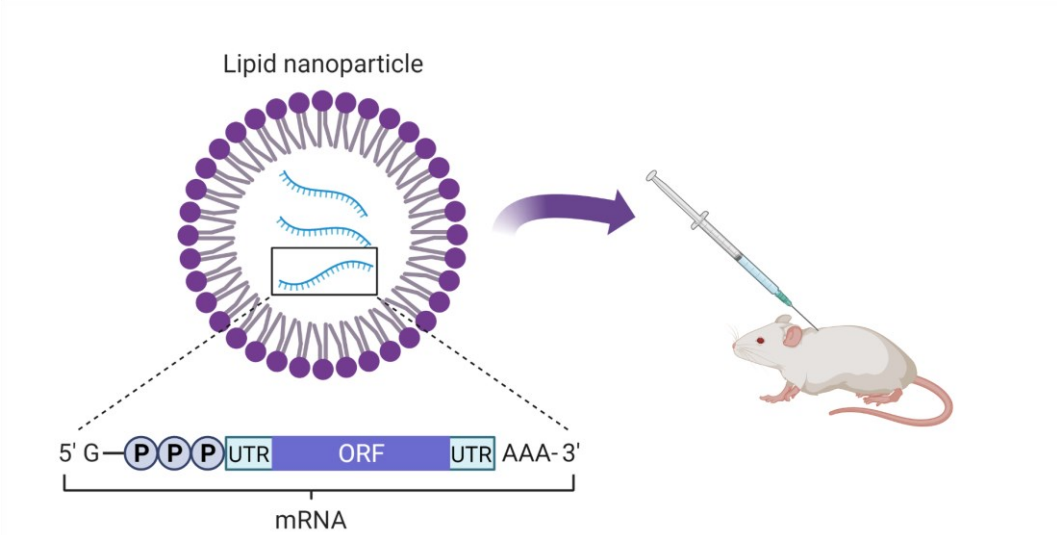

Figure 2: The structure of a mRNA vaccine. Created with BioRender.com

Due to the inherent instability of mRNA and the necessity for it to cross the cell membrane, it is vital to overcome this limitation using appropriate carriers. They compensate for the negative charge of the molecule, as well as protect from the extracellular ribonucleases present in the tissue. Exosomes, ionisable lipids, or lipid-derived nanoparticles are currently the most promising means of transporting the naked mRNA into the cells (Kauffman et al., 2015). However, novel carriers are also being explored. These include polymeric materials, polyamidoamine or polypropylenimine-based dendrimers, and cell-penetrating peptides, as reviewed by Kowalski et al. (2019).

Compared to the DNA-based platforms, mRNA is less stable and more immunogenic due to the number of cellular pathways that react to foreign RNA, such as TLRs and RIG-I-like receptors (Hornung et al., 2006). This platform is thus successful at mimicking the naturally occurring viral infection. Moreover, the

probability of random genomic integration is low, and the antigen expression is short-term as mRNA is quick to degrade in the cell (Maruggi et al., 2019).

Overall, synthetic mRNA vaccines have shown great immunogenic potential and tolerability, as demonstrated by the recent success of Comirnaty and Spikevax, both carrying the sequence for S protein of the SARS-CoV-2 virus.

Self-replicating RNA vaccines are based on the positive RNA strand of viruses. By replacing the coding genes with an antigen of interest and keeping the viral RNA polymerase, it is possible to amplify the amount of produced protein, thus generating a more robust immune response with less vaccination material. Vogel et al. (2018) found that the decrease in the required material could be as high as 64-fold, demonstrating the economic superiority of this platform as well.

4.5 Cell-based platforms

This platform employs the ability of dendritic cells to present antigens and stimulate the immune response. Unlike the other platforms mentioned in this thesis, the dendritic cells are manipulated *ex vivo*, establishing the antigenic specificity, and reinfused to the vaccinated individual.

However, this approach is economically demanding, time-consuming, and could pose problems when scaling up the production for global deployment.

5. Non-cognate ligand strategy

This innovative approach is being advanced at the Laboratory of Ligand Engineering IBT CAS. It is based on the directed evolution of high-affinity protein binders from a complex protein combinatorial library. The proteins are first displayed using a complex portfolio of probable binders and then selected based on the highest affinity for paratopes of well-established neutralising antibodies. The result is a purified protein, which mimics the epitope of the agent to be neutralised and elicits the generation of bNAbs *in vivo*, with their antigen-binding site highly specific for this epitope (Klasse, 2019).

Kosztyu et al. (2019) – see figure below - recently demonstrated that this approach is feasible for developing a prophylactic HIV vaccine. Using proteins derived from the albumin-binding domain (ABD) scaffold of a streptococcal protein G, they identified the highest-affinity binders of an established human anti-HIV gp120 monoclonal antibody VRC01.

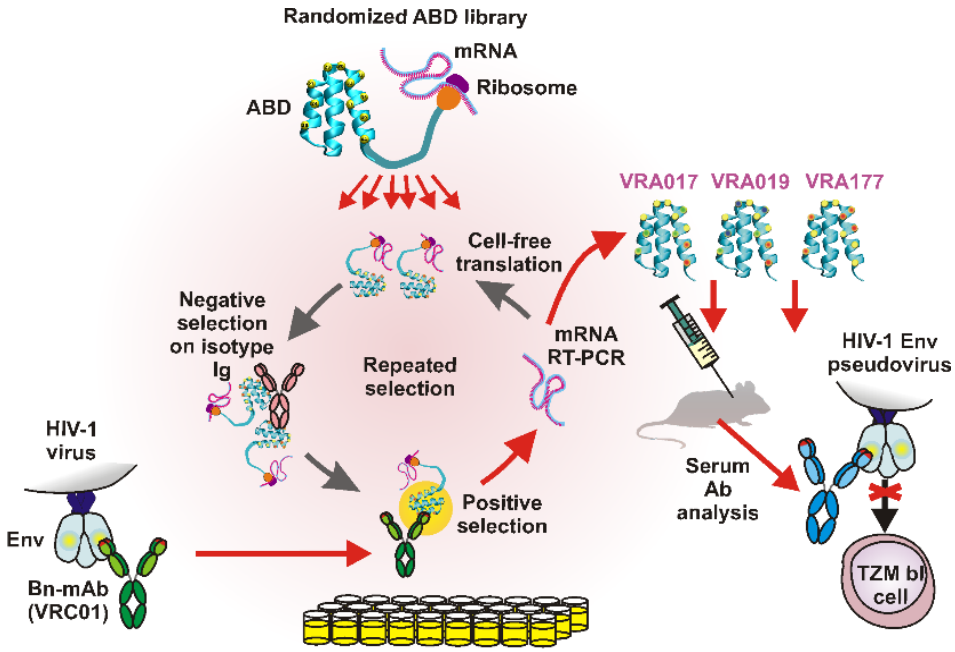

Figure 3: Scheme illustrating the generation of ABD-derived protein mimotopes targeted to paratope of bNAb VRC01 using the proposed NCLS. (Kosztyu et al., 2019)

This antibody is highly potent, has a broad range of activity, and is directed to the CD4-binding site of the virion, which is particularly vulnerable since it cannot be entirely masked by the glycan shield or conformational changes (Wu et al., 2010). The most potent binders were injected into experimental mice with Freund adjuvant, which elicited the generation of Env-binding serum antibodies. In the case of HIV, the *env* gene possesses tremendous genetic variability, and the presence of NAbs exerts systemic selective pressure for new variants, thus presenting an extraordinary challenge (Kwong et al., 1998).

Another study has recently supported the viability of the proposed NCLS. Kuchař et al. (2021) demonstrated the generation of a collection of mimotopes targeted to paratope of HIV-1 broadly neutralising antibody 10E8, specific for MPER Env epitope of gp41. The binding proteins called Myomedins, when used as immunogens, elicited antibodies in murine sera that were able to neutralise a broad portfolio of pseudoviruses in vitro (see figure below).

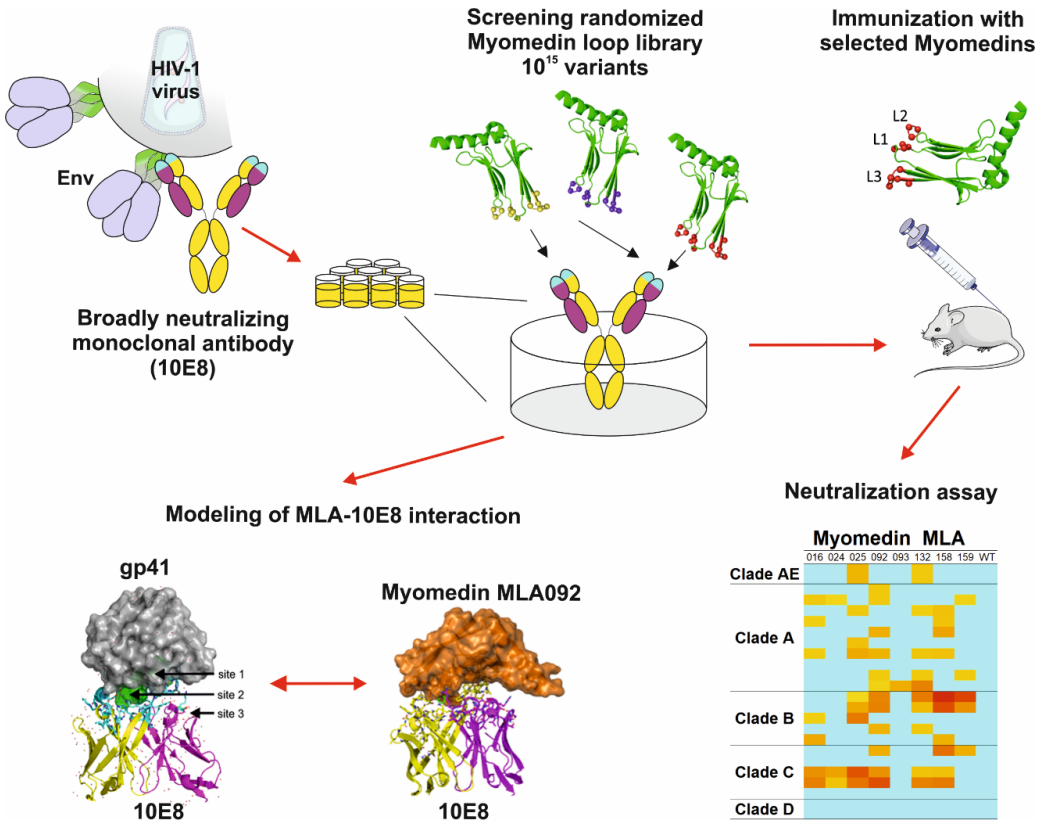

Figure 4: The illustration of the NCLS used for the selection of Myomedin mimotopes targeting bNAb 10E8. (Kuchař et al., 2021)

The non-cognate ligand strategy could overcome long-standing problems connected with the low efficacy of currently tested HIV vaccines caused by immunised subjects not producing a sufficiently broad portfolio of NAbs. Not needing to bypass the low immunogenicity of glycosylated sugars of the shielded neutralisation epitopes, which exhibit a high degree of variability in a native virion, various mimicking proteins can generate bNAbs against the viral surface without additional boosting. Moreover, a suitable combination of candidate proteins could be used to immunise the subjects against various clades of the virus or target more than one conserved region, effectively preventing viral entry. There are sufficient reasons to expect an exciting future in this field with further refinement of the protein binders and assessment of their immunogenicity and reactivity.

6. Current development

As previously mentioned, multiple challenging viruses remain in the field of vaccinology. These employ various forms of immune escape, such as hypervariability of the external surface, antibody-mediated enhancement of the viral entry, or expression of proteins directly interfering with the immune response through a multitude of signalisation cascades.

6.1 HIV

The epidemic of HIV/AIDS still represents a significant global health challenge, especially in African regions. It is estimated that in 2018 over 37 million people were affected by the condition. Moreover, 1.7 million new infections were reported with 700 000 deaths even when therapeutic antiretrovirals were available.

HIV, from the family Retroviridae, is an RNA virus encoding for reverse transcriptase and integrase, allowing it to integrate into the host genome. The genome contains nine genes coding for 16 proteins - structural proteins Gag, Pol, and Env; accessory proteins Nef, Vif, Vpu, and Vpr; and regulatory proteins Tat and Rev.

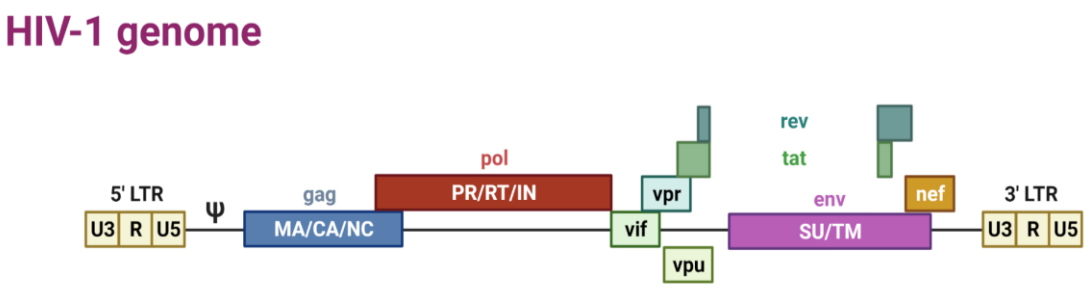

Figure 5: The genome of HIV-1. Reprinted from "HIV-1 Genome and Structure", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

To elicit the antibody response, there is one main target - Env protein, trimeric spike protein, responsible for viral entry into CD4+ cells. The full trimer consists of three gp120 protein units, a transmembrane domain containing gp41 subunits, cleaved post-translationally from the gp160 polypeptide. The outer layer of Env is

heavily glycosylated, thus limiting the neutralisation possibilities by the antibodies. This protein exists in three conformations, open, intermediate, and closed, with varying sensitivity levels to nAbs (Munro et al., 2014). The conformation also changes after ligation to CD4, exposing the binding site for co-receptor CCR5 (Lee et al., 2017). The AIDSVAX trial, targeting gp120, did not successfully prevent infection, even when inducing antibody response (Pitisuttithum et al., 2006). This clinical trial proved that attempts at generating bNAbs against HIV must employ advanced techniques.

A subset of infected patients does not develop AIDS, even without antiretroviral therapy, as they have solid CD8+ responses and an ability to contain the viral replication (Betts et al., 2006). Thus, there have been attempts to develop vaccines based on this finding. T cells primarily target conserved regions within structural proteins, such as Gag protein; therefore, there is no need to bypass the elusive nature of Env.

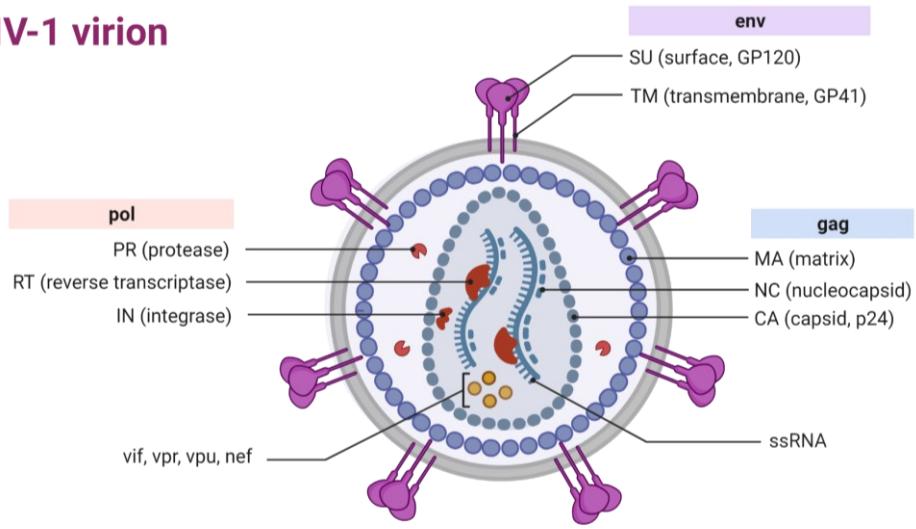

Figure 6: The structure of a mature HIV-1 virion. Reprinted from "HIV-1 Genome and Structure", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

Indeed, a subsequent trial, based on AIDSVAX, was commenced. Rerks-Ngarm et al. (2009) used a prime-boost strategy, employing a viral vector to elicit T cell response. Subjects were primed with a recombinant canarypox vector, expressing Env, Gag and Pro, followed by a booster of a recombinant gp 120 subunit booster. This regimen achieved modest efficacy of 31.2% in comparison to the placebo.

Interestingly, the subjects who contracted HIV during the study's duration showed no viraemia or CD4+ T cell count alterations. This trial remains one of the most successful and paved the way for future studies and clinical trials, such as Mosaico (NCT03964415) by Janssen. This trial uses a vector prime-boost strategy, with the first dose being human adenovirus (Ad26), expressing four mosaic HIV immunogens from various HIV strains, followed by boosting with a gp140 in aluminium phosphate adjuvant. However, a similar clinical trial, Imbokodo (NCT03060629), was abandoned in 2021, achieving an efficacy of only 25%.

With the emergence of the mRNA-based platform during the COVID-19 pandemic, encouraging results were acquired for HIV. Zhang et al. (2021) showed that mRNA vaccine expressing HIV Env protein and simian immunodeficiency virus (SIV) Gag proteins can generate VLPs *in vivo* and thus induce the production of bNAbs in rhesus macaques. In addition, there are three mRNA vaccines for HIV in Phase I clinical trials (NCT05217641). Employing an mRNA-based strategy for the development of an HIV vaccine shows promise also in terms of time, cost, and logistics in the manufacturing process. Additional clinical trials are displayed in the table on the next page.

Table 1: Recent developments in prophylactic HIV-1 vaccine clinical trials

Vaccine	Commencement	Interventions	Type	Phase	Number of participants	Status	Results published	Note	Reference
mRNA based vaccines, presenting Env protein	11.2.2022	BG505 MD39.3 mRNA BG505 MD39.3 gp151 mRNA BG505 MD39.3 gp151 CD4KO mRNA	mRNA-based	I	108	Recruiting	No		NCT05217641
Chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost	25.11.2021	AdC6-HIVgp140 AdC7-HIVgp140 CH505TF gp120	Vector-based, protein-based	I	34	Recruiting	No		NCT05182125
mRNA based vaccines, presenting HIV-1 proteins	12.11.2021	Core-g28v2 60mer mRNA Vaccine eOD-GT8 60mer mRNA Vaccine	mRNA-based	I	56	Recruiting	No	Moderna, no further details disclosed	NCT05001373
Ssingle mosaic prime ChAdOx1.tHIVconsv1 (C1) and a dual boost of MVA. tHIVconsv3 (M3) and MVA.tHIVconsv4 (M4) administered simultaneously.	16.8.2021	ChAdOx1.tHIVconsv1 MVA.tHIVconsv3 MVA.tHIVconsv4	Vector-based	I	88	Recruiting	No		NCT04553016
▸ Polyvalent env (A,B,C, A/E)/gag protein DNA vaccine ▸ Polyvalent gp120 (A,B,C, A/E) protein vaccine	16.06.2021	env (A,B,C,A/E)/gag (C) DNA Vaccine gp120 (A,B,C,A/E) Protein Vaccine	protein-based	I	42	Recruiting	No		NCT04927585
▸Alphavirus DNA replicon; genes coding for the viral capsid and envelope have been replaced by the sequences encoding HIV-1 gp140 ▸ Recombinant CN54gp140 is a HIV-1 envelope protein from the clade C strain. ▸ DNA-HIV-PT123 HIV vaccine includes three DNA plasmids that encode clade C Gag Env, and CN54 Pol-Nef	1.6.2021	Drep-HIV-PT1 CN54gp140+MPLA-L DNA-HIV-PT123 HIV	Vector-based, DNA-based	I	70	Not yet recruiting	No		NCT04844775
CMV-based vaccine	28.12.2020	VIR-1111	Vector-based	I	26	Recruting	No	No further details have been disclosed as to the construction of the platform	NCT04725877
Recombinant Env mimicking proteins	13.01.2020	HIV-1 BG505 SOSIP.664 gp140 with TLR agonist	Protein-based	I	105	Recruiting	No		NCT04177355
DNA-HIV-PT123 HIV vaccine includes three DNA plasmids that encode clade C Gag, Env, and CN54 Pol-Nef. AIDSVAX® B/E is a bivalent HIV gp120 glycoprotein encompassing both subtype B and subtype E proteins. Recombinant CN54gp140 is a HIV-1 envelope protein from the clade C VA-CMDR is a vaccina vector that has been genetically engineered to express the HIV-1 genes env/gp160 subtype E and gag and pol subtype A	1.1.2020	DNA/AIDSVAX DNA/CN54gp140 + MVA/CN54gp140	recombinant glycoprotein/vector-based	II	1668	Not yet recruiting	No	Comparing each of two experimental combination vaccine regimens - DNA/AIDSVAX and DNA/CN54gp140 + MVA/CN54gp140 with placebo control.	NCT04066881
Tetravalent vaccine composed of Ad26.Mos1. Gag-Pol, Ad26.Mos2.Gag-Pol, Ad26.Mos1.Env, and Ad26.Mos2S.Env. Clade C and Mosaic gp140 HIV bivalent vaccine contains: Clade C gp140, HIV-1 Env gp140 of Clade C, Mosaic gp140, HIV-1 Env gp140	31.10.2019	Ad26.Mos4.HIV Clade C and Mosaic gp140 HIV bivalent vaccine	Vector-based	III	3900	Active	No	Mosaico study by Janssen; a similar trial, Imbokodo, failed. Expected completion in March 2024	NCT03964415

6.2 Hepatitis C virus

Discovered by Choo et al. (1989), the hepatitis C virus is a global health issue, with approximately 1% of the world's population infected. Chronic infection results in liver cirrhosis and is linked with hepatocellular carcinoma. Hepatitis C is currently the most prevalent blood-borne viral infection. HCV is an enveloped, positive-strand RNA virus with a genome approximately 9,6 kb long, belonging to the Flaviviridae family. The genome encodes a polyprotein that proteolytically cleaves into structural proteins core, E1, and E2 and non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

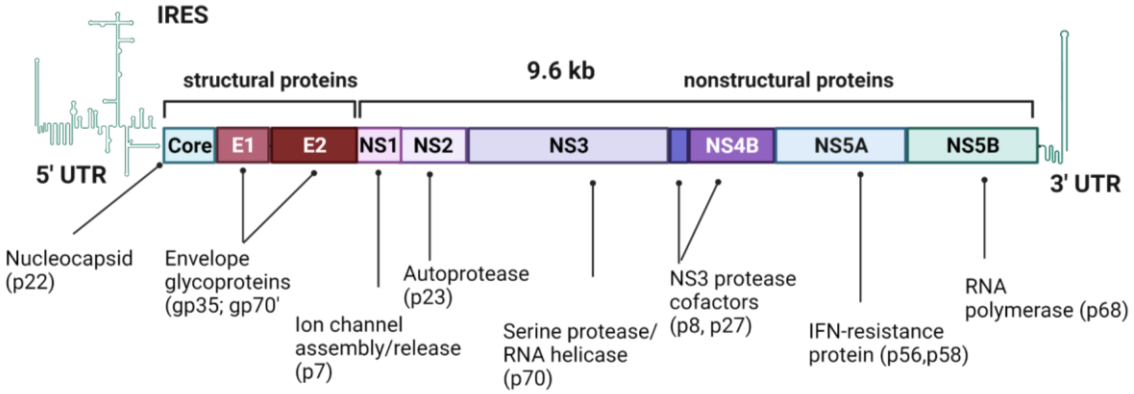

Figure 7: The structure of the HCV genome and its encoded proteins. Created with BioRender.com, based on Kumthip and Maneekarn (2015)

Eight genotypes of the virus have been discovered to date, each with a sequential variation of 30%. These genotypes are further divided into 90 serotypes. Due to the errors in viral replication by the NS5B RNA-dependent RNA polymerase, there is a possibility of generating a diverse array of variants resistant to cellular and humoral immunity (Liu et al., 2010). This variability makes developing a functional vaccine particularly challenging, using ordinary approaches with little to no success. Moreover, attenuating HCV has been nearly impossible due to the inability to culture the virus in cell lines (Thomas and Liang, 2016). As of 2013, direct-acting antiviral (DAA) therapy is available, with virus clearance rates approaching 70% (Liang and Ghany, 2013). However, this treatment is economically demanding, with costs approaching 27 000 USD. Another concern is the emergence of DAA-resistant strains, though combined DAA therapy mitigates the risk substantially (Raj et al., 2017). In addition, successful treatment with DAA does not prevent reinfection, which is a

particular risk among intravenous drug users. If the 2030 target to eradicate HCV by the WHO is to be met, it is essential to develop new prophylactic agents.

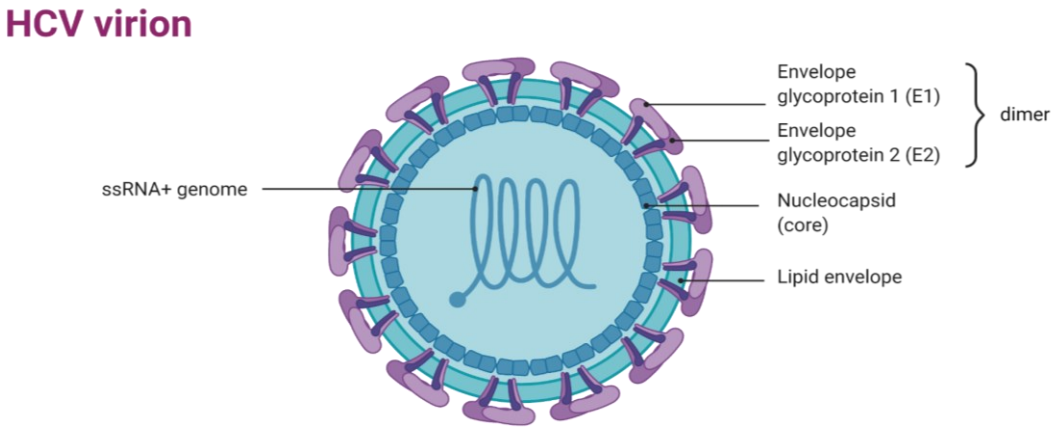

Figure 8: Structure of the HCV virion (Created with BioRender.com.)

Most of the vaccines aim to raise neutralising antibodies for the viral surface. However, Multiple studies show a considerable involvement of both CD8+ and CD4+ T cells. There is indirect evidence that spontaneous recovery is linked to genes coding for MHC II, which plays a role in presenting the viral peptides to T cells (Duggal et al., 2013). However, as mentioned in the paragraph above, the T cells recognise only a specific epitope which varies across a given genotype and serotype of the virus. The development of a vaccine protecting from viral entry is complex, as the E2 protein of the viral envelope contains a hypervariable region that can shield more conserved epitopes, thus providing the virus with the possibility to escape the immune response. There have been multiple vaccines in development, which aim not to induce sterile immunity but to lower the morbidity of the virus by reducing the post-acute sequelae and the possibility of further transmission. For example, NS proteins are more conserved across HCV genotypes and are also the dominant targets of CD8+ T cells. Von Delft et al. (2018) used a computational approach to target the most conserved HCV epitopes and encoded these antigens into a simian adenoviral vector (ChAdOx1), achieving HCV-specific T cell response in a pre-clinical mouse model. A possible limitation of this approach is the lack of immunological pressure for mutations in highly conserved epitopes, rendering them irrelevant.
A similar approach targeting nonstructural proteins was employed by Page et al. (2021) using a prime-boost strategy, first with a recombinant chimpanzee

adenovirus 3 vector (ChAd3) and boosted by a recombinant vaccinia Ankara (MVA). Although the regimen showed excellent tolerability and achieved a T cell response, it could not stop the progression of the disease to the chronic stage. Possible reasons for this might be the presence of vector cross-reactive antibodies, thereby lower immunogenicity, as well as the lack of envelope proteins present in the vaccination material. Several other vaccination candidates failed to confer protection, even when inducing T cell responses. Therefore, it is crucial to study the virus's life cycle and its associated immunology further. Even considering the hypervariable surface of the HCV virion, Merat et al. (2016) demonstrated the feasibility of bNAbs isolated from individuals that cleared the infection without any treatment. Combinations of various bNAbs were able to alleviate established infection by heterologous HCV strains.

There are, however, mechanisms by which HCV avoids humoral immunity, including the induction of antibodies that interfere with neutralising antibodies (P. Zhang et al. 2009) and previously mentioned shielding of the envelope glycoproteins epitopes by glycosylation of amino acids. For these reasons, it seems unlikely that the antibody-mediated vaccine can successfully induce sterilising immunity. Table below depicts recent developments in clinical trials, of which none made it to Phase III.

Table 2: Recent developments in HCV prophylactic vaccine clinical trials

Vaccine	Commencement	Interventions	Type	Phase	Number of participants	Status	Results published	Note	Reference
▸ Attenuated chimpanzee adenovirus (ChAd) vectored vaccine against HCV NS protein ▸ Modified Vaccinia Ankara (MVA) vectored vaccine against HCV NS protein	4.12.2017	ChAd3-hliNSmut MVA-hliNSmut	Vector-based	I	25	Recruiting	No	3 groups - lower dose, higher dose, and higher dose with DAA history	NCT03688061
Novel candidate vaccines against HIV ('HIV.consv') and HCV ('NSmut')	1.10.2014	AdCh3NSmut1 MVA-NSmut	Vector-based	I	33	Completed	Yes	Focused on simultaneus administration of the agens	NCT02362217
Prime-boost strategy with a chimpanzee adenovirus 3 followed by a modified Vaccinia Ankara vectored vaccine encoding NS proteins	6.3.2012	ChAd3NSmut1 MVA-NSMut	Vector-based	I & II	548	Completed	Yes	Placebo-controlled, provided T cell response, lowered peak virus RNA, did not prevent chronic infection	NCT01436357
Autologous dendritic cells transduced with Ad encoding NS3 HCV protein	1.5.2011	DCs	Cell-based	I & II	5	Terminated	No	Therapeutic vaccine, no further information, the study has stopped early	NCT02309086
Synthetic peptide derived from HCV E1 and HCV E2	1.3.2011	Cenv3	Peptide-based	I & II	50	Unknown	No	Also testing for therapeutic potential in Phase II	NCT01718834

7. Conclusion

As mentioned in the introduction, there remains no doubt that vaccination is one of the core features of modern preventative medicine. The field of vaccinology has seen a plethora of incredible advancements, from inoculation of pus to synthetically engineered mRNA vehicles. This thesis aimed to introduce current advances in the field and discuss their applications, advantages, and disadvantages.

Whole-virus-based approaches confer significant prophylaxis levels, especially when attenuating the virus, but the production is logistically demanding and laborious. There is also a risk that the virus can revert to its more virulent form. In terms of enhancing the antigen yield, protein subunit vaccines represent a more rational and more readily deployable approach, however, at the cost of lower immunogenicity. Due to the repetitive pattern of antigen display of virus-like particles, these represent the most robust platform for eliciting neutralising antibodies where the invariable viral surface permits it. In order to stimulate also the adaptive immune system, vector-based and nucleic acid-based vehicles offer the most advantages, with mRNA vaccines showing the greatest promise and possibly even revolutionising the whole field - especially when it comes to evasive and emerging pathogens. Non-cognate ligand strategy can target viruses, which use their glycan or lipid envelopes to shield neutralisation targets. This approach is being tested for use in developing a preventative vaccine against infection by HCV.

Interestingly, one repetitive theme was prevalent in most of the referenced works - the exact mechanisms of developing the immune response after vaccination remain unknown, especially in the case of HIV. Therefore, it is of paramount importance to study the nuances of the viral life cycles and immunological interactions. It is no easy undertaking, but given the enormous mistrust of the public, undoubtedly one worth considering, even though clinical trials show promising results.

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