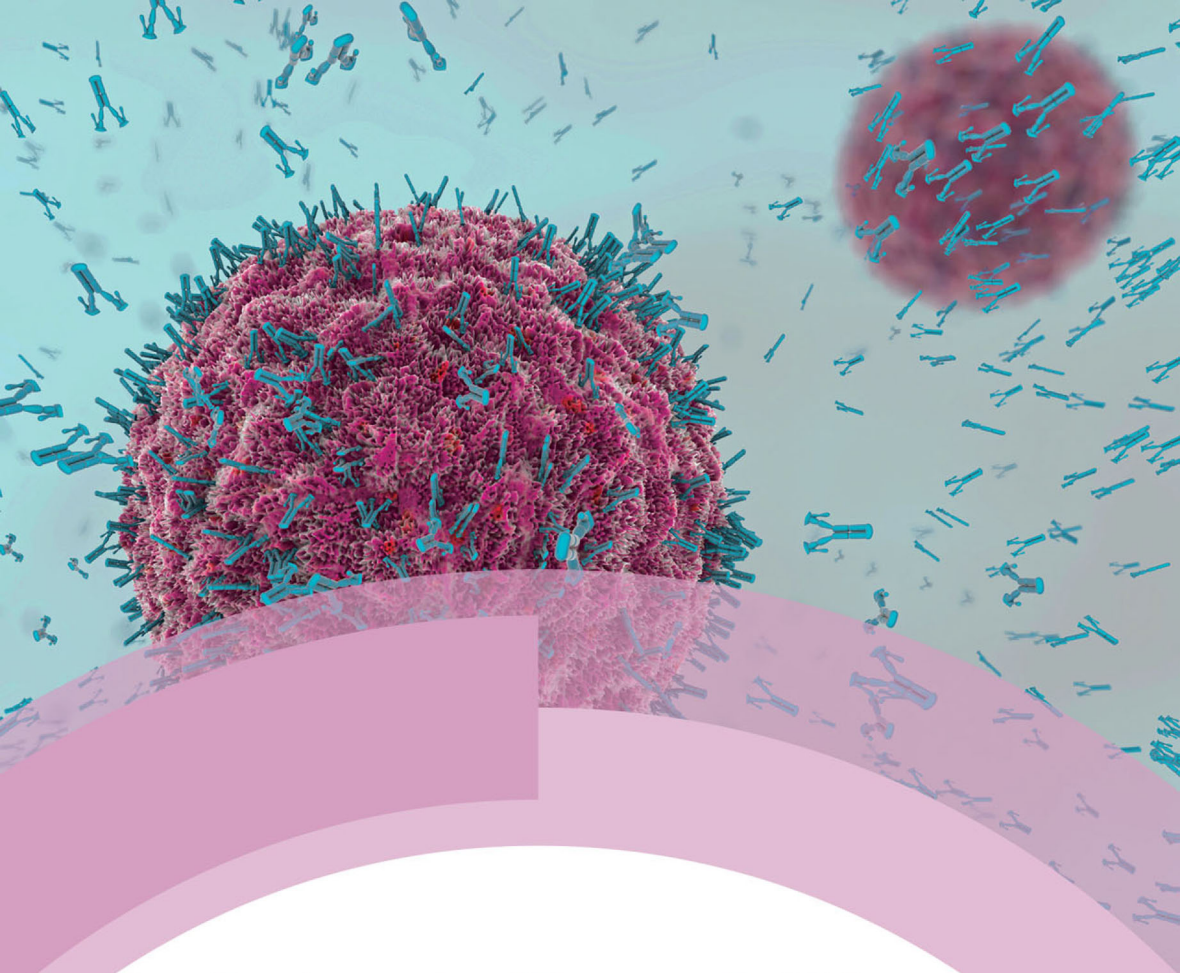

Drug Development and Pharmaceutical Science

Vaccine Development

From Concept to Clinic

Edited by A. Krishna Prasad

ROYAL SOCIETY OF CHEMISTRY

Vaccine Development
From Concept to Clinic

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ROYAL SOCIETY OF CHEMISTRY

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Dedication

This book is dedicated to **Covid-19 Heroes.**

For many of us, our images of heroes are in mythological stories, comics, movies, sports and so on...

The COVID-19 pandemic has taught us that the **REAL HEROES** are those who sacrificed everything to keep the world functioning so that many of us can stay safe and healthy. This allowed many of us to work from the comfort of our home. Many of these heroes had to go to work like any other day.

Healthcare workers in hospitals who took care of the sick and dying, risking their own lives...

Firefighters, teachers, municipal staff, grocery store workers, food and essential goods delivery drivers, postal workers, construction workers...

Researchers and technicians who worked day and night in the laboratories and manufacturing plants to produce new vaccines and drugs at breakneck speed to put an end to this pandemic...

Small businesses who endured economic hardship...

As a small token of my appreciation to these heroes, I pledge to donate all the financial compensation I receive from the sales of this book to organizations such as Direct Relief and World Kitchen, who are doing incredible service across the globe helping victims of natural disasters and wars. Thank you.

A. Krishna Prasad
Chapel Hill, North Carolina, USA

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Foreword

Throughout the COVID-19 pandemic, the general public received comprehensive and publicly available information on the clinical trials of different vaccines, including mRNA, adenovirus-vector, particle, and other types of new vaccine technologies. Open-source US Food and Drug Administration (FDA) and Centers for Disease Control (CDC) meetings as well as summits, events, and meetings organized by the UK Government, the European Union, and World Health Organization shaped a new generation of both armchair experts and biomedical scientists interested in how vaccine effectiveness is measured or how safety is monitored.

However, the processes by which new vaccines are first developed, tested in preclinical models, and transitioned to the clinic remain obscure to most individuals and even scientists trained in basic biochemistry, molecular biology, microbiology, and immunology. Moreover, there are not many volumes devoted to how different vaccine technologies are selected or prioritized depending on whether they are intended for a viral, bacterial, fungal, or parasitic disease target.

Vaccine Development: From Concept to the Clinic informs the reader about the essential elements of vaccine design and selection, together with some fundamental and mission-critical components that are required by national regulatory authorities. They include antigen selection, CMC (chemistry, manufacturing, and control), bioprocess development, adjuvant selection and formulation, and quality control and assurance. These aspects are not typically taught during doctoral training programs in the biomedical sciences, especially in university settings. In fact, they are not easily accessible outside the confines of industry. The bottom line: unless you are hired to work for a biopharmaceutical company, it is not easy to access or incorporate these aspects into your training and daily activities.

More than 20 years ago I expanded my research laboratory – then conducting fundamental work on helminth molecular biology and immunology – to actually

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attempt to make vaccines for human hookworm infection and schistosomiasis.[1] These two parasitic worm infections rank among the most common afflictions of people who live in extreme poverty in low- and middle-income countries (LMICs).

How does one transition from a basic biomedical science laboratory to one that might produce a new vaccine for clinical trials? In my case, I was fortunate to have my first vaccine development laboratory at George Washington University, located in Washington, DC, close to the Walter Reed Army Institute of Research (WRAIR). WRAIR has a storied history of producing vaccines for the military, and also for tropical infections. For instance, the work conducted at WRAIR provided the necessary discovery and development steps to help launch the RTS,S malaria vaccine, now produced by GSK as Mosquirix.[2] Some of those WRAIR scientists, who also had previous ranks in the US military, helped to mentor our group so that we could develop first-generation helminth vaccines and even submit successful investigational new development (IND) applications to our US FDA. Today, our group is based at the Texas Medical Center, the world's largest medical center or one of the first world medical cities. At our Texas Children's Hospital Center for Vaccine Development, we continue to develop new vaccines for poverty-related neglected parasitic infections. However, we have also turned our attention to using similar approaches for the development of coronavirus vaccines. During the pandemic our research group developed a recombinant protein COVID-19 vaccine based on microbial fermentation in yeast.[3–5] We then transferred this technology to several LMIC vaccine producers, including Biological E. in India, where they scaled up and produced CORBEVAX that underwent successful clinical testing.[6,7] Now, CORBEVAX has been given to millions of adolescents and other individuals in India, and was recently approved in Botswana, becoming one of the first COVID-19 vaccines specifically designed for LMICs.[8]

When I first began my transition from a pediatric scientist and biochemist to a true vaccinologist, there was no book or primer to guide us through the steps required for this arduous journey. Therefore, I was thrilled when Dr Krishna Prasad asked me to write the Foreword for this new book. I believe that *Vaccine Development: From Concept to the Clinic* helps fill a void in terms of literature in this field. It should become a valuable aid for young scientists seeking to understand how their basic science discoveries might one day translate into a new vaccine or vaccine technology tested in the clinic. It could become especially useful for scientists working in LMICs who especially need access to this kind of information. In such a case, a book such as this one could become an important volume to help build global vaccine capacity and address inequity.

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CHAPTER 1

Vaccine Development: From Concept to Clinic

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1.1 Introduction

A vast body of clinical experience has supported the introduction of several vaccines for routine use across the globe, resulting in a dramatic impact on public health. Research and development efforts continue to translate this impact and find scientific and novel technological innovations to target many diseases to address unmet medical needs. SARS-CoV-2 represents a new historical milestone in vaccine design and immunotherapeutic strategy development achieved at breakneck speed. The COVID-19 pandemic demonstrates the need for groundbreaking cross-disciplinary research efforts in immunology, vaccinology, infection, and epidemiology. It is important that vaccines meet various regulatory requirements for safety, stability, quality, and efficacy. The primary motivation of this book is to provide a comprehensive forum for the presentation of various components that define the development and control strategies to produce safe and stable vaccines that elicit consistent and robust immunogenic responses. The book aims to cover the landscape comprising preclinical safety considerations, clinical trial design and development, high-throughput clinical assay development, mechanisms of vaccine adjuvants, process development, and also several aspects pertaining to the chemistry, manufacture, and control (CMC) of vaccines. These disciplines are closely tied to regulatory aspects required for

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the commercial licensure of vaccines. While the chapters are designed with the intent to cover all major aspects of vaccine development, a book of this size cannot capture information on all supporting topics and allied fields in infinite detail. We hope that this book will serve as a useful reference providing valuable insights into the design, development, manufacture, and licensure of these complex products.

1.2 Preclinical Safety Assessment Considerations

It is important that vaccines meet various regulatory requirements for safety, stability, quality, and efficacy. In addition to the drug substance antigens (active ingredients), adjuvants, excipients, and stabilizers may be included in the final formulation (drug product) to elicit a robust immune response. To satisfy the safety requirements of vaccines, preclinical toxicological studies are typically conducted to support various clinical studies. Successful preclinical toxicology studies often rely on a variety of factors, including suitable study designs, relevant animal models, and dosing strategies. Chapter 2 by Clarke reviews the non-clinical safety studies to support the development of vaccines against infectious disease and the types of studies that may be required and issues to be considered in the design of the studies.

1.3 Clinical Trials in the Development of Vaccines

Clinical trials are a critical feature of vaccine development to demonstrate safety and efficacy for regulatory submissions in addition to supporting recommendations by various advisory groups for licensure. There is no simple formula for a vaccine clinical program and its component clinical trials. All of the trials must be intelligently and prospectively designed based on a variety of factors, including preclinical information, applicable correlates, epidemiology, licensed vaccines and other medical interventions, target product profile, and dynamics of clinical data. The clinical development process may need multi-country trials with variable settings, timelines, labeling requirements, infrastructure, and regulatory requirements. These settings and requirements may add additional complexity to the process of carrying out multi-national clinical trials. Chapter 3 by Lockhart and Gruber outlines many common features of vaccine clinical trials, including the assessment of tolerability and also adverse events to assess safety, use of immune responses as a biomarker for biological effect, prevention of naturally occurring infectious disease as a primary efficacy endpoint, use of indicative markers of efficacy short of full prevention of natural disease, and the need to use clinical data to confirm manufacturing consistency. The chapter provides a chronological and comprehensive narrative of novel and groundbreaking clinical trials, particularly those leading to marketing approvals for new categories of vaccines.

There is tremendous interest in the development of therapeutic vaccines, which, unlike their prophylactic counterparts, are intended to treat existing

diseases. A wide variety of diseases are potential therapeutic targets, which include both infectious diseases such as human immunodeficiency virus (HIV) and latent tuberculosis (TB) infection, in addition to non-infectious diseases, such as cancer, neurodegenerative diseases, and substance abuse. A clear understanding of the delivery technology, epidemiology, and relevant animal models are critical to translate the potential of a therapeutic vaccine. Clinical trials of therapeutic vaccines employ many of the general principles of prophylactic vaccine development, yet there is a need to demonstrate a positive benefit–risk profile with clinical efficacy and safety endpoints in patients with underlying disease. Clinical therapeutic vaccine trials include immune biomarker and relevant disease clinical modification endpoints, similar to those studied in therapeutic trials of small and large molecules. There is a rich pipeline of therapeutic vaccines currently in clinical development, primarily to target cancer. Technological advances in translational science and delivery methods in conjunction with clinical strategies hold promise for advancing the therapeutics of infectious and other non-communicable diseases. In Chapter 4, Alemayehu and Knirsch discuss the various clinical complexity challenges associated with the development of therapeutic vaccines, including biomarkers, the potential role of adaptive trial design, and statistical and regulatory considerations.

1.4 Pathogenesis and Immunogenicity of SARS-CoV-2 and Vaccine Programs Against COVID-19

The novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in coronavirus disease 2019 (COVID-19) that led to millions of deaths accompanied by devastating social and economic crises worldwide on an unprecedented scale. Researchers across the globe have been evaluating multiple strategies to develop efficient vaccines against SARS-CoV-2, which is essential to reduce morbidity and mortality.[1] SARS-CoV-2 represents a new historical milestone in vaccine design and immunotherapeutic strategy development. COVID-19 vaccine candidates entered clinical trials in less than 6 months from the first identification of the pandemic in Wuhan, China, and received emergency use authorization (EUA) in less than 1 year since the beginning of the COVID-19 outbreak. This exemplifies a record-breaking speed in vaccine development history (Figure 1.1).

Figure 1.1A shows the typical vaccine development timeline which could be spread over nearly 10 years, in most cases. As illustrated in a significant contrasting scenario, Figure 1.1B shows a compressed fast-track vaccine development as exemplified by candidates in a pandemic emergency such as COVID-19 vaccines. As of April 5th 2022, the World Health Organization (WHO) has noted 153 vaccine candidates that had progressed to clinical evaluation, and over 196 candidates that were in preclinical development.

The rapid development of vaccines against COVID-19 disease was spurred primarily by (i) the timely release of the viral genomic sequence, (ii) innovation in vaccine research, (iii) multi-national scientific collaborative efforts,

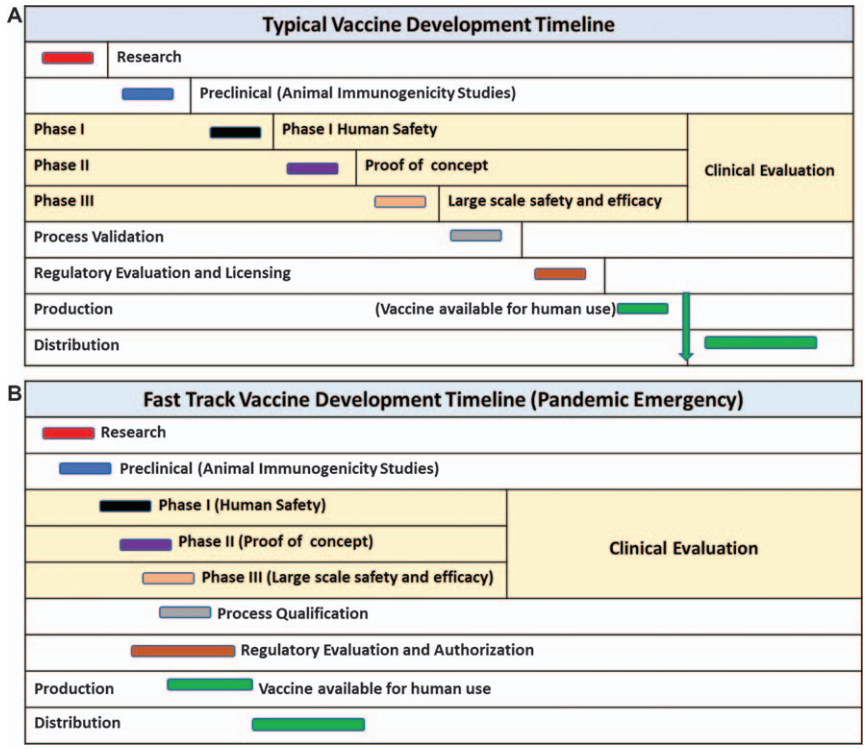

Figure 1.1 Vaccine development timeline.

(iv) smart but risky investments from the industry, (v) generous funding from governments and global non-profit organizations, (vi) the urgent market dynamics, and (vii) rigorous review of the clinical data at an unprecedented pace by regulatory agencies. Critical to the successful authorization of many vaccine platforms, Operation Warp Speed (OWS) has invested an estimated US$18 billion, primarily in the late-phase clinical development and to support production activities of COVID-19 vaccines, and has signed purchase agreements for several hundred million doses.[2] In Chapter 5, Paschall and Avci review the current approaches to vaccine design, particularly immunological considerations such as immune and inflammatory response to infections. Of note, in considering these achievements, is how far translational studies in vaccine research have advanced prior to the COVID-19 pandemic.

Without these innovations that formed a solid foundation in understanding how to generate an effective immune response and overcome immune evasion, research would not have been able to design vaccines so rapidly. The COVID-19 pandemic demonstrates the necessity for groundbreaking cross-disciplinary research efforts in immunology, vaccinology, infection, and epidemiology. Jeyanathan *et al.* discussed the immunological considerations for COVID-19 vaccine development and the ways in which

these strategies may evolve over the next few years.[3] In a recent review, Pollard and Bijker provided an overview of the history, development, immunological basis, and remarkable impact of vaccines on infectious diseases to provide insight into the key issues facing immunologists today.[4] Many of these scientific research and technological advances and also the distribution efforts are being supported heavily by organizations such as the National Institutes of Health (NIH), government agencies, COVAX, Coalition for Epidemic Preparedness Innovations (CEPI), the Bill & Melinda Gates Foundation (BMGF), GAVI, and WHO, to name just a few. More than 2 years from the administration of the first experimental COVID-19 vaccine doses to humans in March 2020, there is now a global race against SARS-CoV-2 to administer vaccines, with uneven levels of rollout in different countries.[5] Despite all the innovations, advancements in vaccine development, and progress in public health, failure to provide global equity in the prompt distribution of SARS-CoV-2 vaccines may result in a doubling of mortality, particularly in poor countries.[6] Of the more than 11 billion vaccine doses that have been administered, 70% of the share belonged to high- and upper middle-income countries. In stark contrast, as of March 19th 2022, 79% of people in high-income countries had received at least one dose of a COVID-19 vaccine compared with only 14% in low-income countries.[7] There is an urgent need to address global vaccine inequity to minimize the risk of facing a continuous onslaught from newer SARS-CoV-2 mutant variants. COVID-19 vaccines significantly reduce the risk of infection and transmission, even against the highly transmissible new variants, such as the Omicron variant.[8]

1.5 High-throughput Assays for Clinical Development

Several clinical assays are required to measure immune responses to demonstrate that a candidate vaccine can elicit a robust immune response. The development and validation of clinical assays require the integration of state-of-the-art technologies, customized reagents, and robust-quality management systems. In Chapter 6, Harris and Giardina provide a survey of the issues and challenges in performing clinical immunoassays that support late-stage vaccine development. Vaccine clinical studies can be of long duration compared with non-vaccine drug studies, and therefore fit-for-purpose methods must include an understanding of how much assay variability can be tolerated within a study, between studies, and throughout a clinical program. High-throughput testing environments are often developed when very large numbers of samples need to be tested over extended periods. Liquid-handling robotics can improve assay precision and repeatability and, in some cases, might also improve testing capacity. Utilizing the high-throughput testing environment for late-stage assay development and scale-up minimizes the risk of unexpected changes in assay performance when the assay is scaled up to full testing capacity. Chapter 6 also provides an overview of the high-throughput testing environment from the perspective of vaccine clinical program support. Areas of focus will include

liquid-handling robotics, assay validation, long-term maintenance (*i.e.* assay life cycle), sample-handling procedures and chain-of-custody, proper data handling, change control, and inspection readiness. Each area of focus will include special considerations for the high-throughput testing environment.

1.6 Complexity in the Development of Multivalent Vaccines: Virus-like Particle-based Vaccines

Many of the currently commercially licensed vaccines typically comprise an antigen (immunogen) and an adjuvant. In the case of ''multivalent vaccines'' targeted against microbial diseases, the product contains antigens (immunogens) from many serotypes within a given serogroup of the microorganism and, optionally, an adjuvant. In the case of ''multi-component vaccines'', the product may contain antigens from many serotypes of the microorganism in addition to one or more heterogeneous antigens and an adjuvant. The development of multivalent and multi-antigen vaccines poses significant preclinical, clinical, process, analytical, and formulation development, manufacturing challenges, and regulatory hurdles.

An example of a multi-antigen vaccine is Merck's VAXELIS®. VAXELIS® is a suspension composed of diphtheria and tetanus toxoids and acellular pertussis, inactivated poliovirus, *Haemophilus influenzae* type b conjugate and hepatitis B vaccine. The vaccine is formulated to contain a heterogeneous mixture of three distinct immunogenic components such as (i) protein antigens, (ii) inactivated virus, and (iii) a glycoconjugate: diphtheria toxoid, tetanus toxoid, acellular pertussis antigens, detoxified pertussis toxin (PT); inactivated polioviruses; and a saccharide antigen of *H. influenzae* type b covalently bound to the outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B, and hepatitis B surface protein antigen (HBsAg). Each dose contains aluminum phosphate as the adjuvant.

Several multivalent vaccines have been approved for commercial use. Some examples among multivalent carbohydrate-based vaccines[9] include pneumococcal (Pfizer's Prevnar13® and Prevnar20™; GSK's Synflorix® and Vaxneuvance™) and meningococcal conjugate vaccines (Sanofi's Menactra® and MenQuadfi™; GSK's Menveo® and Nimenrix®) as described in Chapter 3 by Lockhart and Gruber and Chapter 9 by Krishna Prasad. Among protein-based multivalent vaccines, two products using a virus-like particle (VLP) platform have been licensed for commercial use. To date, three human papilloma virus (HPV) vaccines have been developed and approved: GARDASIL®, a quadrivalent vaccine, and GARDASIL® 9, a nonavalent vaccine, both made by Merck & Co., Inc. (MSD) (Kenilworth, NJ, USA), and Cervarix®, a bivalent vaccine made by GlaxoSmithKline (GSK). Prophylactic HPV vaccines are based on the use of the L1 protein expressed in heterologous expression systems to create self-assembled non-infectious VLPs that display immunodominant type-specific neutralizing epitopes.

In Chapter 7, Kosinski *et al.* focus on the key stages of the development of the fermentation, purification, and formulation processes used to support the

clinical program and subsequent commercial manufacture of GARDASIL®. Cross-functional collaboration between product development teams was critical for the establishment of a commercially sustainable platform manufacturing process. Concurrent with these processes, a ''toolbox'' of assays was developed to ensure process robustness and consistency for key VLP attributes, such as size, disulfide bond content, antigenicity, and stability. A multivalent vaccine poses additional complexity for process development, as amino acid sequence differences between HPV genotypes lead to differences in fermentation and process performance. HPV type differences also impact key process and product challenges such as aggregation during fermentation and the thermal stability of the purified VLPs. Normalization of process parameters across HPV types was pursued where possible during development to reduce operational complexity during commercial manufacture.

1.7 Cell Culture-based Influenza Vaccine Development

Conventionally, the production of seasonal and pandemic influenza vaccines involves viral passage in chicken eggs. Haemagglutinin (HA) as the major vaccine antigen and virus surface protein needs to mutate, so that it can facilitate viral growth in eggs. These mutations may change the antigenicity of HA and thereby decrease vaccine effectiveness (VE). In Chapter 8, Rajaram *et al.* provide a comprehensive overview of the advantages of cell culture technology over conventional egg-based methods. Cell-based vaccines are free of egg proteins, additives, and antibiotics. More importantly, the production of vaccine viruses in cells avoids the need for virus replication in eggs and egg-adaptive mutations, and therefore viruses remain antigenically comparable to wild-type strains. Antigenic characterization performed by WHO collaborating centers showed that cell-derived A/H3N2 viruses match circulating strains more closely than egg-derived A/H3N2 viruses. VE data also demonstrate that cell-derived vaccines are more effective than egg-derived vaccines in preventing influenza-like illness and influenza-associated hospitalizations in individuals of all ages, including those $\geq$65 years old, confirming the advantages of cell culture technology.

1.8 Conjugate Vaccines: Design and Development Considerations

Conjugate vaccines have proven to be a tremendous success story since their introduction more than 30 years ago. In Chapter 9, Krishna Prasad describes the unique and complex challenges associated with the development of conjugate vaccines. Recently, conjugate vaccines have been demonstrated not only to be effective in preventing many bacterial diseases but also to have significant potential to curb antibiotic use to target antimicrobial resistance (AMR). By reducing the incidence of typhoid fever, the introduction of typhoid conjugate vaccine has been estimated to reduce the use of antibiotics significantly. Vaccination of women during pregnancy (maternal immunization)

has emerged recently as an effective prevention strategy to address infant morbidity and mortality. Following maternal immunization, the antibodies are transferred from mother to fetus transplacentally during pregnancy (or *via* breast milk, following delivery), providing protection against infections during infancy, a period of high risk for diseases in the infant. Several clinical trials with conjugate vaccines with group B *Streptococcus* (GBS) capsular polysaccharide coupled to protein antigens induced stronger immune responses than capsular polysaccharide alone. Recent studies support that maternal antibody thresholds raised against capsular polysaccharide antigens could be used as immunological correlates of protection. A significant proportion of the success and impact of the conjugates is attributed to glycoconjugates as commercially licensed prophylactic vaccines. However, small-molecule and peptide–protein conjugate vaccines also have a significant role to play in meeting the unmet need for many diseases primarily as therapeutic vaccines. The choice of the chemistry route to produce a conjugate vaccine is only the first minimal step towards the generation of an optimal conjugate construct. A ''constellation'' of key attributes ultimately defines this optimal conjugate construct. The glycoconjugate vaccine constructs typically are a heterogeneous mixture of molecules and are rarely produced as single molecules. In other words, ''process is the product'' essentially is the success quality mantra for glycoconjugate vaccines. Therefore, early development studies should consider several factors such as stability, consistency of critical and key quality attributes, and manufacturability during the design and development of conjugate vaccines.

1.9 Vaccine Adjuvants

Many vaccine antigens may not stimulate the immune system effectively and often require adjuvants to elicit a robust immune response. The inclusion of adjuvants may allow a lower dose of the vaccine antigen to be administered and may lower the risk for human safety. In Chapter 10, HogenEsch *et al.* provide a comprehensive overview of the current understanding of the mechanisms of action of adjuvants, which is crucial for further rational improvements and informed clinical evaluation. The chapter focuses primarily on the critical role that adjuvants play in processes that are necessary to enhance the immune response, including (i) activation of innate immune cells by engaging pattern recognition receptors, (ii) recruitment and activation of antigen-presenting cells at the injection site, (iii) modulation of antigen uptake and processing, (iv) transport of antigen to the draining lymph node, (v) alteration of the kinetics of lymphocyte migration through lymph nodes to enhance the opportunity for antigen-presenting cell–lymphocyte interactions, and (vi) germinal center formation and persistence. Although the focus is primarily on adjuvants that are part of the currently licensed human vaccines, information from studies with experimental adjuvants that are in different stages of clinical development is also included.

1.10 Development Considerations for Final Dosage Forms: Mucosal Bacterial Vaccines

Mucosal vaccines do not require the use of aseptic techniques in the manufacturing process and have several advantages due to the simplicity associated with vaccine storage and transportation, resulting in a lower cost of goods sold (COGS).

Mucosal surfaces are the initial sites of infection and mucosally administered vaccines can provide protection as the first line of defense. In contrast to systemic administration, mucosal immunization can stimulate cellular and humoral immune responses at both systemic and mucosal levels to induce broad-spectrum and long-lasting immunity. The induction of adaptive immunity at mucosal sites, mediated by secretory antibodies and tissue-resident T cells, has the potential to prevent infection and transmission.[10] Mucosal immunity could be an effective weapon to achieve herd immunity faster by curbing the spread of infectious disease. Mucosal vaccination could induce local neutralizing antibody in addition to T cell-mediated immune responses while the systemic immunity is still produced. To elicit the local mucosal immunity, the immunogen needs to pass through this mucus layer and also the epithelium cells in the upper layer of the mucosal membrane to be captured by the antigen-presenting cells (APCs) such as dendritic cells (DCs), which could result in a low bioavailability of mucosal vaccine. Second, the proteolytic and acidic conditions associated with the exterior environment of the mucosal surface such as the gastrointestinal (GI) tract can degrade the vaccine.

In Chapter 11, Zhang and Patel review the developmental aspects of formulation and dosage forms which are crucial for mucosal vaccines. In the past three decades, significant efforts have been made by researchers to develop novel delivery methods for mucosal drugs. These include alternative formulation strategies involving microparticles, nanoparticles, emulsions, liposomes, mucoadhesive polymers, permeation enhancers, and new mucosal adjuvants. In addition, new mucosal administration routes such as buccal, sublingual, intranasal, and virginal delivery have been evaluated as alternatives to the traditional oral delivery through the GI tract. Most of these alternative routes of administration of vaccines are still in development and have not been licensed. Currently, there are only two licensed oral mucosal vaccines: cholera and typhoid. Chapter 11 has its focus primarily on the mucosal dosage forms, especially for oral mucosal delivery of whole-cell bacteria from the perspective of process development and final dosage forms during manufacture and commercialization.

1.11 Exploiting Glycans for Vaccine Design

Several bacterial and viral pathogens often display an array of glycoconjugates on their surfaces. Some of these glycoconjugates have been connected to various roles, such as innate immune evasion, adhesion, and signaling.

These glycan moieties are often critical mediators of pathogenesis and host–cell interactions. Owing to their surface orientation and the ease of antibody binding, there have been several research studies aimed at exploiting them as antigenic glycans in vaccine development. In Chapter 12, Middleton and Avci review the research studies involving glycan antigens as potential vaccine candidates. Although the capsular polysaccharide (CPS) is a major surface antigen of many bacterial pathogens (see Chapter 9), there are additional potentially conserved glycan epitopes to target in vaccine design against bacterial, viral, and parasitic infection. The envelope spike protein of human immunodeficiency virus-1 (HIV-1) is highly glycosylated. The glycans on the gp120, a component of the envelope spike protein of HIV-1, have been considered as a shield, masking peptide epitopes targeted by immune responses. The gp120 glycans are major targets of broadly neutralizing antibodies. A novel strategy is to exploit the gp120 glycopeptides as MHCII-presented T-cell epitopes to drive robust T-cell immunity. The authors have recently demonstrated that gp120 glycans can directly influence cellular and humoral immune responses through stimulation of CD4 + T cells.

1.12 Public–Private Partnerships for Vaccine Development

Partnerships between public sector agencies and emerging market manufacturers, often facilitated by support from foundations and governments, have long strived towards the development and commercialization of vaccines to address the public health needs of low- and middle-income countries (LMICs). Technology transfer for vaccines is difficult under any circumstances, however, and not surprisingly the success record of such partnerships is mixed. In Chapter 13, Alderson *et al.* present a critical look at how three different partnerships with one private and one state-owned LMIC vaccine manufacturer have succeeded, and the pivotal role played by the global health organization PATH. The vaccines include a meningococcal A conjugate (MenAfriVac®), a decavalent pneumococcal conjugate (PNEUMOSIL®), and a Japanese encephalitis vaccine (CD.JEVAX®). The authors highlight the innovative strategies and approaches taken, what the public sector and local manufacturers brought to the table, resulting benefits to each, and the evolving roles of funding agencies. Future partnerships may benefit from the ''lessons learned'' in these three successful projects. A recent white paper[11] by Greene *et al.* at the Duke-Margolis Center for Health Policy highlighted several key opportunities and strategies for public–private partnerships to support improved efficiency and equity in COVID-19 vaccine distribution, and also considerations for building multi-sector approaches to address emerging challenges. For example, private sector organizations, such as healthcare providers and health plans, can play unique roles in ensuring that populations at additional risk of poor outcomes from COVID-19 have equitable access to vaccines.

1.13 Structure-based Vaccine Design: The New Frontier

A new era in vaccine antigen design has advanced to the forefront supported by a new toolbox comprising atomic-level structural information on viral surface proteins, the capacity for precision engineering, and the ability to produce monoclonal antibodies at a rapid speed. Research efforts centered around HIV-1 vaccine development have been catalysts behind this new toolbox of technologies applicable to other modalities. These research efforts spurred vaccine development targeting respiratory syncytial virus (RSV). Protection against human RSV remains a significant unmet need potentially addressable by maternal immunization. Several vaccines using structure-based vaccine design[12,13] have now been advanced into clinic, possibly representing the first wave of candidates with significant promise.

The combination of decades of research efforts, the discovery by the NIH of cryo-electron microscopy, and computational molecular modeling culminating in structure-based vaccine design resulted in the development of several vaccine candidates against RSV. The RSV F protein is in a ''pre-fusion'' form when present on the surface of the virion, but as it interacts with the host cell it rearranges into an inactive ''post-fusion'' form. The F protein in its post-fusion form cannot elicit potent antibodies owing to the poor binding of the critical immunogenic epitopes (displayed on the pre-fusion form). The pre-fusion form is also unstable and has a strong tendency to revert to the post-fusion form. Historic failures of a successful vaccine have been hampered by this instability of the pre-fusion F protein. A recent phase 1/2 study evaluated a bivalent prefusion F vaccine (RSVpreF) with antigens developed using structure-based vaccine design from RSV subgroups A and B.[14] Adults 18–49 years old were randomized to receive placebo or 60, 120, or 240 μg of RSVpreF with or without $Al(OH)_3$. All RSVpreF formulations induced virus-neutralizing titers higher than those associated with the protection of high-risk infants by palivizumab, the only prophylactic currently available for RSV. Geometric mean fold rises (GMFRs) across RSVpreF doses/formulations were also significantly greater than those historically elicited by post-fusion F vaccines. GMFRs were 3.9–5.2 and 3.7–5.1, respectively, at 12 months post-vaccination. RSVpreF is currently being evaluated in a pivotal phase 3 study for maternal immunization.

Potential areas of expansion of this structure-based vaccine design concept could include germline targeting, epitope focusing, and enhanced display such as multivalent nanoparticle incorporation. A recent review by Ward and Wilson[12] highlighted some of the recent advances in these areas as we progress towards the next generation of immunogens for vaccine development.

1.14 Vaccines to Target Antimicrobial Resistance

Antimicrobial resistance (AMR) has grown to be a major threat to global public health. According to the Centers for Disease Control and Prevention

(CDC), each year in the USA alone at least 2.8 million antibiotic-resistant infections occur, and more than 35 000 people die as a result. Combating AMR requires multifaceted efforts in both the healthcare and veterinary sectors. A recent study focused on global burden[15] provided details on estimated deaths and disability-adjusted life-years (DALYs) attributable to and associated with bacterial AMR for 23 pathogens and 88 pathogen–drug combinations in 204 countries and territories in 2019. Based on the predictive statistical models from this study, there were an estimated 4.95 million deaths associated with bacterial AMR in 2019, including 1.27 million deaths attributable to bacterial AMR. The six leading bacteria for deaths associated with AMR (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) were responsible for more than 900 000 deaths attributable to AMR and 3.57 million deaths associated with AMR in 2019.

The potential role of vaccines as tools to reduce AMR has been underrecognized, even though their effectiveness in targeting several preventable bacterial infectious diseases and AMR is well documented.[16,17] The value of vaccines as an additional modality to combat AMR globally needs more focused efforts, funding, and resources supported by public–private partnerships comparable to the recent COVID-19 vaccine research and development. As mentioned in Chapter 9 by Krishna Prasad, vaccines such as the recently licensed typhoid conjugates have been estimated to reduce the use of antibiotics significantly.[18]

1.15 Technologies Revolutionizing Vaccines

1.15.1 Vaccines Based on Nucleic Acids

Nucleic acid-based vaccines (DNA or RNA) which use genetic material from a disease-causing pathogen have the potential to elicit an immune response against the antigens. In both cases these vaccines can provide instructions to produce specific proteins from the pathogen, which the immune system recognizes as an antigen. DNA- and RNA-based vaccines have been under development for decades against several therapeutic and many infectious diseases, such as influenza and Zika virus prior to the COVID-19 pandemic. Several DNA vaccines are licensed for animal use, including a horse vaccine against West Nile virus. The development of a vaccine for the prevention and transmission of the COVID-19 pandemic disease has been of paramount importance. Developing and testing a new vaccine typically take several years. However, in less than 1 year after the genetic sequence of the SARS-CoV-2 virus was published, two pharmaceutical companies applied for US Food and Drug Administration (FDA) emergency use authorization of vaccines that were highly effective against the virus.

The first messenger ribonucleic acid (mRNA)-based vaccines were approved in December 2020 by the FDA through emergency use authorization in the USA.[19] These vaccines are based on the mRNA vaccine platform and were

developed by Pfizer/BioNTech and Moderna. These represent a new class of products, comprising synthetic mRNA strands encoding the SARS-CoV-2 spike glycoprotein, packaged in lipid nanoparticles (LNPs) to deliver mRNA to cells. Safety and efficacy trials reported high efficacy rates of 94–95% after two interval doses, in conjunction with limited side effects and a low rate of adverse reactions. Current data demonstrate that the currently approved mRNA-based COVID-19 vaccines are safe and effective for most of the population. This breakthrough technology, which creates immunity using a different mechanism compared with traditional vaccines, is one reason why the COVID-19 vaccine research, testing, manufacture, clinical development, and regulatory procedures took less than 1 year. However, decades of pioneering research efforts and incremental technological advances by multiple groups and organizations including both academia and industry have supported mRNA vaccine development.[20] This is one reason why scientists were able to start testing such vaccines against COVID-19 at breakneck speed.

One of the advantages of the mRNA-based vaccines, particularly during pandemics, is that it is relatively quick and easy to design a vaccine against any of its protein antigens. For instance, the mRNA vaccines against COVID-19 entered clinical trials within a few months of the SARS-CoV-2 genome being sequenced. Once DNA encoding the antigen has been chemically synthesized, it is inserted into a bacterial plasmid with the help of specific enzymes. Multiple copies of the plasmid could be produced on a large scale, before being isolated and purified. RNA-based vaccines are relatively straightforward to produce, using well-established chemical synthesis procedures.

One drawback to mRNA vaccines is their instability – they can degrade at high temperatures, which is why the current vaccines are stored at freezing temperatures. Another drawback of mRNA-based vaccines is their high price coupled with storage requirements at freezing temperatures, so that populations in low- and middle-income countries (LMICs) unfortunately have limited access to these technological advances. Ongoing technological advances to address stability, shelf life, and cost of goods sold (COGS) from the next generation of mRNA-based COVID-19 vaccines may alleviate some of these challenges.[21,22]

The emergency use authorization of the first DNA-based COVID-19 vaccine encoding the SARS-Cov-2 spike protein (ZyCoV-D from the Indian pharmaceutical company Zydus Cadila, now Zydus Lifesciences) by India's regulator represents a significant milestone for this class of vaccines.[23] Peer-reviewed data describing the safety and efficacy of this DNA-based vaccine have not yet been published. DNA vaccines could be a valuable alternative if they can overcome many historical limitations, including (i) optimal delivery to antigen-presenting cells (APCs) and (ii) concerns related to potential genotoxicity risks due to chromosomal integration. The advantages of DNA-based vaccines are (i) their high stability, (ii) durability of response, including enhanced T-cell immunity, and (iii) ease of manufacture, features particularly advantageous to LMICs.[23]

1.15.2 VLPs Produced from Plants

Health Canada has recently granted approval for COVIFENZ®, a recombinant plant-based virus-like particles (VLPs) COVID-19 vaccine. This VLP-based vaccine is indicated for active immunization to prevent COVID-19 in individuals 18–64 years of age.[24] COVIFENZ® uses coronavirus-like particle (CoVLP) technology with the vaccine composed of recombinant spike (S) glycoprotein expressed as VLPs co-administered with GSK's AS03 adjuvant. The vaccine is stored at 2–8 °C.

1.16 Vaccines Targeting Latent Viruses

Viruses depend primarily on the host cell for replication. Typically, an active replication of the viral genome results in a lytic cycle represented by the release of new progeny virus particles in the host cell. Another mode of virus infection is the latent phase, a process in which the virus does not replicate. Reactivation is the process by which a latent virus switches to a lytic phase of replication.[25] Reactivation may be caused by several external and/or internal cellular stimuli. Several viruses are known to cause latent infections, which include herpesviruses such as herpes simplex virus (HSV), varicella zoster virus (VZV), which causes shingles, Epstein–Barr virus (EBV), human cytomegalovirus (hCMV), and human herpesvirus 6 (HHV6), in addition to other classes of viruses such as parvovirus and adenovirus.

One of the lasting and signature characteristics of viruses belonging to the *Herpesviridae* family is the establishment of lifelong infections *via* latency.[26] α-Herpesviruses (such as VZV and HSV) are neurotropic pathogens and efficiently invade the peripheral nervous system, resulting in lifelong latency in neurons resident in peripheral ganglia. Herpesviruses have been linked to a variety of incapacitating neuronal diseases such as congenital birth defects and hearing loss (CMV), shingles (VZV), and multiple sclerosis (EBV). Therefore, vaccines targeting herpesviruses can potentially address many of these crippling diseases caused by herpesviruses such as VZV, CMV, EBV, HSV, and HHV6.

1.16.1 Vaccines Targeting Shingles

Varicella, caused by the varicella zoster virus (VZV), is not a benign illness and can be fatal, but most people survive the initial infection. The rash caused by shingles typically disappears. However, the virus is not entirely removed from the human body and can remain dormant in the dorsal root ganglia, the neuronal complex that runs parallel to the spine. By age 55 years, 30–40% of people may lose immunity against VZV, and the virus can reactivate. When the virus escapes from the dorsal root ganglion along the spine, it follows the path of a single nerve group, on only one side of the body. Shingles is characterized by a blistering rash, caused by the same virus as chickenpox, varicella zoster, which lies latent for decades in the