Journal of Drug Research and Development
ISSN 2470-1009 | Open Access

REVIEW ARTICLE

Volume 8 - Issue 2

Challenges of Developing Novel Vaccines and Large Scale Production Issues

Fikadu Ejeta*

School of Pharmacy, College of Medicine and Health Sciences, Mizan-Tepi University, Ethiopia

***Corresponding author:** Fikadu Ejeta, School of Pharmacy, College of Medicine and Health Sciences, Mizan-Tepi University, Ethiopia, Tel: +251910136034; E-mail: fikaduejeta@gmail.com

Received: 19 Jul, 2022 | **Accepted:** 01 Aug, 2022 | **Published:** 08 Aug, 2022

Citation: Ejeta F (2022) Challenges of Developing Novel Vaccines and Large Scale Production Issues. J Drug Res Dev 8(2): dx.doi.org/10.16966/2470-1009.171

Abstract

Live attenuated and inactivated pathogens, as well as subunit vaccinations, can give long-term protection against a variety of deadly diseases. Despite this progress, vaccine development for a number of infectious diseases, particularly those that are more capable of evading the adaptive immune response, remains a serious issue. Furthermore, the fundamental impediment to the greatest uptake of virus vaccines isn't usually the efficacy of conventional procedures, but rather the requirement for more fast research and large-scale manufacture. As a result, the development of more powerful and adaptable vaccine platforms is critical. The complexities of developing the manufacturing process, formulation, and analytical assays, as well as the problem of scientific assay optimization, are the most well-known barriers to vaccine development failures or delays.

Scientists argue that the extremely concentrated state of global vaccine manufacturing capacity limits large-scale vaccine production. At the moment, only a few countries have the capacity to make vaccines on their own. Scaling up vaccine production is difficult, and a shortage of manufacturing sites is limiting global vaccine availability. Vaccine manufacturing and the development of breakthrough technologies capable of producing huge quantities of vaccines against known and undiscovered infections are difficult tasks nowadays. Vaccines can be made as suspensions, emulsions, or freeze-dried powders with a variety of adjuvants. However, many of those manufactured vaccines face multiple problems from a pharmaceutical standpoint, including the risk for acute hypersensitivity reactions, the need for extremely cold storage temperatures, and handling and delivery requirements. These requirements should limit vaccine supply to different populations, which has a negative impact on health equity. In the production of vaccines during upstream and downstream processes, new facilities, equipment, and enabling technology may be required, some of which may have an impact on how existing vaccines are manufactured. Despite these advancements, long-standing difficult circumstances will persist or worsen. Despite the fact that the number of individuals required is significantly less than in massive phase 3 studies, pharmacokinetic investigations can be logistically challenging and expensive. Even if there are challenging scenarios for implementing new pharmacokinetic models, there may be significant value in doing so, even in the context of an approved medication.

The use of this ethically complex and contentious method for vaccine evaluation would necessitate interdisciplinary, global oversight to ensure that the results are rigorous and justify the potential dangers to participants and their communities.

Keywords: Novel vaccines; Formulations; Manufacturing; Scale-up

Introduction

Biological products are defined by the United States Food and Drug Administration (FDA) as a varied product category that includes proteins, monoclonal antibodies, and vaccines, and are used for the diagnosis, prevention, treatment, and cure of diseases and medical conditions [1]. Traditional vaccine techniques, which include live attenuated and inactivated viruses, as well as subunit vaccines, can give long-term protection against a variety of hazardous diseases [2]. Despite this progress, developing vaccines for a variety of infectious diseases, particularly those that are more capable of evading the adaptive immune response, remains a serious issue [3]. Furthermore, the fundamental impediment to the greatest uptake of virus vaccines isn't usually the efficacy of conventional procedures, but rather the requirement for more fast research and large-scale manufacture. As a result, the development of more powerful and adaptable vaccine platforms is critical [4]. With the discovery of mRNA, nucleic acid therapies have arisen as viable alternatives to traditional immunization techniques [5]. New technology must overcome some of the hurdles in order to be effective in combating the epidemic. The newly developing diseases are having serious challenges with pandemic preparedness, which appears to be unpredictable. As shown with the human immunodeficiency virus with human immunodeficiency virus, as well as Severe Acute Respiratory Syndrome, Middle East Respiratory Syndrome Coronavirus, and SARS-CoV-2 virus, the appearance of previously undiscovered infections in the population

is becoming more widespread [6]. Pandemic viral outbreaks show a pathogen's potential to evolve and adapt to a new host or environment, with unanticipated implications on the pathogen's immunogenicity and the severity of the signs and symptoms it causes. As evidenced by recent epidemics and pandemics, RNA viruses are the most vulnerable to such events, as their tremendous mutation rates necessitate adaptation [7].

Formulation of Vaccines and Challenges

Vaccine development is particularly challenging when compared to the development of many product modalities in conjunction with small molecules [8]. The complexities of developing the manufacturing process, formulation, and analytical assays, as well as the problem of scientific assay optimization, are the most well-known barriers to vaccine development failures or delays [8,9]. Ideally, vaccines may be given without the need for injection, eliminating the need for needles, syringes, and trained medical workers [10,11]. In addition to the logistical advantages of non-parenteral immunization [11], there are valid immunological arguments in favor of this method [11,12].

Proteins, unlike smaller particles, are macromolecules with labile bonds and distinctive side-chain orientations that contain primary, secondary, tertiary, or even quaternary structures. Protein denaturation and breakdown can be caused by harsh biological conditions such as proteolytic and violent stomach pH, which can impair biological activities and potentially cause immunogenicity. As a result, employing delivery mechanisms and/or immuno-stimulants is critical for inducing powerful and long-lasting immune safety following mucosal vaccination. Eyles JE, and colleagues found that *Yersinia pestis* F1 and V subunit antigens can induce humoral and mucosal immunity in mice after intranasal administration while co-encapsulated in poly-lactic acid microspheres or co-administrated with the non-toxic pentameric B subunit of Cholera Toxin (CTB) [13-15] as adjuvants, but not with free soluble F1 and V forms. As a result, CTB improves the systemic response to microencapsulated antigens, particularly V, despite the fact that the impact is less significant than when free subunits are administered intra nasally. This could be reflective of a situation in which CTB considerably improves the trans-epithelial flux of free antigen but only modestly improves the uptake of microencapsulated material [15]. Garinot M, et al. developed and compared three alternative vaccine delivery systems made up of variable ratios of Poly Lactic-Co-Glycolic Acid (PLGA), PLGA-Poly Ethylene Glycol (PEG), and Poly-Caprolactone (PCL)-PEG with the goal of boosting oral vaccination efficiency [16-18]. The suppression of β1 integrin transport by anti-1 integrin confirmed the RGD-labeled nanoparticles' selective targeting of β1 integrins in co-cultures. A formulation with a lower proportion of PEG did not produce the same outcome. This could be explained by the ligand's greater accessibility on the nanoparticle surface, which is attributable to the reduced amount of PEG in the formulation (30%) compared to the formulation (55%). Finally, the study found that using an RGD ligand on the nanoparticle's surface with the purpose of achieving M cells caused a mild growth in a variety of mice that produced IgG after immunization, supporting the importance of focusing on the carriers. However, while the RGD ligand was used, no increase in IgG product in serum was seen. This could be due to the peptide's partial breakdown during its transit through the gastrointestinal tract. A non-peptidic 1 integrin ligand may be employed and grafted onto nanoparticles in the area of RGD peptides to avoid ligand degradation inside the belly or inside the gut. It is also possible to investigate the addition of a mucosal adjuvant to the system [18-21]. The effect of freeze-thawing on an aluminum adjuvant and an aluminum adjuvanted native Outer Membrane Vesicle (nOMV) vaccine formulation was studied by Mensch, et al. specifically, employing static light scattering, micro-flow imaging, and cryoelectron microscopy analysis, describing the freeze/thaw-induced agglomeration. Furthermore, the vaccine formulation was evaluated using 0-9% v/v propylene glycol as an excipient to prevent agglomeration caused by freeze/thaw. The results reveal that using 7% v/v propylene glycol as a formulation excipient is efficient in preventing nOMV vaccine formulation agglomeration, which is induced by freeze-thawing Aluminum salt adjuvant vaccines can be successfully converted from suspension to dry powder utilizing a very low concentration of trehalose (2% w/v) as an excipient without causing particle aggregation or diminishing vaccine immunogenicity. Furthermore, following repeated freezing and thawing, the dry vaccine powder did not agglomerate [22]. This thin-film freeze-drying approach [23] is expected to be used to create new vaccines or reformulate existing vaccinations that could be adjuvanted with aluminum salts into dry vaccine powder to prevent vaccine particle aggregation [24]. With the goal of evaluating the protection and immunogenicity of formulation, Logunov DY, and his colleagues developed a heterologous COVID-19 vaccine that includes components, a recombinant adenovirus type 26 (rAd26) vector, and a recombinant adenovirus type 5 (rAd5) vector, each carrying the gene for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [25,26] spike glycoprotein (rAd26-S and rAd5-S). Thus, using a heterologous prime-boost immunization with rAd26-S for priming and rAd5-S for boosting to elicit a robust immune response and overcome the immune response formed by the additives of the vaccine is an effective method to elicit a robust immune response and overcome the immune response formed by the additives of viral vector the vaccine. For a more precise evaluation of the influence of pre-existing immunity on vaccination, the authors suggested more frequent observation and analysis [26]. Pfizer-BioNTech COVID-19 (BNT162b2) vaccine (Pfizer, Inc; Philadelphia, Pennsylvania), a lipid nanoparticle-formulated, nucleoside-changed mRNA vaccine encoding the prefusion spike glycoprotein of SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19), has received an Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA) [27,28]. The following components are used in the Pfizer-BioNTech COVID-19 Vaccine: 2 [(polyethylene glycol)-2000], mRNA, lipids ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), mRNA, lipids ((4 hydroxybutyl) azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate potassium chloride, monobasic potassium phosphate, sodium chloride, dibasic sodium phosphate dihydrate, and sucrose [28,29].

The vaccine may be appropriate for implementation by responsible bodies and people capable of acting (taking action) in the implementation, notwithstanding the difficulty of storing the vaccine at an extremely low temperature and handling and delivery circumstances. These circumstances should limit the availability of the Pfizer-BioNTech COVID-19 vaccination to a small number of people [30]. Health equity may not prevail as a result of this. As a result, efforts must be taken to overcome these difficult situations and increase fitness equity [27,30]. The development of a vaccine, on the other hand, necessitates additional consideration, particularly in the case of combination vaccines [31]. Various manufacturers clearly produce and construct combination vaccinations with different antigen levels [32]. COVID-19 Vaccine AstraZeneca suspension for injection (ChAdOx1-S [recombinant]) formulated as a suspension containing Chimpanzee Adenovirus encoding the SARS-CoV-2 Spike glycoprotein ChAdOx1-S, L-histidine, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80 (E 433), ethanol, sucrose [29,33]. The delivery of these vaccines raises

numerous problems and challenges. COVID-19 vaccines are likely to be made available to all countries in the globe, at least for their priority group [34] to control SARS-CoV-2, due to a number of producers, very big global funding for production, and collaboration in procurement and distribution [35].

A larger number of vaccine products than ever are currently on the market, and these are being taken up by more recipients Despite this, in low-income countries, over a third of deaths occur in children, and the predominant cause is infectious disease. Encouragingly, a recent report from the Pharmaceutical Research and Manufacturers of America listed 145 new vaccines undergoing clinical trials testing, with many targeting infections for which there is no current vaccine. The scientific, financial and ethical challenges are considerable; however, important breakthroughs continue to be made [36].

Vaccine Manufacturing and Challenges

The production of viral vaccines in cell culture can be accomplished with primary, diploid, or continuous (transformed) cell lines [37]. Each cell line, each virus type, and each vaccine preparation require the specific design of upstream and downstream processing. The medium should be chosen in addition to the production container, culture conditions, and operating procedures. Many viruses can only multiply at high titers in naturally developing cells, although a rising number of suspension cell lines are being studied for future usage, comparable to recombinant protein manufacturing procedures [37,38]. There are some critical challenges to address with the established order of large-scale viral synthesis in bioreactors [37]. As an example upstream processing of cell culture-derived influenza virus production is described in more detail for adherently growing and for suspension cells. In particular, use of serum-containing, serum-free, and chemically defined media as well as choice of cultivation vessel are considered [39] with a certain chemical composition, as well as the culture vessel selection [37]. The influenza H1N1 A virus was manufactured using Madin-Darby canine kidney cells (MDCK) suspension cell culture [39,40], were evaluated for the manufacturing of the influenza H1N1 A virus. Except for the use of commercially available serum-free medium for suspension culture, the suspension MDCK cell line grew in the serum-free medium MDCK-SFM2 (serum-free medium) much like it did in adherent serum cultures. The generation of H1N1 influenza virus increased considerably during suspension culture in MDCKSFM2. The infectious viral concentration to total virus concentration ratio is low. Further optimization of medium components and operation procedures is expected to address this issue [41]. As a result, the serum-free suspension culture method [40,42,43] has a lot of potential for environmentally friendly influenza vaccine production [44,45]. In the case of influenza A outbreak, virus that causes the omission of washing and medium exchange procedures before to infection and causes a transition to a serum-free medium [46] is reported. In place of serum and peptone, soy hydrolysate is utilized in the serum-free medium. It also has greater glucose concentrations, lower salt content materials, and higher amino acid concentrations, as well as a different osmolality (260 mmol/kg) and pH stability. Although no medium exchange is required prior to infection, viral titers of equivalent variety have been produced using the Synthetic Complete (SC) medium [40]. The method is simplified by skipping the medium exchange phase, which eliminates the sterility issues associated with the washing steps. The usage of these serum-free media, on the other hand, must be similarly assessed in terms of batch-to-batch stability, repeatability in cell attachment, and impacts on inactivated vaccine downstream processing. More experiments and mathematical models could help researchers better understand the hurdles and bottlenecks in cell metabolism and viral replication, which could lead to higher cell densities and virus yields [40,47]. Isolation of current influenza A (H3N2) viruses in eggs is becoming increasingly difficult, limiting the number of viral candidates that can be examined for vaccine manufacturing. Alternative procedures, such as the use of viruses isolated in permitted cell lines for further replication in both cell-based and egg-based influenza vaccine manufacture, should be created, tested, and reviewed [48]. The aim to adopt more efficient and technologically superior production procedures is driving changes in the manufacturing of such vaccines. As a result, vaccinations that are as good as or better than their predecessors could be developed [49] utilizing modern techniques at a size and pace that matches demand. Finally, vaccines will be created in the fight against diseases for which traditional techniques have failed. In some circumstances, new enabling technology may be required, some of which may have an impact on the manufacturing of existing vaccines. Despite these advancements, long-standing difficult circumstances will persist or worsen. These include overcoming the inertia of alternative well-established production techniques, dealing with complex regulatory challenges connected to releasing new types of vaccine products for healthy individuals, and dealing with the increasing expenses of making complicated new vaccines [49].

The final virus product utilized in the vaccination program must be thoroughly described and have enough amount, purity, and potency to be effective [50,51]. Chromatographic methods are quick, repeatable, and precise. Because the resolving strength of High-Performance Liquid Chromatography (HPLC) allows the separation of intact virus particles from different cellular contaminants or virus particle fragments, they can't be used to measure infective viral particles, but they're a great opportunity for standard techniques in relation to general virus particle quantization. As a result, HPLC approaches can be used to track the impurity/purity profiles of viral items in DSP as well as the USP [52-55]. Analytical techniques such as High-Performance Liquid Chromatography (HPLC) can be utilized as fingerprinting techniques in Downstream Processing (DSP), providing instant information regarding the efficiency of a certain procedure step and allowing for speedier DSP development. The DSP process includes a number of purification procedures. An aliquot of processed viral material must be collected and injected into an HPLC analytical column after each step. In addition to the impurity profile of components present in the processed sample (e.g. DNA, host cell proteins), the elution profile provides a snapshot of a specific purification phase and an approximate estimate of the virus content material as described in figure 1 [56,57]. Demanding scenarios in the manufacturing of new sophisticated particles are often related to target-specific low productivities, loss of solid producers, and the absence of suspension cell traces. Despite the success of CHO cell use in recombinant protein production, the CHO genome is still not fully sequenced and thus not accessible [58,59], but once available, they will simplify the establishment of fully integrated production strategies. Furthermore, various challenges arise during the production of those particles, including the co-production of empty capsids, viral particles that encapsulate an extraordinary genetic material, broken/disassembled particles, virus-DNA aggregates, and extracellular vesicles in the case of viral products [60]. As a result, custom-made purifying procedures must be developed, taking into account the unique features of each product. Although there isn't a "one-size-fits-all" production platform for remarkable bio-therapeutic particles, the methodologies do share some characteristics. The final goal for downstream processes is to expand purification technologies that are as robust as those used today for vaccine products. Purification platforms with a reduced number of purification steps will result in

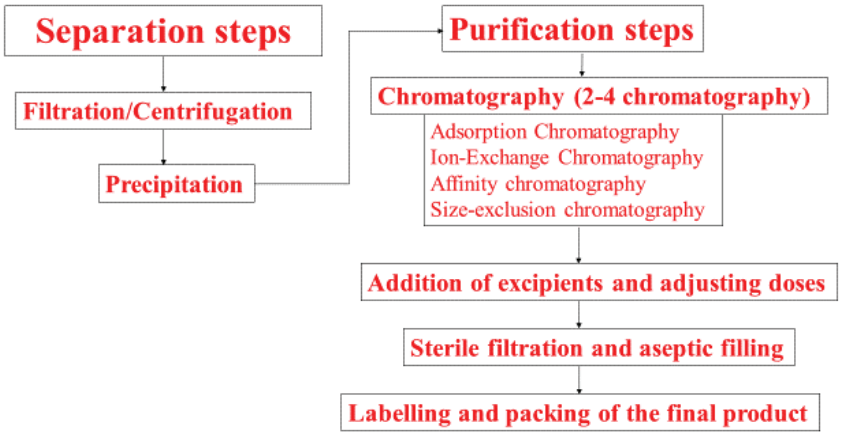

Figure 1: General production process to produce a finished-product (protein) biopharmaceutical.

a higher average process yield, lowering manufacturing costs and allowing for faster dissemination of those unique and promising modalities. The lack of affinity resins for mAbs, such as protein A, is one unmet requirement. There are just a few options, and they are no longer cost-effective enough to be made single-use. As the industry matures, there is a strong prospect of expanding into other matrices and ingredients for those large bio-therapeutic particles. Simultaneously, the diversity and scope of single-use alternative implementation is becoming obvious and prevalent. Because the requirement to clean, sterilize, and validate the equipment/materials is reduced, the usage of such compounds in all production and purification procedures is gaining support [61].

Scientists argue that the extremely concentrated state of global vaccine manufacturing capacity limits large-scale vaccine production. At the moment, only a few countries have the capacity to make vaccines on their own. Scaling up vaccine production is difficult, and a shortage of manufacturing sites is limiting global vaccine availability. Vaccine manufacturing and the development of breakthrough technologies capable of producing huge quantities of vaccines against known and undiscovered infections are difficult tasks nowadays. Table 1 containing the main difficulties concerning vaccine production or purification describes some of these challenges.

Vaccines Pharmacokinetic Issues

To estimate the quantity of vaccine adjuvants in mechanically or toxicologically acceptable target tissues, Pharmacokinetic (PK) and biodistribution studies can be used [62-64]. Tegenge MA, and colleagues studied a comparison of the pharmacokinetics and biodistribution features of radiolabeled squalene 336 hours (14 days) following intramuscular injection of adjuvanted H5N1 influenza vaccinations in mice [65]. Experimental squalene-in-water (SQ/W) emulsion (AddaVax®) and adjuvants system (AS03®) that contained squalene and -tocopherol inside the oil phase of the emulsion were among the adjuvants tested. The initial exponential degradation of the enzyme has a half-life of 1.5 hours for AS03 and 12.9 hours for AddaVax [66]. After AS03 injection, the concentration of tagged squalene in lymph node discharge was 10 times higher than after AddaVax injection (1-6 hours). The peak concentration of squalene inside the spleen (immune organ) AS03 is 7 times higher than AddaVax after injection, and the area-under-concentration curve was as long as 336 hours (AUC0-336hr) [67]. For toxicologically significant target tissues, including as the spinal cord, brain, and kidneys, the maximal systemic tissue concentration of squalene from the two adjuvants, without or with antigen, remained below 1% of the injected dose [68]. The pharmacokinetics of AS03 was changed so that the presence of H5N1 antigen had no effect on them. This shows a quick fall of AS03 from the quadriceps muscle tissues of mice [67], with enhanced transfer to mechanistically applicable tissues in the nearby lymph nodes, in comparison to the standard SQ/W emulsion adjuvant [65], with improved transfer to mechanistically applicable tissues in the local lymph nodes [67,69]. The amount of possible toxicological target tissues exposed in the systemic tissues was extremely low [67,69,70].

The formulations of peptide-based anticancer vaccines that are being tested in clinical trials are typically associated with low potency. Mehta NK, et al. demonstrated that optimizing vaccination immunogenicity in mice by pharmacokinetically tailoring peptide vaccine responses by fusing peptide epitopes to carrier proteins. In vaccinated mice, the service protein transthyretin promotes effective antigen uptake in draining lymphatics from the injection site, antigen payload protection from proteolytic degradation, and antigen presentation in uninflamed distal lymphoid organs at the same time. Optimizing those aspects can boost vaccine immunogenicity by up to 90 percent while also improving responses to viral antigens, tumor-related antigens, oncofetal antigens, and shared neoantigens. Protein-peptide epitope fusions are a simple and generalizable strategy to augment T-cell responses generated by subunit vaccines *via* way of means of subunit vaccines [71,72]. Adjuvants are added to vaccination formulations to increase the importance of the immune response and/or adjust its quality [73,74]. The oldest and most widely used adjuvants are aluminum salts, although a novel family of squalene-containing vaccine adjuvants has arisen in recent years. The identification of those new adjuvant structures was aided in part by developments in proteomics and pathogen genome sequencing, which assisted in the rational design of purified antigens [73-77]. In positive emulsion vaccine adjuvants, squalene is employed in the oil phase. Its fate as a vaccine component after intramuscular (IM) injection in humans, however, remains unknown. The optimum pathways for employing squalene-containing adjuvants to augment the immune

Table 1: Major challenges during vaccine productions.

Viral production methods	Common problems
Cell culture suspension	✓ Contamination by bacteria, fungi, and yeast ✓ Mycoplasma contamination ✓ Chemical contamination
Serum-free medium	✓ The concentration of serum proteins is generally much higher than the protein produced by mammalian cells in vivo. ✓ The abundantly available nutrients in fetal bovine serum overfeed the cells, they become too large, proliferate faster, and the morphology is atypical. ✓ Serum proteins are a significant contaminant and may unwantedly interact with your component of interest. ✓ If the target protein is related to a serum protein, you can't separate the two easily. ✓ On top of that, harvesting serum from unborn calves in slaughterhouses poses ethical concerns.
HPLC for virus purification	✓ Sample handling ✓ Inefficient sample extraction from a matrix is a frequent source of difficulty ✓ Different brands of Mobile phase ✓ Difference in pumps to pressurize a mobile phase ✓ Variation in Injectors ✓ Variations in Columns and detectors

response are still unknown. Adjuvant pharmacokinetic data following Intramuscular (IM) administration is few and limited [62,78,79]. It's controversial if the squalene-containing vaccination adjuvant has a local or systemic immune effect [80]. Because experimental pharmacokinetic research isn't currently required as part of the global vaccination regulatory registration framework [81-83], there is a scarcity of published data on vaccine pharmacokinetics [78]. On pathology, immunology, and vaccine research, Controlled Human Infection Model (CHIM) experiments in human volunteers exposed to infectious microorganisms (also known as challenge agents) are critical. As has been demonstrated for malaria and typhoid vaccine development, microbial challenge research is excellent for providing evidence of suggestions for therapeutic interventions and may greatly reduce the time required to reach phase three research of a clinical trial [84-87]. The administration of a known dose of SARS-CoV-2 in a carefully controlled context has been recommended as a model to allow for a quick preliminary assessment of vaccination efficacy and early deselection of vaccine candidates [88,89]. A COVID-19 CHIM model has a number of advantages over studies that rely on spontaneous community transmission, which is difficult to forecast, as well as behavioral changes and public health interventions. CHIM research necessitates the controlled delivery of a standardized inoculum, ideally prepared using acceptable manufacturing techniques, as well as great care to prevent the challenge strain from spreading in the population [90]. Even though the number of people required is significantly less than in major phase 3 clinical trials, these studies may be logistically difficult and expensive for participants. Even though there are challenging circumstances for implementing a CHIM for SARS-CoV-2 [89], there may be significant value in doing so, even in the context of an authorized product. The use of this ethically complex and contentious method for vaccine evaluation would necessitate interdisciplinary, global oversight to ensure that the results are rigorous and justify the potential dangers to participants and their communities [91].

Conclusions

Vaccines can be made as suspensions, emulsions, or freeze-dried powders with a variety of adjuvants. However, many of those manufactured vaccines face multiple problems from a pharmaceutical standpoint, including the risk for acute hypersensitivity reactions, the need for extremely cold storage temperatures, and handling and delivery requirements. These requirements should limit vaccine supply to different populations, which has a negative impact on health equity. In the production of vaccines during upstream and downstream processes, new facilities, equipment, and enabling technology may be required, some of which may have an impact on how existing vaccines are manufactured. The number of people required is significantly high in clinical trials; these studies may be logistically difficult and expensive for participants. Even though there are challenging circumstances for implementing a vaccine pharmacokinetic study protocol, there may be significant value in doing so, even in the context of an authorized product. The use of this ethically complex and contentious method for vaccine evaluation would necessitate interdisciplinary, global oversight to ensure that the results are rigorous and justify the potential dangers to participants and their communities.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Conflict of interest

The author declares no conflict of interest.

Funding

Not applicable.

Authors' contributions

Author made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval

of the version to be published; has agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

References

1. USFDA, Biological Product Definitions.
2. Plotkin SA (2009) Vaccines: The fourth century. Clin Vaccine Immunol 16: 1709-1719.
3. Rodrigues CMC, Pinto MV, Sadarangani M, Plotkin SA (2017) Whither vaccines? J Infect 74: S2-S9.
4. Pardi N, Hogan MJ, Porter FW, Weissman D (2018) mRNA vaccines-a new era in vaccinology. Nat Rev Drug Discov 17: 261-279.
5. Jirikowski GF, Sanna PP, Maciejewski-Lenoir D, Bloom FE (1992) Reversal of diabetes insipidus in Brattleboro tats: Intrahypothalamic injection of vasopressin mRNA. Science 255: 996-998.
6. Wang J, Peng Y, Xu H, Cui Z, Williams RO 3rd (2020) The COVID-19 vaccine race: Challenges and opportunities in vaccine formulation. AAPS PharmSciTech 21: 225.
7. Rauch S, Jasny E, Schmidt KE, Petsch B (2018) New vaccine technologies to combat outbreak situations. Front Immunol 9: 1963.
8. World Health Organization (2017) Guidelines on clinical evaluation of vaccines: regulatory expectations, Annex 9, TRS No 1004: Replacement of Annex 1 of WHO Technical Report Series, No. 924. WHO Technical Report Series 924: 503-574.
9. Heaton PM (2020) Challenges of developing novel vaccines with particular global health importance. Front Immunol 11: 517290.
10. Alvarez ML, Cardineau GA (2010) Prevention of bubonic and pneumonic plague using plant-derived vaccines. Biotechnol Adv 28: 184-196.
11. Ogra PL, Faden H, Welliver RC (2001) Vaccination Strategies for Mucosal Immune Responses. Clin Microbiol Rev 14: 430-445.
12. Husband AJ (1993) Novel vaccination strategies for the control of mucosal infection. Vaccine 11: 107-112.
13. Kelly SH, Cossette BJ, Varadhan AK, Wu Y, Collier JH (2021) Titrating polyarginine into nanofibers enhances cyclic-dinucleotide adjuvanticity *in vitro* and after sublingual immunization. ACS Biomater Sci Eng 7: 1876-1888.
14. Kelly SH, Wu Y, Varadhan AK, Curvino EJ, Chong AS, et al. (2020) Enabling sublingual peptide immunization with molecular self-assemblies. Biomaterials 241: 119903.
15. Eyles JE, Sharp GJE, Williamson ED, Spiers ID, Alpar HO (1998) Intra nasal administration of poly-lactic acid microsphere co-encapsulated *Yersinia pestis* subunits confers protection from pneumonic plague in the mouse. Vaccine 16: 698-707.
16. Gullberg E, Leonard M, Karlsson J, Hopkins AM, Brayden D, et al. (2000) Expression of specific markers and particle transport in a new human intestinal M-cell model. Biochem Biophys Res Commun 279: 808-813.
17. Gullberg E, Keita AV, Salim SY, Andersson M, Caldwell KD, et al. (2006) Identification of cell adhesion molecules in the human follicle-associated epithelium that improve nanoparticle uptake into the Peyer's patches. J Pharmacol Exp Ther 319: 632-639.
18. Garinot M, Fiévez V, Pourcelle V, Stoffelbach F, des Rieux A, et al. (2007) PEGylated PLGA-based nanoparticles targeting M cells for oral vaccination. J Control Release 120: 195-204.
19. Eldridge JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, et al. (1990) Controlled vaccine release in the gut-associated lymphoid tissues I. Orally administered biodegradable microspheres target the Peyer's patches. J Control Release 11: 205-214.
20. Shakweh M, Ponchel G, Fattal E (2004) Particle uptake by Peyer's patches: a pathway for drug and vaccine delivery. Expert Opin Drug Deliv 1: 141-163.
21. Moldoveanu Z, Huang WQ, Kulhavy R, Pate MS, Mestecky J (2005) Human male genital tract secretions: both mucosal and systemic immune compartments contribute to the humoral immunity. J Immunol 175: 4127-4136.
22. Clausi AL, Morin A, Carpenter JF, Randolph TW (2009) Influence of protein conformation and adjuvant aggregation on the effectiveness of aluminum hydroxide adjuvant in a model alkaline phosphatase vaccine. J Pharm Sci 98: 114-121.
23. Shah UV, Karde V, Ghoroi C, Heng JYY (2017) Influence of particle properties on powder bulk behaviour and processability. Int J Pharm 518: 138-154.
24. Li X, Thakkar SG, Ruwona TB, Williams RO 3rd, Cui Z (2015) A method of lyophilizing vaccines containing aluminum salts into a dry powder without causing particle aggregation or decreasing the immunogenicity following reconstitution. J Control Release 204: 38-50.
25. Dong E, Du H, Gardner L (2020) An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 20: 533-534.
26. Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatullin AI, Shcheblyakov DV, et al. (2020) Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet 396: P887-P897.
27. Food and Drug Administration (2020) Vaccines and Related Biological Products Advisory Committee Meeting February 26, 2021 FDA Briefing Document. Janssen Ad26.COV2.S (COVID-19) Vaccine, FDA 1-53.
28. CDC (2021) COVID-19 Vaccine Emergency Use Authorization (EUA) Fact Sheets for Recipients and Caregivers. Centers for Disease Control and Prevention 1-6.
29. England PH (2021) COVID-19 vaccination programme Information for healthcare practitioners. 1-40.
30. Oliver SE, Gargano JW, Scobie H, Wallace M, Hadler SC, et al. (2021) The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Janssen COVID-19 Vaccine - United States, February 2021. MMWR Morb Mortal Wkly Rep 70: 329-332.
31. Wen EP, Ellis R, Pujar NS (2015) Vaccine development and manufacturing. John Wiley & Sons.
32. Tafreshi SH (2020) Efficacy, safety, and formulation issues of the combined vaccines. Expert Rev Vaccine 19: 949-958.
33. European Medicines Agency (2021) COVID-19 Vaccine AstraZeneca: Product Information as approved by the CHMP on 29 January 2021, pending endorsement by the European Commission.
34. World Health Organization (2021) WHO Concept for fair access and equitable allocation of COVID-19 health products. World Health Organization, Geneva.
35. Knoll MD, Wonodi C (2021) Oxford-AstraZeneca COVID-19 vaccine efficacy. Lancet 397: 72-74.
36. Oyston P, Robinson K (2012) The current challenges for vaccine development. J Med Microbiol 61: 889-894.

37. Genzel Y, Rödig J, Rapp E, Reichl U (2014) Vaccine production: Upstream processing with adherent or suspension cell lines. Methods Mol Biol 1104: 371-393.

38. Gomez PL, Robinson JM, Rogalewicz JA (2013) Vaccine manufacturing. Vaccines 2013: 44-57.

39. Genzel Y, Olmer RM, Schäfer B, Reichl U (2006) Wave microcarrier cultivation of MDCK cells for influenza virus production in serum containing and serum-free media. Vaccine 24: 6074-6087.

40. Genzel Y, Fischer M, Reichl U (2006) Serum-free influenza virus production avoiding washing steps and medium exchange in large-scale microcarrier culture. Vaccine 24: 3261-3272.

41. Huang D, Xia-Hou K, Liu XP, Zhao L, Fan L, et al. (2014) Rational design of medium supplementation strategy for improved influenza viruses production based on analyzing nutritional requirements of MDCK Cells. Vaccine 32: 7091-7097.

42. Zhang L, Chen Q, Ma Y, Sun J (2020) Microfluidic Methods for Fabrication and Engineering of Nanoparticle Drug Delivery Systems. ACS Appl Bio Mater 3: 107-120.

43. Genzel Y, Vogel T, Buck J, Behrendt I, Ramirez DV, et al. (2014) High cell density cultivations by alternating tangential flow (ATF) perfusion for influenza A virus production using suspension cells. Vaccine 32: 2770-2781.

44. Huang D, Peng WJ, Ye Q, Liu XP, Zhao L, et al. (2015) Serum-free suspension culture of MDCK cells for production of influenza H1N1 Vaccines. PLoS One 10: e0141686.

45. Lohr V, Rath A, Genzel Y, Jordan I, Sandig V, et al. (2009) New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: Studies on growth, metabolism and virus propagation. Vaccine 27: 4975-4982.

46. Fishbein DB, Yenne KM, Dreesen DW, Teplis CF, Mehta N, et al. (1993) Risk factors for systemic hypersensitivity reactions after booster vaccinations with human diploid cell rabies vaccine : A nationwide prospective study. Vaccine 11: 1390-1394.

47. Genzel Y, Behrendt I, König S, Sann H, Reichl U (2004) Metabolism of MDCK cells during cell growth and influenza virus production in large-scale microcarrier culture. Vaccine 22: 2202-2208.

48. Donis RO, Influenza Cell Culture Working Group, Davis CT, Foust A, Hossain MJ, et al. (2014) Performance characteristics of qualified cell lines for isolation and propagation of influenza viruses for vaccine manufacturing. Vaccine 32: 6583-6590.

49. Ulmer JB, Valley U, Rappuoli R (2006) Vaccine manufacturing: Challenges and solutions. Nat Biotechnol 24: 1377-1383.

50. World Health Organization (2013) Annex 4 Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines: Replacement of Annex 2 of WHO Technical Report Series, No. 786 and Annex 4 of WHO Technical Report Series, No. 889. WHO Technical Report Series No. 978, 2013: 189-240.

51. FDA (2004) Guidance for Industry Guidance for Industry PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. The U.S. Food and Drug Administration (FDA).

52. Whitfield RJ, Battom SE, Gilham DE, Ball PD (2009) Rapid high-performance liquid chromatographic analysis of adenovirus type 5 particles with a prototype anion-exchange analytical monolith column. J Chromatogr A 1216: 2725-2729.

53. Transfiguracion J, Bernier A, Arcand N, Chahal P, Kamen A (2001) Validation of a high-performance liquid chromatographic assay for the quantification of adenovirus type 5 particles. J Chromatogr B 761: 187-194.

54. Transfiguracion J, Bernier A, Voyer R, Coelho H, Coffey M, et al. (2008) Rapid and reliable quantification of reovirus type 3 by high performance liquid chromatography during manufacturing of Reolysin®. J Pharm Biomed Anal 48: 598-605.

55. Klyushnichenko V, Bernier A, Kamen A, Harmsen E (2001) Improved high-performance liquid chromatographic method in the analysis of adenovirus particles. J Chromatogr B 755: 27-36.

56. Wolff MW, Reichl U (2008) Downstream processing: From egg to cell culture-derived influenza virus particles. Chem Eng Technol 31: 846-857.

57. Urbas L, Jarc BL, Barut M, Zochowska M, Chroboczek J, et al. (2011) Purification of recombinant adenovirus type 3 dodecahedric virus-like particles for biomedical applications using short monolithic columns. J Chromatogr A 1218: 2451-2459.

58. Jenkins N, Barron N, Alves P (2012) Proceedings of the 21st Annual Meeting of the European Society for Animal Cell Technology (ESACT), Dublin, Ireland, June 7-10. 2009. ESACT Proceedings.

59. Wurm FM (2004) Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 22: 1393-1398.

60. Steppert P, Burgstaller D, Klausberger M, Kramberger P, Tover A, et al. (2017) Separation of HIV-1 gag virus-like particles from vesicular particles impurities by hydroxyl-functionalized monoliths. J Sep Sci 40: 979-990.

61. Moleirinho MG, Silva RJS, Alves PM, Carrondo MJT, Peixoto C (2020) Current challenges in biotherapeutic particles manufacturing. Expert Opin Biol Ther 20: 451-465.

62. Ott G, Barchfeld GL, Chernoff D, Radhakrishnan R, van Hoogevest P, et al. (1995) MF59. Design and evaluation of a safe and potent adjuvant for human vaccines. Pharm Biotechnol 6: 277-296.

63. Segal L, Wouters S, Morelle D, Gautier G, Le Gal J, et al. (2015) Non-clinical safety and biodistribution of AS03-adjuvanted inactivated pandemic influenza vaccines. J Appl Toxicol 35: 1564-1576.

64. Dupuis M, McDonald DM, Ott G (1999) Distribution of adjuvant MF59 and antigen gD2 after intramuscular injection in mice. Vaccine 18: 434-439.

65. Tilvis RS, Miettinen TA (1982) Fate of intravenously administered squalene in the rat. Biochim Biophys Acta 712: 374-381.

66. Gylling H, Miettinen TA (1994) Postabsorptive metabolism of dietary squalene. Atherosclerosis 106: 169-178.

67. Tegenge MA, Von Tungeln LS, Mitkus RJ, Anderson SA, Vanlandingham MM, et al. (2016) Pharmacokinetics and biodistribution of squalene-containing emulsion adjuvant following intramuscular injection of H5N1 influenza vaccine in mice. Regul Toxicol Pharmacol 81: 113-119.

68. Tilvis RS, Miettinen TA (1983) Dietary squalene increases tissue sterols and fecal bile acids in the rat. Lipids 18: 32-36.

69. Garçon N, Vaughn DW, Didierlaurent AM (2012) Development and evaluation of AS03, an Adjuvant System containing α-tocopherol and squalene in an oil-in-water emulsion. Expert Rev Vaccines 11: 349-366.

70. Tegenge MA, Von Tungeln LS, Anderson SA, Mitkus RJ, Vanlandingham MM, et al. (2019) Comparative pharmacokinetic and biodistribution study of two distinct squalene-containing oil-in-water emulsion adjuvants in H5N1 influenza vaccines. Regul Toxicol Pharmacol 108: 104436.

71. Hanson MC, Crespo MP, Abraham W, Moynihan KD, Szeto GL, et al. (2015) Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants. J Clin Invest 125: 2532-2546.

72. Mehta NK, Pradhan RV, Soleimany AP, Moynihan KD, Rothschilds AM, et al. (2020) Pharmacokinetic tuning of protein-antigen fusions enhances the immunogenicity of T-cell vaccines. Nat Biomed Eng 4: 636-648.

73. Pulendran B, Ahmed R (2011) Immunological mechanisms of vaccination. Nat Immunol 12: 509-517.

74. Levitz SM, Golenbock DT (2012) Beyond empiricism: Informing vaccine development through innate immunity research. Cell 148: 1284-1292.

75. Sette A, Rappuoli R (2010) Reverse vaccinology: Developing vaccines in the era of genomics. Immunity 33: 530-541.

76. Rappuoli R, Mandl CW, Black S, De Gregorio E (2015) Vaccines for the twenty-first century society. Nat Rev Immunol 11: 865-872.

77. Tetsutani K, Ishii KJ (2012) Adjuvants in influenza vaccines. Vaccine 30: 7658-7661.

78. Tegenge MA, Mitkus RJ (2013) A physiologically-based pharmacokinetic (PBPK) model of squalene-containing adjuvant in human vaccines. J Pharmacokinet Pharmacodyn 40: 545-556.

79. Elmgren L, Li X, Wilson C, Ball R, Wang J, et al. (2013) A global regulatory science agenda for vaccines. Vaccine 31: B163-B175.

80. Valensi JP, Carlson JR, Van Nest GA (1994) Systemic cytokine profiles in BALB/c mice immunized with trivalent influenza vaccine containing MF59 oil emulsion and other advanced adjuvants. J Immunol 153: 4029-4039.

81. World Health Organization (2005) Annex 1-WHO guidelines on non clinical evaluation of vaccines. WHO Technical Report Series, No. 927, 2005: 31-63.

82. Wolf JJ, Kaplanski CV, Lebron JA (2010) Nonclinical safety assessment of vaccines and adjuvants. Methods Mol Biol 626: 29-40.

83. Sun Y, Gruber M, Matsumoto M (2012) Overview of global regulatory toxicology requirements for vaccines and adjuvants. J Pharmacol Toxicol Methods 65: 49-57.

84. The Academy of Medical Sciences (2005) Microbial Challenge Studies of Human Volunteers: A guidance document from the Academy of Medical Sciences.

85. Cooper MM, Loiseau C, McCarthy JS, Doolan DL (2019) Human challenge models: tools to accelerate the development of malaria vaccines. Expert Rev Vaccines 18: 241-251.

86. Meiring JE, Giubilini A, Savulescu J, Pitzer VE, Pollard AJ (2019) Generating the evidence for typhoid vaccine introduction: Considerations for global disease burden estimates and vaccine testing through human challenge. Clin Infect Dis 69: S402-S407.

87. Kirchhelle C, Pollard AJ, Vanderslott S (2019) Typhoid-From Past to Future. Clin Infect Dis 69: S375-S376.

88. Eyal N, Lipsitch M, Smith PG (2021) Human challenge studies to accelerate coronavirus vaccine licensure. J Infect Dis 221: 1752-1756.

89. Baay M, Neels P (2020) SARS-CoV-2 controlled human infection models: Ethics, challenge agent production and regulatory issues. Biologicals 67: 69-74.

90. World Health Organization (2020) Feasibility, potential value and limitations of establishing a closely monitored challenge model of experimental COVID-19 infection and illness in healthy young adult volunteers. WHO, Geneva.

91. Hodgson SH, Mansatta K, Mallett G, Harris V, Emary KRW, et al. (2021) What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. Lancet Infect Dis 21: e26-e35.