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Recombinant vaccines: Current updates and future prospects

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ABSTRACT

Recombinant technology-based vaccines have emerged as a highly effective way to prevent a wide range of illnesses. The technology improved vaccine manufacturing, rendering it more efficient and economical. These vaccines have multiple advantages compared to conventional vaccines. The pandemic has heightened awareness of the advantages of these vaccine technologies; trust and acceptance of these vaccines are steadily growing globally. This work offers an overview of the prospects and advantages associated with recombinant vaccines. Additionally, it discusses some of the challenges likely to arise in the future. Their ability to target diverse pathogen classes underscores their contributions to preventing previously untreatable diseases (especially vector-borne and emerging diseases) and hurdles faced throughout the vaccine development process, especially in enhancing the effectiveness of these vaccines. Moreover, their compatibility with emerging vaccination platforms of the future like virus-like particles and CRISPR/Cas9 for the production of next-generation vaccines may offer many prospects. This review also reviewed the hurdles faced throughout the vaccine development process, especially in enhancing the effectiveness of these vaccines against vector-borne diseases, emerging diseases, and untreatable diseases with high mortality rates like AIDS as well as cancer.

KEYWORDS: Malaria; Dengue; Emerging diseases; Immunoinformatics; Virus-like particles; CRISPR-Cas9

1. Introduction

Vaccines have progressed beyond their original purpose of preventing infectious diseases. Presently, they are utilized to combat various circumstances in variety of human health conditions[1]. Vaccines have notably extended the average human lifespan and reduced infant mortality rates. It is estimated that vaccines prevent approximately 6 million deaths each year[2]. This is especially evident in the current scenario where vaccination has significantly reshaped the trajectory of the continuing COVID-19 pandemic, preserving over 16.5 million deaths globally[3,4]. More than half of the vaccines created to combat COVID-19 were based on recombinant technology, a relatively recent approach for vaccine generation[4]. In contrast to the traditional vaccine, which is sometimes limited by factors such as unpredictable adverse effects, unavailability, and challenging costs, recombinant vaccines offer cost-effectiveness and time efficiency, and it also confers protection against a broad spectrum of diseases[1,4,5]. Furthermore, their ability

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to remain relatively more stable at standard ambient temperature reduces cold chain dependencies, making immunization much more affordable and accessible (especially in third-world nations)[5].

Recombinant vaccine's success significantly depends on selecting correct cellular factories (the expression systems). It includes common systems like bacterial-based (*e.g.*, *Escherichia coli*), yeast-based (*e.g.*, *Saccharomyces cerevisiae*) and mammalian/insect cells-based systems. They are reliable and there are unique advantages offering opportunity to fulfill diverse needs[6]. Vaccine types include subunit, virus-like particles (VLPs), DNA vaccines and mRNA vaccines, and each tailored to elicit specific immune reactions with minimized side effects[7]. Employing viral vectors (like adenoviruses, lentiviruses, and vesicular stomatitis viruses) renders higher gene delivery. Whereas adenoviral vectors offer comparatively more transduction efficiency than others, lentiviral vectors offer benefits of targeting dividing and non-dividing cells similarly, while vesicular stomatitis viruses-based systems provide safety and strong immune responses[8]. The combination of vaccine types, expression systems, and chosen vectors define the versatility, acceptability and success of recombinant vaccines.

The years since 2020 have bound a significant rise in the advancement of recombinant vaccines, surpassing the limited number of traditional vaccine approaches. Apart from SARS-CoV-2, these vaccines have evidently shown different degrees of promising results against a wide range of infectious and non-infectious diseases (malaria, dengue, Chikungunya, cancer and AIDS), which would otherwise be impossible to generate any potent vaccines for these disease a few decades ago[4,5]. These newer methodologies proved to be successful in breaking barriers for researchers. While it was almost impossible over the course of years, a range of strategies have been employed to produce such types of vaccines[9,10]. These include CRISPR/Cas9, immune-informatics, VLPs, prime-boost, and trained immunity approaches. These techniques are being efficiently applied to construct recombinant vaccines for a diversity of illnesses[9,10]. The present review aims to address the future prospects and potential challenges associated with this vaccine technology with the main perspective of how they may be expected to change the course of prevention of diseases that are either prevalent or emerging as a threat in the future.

2. The role of recombinant vaccines in the COVID–19 pandemic

The COVID-19 pandemic raised global vaccine demand with the need for vaccines with different types of vaccine candidates around the world. A huge chunk of vaccines developed against COVID-19 have utilized recombinant technology attributed to their inherent advantages making them suitable choices[10,11]. Figure 1 shows the percentage of recombinant COVID-19 vaccines administered globally and in different countries.

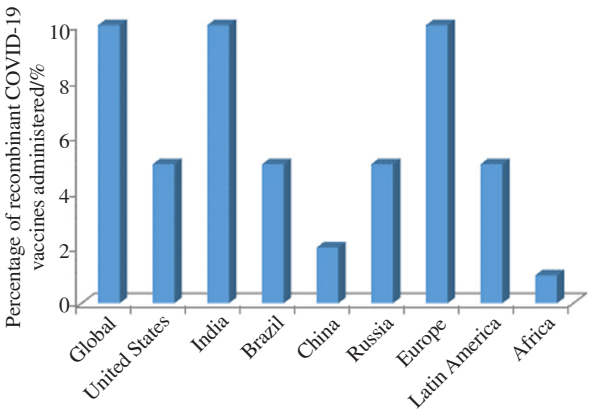

Figure 1. The percentage of recombinant COVID-19 vaccines administered globally.

Within a year of the pandemic, two different recombinant vaccines were developed namely Ad5-nCoV in early 2020 and AZD1222 in the later part of the year (in contrast, conventional vaccines were in early stages of development)[12]. The Ad5-nCoV (Institute of Biotechnology, affiliated with the Academy of Military Medical Sciences, China) utilized an aerosolized vector of adenovirus type-5 for the purpose to get rid of any adverse side effects in human[13]. The safety and immunogenicity analysis exhibited favorable outcomes. The investigation is warranted for the efficacy and cost-effectiveness of aerosol immunization[14]. Whereas, AZD1222 (AstraZeneca and Oxford University, UK utilizing the whole spike protein of the SARS-CoV-2 virus expressed through ChAdOx1 nCoV-19 vector) 28-day evaluation demonstrated that the safety profile of ChAdOx1 nCoV-19 was deemed adequate[15]. Subsequently, the phase 3 trial yielded significantly improved outcomes, as the vaccine demonstrated comparable immunogenicity across all age cohorts and exhibited enhanced tolerability and efficacy (70.4%) among older participants in comparison to their younger counterparts[16].

Later, the recombinant vaccines portfolio were improved by the addition of more vaccines (total seven new vaccines were added) with the aim of improving accessibility to resource limited regions of the world (Afghanistan, Ethiopia, Madagascar, Niger, Somalia *etc*.)[16,17]. The Gamaleya Research Institute in Russia developed a heterologous adenoviral vector-based vaccine known as Sputnik V (adenovirus vectors of type 26 and type 5, which were engineered to contain SARS-CoV-2 spike gene)[17]. The results indicated that this course of vaccination exhibited an efficacy rate of 91.6% in preventing symptomatic cases of COVID-19[18]. The freeze-dried formulation of this vaccine, which is developed using recombinant technology, demonstrates utility in the global administration of vaccines across diverse climatic conditions, necessitating storage at temperatures ranging from 2 ℃ to 8 ℃[19].

In 2021, more advanced recombinant vaccines started developing. VXA-CoV2-1 (Vaxart Inc, USA) demonstrates promise as an oral vaccination formulation (the oral formulation of the medication enhances accessibility by obviating the need for cold storage during transportation and refrigeration upon delivery)[20]. The virus attacks the body *via* the oral and nasal routes. Consequently, an oral vaccine may trigger the immune system to neutralize the infectious agent at the mucosal level only. Oral immunizations are known to generate significantly more robust mucosal immunity in comparison to injectable vaccines, and this is essential to suppressing the viral infection in the upper airways and thereby avoiding the progression to an advanced stage of COVID-19 disease. The lack of mucosal defense provided by the production of local secretory immunoglobulin A (IgA) antibodies heightens the likelihood of SARS-CoV-2 transmission from vaccinated individuals who remain susceptible to infection with the potential of transmitting the virus. Plenty of evidence shows that oral or nasal vaccination generates a potent efficient immune response[21]. Also, Nuvaxovid (No-vavax, USA) involved the utilization of the genetic sequence of the initial SARS-CoV-2 strain. Choosing the first viral sequences is a pivotal stage in vaccine manufacturing, since it strongly influences both the safety and effectiveness of the final vaccine product. Novavax employed its recombinant nanoparticle technology to generate antigens derived from the S protein of the coronavirus in the preparation of this vaccine[22]. Two significant advantages of these vaccines are their rapid production and cost-effectiveness. Furthermore, it is worth noting that these immunizations exhibit stability throughout the temperature range of 2 ℃ to 8 ℃[23]. Evidently, recombinant vaccines have a major role in controlling the pandemic of COVID-19. Figure 2 represents the administration of various recombinant COVID-19 vaccines all over the world. Additionally, the pandemic has also helped the realization of the potential of recombinant vaccines. Moreover, the global population is now aware of the existence and the significance of such vaccines[24,25].

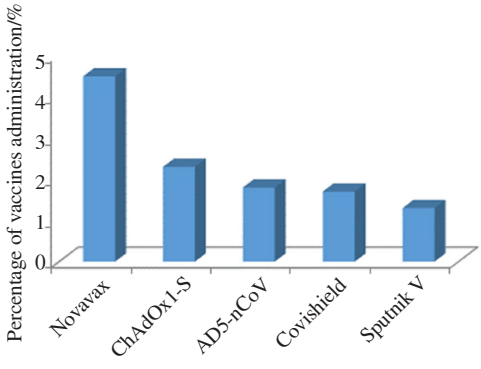

Figure 2. The statistics of various recombinant COVID-19 vaccines administration all over the world.

As of January 2024, the pandemic is largely under control from its peak in early 2020 with reports of minor sporadic incidences. Notably, a sustained decline in new cases and fatalities has also been observed since the onset of 2023. Omicron derived subvariants are globally predominant with high transmissibility and known to cause milder illness in vaccinated individuals. With more than 13 billion doses administered worldwide, vaccination along with mixed immunity is a key mitigator of severity of COVID-19[25,26].

3. Recombinant vaccine for various diseases

Recombinant technology has allowed generation of vaccine against human diseases/disorders that are otherwise impossible to achieve through conventional vaccine platform[27]. A plethora of intrinsic technological advantages allow the recombinant platform to raise vaccine-induced immunity against complex, multistage, disease-causing pathogens transmitted by various vectors especially mosquitoes[28].

3.1. Malaria

Malaria is a parasitic disease, caused by *Plasmodium* species transmitted through the female *Anopheles* mosquito with the ability of transmitting infections. It disproportionately affects regions with inadequate sanitation conditions. This lethal pathogen has claimed millions of lives, with a significant impact on African and Asian nations[29,30]. During childhood, malaria can cause devastating consequences. However, the development of a malarial vaccine holds great promise in the fight against this pervasive threat[31].

Harnessing recombinant technology in the fight against malaria holds the promise of revolutionizing our ability to combat this deadly scourge. In light of this, various recombinant vaccine contenders have emerged, targeting specific stages of the parasitic lifecycle[32]. A prominent strategy involves aiming the pre-erythrocytic or pre-blood stage. The most well-received vaccine available is Glaxo-SmithKline Inc RTS, S/AS02[33,34]. It incorporates two peptides (RTS and S) which are produced simultaneously through recombinant expression in *Saccharomyces cerevisiae*. While providing moderate to limited protection against *Plasmodium (P.) falciparum* sporozoite infection in humans, it marked itself as the first potential malarial vaccine in the market. A more potent version of this vaccine, RTS, S/AS02, elevated CMI responses in both malaria-affected and unaffected individuals[33,35]. A distinct phase 3 clinical trial which encompassed 6 000+ infants (aged 6-12 weeks) in countries of Africa unveiled post-vaccination clearance rates of up to 46%. Recommended for administration in children living in high-risk zones (Kenya, Ghana and Malawi) since mid-year 2021 and estimated 2.3 million doses,

demonstrated excellent safety and efficacy[36–38].

Another vaccine, R21/Matrix-M, recommended for children since October 2023, exhibits high efficacy, particularly during high transmission seasons, with a 75% reduction in symptomatic cases[39]. It consists of a fusion of the R21 virus-like protein derived from the circumsporozoite protein (PfCSP) of *P. falciparum*, linked to the *N*-terminus of HBsAg[40,41]. In May 2021, the findings from a phase 2b clinical trial evaluating the safety and efficacy in the regions of West Africa displayed promising outcomes with overall 77% efficacy (becoming the first to meet WHO's 75% target). Furthermore, no serious adverse effects were recorded[41,42].

Owing to the high demand, vaccine supply (especially for RTS,S/AS01) is reported to be lagging. However, Gavi-the Vaccine Alliance has worked upon an effective production scale-up plan to fulfil the ever-increasing demand. Both candidates demonstrated significant positive efficacy, with RTS,S/AS01 reducing severe malaria by 30% and overall malaria cases by 40%, while R21/Matrix-M is a step ahead with up to 66% reported efficacy post-three doses, with the potential of reaching as high as 75% with a fourth booster shot. To enhance malarial vaccine access, recently more than 18 million doses of RTS,S/AS01 were sent to 12 high-burden regions of the African continent for 2023-2025. Big manufacturers like Bharat Biotech aim at making RTS,S/AS01 to improve vaccine availability and consequently make it more affordable. Moreover, trials and research for future malaria vaccines aim at targeting multiple if not all parasites life cycle stages to seek higher efficacy and broader protection[43].

Aside from this, extensive research explores the feasibility of creating vaccines targeting the blood stage of the parasite[44]. However, efficacy of these erythrocytic vaccines is comparably lower than pre-erythrocytic. This is attributed to the technological and other limitations-like a lack of sustained immune system response, the need for several booster dosages across a brief period and tremendous variations among the parasite species[34,45]. The SPf66, one of the pre-erythrocytic contender, consisted of a multistage peptide mixture with an alum adjuvant. However, further advancement of SPf66 was halted due to multiple studies revealing its very significantly lower efficacy and requiring further work to improve its overall efficiency. The current research is aiming at improving the efficiency of such pre-erythrocytic contender by studying new delivery platforms and working upon finding new and more suitable adjuvants[46].

As of now, several surface-exposed antigens on blood-stage parasites are under investigation, including PfAMA1 (Apical Membrane Antigen 1), PfMSP-1 (Merozoite Surface Protein), 2 and 3[47,48]. Despite achieving sufficient antibody titers, these candidates didn't deliver effective protection against some fatal strains (especially *P. falciparum* and *P. vivax*)[49].

The last group of vaccines intends to hinder the transmission stage and aren't designed to directly provide personal protection. Instead, their objective is to interfere with the development of gametocytes, leading to reduction in the infection rates[50]. The protective immunity of two transmission-blocking vaccine candidates, namely Pfs230 (pre-fertilization antigen) and Pfs25 (post-fertilization antigen), was observed to have been significantly less in human clinical studies[51,52]. However, in an ongoing study, GSK's AS01 adjuvant is being utilized with both Pfs230 and Pfs25 to elicit stronger responses[52,53].

Such headway in malarial vaccine creation has also presented certain undeniable obstacles primarily due to the intricate parasites nature (the main mediator of this natural occurrence may be primarily tied with multiple attributable factors including a complex digenic cycle of life, a high level pathogen's genetic as well as antigen plurality and the capacity to circumvent the immunity of the host), which has resulted in unsatisfactory immunity in patients[54,55]. It may be expected with further research and improvement in the upcoming developments in recombinant technologies, research fraternity will be able to develop malaria vaccine(s) within a decade[55–57].

3.2. Dengue

Dengue, a viral disease transmitted primarily by *Aedes* mosquitoes, is a significant global health concern; attributed to the dengue virus (DENV), which is a viral member of the Flaviviridae family, also comprising of Zika and Japanese encephalitis viruses. Like malaria, dengue is also prevalent in tropical and subtropical regions and it also affects millions of people annually and more than 5500 deaths are reported globally from many countries[58]. Its rapid spread and potential for outbreaks make it a critical focus of research and public health efforts worldwide[59].

DENV serotypes (DENV1-4) continue to pose a significant public health threat, with serotype 2 (DENV-2) being the most prevalent and deadly[60]. Repeated infections potentially trigger severe manifestations in some patients, such as haemorrhagic fever caused by dengue or dengue shock syndrome phenomena, commonly referred to as antibody-dependent enhancement, complicating vaccine development. Recombinant vaccine technology allows scientists to design vaccines that target specific components of the dengue virus, reducing antibody-dependent enhancement risk[61].

Dengvaxia, developed by Sanofi Pasteur, is a recombinant vaccine designed to provide protection against all dengue serotypes[62]. The ongoing development of vaccine candidates mostly revolves around the utilization of the viral surface glycoprotein E (viral surface protein facilitating DENV-host cell interaction; an important event in dengue infection)[63]. In recent years, an exemplary illustration that stood out was V180 (DEN-80E), a prospective tetravalent vaccination candidate that has effectively concluded phase 1 clinical trials and has demonstrated favorable tolerability in several investigations[64,65].

Aside from surface proteins, RdRp-derived peptides also hold potential for vaccine development. In this regard, Wahongan and

team employed immunoinformatics to construct a potential vaccine based on the epitope MGKREKKLGEFGKAKG, demonstrating persistent binding to dendritic cell receptors[66]. It is anticipated to trigger an immunological response, positioning it as a candidate for a universal dengue virus vaccine. Likewise, malaria, significant hindrances with DENV vaccine production arise from complex viral structure (as observed with other flaviviruses) and a lack of well-versed studies on the infection[67].

Developing and producing affordable recombinant vaccines against these two diseases are vital for ensuring accessibility to populations in endemic regions, particularly because these diseases are more prevalent in developing countries[68]. Consequently, securing funding for vaccine development and fostering collaborations among researchers, pharmaceutical companies, and governments can be formidable tasks but are absolutely essential for progress. Cost-effectiveness is another crucial factor in this equation, as it directly impacts the feasibility of vaccine production and distribution in resource-constrained settings[67,68]. Addressing these challenges is pivotal in the fight against diseases like malaria and dengue, which disproportionately affect vulnerable populations in low-resource regions.

3.3. Recombinant vaccine against emerging Zika, and Chikungunya

Zika and Chikungunya first originated in the African continent. Their frequent outbreaks occur around the world especially in tropical regions due to supportive climatic conditions for both the disease vectors and mosquitoes. Zika gained international attention when it emerged outside of its usual regions. Major outbreaks occurred in the Pacific Islands in 2007 and in the Americas, particularly Brazil, in 2015 and 2016[69].

Chikungunya began to spread to new regions starting in the early 2000s. Large outbreaks occurred in the Indian Ocean islands, India, and Southeast Asia. In late 2013, the virus was introduced to the Caribbean, marking its first sustained transmission in the Americas. It subsequently spread to numerous countries in the Western Hemisphere[70].

3.3.1. Zika

The Zika virus is recognized as a mosquito-borne virus of the Flaviviridae family, with other prominent viruses such as dengue, West Nile, and Yellow Fever. The Zika virus (ZIKV) was first identified in 1947 in the Zika Forest situated in Uganda, Africa. The virus was initially found in a rhesus monkey and later in mosquitoes[69]. Different contender vaccines have been introduced to protect against ZIKV, several of which are mainly based on the pre-membrane-envelope (prM-E) viral proteins[71].

Currently, Austrian researchers are actively recruiting individuals to participate in a study evaluating the efficacy and safety of a novel measles-based Zika vaccine called MV-ZIKV-RSP[58,71]. This vaccine has recently progressed to phase 1 clinical trial. Additional candidate (ChAdOx1-EDIII) is also under phase 1 clinical trial. This vaccine candidate encodes the prM-E protein of ZIKV, with the exclusion of its transmembrane region[72].

The administration of another recombinant rhesus adenovirus serotype 52 (RhAd52) vector-based vaccine in rhesus monkeys resulted in the activation of cellular immune responses specific to the E protein of ZIKV, as well as the production of neutralizing antibodies specifically targeting ZIKV[73,74]. Additionally, comprehensive immune response conferred complete protection for a year post-vaccination.

In another study, the capsid protein of the ZIKV strain PRVABC59 was integrated into a vaccine known as VSV-ZikaE260-425, which is based on the vesicular stomatitis virus (VSV). This recombinant vaccine elicited robust humoral responses specific to the ZIKV in mice that were immunized. These findings suggest that the VSV-ZikaE260-425 vaccine offers enhanced protection against ZIKV infection[75].

Another proposal based on the Modified Vaccinia Ankara (MVA) vector showed promising results. When mice with a fully functioning immune system were administered a solitary dosage of the MVA-ZIKV-NS1 vaccine, it elicited strong immune responses both in terms of antibodies and immune cells. Furthermore, this vaccination provided complete protection, with a 100% survival rate, against a lethal dose of ZIKV that was administered directly into the brain[75,76].

Research findings have indicated that the introduction of ZIKV prM-E genes into chimeric DENV-2 and Japanese encephalitis virus SA14-14-2 vaccine has resulted in the safeguarding of mice against ZIKV infection. This intervention has been observed to elicit robust and durable immune responses[76]. The protective efficacy of ZIKV E90, derived from the E protein of the ZIKV FSS13025 strain, was seen against both the foetal and neonatal manifestations of the illness. Significantly, mice exhibited continued resistance to ZIKV infection even 140 days post-administration of the E90 vaccine[76,77].

There is a clear indication that extensive research efforts are being dedicated to the development of a very effective vaccine for the ZIKV through the utilization of recombinant technologies. Nevertheless, a significant limitation lies in their reduced immunogenicity; hence research on appropriate adjuvants may help to boost their immunogenicity[77].

3.3.2. Chikungunya

Chikungunya is a disease caused by the virus known as Chikungunya (CHIKV), belonging to the Togaviridae family. The disease virus reaches to human beings by the bite of infected *Aedes* mosquitoes (specifically *Aedes aegypti* and *Aedes albopictus*). This disease caused significant disruption in India recently[70].

Much like dengue and Zika, CHIKV doesn't have any vaccinations approved for use in humans. However, certain potential candidates are presently undergoing clinical trials[78]. Presently, MV-CHIK

based on measles vector, is one of the vaccine options that have progressed to phase 2 clinical trials (most advanced stage among all the vaccines). It was evaluated in a dose-escalation phase 1 clinical trial, and it performed well with a seroconversion rate ranging from 44% to 92% with a single dose and increasing to 100% following a second immunization[78].

Vaxart Inc (USA) is actively developing an oral adenovirus-based vaccine that expresses the structural proteins of the CHIKV. Early findings from clinical trials are promising, revealing that a single dose of the vaccine induces significant antibody blood titers. Notably, the vaccine has demonstrated efficacy in protecting against virally-induced inflammation of foot-pads in some animal subjects. The vaccine's thermostability and the potential for easy delivery to individuals at high risk provide additional strengths, strongly supporting the rationale for further development[76]. As Vaxart Inc is working on the development of an oral adenovirus-based Chikungunya vaccine, ensuring clinical efficacy, assessing long-term immunity and safety, optimizing dosage, and navigating regulatory approvals are key considerations. Additionally, addressing global manufacturing scalability, fostering public acceptance, and establishing equitable distribution strategies are pivotal for successful deployment.

Another pair of adenovirus-based investigations with poor replication: two different candidates namely-CHIKV-(ChAdOx1 sCHIKV and ChAdOx1 sCHIKV C) were subsequently produced. Mice that had received either of the two vaccines displayed a high T-cell response frequency and strong humoral response within two weeks following inoculation[79]. Further research will be required to fully establish its immunity.

In a recent study, researchers successfully demonstrated the immunogenicity and protective efficacy of a single vaccination using a multi-pathogen SCV vaccine (SCV-ZIKA/CHIK). This vaccine contains the structural gene cassettes of both CHIKV and ZIKV. The effectiveness of the vaccine was evaluated through a series of challenge tests conducted on mice. The results indicated that the vaccine provided robust immune responses and conferred protection against both CHIKV and ZIKV infections[76].

The epidemiology of Chikungunya is dynamic, and outbreaks can occur in different regions (especially in countries of South America like Costa Rica, Nicaragua, Honduras, El Salvador). Researchers need to consider the changing epidemiology when designing and testing vaccines. CHIKV exists as multiple genotypes and has undergone genetic changes over time. Developing a vaccine that covers the genetic diversity of CHIKV is challenging. Researchers must consider the potential for antigenic variation. Although there has been noteworthy progression thanks to recombinant technology, due to fewer number of studies and failures after phase 2 trials, Chikungunya still remains without a successful vaccine[80].

4. Novel and promising upcoming recombinant vaccine approaches

4.1. CRISPR/Cas9

The utilization of CRISPR/Cas9-a recent revolutionary genomic editing approach, has extensive application in vaccine research due to its notable attributes (efficacy, adaptability, ease of use, and cost-effectiveness in comparison to alternative methodologies)[81]. The MAVERICK approach, denoted as mosaic variant enrichment *via* CRISPR-Cas9 knock-in, represents an evolving paradigm employing CRISPR-Cas9 for precision genome manipulation in diverse organisms, particularly plants. Recent strides encompass the concurrent introduction of multiple predetermined mutations, thereby augmenting the intricacy of genetic editing. Enhanced operational efficiency has been achieved, yielding heightened quantities of targeted mutants while mitigating undesired offtarget modifications[82,83].

Beyond botanical applications, MAVERICK's scope extends to encompass gene therapy, antibiotic resistance research, and metabolic engineering. Persisting challenges involve the identification of optimal target sites, a predicament addressed through ongoing efforts in computational tools and refined Cas9 variants. Additionally, the optimization of homologous recombination pathways remains paramount for the successful implementation of desired mutations *via* MAVERICK. Overcoming impediments in the efficient delivery of Cas9 protein and guide RNA represents a critical focal point, driving research endeavors towards pioneering improved delivery methodologies in various organisms[82,83].

The utilization of a genome-wide CRISPR/Cas9 screening approach to discern and categorize host limiting features of the H1N1 influenza virus within a cell-based vaccine production platform has presented the potential for enhanced vaccine manufacturing capabilities in subsequent periods[81]. Multiple studies have provided confirmation about the applicability of the CRISPR/Cas9 system in the creation of recombinant vaccines targeting avian herpes viruses, especially in the manipulation of viral genomes and the progress made in the development of recombinant orthopoxvirus (OPXV) vaccines[84].

CRISPR/Cas9 system-based products are reported to elicit a robust immunological response by stimulating T cells, thereby inhibiting or impairing supplementary mechanisms of defense against viral infections. However, a significant concern associated with vaccines is the gradual degradation of the antibody's efficacy over time mainly attributed to short antibody half-life and interindividual immune response variability. This may be tackled by using booster doses or any other similar intervention method[85]. This could be an interesting domain and can be addressed in future research. CRISPR-Cas9, as a cutting-edge gene-editing tool, offers the potential in

combating diseases like malaria, dengue, Zika, and Chikungunya. It can modify mosquito genes to limit disease transmission and aid in understanding host-pathogen interactions[86]. In Zika and Chikungunya research, it aids in replication studies and vaccine development. However, ethical and safety concerns require careful consideration.

4.1.1. Challenges

Numerous studies have also examined the adverse consequences associated with CRISPR-Cas9 genome editing. The primary topic of discussion around CRISPR-Cas9 technology revolves around the potential occurrence of off-target mutations, which could result in significant consequences such as the impairment of functionality in genes not intended to be targeted[87,88].

4.1.2. Case example

Recently, scientists successfully generated a CRISPR technology based recombinant phage using against the pandemic virus, by introducing certain gene targets. The efficacy of this alternative vaccine approach has been evaluated through experimental trials conducted on animal models[89,90]. Figure 3 represents the schematic diagram showing various steps of novel approaches of recombinant vaccine production.

4.2. Nanostructured virus-like particles (VLPs)

Nanotechnology has facilitated the development of vaccines utilizing nanomaterials possessing encapsulating qualities and notable characteristics in terms of their size and surface similarities to the viruses[91,92]. These attributes enable them to effectively transport antigens and serve as immunostimulants. Immunogenic VLPs based vaccines can be obtained by synthesizing the edited viral genome with the mean of a recombinant technology[92,93].

These VLPs do not possess infectious genetic material and exhibit repeating subunits on their surface, which ensures their safety and promotes the development of strong immune responses[94,95]. Therefore, it is possible to generate VLPs which maintain the antigenic features of the viral capsid and possess the potential to self-synthesize[96].

VLPs are a versatile platform for vaccine development against diseases like dengue and Zika. They can be engineered to display viral antigens, stimulating disease free protective immune responses[97,98]. In addition, VLPs offer disease specific customization, accommodating antigens from different strains or serotypes, a critical advantage for diseases with multiple variants. Customized VLPs-based vaccines therefore hold promise for broader protection against evolving and emerging pathogens[99,100].

4.2.1. Advantages

Proteinonly vaccines due to the self-assembly of recombinant proteins have been used for a long time and is a way to the requirements of a vaccine formulation ideal. In addition, it avoids the use of non-protein nanoparticles as carriers, which could be a source of concern due to their potential secondary toxicity. However,

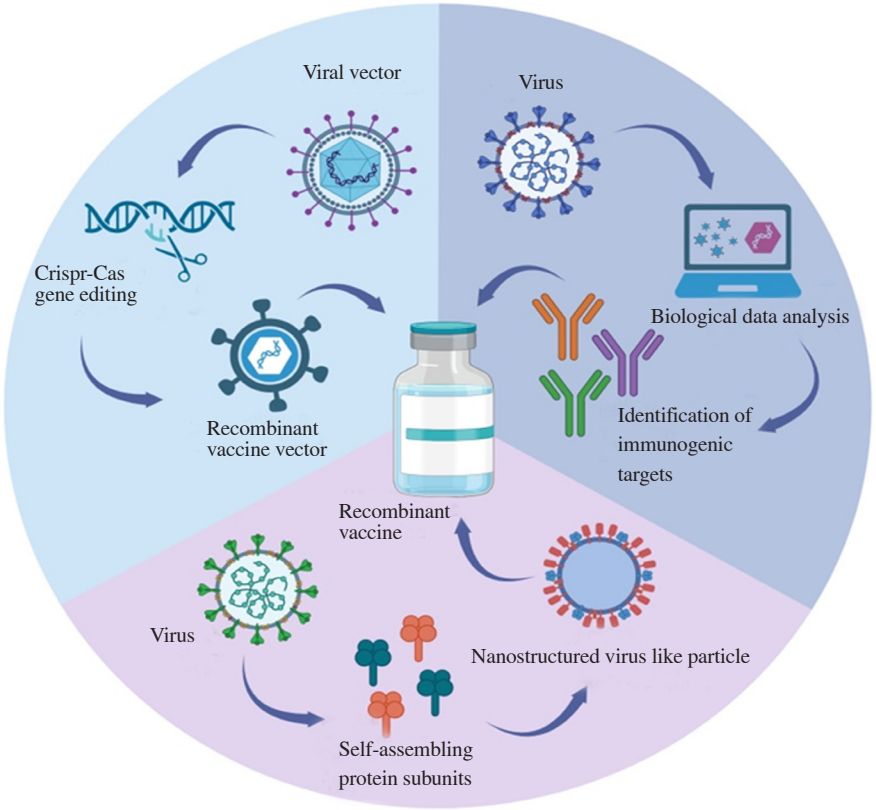

Figure 3. Various steps of novel approaches of recombinant vaccine production.

VLPs have an important limitation that they generate relatively small yields, which boosts the expense and renders them less attractive for economically deprived regions of the world. Consequently, they're doubtful to prove to be a viable option for battling worldwide pandemics such as COVID-19[101].

4.2.2. Case examples

The hepatitis B (HBV) virus vaccine was the initial recombinant vaccination to be approved for human use. The product was manufactured in 1985 with recombinant technology and afterwards incorporated into VLPs. The commercial viability of the VLP platform is demonstrated by its current market presence, with two formulations already available for the treatment of human papillomavirus (HPV) and two formulations for HBV[102].

Novavax (USA) has developed a nanoparticle recombinant vaccine utilizing the spike protein of SARS-CoV-2. The vaccine successfully completed phase 3 clinical trials, demonstrating an efficacy rate of 89.7%. It also showed cross-protection against the variant antigens. Its approval has marked a significant milestone in vaccine technologies against COVID-19[103,104].

4.3. Immunoinformatics approach

The bioinformatics approach is a simpler method for designing *in-silico* vaccines. Immunoinformatics is connected to the production of recombinant vaccines, serving as a critical bridge between computational biology and vaccine development. This interdisciplinary fields harnesses the power of bioinformatics and genomics to streamline and optimize the vaccine discovery process. One of its primary contributions is the prediction of potential antigens within pathogenic genomes, enabling the selection of suitable targets for recombinant vaccine candidates[105]. As shown in Figure 1, 10% recombinant vaccines have been administered globally. Of these, the highest (10%) vaccines have been administered in each Indian and European population as compared to least in Africa (1%).

Moreover, immunoinformatics aids in the identification of immunodominant epitopes, guiding the design of vaccines that specifically target essential regions of pathogens to induce robust immune responses. It also plays a pivotal role in assessing the immunogenicity of antigenic sequences, prioritizing those most likely to provoke effective immune reactions[106]. Major histocompatibility complex (MHC) binding prediction, a core component of T-cell epitope identification, is facilitated by immunoinformatics tools, ensuring the recombinant vaccine's ability to stimulate cellular immunity. Additionally, immunoinformatics contributes to reverse vaccinology by analyzing pathogen genomes for potential vaccine targets and addresses antigenic diversity, essential for diseases like dengue and Zika[107].

Furthermore, by employing immunoinformatics, researchers can customize vaccines for specific populations or genotypes based on genetic variations, contributing to precision vaccinology. The advantages of immunoinformatics are substantial. It not solely accelerates both the speed and efficacy of the vaccine development process, but also minimizes the overall expense at various phases, particularly by dramatically reducing laboratory expenditures[108]. Additionally, it supports post-licensure vaccine surveillance by monitoring vaccine performance and safety in real-world populations.

4.3.1. Case examples

Potential candidates for vaccine were rapidly developed by computer-aided screening software/servers. Several vaccines have been designed following these *in silico* approaches, *e.g.* COVID-19, *Salmonella typhi*, dengue and cancer[109]. However, the *in vivo* and *in vitro* experiments would further substantiate the results of this study[110]. Figure 4 shows various advantages of using novel approaches of recombinant vaccine production.

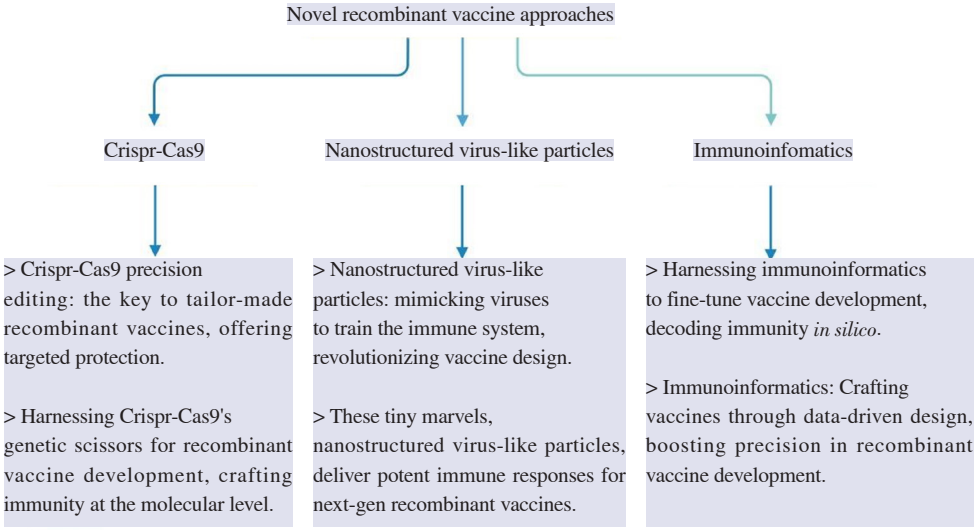

Figure 4. Various advantages of using novel approaches of recombinant vaccine production.

5. Recombinant vaccines against cancer and HIV

5.1. Cancer

The use of recombinant classes of vaccines in the cure of a variety of malignancies is filled with numerous major barriers. Nevertheless, the present initiatives are focused on finding remedies. Many forms of recombinant vaccines are researched, with DNA and dendritic cell vaccines showing some potential[111]. Individually tailored immunizations created specifically for individual cancers based on their own for tumor-associated antigens (TAAs) characteristics could possibly offer a ray of hope. They possibly can deliver the genetic instructions for TAAs capable of establishing lasting immunity. However, they face difficulties in effectively delivering related challenges[112]. Two significant examples are the HER2 expression vaccine (RG7420), created to especially targets the HER2 protein in some types of breast cancer and the GVAX vaccine created to target numerous prostate cancer TAAs[113].

The dendritic cell-based vaccines, while showing possibilities, are now in the preliminary stages of research. The use of Sipuleucel-T (provenge) for prostate cancer involves utilizing the patient's dendritic cells[114]. On the other hand, DCVax-L is being investigated for its efficacy in managing multiple malignancies by dendritic cells that are loaded with several TAAs found from the patient's tumor. This strategy exhibits the possibility of future advances[115].

It looks like cancer shots simply might not be adequate to successfully battle malignancies. The combination of vaccinations with checkpoint inhibitors or any other therapeutic intervention requires a thorough analysis[111,116].

5.2. HIV

Recombinant vaccines represent a strategic approach to combat HIV, equipping the immune system for recognition and neutralization rather than a direct attack. Several contenders in this arena include subunit vaccines presenting purified HIV proteins, DNA vaccines injecting the DNA blueprint for antigens, viral vector vaccines employing modified viruses, and innovative mosaic vaccines targeting multiple conserved regions of HIV[115,117].

Subunit vaccines produce immune-mediated reactions, yet they lack significant and long-term antibody responses. Viral vector-based vaccines possess excellent antigen delivering potency, yet they face problems related safety hazards. Mosaic vaccines, which are in the preclinical phase of the study, have great potential for providing comprehensive resistance by attacking numerous conserved RNA sequences of HIV[118-121].

Despite significant advancements in vaccine research, creating of a viable AIDS vaccine seems to be a time-consuming task owing in part to the virus's vast genetic diversity, fast mutation rate, and the current under-research vaccine's failure to stimulate the production of lasting neutralizing antibodies which offer resistance. The ongoing challenges associated with optimizing vaccine management and ensuring effective immune-responses in many different groups highlight the need for continuous research and creative solutions[119].

6. Conclusions

This review highlights the significance of recombinant vaccines as a key technology in preventing diseases on a global scale. Over the past few decades, there has been substantial progress in reducing health risks through recombinant vaccines. The use of recombinant vaccines has the potential to revolutionize disease prevention, making it imperative to continue research and collaboration to overcome obstacles (scale-up and production-related challenges, achieving overall cost-effectiveness and eliminating challenges pertaining to endemic diseases of Africa continent) and fully realize their potential (especially treating new human ailments like vector borne diseases, emerging diseases, AIDS, cancer and many more)[122]. This comprehensive perspective on recombinant vaccines provides valuable insights for policymakers, scientists, and healthcare professionals who are keen on advancing vaccination strategies. Moreover, emerging vaccination platforms like virus-like particles, Crispr-Cas9 technology, and immune-informatics are expected to enhance the effectiveness of recombinant vaccines in the near future[123]. In the years to come, the future of recombinant vaccines will depend on ongoing dedication to research, teamwork, and innovative approaches.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

VK, and AV collected the literatures and prepared the manuscript. VK, SK, and SKM designed the original idea, collected the literatures and prepared the manuscript. HNS, RS, PG, and SKS reviewed and edited the manuscript. All the authors approved the final version of the manuscript.

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