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Exploring future perspectives and pipeline progression in vaccine research and development

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Keywords: Vaccine R&D; vaccines pipeline; new vaccine platforms
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Abstract

Introduction. *The COVID-19 pandemic had a profound impact on vaccines' Research and Development, on vaccines' market, and on immunization programmes and policies. The need to promptly respond to the health emergency boostered resources' allocation and innovation, while new technologies were made available. Regulatory procedures were revised and expedited, and global production and distribution capacities significantly increased. Aim of this review is to outline the trajectory of research in vaccinology and vaccines' pipeline, highlighting major challenges and opportunities, and projecting future perspectives in vaccine preventables diseases' prevention and control.*
Study Design. *Narrative review.*
Methods. *We comprehensively consulted key biomedical databases including "Medline" and "Embase", preprint platforms, including"MedRxiv" and "BioRxiv", clinical trial registries, selected grey literature sources and scientific reports. Further data and insights were collected from experts in the field. We first reflect on the impact that the COVID-19 had on vaccines' Research and Development, regulatory frameworks, and market, we then present updated figures of vaccines pipeline, by different technologies, comparatively highlighting advantages and disadvantages. We conclude summarizing future perspectives in vaccines' development and immunizations strategies, outlining key challenges, knowledge gaps and opportunities for prevention strategies.*
Results. *COVID-19 vaccines' development has been largely supported by public funding. New technologies and expetited authorization and distribution processes allowed to control the pandemic, leading vaccines' market to grow exponentially. In the post-pandemic era investments in prevention are projected to decrease but advancements in technology offer great potential to future immunization strategies. As of 2023, the vaccine pipeline include almost 1,000 candidates, at different Research and Development phase, including innovative recombinant protein vaccines, nucleic acid vaccines and viral vector vaccines. Vaccines' technology platforms development varies by disease. Overall, vaccinology is progressing towards increasingly safe and effective products that are easily manufacturable and swiftly convertible.*

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Conclusions. *Vaccine research is rapidly evolving, emerging technologies and new immunization models offer public health new tools and large potential to fight vaccines preventables diseases, with promising new platforms and broadened target populations. Real-life data analysis and operational research is needed to evaluate how such potential is exploited in public health practice to improve population health.*

Introduction

The COVID-19 pandemic has propelled vaccine research into an unprecedented era of challenges and opportunities, sparking a global initiative to swiftly develop, produce, and distribute effective vaccines. The health emergency underscored the critical need to expedite advancements in scientific frontiers, accelerating efforts to find innovative preventive solutions. The emergence of SARS-CoV-2 has triggered a race against time within the scientific community, driving the development and manufacturing of new vaccine platforms. The substantial increase in both public and private investments in vaccine research has reshaped the scientific landscape, resulting in a significant overhaul of processes and authorization procedures. Notably, the adoption of practices like the rolling reviews contributed to expedite vaccine approval while upholding stringent safety and efficacy standards (1). Engagement from various stakeholders, including governments, academic institutions, pharmaceutical companies, and international organizations, has fostered extensive collaboration in Research and Development (R&D), together with an unprecedented sharing of knowledge. Regulatory authorities have intensified their involvement and interaction with sponsors, governments had to re-evaluate the importance of their preparedness for pandemics, leading them to prioritize investments and seek ways to accelerate the development of new medicines. Companies have also reconsidered the structure of their R&D initiatives, focusing on primary outcomes rather than burdening trials with secondary endpoints and evaluations. Additionally, they have re-assessed the approaches used in conducting clinical trials, including the application of predictive modeling for the selection of trial sites (2).

Regulatory frameworks

The global pandemic triggered a paradigm shift in international regulatory mechanisms, notably through the implementation of innovative approaches. In Europe, the use of rolling reviews enabled researchers to continuously submit data throughout multiple review cycles as it became accessible, preceding the formal application submission. This approach actually departed from the traditional approval pathway, where all data undergoes assessment at the conclusion of clinical trials. Adopted by several health authorities worldwide during the pandemic (3), rolling reviews allowed regulators to continuously evaluate emerging data, expediting the assessment and potential approval of vaccines by expediting the review process (4). In the United States, this approach was not an independent procedure, but rather considered a facet linked to the "Fast Track Designation" under the Food and Drug Administration (FDA) guidance (5). Furthermore, the urgency to address the pandemic prompted regulatory agencies to implement expedited Marketing Authorizations (MAs), such as the EMA's Conditional Marketing Authorization (CMA) and the FDA's Accelerated Approval (6). MAs facilitated the provisional endorsement of vaccines using interim data, conditional upon meeting specific criteria to ensure their safety, effectiveness, and ongoing monitoring. Innovative pragmatic approaches swept vaccine deployment while ensuring continuous evaluation and data gathering post-endorsement to address an unmet medical need (1).

The impact of the COVID-19 pandemic on vaccine global market: manufacturing, volumes, Research and Development

With the introduction of COVID-19 vaccines, the global production of vaccine doses experienced a significant surge, rising from 5.8 billion doses in 2019 to 16 billion doses in 2021 (7). COVID-19 vaccine doses alone accounted for 67% of the global volume in 2021 (7). This unprecedented increase in production had a substantial economic impact. The costs associated with manufacturing and distributing vaccines soared alongside global distribution, prompting a pivotal shift in R&D expenditure. In 2021, the estimated global investments dedicated

to vaccine development saw a substantial increase, rising from approximately 1 billion to 13 billion dollars (8). Furthermore, the production timelines significantly changed: before COVID-19, the average duration from initial Phase I clinical testing to final product approval spanned over nearly a decade. This sharply contrasted with the development timelines of COVID-19 vaccines, completed in less than a year (3). For instance, certain clinical development phases were initiated before the preceding phases had been entirely concluded (2).

Who provides funding?

In pandemic times, substantial investments were facilitated by various funding sources. Before the pandemic, basic vaccine research and early-stage development often received support from the public sector (9). Throughout the pandemic, an unparalleled amount of resources was allocated to finance clinical trials, expand manufacturing capabilities, and establish Advance Purchase Agreements (APAs). A study requested by the European Parliament's Policy Department for Economic, Scientific and Quality of Life Policies reported that governments, primarily the US (with some not-for-profit entities), massively supported corporate investments, either for R&D, manufacturing, or both, by nearly EUR 9 billion. Governments and other public entities constituted more than 80% of the overall external funds identified. Their support was provided through grants and loans (10). The substantial investments from the public sector, combined with remarkable collaborative initiatives by regulatory bodies, enabled manufacturers to develop vaccines within a 10-month period and simultaneously expand manufacturing capabilities.

Progress and sustainability

Enterprising companies ventured into risk-based investments to support the development of COVID-19 vaccines, a conventional approach in drug development that was unprecedentedly expanded during the crisis (2). Despite the substantial growth in the global vaccine market size due to COVID-19, it is expected that this impact will wane by 2024, with the projected vaccine market size returning to pre-pandemic estimates (11). The resolution of the pandemic crisis and the subsequent departure from emergency regulatory measures and significant public funding will require a more cautious approach to vaccine R&D within companies. This transition calls for a recalibration towards sustainable financial practices. While the extraordinary public funding during the pandemic facilitated swift progress in vaccine development and other medical interventions, companies are now faced with the imperative of establishing a sustainable financial framework for future R&D initiatives. The conclusion of emergency regulatory mechanisms indicates a need for companies to exercise caution and prudence in their R&D pursuits, giving priority to long-term financial sustainability over rapid, resource-intensive breakthroughs. This transition emphasizes the importance of striking a strategic balance between innovation and fiscal responsibility, promoting a renewed focus on cost-effectiveness, efficient resource allocation, and the pursuit of R&D projects that ensure sustained viability and societal benefit in the post-pandemic landscape.

Aim

Within a realm characterized by the rapid pace of scientific advancements and collaborative endeavors, this article explores the evolving perspectives of vaccine R&D. It carefully examines technological progress and assesses the transformative impact of COVID-19 on the trajectory of vaccinology. Our review aims to capture the current landscape of vaccine R&D; this entails mapping its pathways and delineating the current vaccine pipeline, which integrates both established and emerging technologies, while forecasting their present and potential future applications.

Methods

The review integrates research articles and literature reviews, retrieving information from different sources. Searches were conducted in Medline and Embase up to January 2024 using key words and MeSH terms (i.e. vaccines, immunization), and we also referred to the BioRxiv and MedRxiv platforms for unpublished data and supplementary details. In addition, we consulted selected clinical trial registries, publicly available documents, and reports from technical committees at both national and international health level. Further data and insights were collected interviewing experts in the field. We first embark on reconstructing the trajectory of vaccine research, elucidating the overarching direction and the array of tools currently available: this involved delineating the general path of progression and highlighting the available resources. Subsequently, we provide detailed insights into the platforms currently prominent in the vaccine pipeline: our exploration encompass understanding their

functionalities, strengths and limitations, current fields of experimentation, and, whenever feasible, prospects for future applications. Finally, we synthesize the literature findings, consolidating them into a dedicated summary table. The content of this paper was presented during the advanced course "Vaccination in high-risk individuals" organized by the International School of Epidemiology and Preventive Medicine "Giuseppe d'Alessandro" at the "Ettore Majorana" Foundation and International Centre for Scientific Culture on 2023 November 22-25 (12).

Results

Vaccine R&D trajectory

A well-known barrier in vaccine development since its early stages has been the decreasing effectiveness of immunostimulation as antigens are simplified and purified. Earlier attenuated or inactivated whole-organism vaccines provided a significant immunogenicity but were poorly tolerated due to frequent side effects (13,14). As shown in Figure 1, to reduce reactogenicity, vaccines have progressively shifted towards formulations comprising only sections of the microorganism, subunits, or purified antigens (15). However, while vaccines containing a limited set of purified antigens typically demonstrate superior safety profiles compared to live-attenuated and whole-pathogen vaccines, a decrease in their immunogenicity often occurs (13). For instance, purified protein antigen vaccines without adjuvants elicit a modest antibody response with minimal or no T cell response. The incorporation of adjuvants or other enhancers facilitated the reinstatement of immunogenicity in these vaccines, often showcasing significantly enhanced tolerability profiles, compared to conventional whole inactivated organism vaccines. These adjunctive components serve to amplify and fine-tune the body's immune reaction, compensating for the lower immunogenicity of certain novel vaccine technologies. Thus, the strategic use of these enhancers becomes essential in maximizing vaccines' effectiveness, ensuring resilient and comprehensive immune protection.

Adjuvants for Vaccine Platforms

The role of adjuvants in vaccine formulations has long been recognized. Alum-adjuvanted vaccines were approved over 70 years ago during the development of vaccines for diphtheria, tetanus, pertussis, and poliomyelitis. This type of adjuvant preferentially stimulates CD4 cells. Since then, adjuvants have evolved over time, progressing towards formulations that are less reactive, while still capable of stimulating both the humoral and cellular arms of the immune system. More recent vaccines, such as those for human papillomavirus and hepatitis B, have benefited from similar but updated adjuvants, like AS04, which is more selective in activating TLR4 and ensuing cellular component of the immune response. Oil-in-water emulsion adjuvants (as well as those constituted by liposomes) such as MF59 and AS03, have been used in the past and reintroduced more recently in innovative forms. For instance, AS02 has been deployed in experimental vaccines for malaria (16). In general, novel adjuvants' formulations comprising emulsions or liposomes involve the incorporation of monophosphoryl lipid A (MPLA). This compound maintains its capacity to trigger the innate immune response through its interaction with TLR-4 (17): liposomes with the addition of MPLA have been tested in malaria vaccines as seen in the instances of AS01 (18) and ALF formulations (19). New adjuvants have evolved to ensure concurrent stimulation of both CD4 and CD8 cells. The enhancement of CD8+ T-cell responses can be further augmented by exogenous components such as saponins. Specifically, the saponin QS21, derived from the bark of the Chilean native tree Quillaja Saponaria, can significantly boost these responses. For instance, the AS01B adjuvant combines MPL with QS21 saponin and has been employed in the Herpes zoster recombinant vaccine. Among recent advancements in adjuvant development, the AS01E stands out. It serves as an adjuvant for recombinant protein vaccines, which have already been employed against COVID-19 (20). This platform effectively merges various principles used in its precursors. Specifically, this compound constitutes a complex where saponin combines with specific fatty acids, namely cholesterol and phospholipids (20). It is already incorporated into several vaccines currently under development, including the R21/Matrix-M malaria vaccine (21) and selected influenza vaccines (22).

Where to next after the pandemic?

As of 2023, the global vaccine pipeline include almost 1000 candidates, the majority being recombinant protein vaccines (22%), mRNA vaccines (18%), inactivated vaccines (14%), viral vector vaccines (14%), and conjugate vaccines (11%) (23). Below, we provide an overview of the vaccine R&D pipeline and its innovative aspects, organized by technology

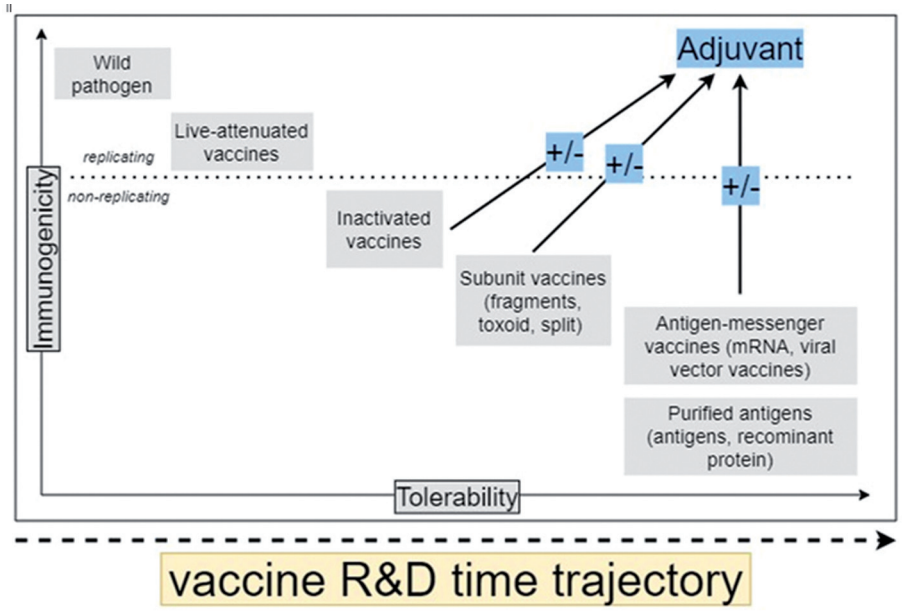

Figure 1 - Vaccine R&D time trajectory

type. We outline the immunological mechanisms, applications, and the preventive areas currently being tested. A summary is reported in table 1.

Recombinant protein vaccines

Recombinant proteins have been used as drugs for decades (24). They are often produced using bacteria, yeast, mammalian, or insect cells as factories for antigens (25). Recent advancements in recombinant protein technology have significantly enhanced efficiency and accessibility, enabling cost-effective production across various microbial and expression host systems (26,27). Despite their advantages, the immune-stimulating potential of subunit vaccines tends to be lower compared to those containing the entire virus. As a result, the administration of

Table 1 - Vaccine platforms, advantages and disadvantages

Vaccine platform	Advantages	Disadvantages
Recombinant protein	Safe and well-tolerated Stable at higher temperatures (2-8°C)	Low immunogenicity Requirement of adjuvant or conjugate to increase immunogenicity
mRNA	Safe and well-tolerated Highly adaptable to new pathogens No need for adjuvants	Immunological instability (over time and depending on new emerging variants) Requirement of complicated cold chain management (-15 to -80°C)
Viral vector	Stronger immune response (preservation of native antigen) Mimicking natural infection Stable at higher temperatures (2-8°C)	Complicated manufacturing process
Inactivated	Safe and well-tolerated Stable at higher temperatures (2-8°C)	Complicated manufacturing process Moderate immune response Requirement of high-dose formulations or adjuvants (under investigation for mucosal vaccines) Less adaptable to new pathogen
Conjugate (polysaccharide)	Longer duration of protection compared to polysaccharide vaccine Stable at higher temperatures (2-8°C)	Complicated manufacturing process

multiple doses and the inclusion of adjuvants are often necessary. During the COVID-19 pandemic, the spotlight has shifted towards recombinant protein vaccines, marking a pivotal moment in vaccination strategies. NVX-CoV2373 is a recombinant vaccine against SARS-CoV-2 in which nanoparticles are mixed with AS01E adjuvant. It requires a standard cold storage (2-8°C) (28,29). Data from phase 3 clinical trials, which led to their commercialization, highlighted an efficacy of NVX-CoV2373 of around 90% against the B.1.1.7 (Alpha) SARS-CoV-2 variant (30,31). Furthermore, as real-world data is accumulating, in a prospective observational study, NVX-CoV2373 protein-adjuvanted vaccine demonstrated less reactogenicity (77.6%) than mRNA vaccines (95.9%) (32). Italian real-world data collected on 21000 subjects showed an estimated effectiveness of a NVX-CoV2373 primary cycle higher than BNT162b2 and similar to mRNA-1273 (around 45%) (33). A decrease in effectiveness based on the circulating variant has also been documented in post-market observational studies of both mRNA COVID-19 vaccines (34). While recombinant technology may still exhibit limitations in terms of long-term efficacy, its advantages in terms of reactogenicity and a high safety profile make such technology extremely advantageous. In March 2023, EMA recommended the approval of PHH-1V as a booster vaccine for COVID-19 (35). PHH-1V is an adjuvanted recombinant protein vaccine that applies recombinant DNA technology to combine two distinct receptor binding domains (RBDs) from the Beta and Alpha variants of SARS-CoV-2. A booster dose of PHH-1V administered at 6 months demonstrated significantly higher neutralizing antibody titers, compared to individuals who received the BNT162b2 mRNA vaccine, showing efficacy against different variants (36,37). The DNA recombinant protein vaccine PHH-1V exhibited also low reactogenicity and achieved significantly superior neutralizing antibody responses, compared to BNT162b2 (38). Notably, PHH-1V does not require deep-freezing for distribution or onsite storage (36): this characteristic facilitates storage and distribution across diverse logistical and healthcare settings. A saponin-adjuvanted recombinant DNA vaccine (RZV), specifically designed for preventing herpes zoster (HZ), has already received approvals from both the FDA and EMA, showing greater effectiveness than the zoster live attenuated vaccine (ZVL) (39,40). Differently from ZVL, its low reactogenicity enables administration to high-risk immunocompromised patients aged 18 and older, expanding vaccine's target population; this expansion positively impacts on preventive strategies aimed at vulnerable individuals, providing an improved tool in the planning phase of public health policies. Additional examples include recombinant protein vaccines against malaria: in October 2023, the World Health Organization (WHO) recommended the R21/Matrix-M malaria vaccine as it was shown to reduce symptomatic cases of malaria by 75% during the 12 months following a 3-dose series in areas with highly seasonal transmission (41). Two recombinant vaccines have already been approved by the FDA and EMA for respiratory syncytial virus (RSV) prevention among elders (42,43), one of which has also been approved for vaccination during pregnancy and for preventing the disease in newborns (44,45). Its characteristics make the recombinant platform attractive for the future: several vaccines using this technology are currently being studied in clinical research, including a quadrivalent seasonal influenza vaccine (46).

mRNA vaccines

The pioneering technology of messenger RNA (mRNA) vaccines has garnered immense recognition, notably highlighted by the 2023 Nobel Prize in Physiology or Medicine awarded to the scientists behind its development. mRNA, encoding a specific protein capable of mimicking the antigen, is delivered through lipid nanoparticles (LNPs) vaccine vehicles and enters cells solely via endocytosis. mRNA vaccines exhibit a self-adjuvant effect as the single-stranded RNA (ssRNA) can be identified by Toll-Like Receptor 7 (TLR7) and TLR8 within endosomes (47), subsequently triggering a cellular immune response in addition to the humoral response activated by the post-translational antigen presentation (48,49), without the need for an adjuvant. Furthermore, the lipids present in the nanoparticle, where the mRNA is carried, can stimulate the production of IL-6, thereby amplifying the CD4+ follicular helper T cell and B cell response (50). Due to their intrinsic ability to activate cellular immunity, this type of vaccine was first tested in an oncological setting, specifically in patients with advanced-stage melanoma, in an initial trial back in 2008 (51). As for recombinant protein vaccines, the advantages of mRNA-based vaccines stem from their proven effectiveness and safety records. Within the fight against SARS-CoV-2, mRNA vaccines have emerged as frontrunners, with BNT162b2 and mRNA-1273 receiving global emergency use authorization. The pandemic context has been a valuable testing ground for this type of vaccines

to assess their resilience. From this perspective, as mentioned earlier, mRNA vaccines share similar results in terms of effectiveness and reactogenicity with recombinant protein vaccines. They also face comparable challenges concerning the duration of effectiveness and efficacy against newly emerging variants: from an observational study in England, effectiveness of a BNT162b2 or mRNA-1273 booster against COVID-19 symptoms declined consistently under 50% at 10 or more weeks (52). In the case of mRNA vaccines, the challenge of immunological stability compounds the logistical issue of storage. It has been demonstrated that the lipid nanoparticle composition of these vaccines is influenced by certain elements, such as pH and temperature. Specifically, very low temperatures are associated with a higher particle concentration and better functionality, whereas exposure to excessively high temperatures compromises the nature of the nanoparticles, causing them to aggregate (53). This necessitates a cold-chain storage for these vaccines, posing organizational challenges both in terms of storage and transportation. For instance, ultra-cold storage requirements slowed down the distribution of COVID-19 mRNA vaccines in low income countries (54). The similar effectiveness of mRNA and recombinant vaccines is biologically proportional to the immune response prompted by both technologies: initial real-world data showed a similar response in both spike-specific CD4+ T cell response and acute and memory CD8+ T cell frequencies (55). Interestingly, observational studies have consistently indicated distinctions between the two mRNA COVID-19 vaccines concerning immune response (56,57) and clinical effectiveness (58,59) in immunocompromised populations with mRNA-1273 associated to better outcomes than BNT162b2. mRNA-1273 and BNT162b2 vaccines were associated with a very low risk of adverse events (60); mRNA-1273 was also found to be correlated to a lower risk of selected adverse events, such as pulmonary embolism, thromboembolic events, myocarditis, pericarditis and acute myocardial infarction, compared with BNT162b2 (61,62). While the incidence of myocarditis and pericarditis appears slightly elevated following mRNA vaccine administration compared to the general population, it remains considerably lower than the risk associated with a SARS-CoV-2 infection (63,64). When considering the broader spectrum of cardiovascular risks posed by COVID-19, the overall benefit-risk assessment strongly advocates for vaccination across all age and gender demographics (65). Overall, mRNA vaccine technology has demonstrated significant reliability. Initially, it provided a tool capable of addressing the pandemic threat, and over time, it has shown excellent efficacy in the medium term (though not entirely in the long term), along with an outstanding safety profile: effectiveness against severe diseases varied between 75% and 90% depending on the predominant variants (34). Not surprisingly, the vaccine pipeline using this technology is rich and extremely promising: a new vaccine (mRNA-1345) for preventing RSV disease in individuals over 60 has shown an 80% efficacy in a phase 3 trial (66). In the near future, a new pan-respiratory vaccine could combine three mRNA vaccines in the same formulation (mRNA-1230): COVID, influenza, and RSV (67). A Phase 1 Study has been started to evaluate the safety and immunogenicity of a mRNA Vaccine (mRNA-1644) against HIV. Furthermore, mRNA vaccines research for cancer treatment experienced a significant acceleration with the implementation of this technology during the pandemic period: the underlying mechanism involves antigen-presenting cells displaying tumour-associated antigens on both MHC class I and MHC class II to activate CD8+ and CD4+ T cells (68). Some trials showed sustained positive responses in cancer patients post mRNA-based vaccine treatment, without encountering uncontrollable toxic effects (69). mRNA vaccines exhibited potential as valuable therapeutic options for upcoming cancer treatments, particularly when used alongside supplementary immunotherapies (70). Administration of mRNA-4157/V940 vaccine as an adjuvant therapy during a 2b phase trial, in conjunction with a monoclonal antibody, decreased the risk of recurrence or death by 44% in individuals with completely removed stage III/IV melanoma (71).

Viral Vector Vaccines

The technology of recombinant vectors used to deliver antigens from a specific microorganism has been employed for a long time (72). Viral vectors are harmless and serve as vehicles to transport genetic information into host cells, prompting the synthesis of antigens that activate the immune response (73): they undergo genetic engineering to incorporate specific genes that encode crucial antigens of pathogens (74). Various viruses, including retrovirus, lentivirus, cytomegalovirus, and adenovirus, have been used as carriers. Among these, adenovirus stands out as the most commonly employed viral vector owing to its extensively documented safety profile and its ability to effectively stimulate the inflammatory

and immune systems (75). Indeed, one advantage of replicating vectors is their mimicry of a natural infection, resulting in the induction of cytokines and co-stimulatory molecules that provide a potent enhancing effect; viral vector vaccines can induce high immunogenicity without the use of an adjuvant, along with enduring immune responses (76). ChAdOx1-S and Ad26.COV2.S, two viral vector vaccines, were among the initial resources employed in the fight against COVID-19 (77). In the case of the ChAdOx1 nCoV-19 vaccine, the genetically modified chimpanzee adenovirus carries the gene responsible for the SARS-CoV-2 spike protein into the nucleus, where it is transcribed into mRNA by DNA polymerase (74). Despite their effectiveness in reducing SARS-CoV-2 complications, this type of vaccine has demonstrated lower immunogenicity compared to its mRNA counterparts. In a prospective cohort study conducted in the Netherlands, four weeks after the completion of the initial vaccination series, individuals who received mRNA-1273 vaccines exhibited the highest levels of neutralizing antibodies against the SARS-CoV-2 wild-type; this was followed by recipients of the BNT162b2 vaccine, whereas considerably lower antibody titres were observed in individuals vaccinated with the adenovirus vector-based vaccines ChAdOx1-S and Ad26.COV2.S (78). In a longitudinal analysis of immune response to four different COVID-19 vaccines, neutralizing antibody titres were also observed to be lower compared to NVX-CoV2373 (55). These findings are consistent with the distinct cellular dynamics triggered by different types of vaccines, showing a lower spike-specific CD4+ T cell response at 6 weeks post-immunization for viral vector vaccines, compared to mRNA vaccines and recombinant protein vaccines (55). Furthermore, viral vector vaccines demonstrated the capability to trigger Th1 cell responses, thereby eliciting strong protective effects (79). Viral vector-based vaccines are associated with more frequent systemic side effects, compared to mRNA-based vaccines (80,81,82). A systematic review reported a higher number of cardiovascular and hemorrhagic events following viral vector-based vaccine administration compared to mRNA-based vaccines, based on data collected from 98 studies (83). Furthermore, vaccine-induced immune thrombotic thrombocytopenia (VITT) has been reported after adenoviral vaccines administration (84,85) and a strong association was found between VITT and adenoviral vector-based vaccines (86,87,88) compared with mRNA-based vaccines (89), mostly among females aged below 60 (90). Nevertheless, viral vector vaccines use is associated with significant logistical advantages, as demonstrated during the pandemic: this type of vaccine is challenging to manufacture but the enhanced molecular stability allows for storage at less extreme temperatures compared to mRNA platforms, facilitating also easier transportation (91,92). Viral vector vaccines represent a large share of the current vaccine pipeline, with over 130 candidates (23). Among these, approximately 80 are composed of adenoviral vectors and are being tested for vaccines against influenza, Ebola and HIV (93).

Inactivated vaccines

Inactivated vaccines, along with live attenuated vaccines, belong to a more traditional type of vaccines and have been widely used in clinical practice for a long time. Inactivated vaccines comprise all pathogen's components but in an inactivated state, making it unable to cause illness in humans. These vaccines are crafted using methods like heat, radiation, or chemical agents such as formaldehyde or β-propiolactone, that disassemble the viral structure and genetic material (94). Notably, inactivated vaccines are widely regarded as safe. However, they typically exhibit relatively lower immunogenicity, potentially resulting in a weaker immune response (95,96). To enhance vaccines' effectiveness high-dose formulations are required (97). Alternatively, adjuvants are often included to elicit a stronger immune response (98,99): influenza adjuvant trivalent inactivated vaccine was more effective in averting influenza-related outcomes compared to high-dose inactivated vaccine (100). VLA2001 (inactivated whole-virus, adjuvanted SARS-CoV-2 vaccine) was the first COVID-19 vaccine to receive a standard marketing authorization in Europe. In a phase 3 trial VLA2001 showed lower reactogenicity and exhibited higher immunogenicity compared to ChAdOx1-S (101). The safety and comprehensive knowledge of these vaccines still make them viable candidates for various platforms: currently, inactivated vaccines for influenza, Zika, and rabies are undergoing trials (102). Storage is permitted at standard temperatures (2-8°C) (28,29). In recent years, inactivated formulations have been employed for the production of mucosal vaccines (e.g., influenza, cholera), and others are currently under experimentation (e.g. against SARS-CoV-2) (103): in this context as well, the use of adjuvants emerges as a potential solution to enhance the efficacy of inactivated vaccines (104). However, uncertainties persist regarding the potential reactogenicity of current adjuvants for mucosal delivery (105).

Conjugate vaccines

Conjugate vaccines are a category within the domain of subunit vaccines, largely used for pneumococcal immunization. They are characterized by a specific composition where a polysaccharide chain is attached to a immunogenic carrier protein (106) in order to enhance immunogenicity and stability (23). This unique configuration allows conjugate vaccines to offer prolonged protection compared to raw polysaccharide vaccines (107,108). They require standard storage (2-8°C) (109), but their manufacturing is a complex process (110). Conjugate pneumococcal vaccines have evolved, progressively targeting a greater number of bacterial serotypes. Recently, PCV15 and then PCV20 have been added to the pool of available conjugate vaccines. In a phase 1/2 trial, V116, an experimental 21-valent pneumococcal conjugate vaccine (PCV), exhibited good tolerance with a safety profile largely similar to PPSV23. Furthermore, it was non-inferior to PPSV23 for the common 12 serotypes and superior for the 9 unique serotypes in V116 (111). Innovative conjugation methods are currently undergoing experimentation: site-specific covalent conjugation could lead to a more reliable conjugation process, allowing the incorporation of a greater variety of serotypes while reducing carrier-mediated immunological interference: VAX-24 exhibited a superior immunological response compared to PPV23 (112,113). Moreover, new Multiple Antigen Presenting System (MAPS) platform, harnessing a high-affinity noncovalent binding technology, showed a robust B-cell and T-cell immune response in animal models (114): a 24-valent pneumococcal MAPS vaccine has completed a Phase 2 trial in older adults (115) demonstrating a stronger antibody response compared to vaccinations with PCV13 and PPSV23 while maintaining a similar safety profile (116), and is currently undergoing a Phase 2 trial in infants (117).

Discussion

Ongoing efforts in vaccine R&D are prominently focused on innovative technologies designed to enhance the effectiveness and resilience of evolving vaccine platforms. These advancements not only show potential in strengthening vaccine efficacy, but also hold promise for addressing organizational challenges that emerged during the pandemic (118-120). The introduction of novel technologies may provide solutions to logistical complexities, particularly in the management of the cold chain, while simultaneously enabling more efficient and widespread immunization campaigns. Moreover, these advancements may set the stage for proactive initiatives targeting various potentially emerging diseases, especially within vulnerable populations, thereby enabling timely and comprehensive preventive approaches.

During the COVID-19 pandemic, technologies such as recombinant protein vaccines and mRNA vaccines have experienced remarkable success. These platforms have undergone extensive development and constitute the cornerstone of the current vaccine pipeline. While recombinant vaccines entered the market later, holding large potential, they have yet to undergo long-term evaluation compared to mRNA vaccines. Despite demonstrating highly reassuring levels of effectiveness and safety, mRNA vaccines present two main challenges to address: the first relates to their high immunological instability, requiring periodic booster doses; the second is an organizational concern regarding the storage of formulations at sufficiently low temperatures to prevent denaturation (121). The forthcoming generation of mRNA vaccines, using self-amplifying mRNA (saRNA), or replicon RNA, holds the potential to overcome these challenges. Replicons share the same mechanism of action as current mRNA vaccines but, additionally, they are linked to a self-amplifying gene that enables them to replicate within the cell: in this way, each replicon can transcribe for proteins, allowing the translation of a greater number of them. Another cutting-edge possibility involves the utilization of circular RNAs (circRNAs), a recent advancement in the mRNA vaccine domain: due to the absence of free ends susceptible to exonuclease degradation, they exhibit enhanced stability compared to linear mRNA vectors (122).

The challenge associated with the stability of newly manufactured vaccines is not only tied to their effectiveness, but also to the organizational aspects of their administration. Currently, innovative technologies ensure the production of safe vaccines but require periodic boosters. Thanks to their robust safety profile, they will facilitate administration to increasingly larger segments of the population, allowing for the prioritization of high-risk patients, regardless of age. The need to vaccinate more people and more frequently is propelling R&D to explore new combined formulations: as mentioned earlier, experimentation is underway for a pan-respiratory vaccine (mRNA-1230), while additional combinations are currently being explored. Notably, a vaccine candidate targeting influenza and COVID-19 (mRNA-1083) has already entered Phase 3 evaluation after

achieving antibody titres similar to, or greater than licensed quadrivalent influenza vaccines and the mRNA-1273 COVID-19 vaccine (123).

The substantial surge in vaccine research driven by the COVID-19 pandemic is set to decelerate. Given the extraordinary historical context and the unprecedented volume of funding, largely supplied from public and governmental sources, the vaccine pipeline has seen significant enrichment in recent years, both in terms of quantity and technological diversity. Platforms like mRNA, originally explored in other fields of medicine such as oncology, have shifted focus to infectious disease prevention, yielding remarkable outcomes within relatively short timeframes. As we transition from an emergency context, innovative technologies will face challenges: it is likely that only the most promising or those with a well-established track record of reliability will persist in use, contributing to a sustainable perspective. New technologies have far exceeded the challenges posed by the pandemic, with recombinant protein and mRNA platforms emerging as dominant players in the vaccine pipeline: they are currently undergoing trials for broader applications across various diseases and domains. However, certain limitations have undeniably emerged: historically, vaccine R&D developed from safer and less reactive platforms, but enhanced safety profiles often correlate with reduced immunogenicity. Especially in highly variable pandemic contexts, where exposure to rapidly evolving viral agents is prevalent, such vaccines have demonstrated limited long-term immunological stability. This highlighted the need for booster administrations and a decline in efficacy against emerging variants. Nonetheless, ongoing advancements are directed towards bolstering the stability and reliability of these tools: emerging technologies, such as experimental self-amplifying mRNA (saRNA) or circRNAs, aim to enhance the stability of mRNA vaccines, prolonging their efficacy over time and enabling more convenient and less resource-demanding transportation and storage methods. Overall, vaccine research is also progressing towards technologies that facilitate highly effective and large-scale public health strategies. In this context, improved safety profiles are poised to broaden the pool of eligible candidates, preventing potential complications from dangerous disease in high-risk individuals across all age groups (124). Additionally, biologically more stable technologies will streamline storage and transportation systems, thereby simplifying organizational and logistical processes. This study presents certain limitations related to the narrative approach of our review. Its objective is to provide context to the current landscape of vaccine research, a field that has undergone significant acceleration amid the ongoing pandemic. Just as pathogens constantly evolve, vaccines necessitate adaptation to enhance effectiveness while upholding safety standards. Our data and reasoning provide insights to public health policymakers, tasked with enhancing the development of preventive strategies targeting broader populations, and ultimately maximizing efficiency in the utilization of both time and resources.

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Riassunto

Opportunità e prospettive future della pipeline vaccinale e dell'innovazione in vaccinologia

Introduzione. La pandemia da COVID-19 ha avuto un profondo impatto sulla ricerca in ambito vaccinale, sul mercato globale, sui programmi e le politiche di immunizzazione. La necessità di far fronte in tempi rapidi all'emergenza sanitaria ha reso necessarie diverse innovazioni: a livello regolatorio le procedure di immissione in commercio sono state riviste e rese più rapide e la capacità di produzione e distribuzione ha visto un incremento significativo. Lo scopo di questa revisione è quello di ricostruire la traiettoria della ricerca in ambito vaccinale, evidenziandone le attuali sfide e le principali criticità.

Disegno dello studio. Lo studio è una revisione narrativa della letteratura.

Metodi. Le evidenze disponibili sono state selezionate consultando i principali database biomedici, preprint server, registri di trial clinici, selezionate fonti di letteratura grigia e rapporti scientifici. Ulteriori dati e approfondimenti sono stati raccolti attraverso la consultazione di esperti nel settore. Abbiamo analizzato l'impatto complessivo della pandemia sulla ricerca e sviluppo in ambito vaccinale, sui quadri normativi e sul mercato. Siamo passati poi ad analizzare l'attuale pipeline vaccinale e le tecnologie ad oggi impiegate. Infine, sono state riassunte le prospettive future nello sviluppo dei vaccini e nelle strategie di immunizzazione, delineandone le principali sfide e opportunità.

Risultati. Lo sviluppo dei vaccini COVID-19 è stato supportato da ingenti finanziamenti pubblici. Lo sviluppo di nuove tecnologie, insieme a processi di autorizzazione ed immissione in commercio più rapidi, hanno permesso di controllare la pandemia, generando una crescita esponenziale del mercato vaccinale globale. Nell'era post-pandemica, gli investimenti in prevenzione sono destinati a decrescere, ma i progressi tecnologici in atto hanno il potenziale per supportare le future strategie di immunizzazione. Nel 2023 la pipeline vaccinale include circa 1000 candidati, tra cui vaccini a proteine ricombinanti, vaccini a base di acidi nucleici e vettori virali, vaccini

inattivati e coniugati. Nella trattazione dettagliamo lo sviluppo delle piattaforme tecnologiche, differenziando malattia infettiva prevenibile e popolazioni target. In generale, la ricerca in ambito vaccinale progredisce verso prodotti sempre più sicuri ed efficaci, di facile produzione e stoccaggio e di agevole conversione.

Conclusioni. La ricerca in ambito vaccinale evolve rapidamente: le nuove tecnologie mettono a disposizione della sanità pubblica nuovi strumenti utili ad estendere la protezione vaccinale. Nuove ricerche basate su real-life data sono necessarie per valutare l'impatto di tale potenziale come strumento di prevenzione per la tutela della salute collettiva.

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