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Chapter 28

Vaccines for Emerging Viral Diseases

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1 INTRODUCTION

Infectious diseases continue to plague mankind and evolve to keep pace with the efforts to control them. Sir William Osler captured the ongoing fear of infectious pathogens when he said, “Humanity has but three great enemies: fever, famine, and war; of these by far the greatest, by far the most terrible, is fever.” Despite the significant impact of antimicrobials and vaccines on public health, there has only been one major human pathogen eradicated—variola virus, the agent of smallpox. In its place have been a series of new and reemerging microbes responsible for isolated infections, regional outbreaks, and global pandemics. Bacterial, fungal, and parasitic pathogens have the capacity to cause widespread epidemics such as the “Black Death” caused by Yersinia pestis in 14th century Europe. However, bacteria, fungi, and parasites are less likely to cause widespread human pandemics at this point in history and are less amenable to vaccine strategies than viral diseases. Focusing on viruses, a catalogue of newly discovered human pathogens from the beginning of the 20th century shows a predictable and nearly linear rate of new agents discovered over time.\(^1\) However, of the more than 100 virus families, only 22 have been associated with human infections, a number that seems to have plateaued.\(^1\) In this chapter we will concentrate on vaccines for emerging viral diseases.
Experiences over the last 3 decades with HIV, SARS, and Ebola have taught us the potential consequences of viral infectious threats on global health, and economic and political stability. Emerging viral infections with pandemic potential can be chronic, persistent, or acute in nature. They can be disseminated by respiratory droplet or other bodily fluids. They can emerge from animal reservoirs and spread via insect vectors, and can be precipitated by changes in climate, animal habitats, human population dynamics, and other ecological events.\(^2\) And they can emerge as a consequence of human activity in the context of deliberate viral modification as a form of biowarfare. We are faced with important questions including what can be done to anticipate these events and how can we best prepare to intervene when new or changing viral threats arrive?

Most current licensed antiviral vaccines utilize live-attenuated or whole-inactivated viruses, although there are now a few examples of effective virus-like particle (VLP) vaccines. In the setting of a new pandemic viral threat and without the advantage of a preexisting understanding of its pathogenesis, growth or attenuating features, it would be difficult to quickly develop traditional live-attenuated or whole-inactivated vaccine approaches due to uncertainty about the safety of attenuating mutations or the production of replication-competent virus in bulk. Therefore, it is more likely that developing vaccines for emerging infections will involve new technologies, some of which have not yet been licensed for human use. Using technologies that can provide a candidate vaccine based on information derived entirely from target gene sequences is safer and more expeditious than procedures requiring virus isolation and growth that require a high level of containment. Therefore, even for virus families for which there are currently licensed vaccines, additional approaches beyond live-attenuated and whole-inactivated products should also be pursued.

Historically, decades have elapsed between when a new virus is discovered and when a relevant vaccine becomes available for human use (Fig. 28.1). In the setting of an epidemic, such protracted vaccine development timelines are incompatible with rapid deployment of a vaccine intervention and therefore not a practical consideration for immediate control of the outbreak. In part because of the 2014 West African Ebola outbreak, emergence of new viral threats to public health are becoming more of a global concern and have more media and political visibility. Fortunately, this is a time of remarkable technical advances in human monoclonal antibody discovery, structure-guided antigen design, and nucleic acid sequencing—making rapid development of biologics more feasible. Therefore, defining new approaches and pathways to efficiently deploy vaccine interventions for emerging infections is a priority for public health agencies, commercial entities, government officials, and nonprofit organizations. However, the key to a rapid vaccine response is advanced preparation.

Several steps are needed to improve preparedness for emerging viral infectious diseases. These can be divided into 4 broad categories: (1) surveillance and discovery, (2) reagent, assay, and animal model development, (3) vaccine
design and product development, and (4) manufacturing, clinical evaluation, and an appropriate regulatory framework.

Depending on features of the virus structure, transmission dynamics, entry requirements, tropism, and replication strategy, a vaccine approach should be proposed, designed, and evaluated in small animals for immunogenicity and protection against challenge. Manufacturing a candidate vaccine for which there is no immediate market poses a significant dilemma because most stages of advanced vaccine development are carried out by large pharmaceutical companies that need to make profit to stay in business. While emerging infectious diseases pose a public health threat, they rarely present a compelling commercial opportunity. Vaccines require a large investment and historically have a relatively low probability of being successful without an extensive iterative process of evaluation and redesign. Therefore, in addition to new biological tools, there needs to be political will to prioritize public funding of advanced vaccine development and new business models for managing this process.3

In this review, we describe the vaccine development efforts for three distinct viruses that collectively capture many of the challenges faced when developing vaccines for emerging viral threats. Ebola, a member of the Filoviridae family, is spread by body fluids and secretions with a relatively low attack rate but causes a systemic disease with high mortality. Chikungunya, an alphavirus in

| Viral pathogen     | Virus discovered | Vaccine developed for human use | Years to vaccine |
|--------------------|------------------|--------------------------------|-----------------|
| Yellow fever virus | 1900             | 1935                           | 35              |
| Polio              | 1909             | 1954                           | 45              |
| Measles            | 1911             | 1957                           | 46              |
| HSV                | 1919             | Not available                   | >96             |
| Influenza          | 1933             | 1945                           | 12              |
| RSV                | 1956             | Not available                   | >59             |
| Dengue virus       | 1960             | Not available                   | >55             |
| Hepatitis B        | 1967             | 1984                           | 17              |
| Rotavirus          | 1973             | 1998                           | 25              |
| Hepatitis A        | 1973             | 1995                           | 22              |
| HPV                | 1974             | 2007                           | 33              |
| HIV                | 1983             | Not available                   | >32             |
| HCV                | 1989             | Not available                   | >26             |

FIGURE 28.1 Time from identification of a viral pathogen to vaccine availability. Vaccine development is a lengthy process often measured in decades. Many steps are required even for a traditional empirical approach including identification of target antigen; assay development; defining seroprevalence and incidence in relevant populations; understanding transmission dynamics and whether there is an intermediate animal host; developing animal models; exploring pathogenesis and defining immune mechanisms of protection; designing vaccine antigens; determining formulation, delivery route and method; preclinical evaluation for safety and immunogenicity; manufacturing; and several years of clinical evaluation and clearing regulatory hurdles. Vaccines can fail at any of these steps, and it is rare for the first attempted concept to become the final product.
the Togaviridae family, is transmitted by mosquito vectors with a high attack rate and causes a systemic disease with low mortality but high frequency of chronic disabling arthritis. Middle East Respiratory Syndrome (MERS CoV), a beta-coronavirus and member of the Coronaviridae family, is spread by respiratory droplets and causes a relatively high mortality in persons with underlying disease. It has a reproductive rate \((R_0)\) of < 1 for person-to-person spread, but occasional “super-spreaders” can infect multiple people. The MERS reservoir, dromedary camels, will continue to be a source of new human infections.

Ebola, Chikungunya, and MERS CoV are representative infectious pathogens with pandemic potential. Use of new technologies to arrive at a more comprehensive understanding of viral structure and pathogenesis has paved the way for rational vaccine design for each of these viruses. Herein we elaborate on the iterative path taken to develop and evaluate candidate vaccines for Ebola, Chikungunya and MERS CoV and the factors that propel or delay progress. We focus our discussions primarily on the candidate vaccines developed at the NIAID Vaccine Research Center, not because they are necessarily the most promising or advanced, but because we are more familiar with the events associated with their development, and the factors impacting advancement and implementation.

2 EBOLA

Ebola is a highly virulent pathogen from the family Filoviridae. Ebolavirus is an enveloped, negative-strand RNA virus whose genome encodes 7 structural proteins including a transmembrane glycoprotein that mediates viral entry into host cells.\(^4\) The surface glycoprotein (GP) mediates viral attachment and entry and is the primary antigenic target for vaccine development. Five species of Ebola have been identified including Zaire (the cause of the 2014 West African epidemic), Sudan, Bundibugyo, Tai Forest, and Reston. Ebola virus disease (EVD) was first recognized in two distinct outbreaks in the Ebola River Valley of the Democratic Republic of Congo (formerly Zaire) and in Sudan in 1976.\(^5,6\) EVD emerged again in 1994 in Gabon and in 1995 in an outbreak involving 315 people in Kikwit, Democratic Republic of Congo (DRC). Since then, sporadic outbreaks have occurred in equatorial Africa, especially the DRC, Republic of Congo, Gabon, Uganda, and Sudan with fatality rates averaging over 50%.\(^7,8\)

After an incubation period of 2–21 days, onset of EVD is manifested by fevers, chills, malaise, and myalgias with onset of gastrointestinal symptoms including nausea, vomiting and diarrhea by days 3–5.\(^8,9\) When fatal, death typically occurs by days 7–12 and can be characterized by hypovolemic shock and multiorgan failure.\(^8,9\) The disease is transmitted human-to-human through direct contact with infected bodily fluids through mucosal surfaces and breaks in the skin.\(^8\)

In Mar. of 2014, Guinea’s Ministry of Health was notified of a highly pathogenic, febrile illness circulating in Gueckedou and Macenta. An epidemiologic evaluation ensued and samples from hospitalized patients were sent to BSL4
labs in France and Germany. Ebola was confirmed by either polymerase chain reaction (PCR), electron microscopy, or from isolation in cell culture. Viral RNA was extracted, sequenced, and compared to available Ebola sequences in GenBank enabling phylogenetic analysis. An Ebola strain was identified with 97% similarity to previously collected Ebola strains in the DRC and Gabon. In turn, the outbreak was traced to a single index case, a 2-year-old boy who died in Dec. 2013 in Gueckedou. The epidemic of EVD that followed has accounted for more cases than all prior EVD outbreaks combined. As of Jul. 2015, over 28,000 suspected and confirmed cases and more than 11,000 deaths have been reported with the majority of cases occurring in the West African countries of Guinea, Liberia, and Sierra Leone. The World Health Organization (WHO) declared the epidemic a public health emergency of international concern in Aug. of 2014. And as the outbreak emerged, the international community responded with an unprecedented effort to accelerate Ebola vaccine development. Prior to 2014, the largest single Ebola outbreak was 425 infections leading to 224 deaths.

The Vaccine Research Center within the National Institute of Allergy and Infectious Diseases performed a series of phase I clinical trials between 2003 and 2009 to evaluate the safety and immunogenicity of GP antigen constructs. These included a DNA vaccine encoding a transmembrane-deleted, secreted version of the glycoprotein, a recombinant human adenovirus serotype 5 (rAd5) vectored vaccine encoding the Ebola GP with one amino acid mutation, and subsequently a DNA vaccine encoding the full-length, wild-type (WT) GP. These phase I studies showed the WT full-length GP was safe and well tolerated. In parallel, studies to define the immunological correlates of protection and optimal antigen delivery approaches were evaluated in nonhuman primates (NHP). In addition to the important role for antibodies targeting GP, it was found that CD8 T cell-mediated immunity was found to be critical for vaccine efficacy in NHP. It was also found that antivector immunity would diminish vaccine potency particularly for the induction of CD8 T cells. Because of the high seroprevalence of Ad5, rare serotype adenovirus vectors were explored including human rAd26 and rAd35 vectors, and chimpanzee-derived rAd vectors.

Chimpanzee adenovirus serotype 3 (abbreviated as ChAd3 or cAd3) encoding the wild-type glycoproteins from both the Ebola Zaire and Sudan species was ultimately chosen as the candidate vaccine vector because of its low seroprevalence and its similar potency and pattern of innate immune response induction to Ad5. This vector was originally produced by Okairos which is now owned by GlaxoSmithKline (GSK). Vector potency comparable to rAd5 was considered to be important for rapid induction of both antibody and CD8 T cells with a single dose. This would facilitate use of the cAd3-Ebola GP vaccine in an outbreak setting using a ring vaccination strategy to achieve rapid short term protection for those at highest risk of infection. Both Zaire and Sudan GP were included in the initial vaccine to protect against both Zaire and Sudan, the most common species responsible for EVD. This replication-defective vaccine, now
called cAd3-EBO, provided 100% protection to nonhuman primates 5 weeks following vaccination in an otherwise lethal Ebola challenge model, and partial protection (50%) to lethal challenge 10 months following vaccination. The cAd3-EBO was also shown to effectively prime for a modified Vaccinia virus Ankara (MVA)-vectored vaccine boost encoding the same glycoprotein inserts, improving survival from lethal Ebola challenge to 100% at 10-months post-boost.\textsuperscript{19} The combination of cAd3-MVA prime-boost produces a much higher magnitude response and might provide more durable protection to health care workers, ambulance drivers, burial workers, and others with ongoing risk of Ebola exposure.

The first quarter of 2015 was targeted for phase I clinical evaluation of cAd3-EBO at the NIH Clinical Center and a Pre-Investigational New Drug (IND) application was submitted to that end in Aug. of 2013. However, in response to the epidemic, the NIH, FDA, IRBs, and others coordinated efforts to consolidate timelines. An IND application was submitted to the FDA on Aug. 15, 2014 and a phase I trial began 18 days later. The cAd3-EBO vaccine was found to be safe and immunogenic in early phase 1 testing of two doses, $2 \times 10^{10}$ and $2 \times 10^{11}$ particle units (PU), and the day 28 postvaccination results were published 3 months later.\textsuperscript{20} All vaccine recipients developed glycoprotein (GP)-specific antibodies; however, GP-specific antibody responses as well as GP-specific T cell responses were greater with the $2 \times 10^{11}$ PU dosing. Antibody titers with the higher dose were in the range associated with protective immunity in the NHP challenge model.

The Zaire GP antigen encoded by cAd3 was derived from the original Mayinga strain of Ebola isolated in 1976. Compared to strains circulating in West Africa it differed in very few GP residues outside the glycan cap, which is cleaved prior to virus entry. Mayinga was also more related genetically to the outbreak strain than the Kikwit strain that had been used in the NHP challenge studies. Therefore, the available cAd3 Ebola Zaire construct was thought to be antigenically relevant to the outbreak strain and suitable for testing. To directly address the West African crisis and to accelerate manufacturing timelines, additional phase I studies of the cAd3 vaccine in monovalent form (encoding the GP from Zaire, not Sudan species) were performed in the United States, United Kingdom, Mali, and Switzerland at doses ranging from $1 \times 10^{10}$ to $1 \times 10^{11}$.\textsuperscript{21} These studies, done in collaboration with GSK, supported advancement of the monovalent vaccine into an on-going phase II/III study in Liberia (NCT02344407) as well as US evaluation of a prime-boost regimen consisting of cAd3-EBO followed by MVA-EBOZ to evaluate durable immune responses (NCT02408913). The monovalent cAd3-EBOZ has also been evaluated as a prime for boosting with a recombinant MVA vector provided by Bavarian Nordic expressing the GP from Zaire, Sudan, and Marburg, and N from Tai Forest at sites in the United Kingdom and Mali. A similar vaccine approach using rAd28 priming and MVA boosting developed by Crucell (now owned by Janssen as part of Johnson & Johnson) has been evaluated in subsequent clinical trials.
In parallel with cAd3-EBO development, a replication-competent, recombinant vesicular stomatitis virus (rVSV) vaccine expressing GP from Ebola Zaire (Kikwit strain) showed promising results in preclinical NHP challenge models.\textsuperscript{22,23} The vaccine was developed by the Public Health Agency of Canada, licensed to BioProtection Systems (a subsidiary of NewLink Genetics), and subsequently licensed to Merck. The donation of 800 vials of this vaccine by the Canadian government to WHO enabled initial evaluation of this candidate vaccine in 150 people at doses ranging from 300,000 to 50 million PFU in phase I trials in Gabon, Kenya, Germany, and Switzerland. Although no life-threatening adverse events were observed, there was evidence of unexpected viral seeding of joints and transient arthritis in addition to vaccine virus positive skin vesicles in some participants. A lower dose of rVSV-ZEBOV (300,000 PFU IM) did not diminish the likelihood of rVSV infecting peripheral tissues. Thirteen of 51 participants developed arthritis and 2 participants developed cutaneous lymphocytic vasculitis with rVSV established as the etiology based on synovial fluid and skin lesion analysis.\textsuperscript{24,25} The vaccine was immunogenic and all vaccine recipients evaluated developed GP-specific antibody responses.\textsuperscript{26} An additional evaluation of this candidate vaccine in two phase I trials in the United States (WRAIR and NIH) supported advancement of the vaccine at 20 million PFU dosing. Compared to 3 million PFU dosing, 20 million PFU resulted in higher IgG and neutralizing antibody titers.\textsuperscript{27} The higher dose vaccine is currently under evaluation in the NIAID-sponsored PREVAIL trial in Liberia, the CDC-sponsored STRIVE study in Sierra Leone, and the WHO-sponsored Ebola ça suffit study in Guinea. Notably, the skin and joint complications were not seen during active follow-up of individuals in the United States or African studies.

Interim results from the Ebola ça suffit phase III trial evaluating the safety and efficacy of rVSV-ZEBOV in an unblinded, cluster-randomized trial in Guinea are encouraging and consistent with vaccine efficacy. The trial utilized a ring vaccination design in which individuals at high risk of infection (contacts and contacts-of-contacts of a lab confirmed case of EVD) were randomized as a cluster to receive either immediate vaccination with rVSV-ZEBOV or delayed vaccination 21 days later. There were no cases of EVD with symptom onset >10 days following randomization in the group receiving immediate vaccination (48 clusters with 2014 subjects) whereas the authors reported 16 cases of EVD in subjects randomized to delayed vaccination (42 clusters with 2380 subjects).\textsuperscript{28}

The pathway to licensure of new vaccines requires evidence of vaccine safety and efficacy in clinical trials. For infectious pathogens that emerge sporadically and with low incidence, such as Ebola, the ability to perform randomized, placebo-controlled, double-blinded efficacy trials remains a limiting factor. The hard fought decline in EVD cases in West Africa is welcome and will hopefully lead to complete control of the epidemic without rebound or reemergence. However, the decline will likely preclude efforts to evaluate vaccine efficacy
of other vaccine candidates during this outbreak. For the cAd3-EBO vaccine, it remains unknown if a path to licensure is feasible in the absence of human efficacy data. Regulators will need to consider if supportive evidence from pre-clinical NHP challenge models, safety data in on-going human trials, and use of alternative surrogate immunogenicity endpoints is adequate to move forward with licensing.

Therefore, several challenging questions remain for regulators and vaccine developers alike. These include determining which Ebola vaccines will be made available for the next Ebola epidemic and what trial design will be used (ie, ring vaccination strategy with delay or community randomization; a step-wedge design with staged vaccination; or a placebo-controlled, randomized study) or whether the initial results from the recent trial will preclude further evaluation of experimental (unlicensed) products. Nonetheless, several important lessons can be extracted from the recent development of Ebola vaccine candidates. Perhaps the most important is that the rapid development and testing of candidate vaccines in 2014 and 2015 was made possible because of years of prior investment into the study of Ebola basic virology and pathogenesis, in part driven by biodefense concerns. Other factors that enabled accelerated vaccine development include: (1) preexisting established animal models, (2) preclinical data from NHP challenge studies, (3) the presence of cGMP vaccine product, and (4) global concern, extensive media coverage and political visibility that helped foster coordination between funding agencies, regulatory authorities, governments, clinical trial sites, laboratories, commercial partners, and publishers.

3 CHIKUNGUNYA

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus of the family Togaviridae transmitted primarily by Aedes aegypti and Aedes albopictus mosquitoes. Three CHIKV clades have been identified and include West African, Asian, and East/Central/South African, each sharing significant amino acid homology. Chikungunya infection manifests as abrupt onset of fever, myalgias, rash, headache, nausea, and arthralgias with illness onset typically 3–7 days following viral transmission. The hallmark arthritis of CHIKV infection can be relapsing, incapacitating, and may persist for months. Severe manifestations of Chikungunya can include myocarditis, hepatitis, and neurologic complications including encephalitis. And although rare, deaths have occurred particularly in the elderly and infants. There are currently no FDA licensed vaccines or treatments specific for Chikungunya.

CHIKV has an 11.8 kB, single-stranded, positive-sense RNA genome that encodes 4 nonstructural proteins involved in virus replication (nsP1-4) and 5 structural proteins including the capsid and envelope glycoproteins E1 and E2. The E1 glycoprotein mediates cell fusion and the E2 glycoprotein interacts with the host receptor. The mature virion diameter is 70 nm and the external surface exhibits trimeric spikes consisting of 240 E2/E1 heterodimers.
Chikungunya virus was discovered at the East African Virology Research Institute in Entebbe, Uganda, now the Uganda Virology Institute (UVRI) and was isolated from a member of the Makonde tribe in Tanzania in the early 1950s. CHIKV has been responsible for outbreaks in Africa and Asia since the 1960s. CHIKV is endemic in tropical and subtropical regions of Africa where it exists as part of an enzootic cycle. However, intermittent epidemics emerge characterized by human-mosquito-human transmission with attack rates that can exceed 50%. CHIKV reemerged in 2005 in an epidemic infecting >272,000 people across several islands in the Indian Ocean. Genetic mutations in the virus including a substitution (A226V) in the E1 glycoprotein that enhanced viral infectivity of the A. albopictus vector contributed both to the 2005 epidemic as well as widespread dissemination of CHIKV into new and temperate climates. The first case of autochthonous transmission in the Americas was reported in 2013 and local transmission has now been reported in over 43 countries or territories in the Americas. A CHIKV epidemic continues in the Caribbean and as of Jul. 2015, >1.5 million suspected CHIKV cases have been reported in the Caribbean, Central America, South America, Mexico, and the US. The CHIKV in the Americas is most similar to the Asian strain and is almost exclusively transmitted by A. aegypti. An East/Central/South African (ECSA) strain has more recently been detected in Brazil, which may make adaptation to A. albopictus more likely. If this occurs, broader spread into North America is possible.

CHIKV viremia peaks on the day of symptom onset with titers reaching $10^9$ viral RNA copies/mL. Both neutralizing activity and induction of IgG3 antibody isotype early in the course of infection are associated with lower risk of chronic disease and persistent arthralgias. Research to date supports an antibody-mediated mechanism of protection from CHIKV infection and passive protection was shown in an otherwise lethal mouse model of CHIKV infection following IgG administration from NHPs who had received a Chikungunya virus-like particle (VLP) vaccine.

Several promising candidate vaccine platforms have been evaluated including formalin-inactivated CHIKV vaccines, recombinant MVA and measles vectored vaccines, chimeric alphavirus vaccines, insect cell-produced VLP vaccine candidates, and DNA vaccine candidates. A live, attenuated CHIKV vaccine was advanced into phase II testing and induced neutralizing antibodies to CHIKV by day 28 in 98% of recipients but was also associated with arthralgias in 8% of subjects. Due to our familiarity with the limitations and obstacles for advancement of a candidate vaccine developed at the NIAID Vaccine Research Center, we will focus the discussion primarily on a mammalian cell-produced VLP vaccine. This candidate vaccine was evaluated in a phase I clinical trial and is currently being advanced into phase II clinical testing. To produce the VLP, Akahata and coworkers transfected 293T HEK cells with expression vectors encoding C-E3-E2-6K-E1 proteins from the West African CHIKV strain 37997. Electron microscopy revealed production of VLPs with
the same morphologic appearance as wild-type CHIKV, characterized by a 65 nm external diameter, 40 nm core diameter, and a structure with E1 and E2 glycoproteins organized into 240 heterodimers and 80 glycoprotein spikes. In preclinical assessment, all NHPs receiving this VLP developed neutralizing antibodies to both heterologous and homologous CHIKV strains. In a challenge model, all NHPs were protected against viremia as well as the postinflammatory sequelae of infection when exposed to intravenous CHIKV challenge 15 weeks after VLP administration.34

The VLP vaccine candidate was found to be safe and immunogenic in a phase I, dose-escalation clinical trial evaluating a dose range of 10–40 µg over a 3 dose regimen (weeks 0, 4, and 20) in 25 healthy adults. Neutralizing antibodies against an outbreak strain from the ECSA clade were identified in all participants 4 weeks following the second vaccination revealing both robust immunogenicity and evidence of cross-reactive neutralizing activity.49

Several advantages of this VLP vaccine include its highly symmetric exterior that resembles wild-type virus, induction of high titer neutralizing antibody, safety profile as a nonreplicating candidate vaccine with low containment manufacturing. Factors contributing to the delay in advanced development of this vaccine include difficulty in establishing a commercial partnership, resources needed for process development and scale-up, and difficulties in establishing clinical trial infrastructure for defining efficacy and immune correlates of protection. Due to the ongoing Chikungunya epidemic in the Americas and the Caribbean, there is an opportunity to obtain an efficacy result in a field trial. Therefore, advancing clinical evaluation of candidate vaccines should be a public health imperative. If field trials are delayed until the outbreak has saturated the region and the disease becomes more sporadic, it will be difficult to ever obtain an efficacy outcome or establish an immunological correlate of protection. This will complicate achieving licensure for general use, and is another example of how public options for manufacturing would facilitate the development of vaccines for emerging infectious diseases.

4 MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses with very large genomes (~30 kb). Endemic human coronaviruses are widespread and include coronaviruses HCOV-229E, OC43, NL63, and HKU1 which generally cause mild respiratory infections including the common cold. However, several features of coronaviruses allow adaptation to new hosts and ecological niches.50 These include large RNA genomes, high frequency of RNA recombination, and infidelity of the RNA-dependent RNA polymerase. Following the 2002 emergence of severe acute respiratory syndrome coronavirus (SARS CoV), a highly pathogenic lineage B betacoronavirus, concerns arose that novel coronaviruses could represent a major public health threat. This was further validated by the emergence of Middle East Respiratory Syndrome coronavirus (MERS CoV).
The first case of MERS CoV was reported in 2012 in a 60-year-old male in Saudi Arabia with acute pneumonia who subsequently died from progressive pulmonary and renal failure. The subsequent rapid international response to the emergence of MERS CoV included genomic sequencing, development of diagnostic assays, surveillance for a zoonotic reservoir, development of animal models, and evaluation of viral pathogenesis to enable rational design of candidate vaccines.

The complete genome sequence of the implicated coronavirus was rapidly identified and made available in GenBank. Genomic sequencing enabled phylogenetic and taxonomic analysis and MERS CoV was identified as a lineage C betacoronavirus, distinct from previously known human coronaviruses and most closely related to HKU4 and HKU5 coronaviruses previously isolated from bats.

Clinical presentation of MERS CoV can range from asymptomatic to a severe respiratory illness that culminates in death. Following an incubation period of 5.2 days (1.9–14.7 days), presenting symptoms can include fever, cough, and dyspnea. Rapid deterioration of respiratory status can occur within just a few days of symptom onset and fatality rates are estimated to be about 35%. Since 2012, there have been 1595 lab-confirmed cases of MERS CoV infection and at least 571 deaths globally. This includes an outbreak of 186 individuals in South Korea initiated by a single index case, a man returning from a trip to Saudi Arabia. The outbreak in Seoul was primarily restricted to hospital settings where 5 individual “super-spreaders” accounted for the majority of infections of other patients and health care providers.

MERS CoV expresses a membrane-anchored trimeric spike (S) protein that consists of a S1 subunit that engages the receptor on the host cell and a S2 subunit that mediates membrane fusion. The receptor binding domain on S1 has served as the primary antigenic target for vaccine development. Similar to the angiotensin-converting enzyme 2 (ACE2) peptidase receptor used by SARS CoV, the MERS CoV target cell receptor is CD26, dipeptidyl peptidase 4 (DPP4), which is a 766 amino acid, type II transmembrane glycoprotein expressed on epithelial and endothelial cells of several organs including lung and kidney. DPP4 has been shown to be a biomarker of IL-13 expression in asthma and is associated with glycemic homeostasis and microvascular complications of diabetes. Transfection of human DPP4 into otherwise nonsusceptible cells from feline, murine and canine species enables MERS CoV infection. Lu et al. subsequently solved the crystal structure of the receptor binding domain of S1 in complex with CD26.

Comparative serologic studies emerged shortly after MERS CoV was first identified to look for zoonotic reservoirs. Evaluation of serum-specific IgG targeting the receptor-binding S1 subunit was performed by protein microarray and confirmed by virus neutralization testing and revealed high-titer neutralizing antibodies were prevalent in camels. Experimental support for the camel as a zoonotic reservoir was performed by Adney and coworkers who inoculated
camels with MERS CoV. The camels subsequently developed upper respiratory symptoms and showed evidence of upper respiratory tract viral shedding in nasal secretions for 7 days postinoculation.\textsuperscript{60} Evidence of zoonotic transmission from camel to humans was further supported from a patient in 2013 who died of MERS CoV following exposure to camels with rhinorrhea. Nasal swabs for both the patient and camels were positive for MERS CoV RNA and subsequent genomic sequencing revealed the isolates to be identical.\textsuperscript{61} However, not all cases of MERS CoV infection have been linked to camel exposure. Additional studies are needed to further define transmission dynamics and intermediate hosts.

Low herd immunity in humans, a highly pathogenic virus and evidence of both zoonotic and human-to-human transmission suggest that outbreaks of MERS CoV will continue to occur and may have pandemic potential. There are currently no FDA approved vaccines for MERS CoV but previous vaccine development efforts for SARS CoV created a foundation for MERS CoV vaccine design. The SARS spike protein is similarly responsible for receptor binding and membrane fusion and is the primary antigen target for neutralizing antibody and vaccine development.\textsuperscript{62}

Candidate MERS CoV vaccines also target the spike protein. Approaches have included subunit proteins, DNA, and gene-based vectors. A recombinant modified vaccinia virus Ankara vaccine expressing the full length MERS CoV spike protein produced neutralizing antibody in immunized mice.\textsuperscript{63} Subsequently, Ma and coworkers focused on the receptor binding domain (RBD) of the MERS CoV spike protein to develop a subunit protein vaccine. Five different receptor binding domain fragments from S1 were individually fused with the Fc of human IgG and each was evaluated for receptor binding affinity, immunogenicity, and neutralizing potential. The S377-588-Fc fragment that contains the stably folded RBD had the highest affinity for DPP4 and induced the highest titer neutralizing antibodies in both mice and immunized rabbits while minimizing exposure to nonneutralizing epitopes.\textsuperscript{64} In addition, two recombinant vaccine candidates have been developed using either a baculovirus-based expression system or a Venezuelan equine encephalitis replicon particle approach.\textsuperscript{65,66}

Potent neutralization was induced in mice and NHPs following immunization with DNA expressing the full-length spike protein followed by a boost with S1 subunit glycoprotein. This strategy elicited responses against both RBD and non-RBD neutralizing epitopes which may decrease the chance of escape mutations. This DNA prime-protein boost regimen provided protection from computerized tomography (CT) defined pneumonia in a NHP viral challenge model.\textsuperscript{67} This work highlights the importance of developing mAbs against the vaccine target antigen in order to define mechanisms of viral neutralization, protein structure, and antigenicity to guide rational vaccine design.

A DNA plasmid-based vaccine encoding full length consensus MERS spike protein was constructed based on available S protein genomic sequences in the
GenBank-NCBI database. The vaccine induced polyfunctional T cell and humoral responses in mice and NHPs, including antigen-specific neutralizing antibodies in mice, macaques, and camels. The vaccine also provided protection from pneumonia in a NHP viral challenge model.68

There are no clinical trial data yet for candidate MERS CoV vaccines. MERS CoV is likely to continue to cause sporadic outbreaks, fueled by camel exposure and the occasional super-spreading event. However, the relatively low R0 suggests that MERS CoV has a relatively low probability of causing a widespread pandemic. Therefore, the target populations for a vaccine when available is not the general population, but groups at high risk of exposure to animal reservoirs, health care workers in endemic regions, and possibly at-risk travelers. With this relatively small market, it will be difficult for commercial organizations to invest in MERS CoV vaccine development. With such low incidence, a field study to evaluate efficacy will be large and difficult to complete. Therefore, questions again are raised about how to achieve advanced development and licensing of a vaccine for this type of emerging virus.

5 CONCLUSIONS

The activities needed to prepare for future pandemic viral threats include a broad spectrum of disciplines and skill sets ranging from logistics and communication to epidemiology and ecology, clinical trials, and highly technical biomedical research programs. Fortunately, relatively recent technological advances have made the prospects of a comprehensive program to systematically prepare for emerging infectious diseases more feasible. If done with forethought, the development of such a program would strengthen existing health care programs, build research infrastructure, and significantly improve the status of global public health. In particular, we should take advantage of new technologies such as high throughput sequencing, isolation of human monoclonal antibodies, structural biology, atomic-level antigen design, molecular biology, and vector biology. These tools can provide the information needed for rapid achievement of optimal expression, immunogenicity, production, and delivery of vaccine antigens for new emerging threats like the current crisis caused by Zika virus (Fig. 28.2).

Due to the new technologies available for surveillance, assay development, vaccine design, animal modeling, and manufacturing, the scientific aspects of vaccine development are not limiting our ability to prepare for emerging viral diseases. The major factors that need to be addressed include (1) the political will to provide the resources necessary to conduct the epidemiology and laboratory work needed to support vaccine development, (2) new business models to create an infrastructure for advanced vaccine development that does not require profit motive, and (3) creative regulatory processes and clinical trial designs to evaluate products for efficacy during outbreaks that are by nature sporadic and causing social chaos. Importantly, achieving solid efficacy data that can support licensure is critical so products can be more readily available during future epidemics.
FIGURE 28.2 A new paradigm for vaccine development. Novel approaches to vaccine design may significantly shorten vaccine development timelines and increase the frequency of success. New technologies that have rapidly evolved over the last 5–10 years make atomic level antigen design feasible and provide mechanisms for rapid, iterative improvements in antigenicity and immunogenicity of candidate vaccines. In particular, high throughput sequencing provides a starting point for vaccine antigens and helps to define the extent of genetic variability. Relatively inexpensive gene synthesis, the ease of isolating and characterizing human monoclonal antibodies (mAbs) against target antigens, the ability to define structures of vaccine antigens in complex with mAbs with desirable functional properties, and the development of assays that evaluate immunogenicity of vaccines in animal models, provide the foundation for rapid development of highly characterized candidate vaccines and their advancement into clinical evaluation. The figure illustrates the steps taken in the case of MERS CoV to rapidly develop the tools needed to generate candidate vaccines. Advanced knowledge of effective vaccine approaches for a particular virus family together with these modern development and design approaches may significantly shorten the time needed to prepare vaccines for field testing in the setting of a new pandemic threat.
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REFERENCES

1. Woolhouse ME, Howey R, Gaunt E, Reilly L, Chase-Topping M, Savill N. Temporal trends in the discovery of human viruses. Proc Biol Sci 2008;275(1647):2111–5.
2. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature 2008;451(7181):990–3.
3. Graham BS, Ledgerwood JE, Nabel GJ. Vaccine development in the twenty-first century: changing paradigms for elusive viruses. Clin Pharmacol Ther 2009;86(3):234–6.
4. Zampieri CA, Sullivan NJ, Nabel GJ. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nat Immunol 2007;8(11):1159–64.
5. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ. 1978;56(2):271–93.
6. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. Bull World Health Organ. 1978;56(2):247–70.
7. Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. Nat Rev Microbiol 2009;7(5):393–400.
8. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet 2011;377(9768):849–62.
9. Chertow DS, Kleine C, Edwards JK, Scaini R, Giuliani R, Sprecher A. Ebola virus disease in West Africa—clinical manifestations and management. N Engl J Med 2014;371(22):2054–7.
10. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, et al. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med 2014;371(15):1418–25.
11. WHO. Ebola virus disease outbreak—Home 2015. Available from: http://who.int/csr/disease/ebola/en/
12. Krause PR, Bryant PR, Clark T, Dempsey W, Henchal E, Michael NL, et al. Immunology of protection from Ebola virus infection. Sci Transl Med 2015;7(286):286ps11.
13. Okware SI, Omaswa FG, Zaramba S, Opio A, Lutwama JJ, Kamugisha J, et al. An outbreak of Ebola in Uganda. Trop Med Int Health 2002;7(12):1068–75.
14. Martin JE, Sullivan NJ, Enama ME, Gordon JJ, Roederer M, Koup RA, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol 2006;13(11):1267–77.
15. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamschikov G, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine 2010;29(2):304–13.
16. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis 2015;211(4):549–57.
17. Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nat Med 2011;17(9):1128–31.
18. Geisbert TW, Bailey M, Hensley L, Asiedu C, Geisbert J, Stanley D, et al. Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge. J Virol 2011;85(9):4222–33.
19. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014;11:1126–9.

20. Ledgerwood JE, DeZure AD, Stanley DA, Novik L, Enama ME, Berkowitz NM, et al. Chimpanzee adenovirus vector ebola vaccine—preliminary report. *N Engl J Med* 2014;373(8):775–6.

21. Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede EB, Payne R, et al. A monovalent chimpanzee adenovirus ebola vaccine—preliminary report. *N Engl J Med* 2015. doi: 10.1056/NEJMoa1411627.

22. Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, Reed DS, Feldmann F, Grolla A, et al. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine* 2008;26(52):6894–900.

23. Geisbert TW, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis*. 2011;204(Suppl 3):S1075–81.

24. Huttner A, Dayer JA, Yerly S, Combescure C, Auderset F, Desmeules J, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. *Lancet Infect Dis* 2015.

25. Ledgerwood JE. Use of low dose rVSV-ZEBOV: safety issues in a Swiss cohort. *Lancet Infect Dis* 2015;15(10):1117–9.

26. Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and Europe—preliminary report. *N Engl J Med* 2015. doi:10.1056/NEJMoa1502924.

27. Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Munoz P, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine - Preliminary Report. *N Engl J Med*. 2015. doi:10.1056/NEJMoa1414216.

28. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al. Efficacy and effectiveness of an rVSV-vectorised vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* 2015;386(9996):857–66.

29. Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks—the globalization of vector-borne diseases. *N Engl J Med* 2007;356(8):769–71.

30. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirus. *Lancet Infect Dis* 2007;7(5):319–27.

31. WHO. Chikungunya 2015. Available from: http://www.who.int/denguecontrol/arbo-viral/other_arboviral/chikungunya/en/

32. Weaver SC, Osorio JE, Livengood JA, Chen R, Stinchcomb DT. Chikungunya virus and prospects for a vaccine. *Expert Rev Vaccines* 2012;11(9):1087–101.

33. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med* 2015;372(13):1231–9.

34. Akahata W, Yang ZY, Andersen H, Sun S, Holdaway HA, Kong WP, et al. A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med* 2010;16(3):334–8.

35. Parola P, de Lamballerie X, Jourdan D, Rovry C, Vaillant V, Minodier P, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. *Emerg Infect Dis* 2006;12(10):1493–9.

36. Panning M, Grywna K, van Esbroeck M, Emmerich P, Drosten C. Chikungunya fever in travelers returning to Europe from the Indian Ocean region, 2006. *Emerg Infect Dis* 2008;14(3):416–22.

37. Kam YW, Simarmata D, Chow A, Her Z, Teng TS, Ong EK, et al. Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance and long-term clinical protection. *J Infect Dis* 2012;205(7):1147–54.
38. Kam YW, Lee WW, Simarmata D, Harjanto S, Teng TS, Tolou H, et al. Longitudinal analysis of the human antibody response to Chikungunya virus infection: implications for serodiagnosis and vaccine development. *J Virol* 2012;86(23):13005–15.

39. Warter L, Lee CY, Thigagarajan R, Grandadam M, Lebecque S, Lin RT, et al. Chikungunya virus envelope-specific human monoclonal antibodies with broad neutralization potency. *J Immunol* 2011;186(5):3258–64.

40. Tiwari M, Parida M, Santhosh SR, Khan M, Dash PK, Rao PV. Assessment of immunogenic potential of Vero adapted formalin inactivated vaccine derived from novel ECSA genotype of Chikungunya virus. *Vaccine* 2009;27(18):2513–22.

41. Harrison VR, Eckels KH, Bartelloni PJ, Hampton C. Production and evaluation of a formalin-killed Chikungunya vaccine. *J Immunol* 1971;107(3):643–7.

42. Garcia-Arriaza J, Cepeda V, Hallengard D, Sorzano CO, Kummerer BM, Liljestrom P, et al. A novel poxvirus-based vaccine, MVA-CHIKV, is highly immunogenic and protects mice against chikungunya infection. *J Virol* 2014;88(6):3527–47.

43. Brandler S, Ruffie C, Combredet C, Brault JB, Najburg V, Prevost MC, et al. A recombinant measles vaccine expressing chikungunya virus-like particles is strongly immunogenic and protects mice from lethal challenge with chikungunya virus. *Vaccine* 2013;31(36):3718–25.

44. Wang E, Volkova E, Adams AP, Forrester N, Xiao SY, Frolov I, et al. Chimeric alphavirus vaccine candidates for chikungunya. *Vaccine* 2008;26(39):5030–9.

45. Metz SW, Gardner J, Geertsema C, Le TT, Goh L, Vlak JM, et al. Effective chikungunya virus-like particle vaccine produced in insect cells. *PLoS Negl Trop Dis* 2013;7(3):e2124.

46. Muthumani K, Lankaraman KM, Laddy DJ, Sundaram SG, Chung CW, Sako E, et al. Immunogenicity of novel consensus-based DNA vaccines against Chikungunya virus. *Vaccine* 2008;26(40):5128–34.

47. Hallengard D, Kakoulidou M, Lulla A, Kummerer BM, Johansson DX, Mutso M, et al. Novel attenuated Chikungunya vaccine candidates elicit protective immunity in C57BL/6 mice. *J Virol* 2014;88(5):2858–66.

48. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. *Am J Trop Med Hyg* 2000;62(6):681–5.

49. Chang LJ, Dowd KA, Mendoza FH, Saunders JG, Sitar S, Plummer SH, et al. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial. *Lancet* 2014;384(9959):2046–52.

50. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012;367(19):1814–20.

51. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, et al. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio* 2012;3(6).

52. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV)—Republic of Korea, 2015. Available from: http://www.who.int/csr/don/23-june-2015-mers-korea/en/

53. Middle East Respiratory Syndrome Coronavirus Outbreak in the Republic of Korea, 2015. Osong Public Health Res Perspect. 2015;6(4):269–78.

54. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev* 2015;28(2):465–522.

55. Brightling CE, Chanez P, Leigh R, O’Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2015;3(9):692–701.
56. Avogaro A, Fadini GP. The effects of dipeptidyl peptidase-4 inhibition on microvascular diabetes complications. *Diabetes care* 2014;37(10):2884–94.

57. Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013;495(7440):251–4.

58. Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature* 2013;500(7461):227–31.

59. Reusken CB, Haagmans BL, Muller MA, Gutierrez C, Godeke GJ, Meyer B, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect Dis* 2013;13(10):859–66.

60. Adney DR, van Doremalen N, Brown VR, Bushmaker T, Scott D, de Wit E, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerg Infect Dis* 2014;20(12):1999–2005.

61. Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, et al. Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med* 2014;370(26):2499–505.

62. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat Rev Microbiol* 2009;7(3):226–36.

63. Song F, Fux R, Provacia LB, Volz A, Eickmann M, Becker S, et al. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. *J Virol* 2013;87(21):11950–4.

64. Ma C, Wang L, Tao X, Zhang N, Yang Y, Tseng CT, et al. Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments—the importance of immunofocusing in subunit vaccine design. *Vaccine* 2014;32(46):6160–6.

65. Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. *Vaccine* 2014;32(26):3169–74.

66. Zhao J, Li K, Wohlford-Lenane C, Agnihotram SS, Fett C, Zhao J, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc Natl Acad Sci USA* 2014;111(13):4970–5.

67. Wang L, Shi W, Joyce MG, Modjarrad K, Zhang Y, Leung K, et al. Evaluation of candidate vaccine approaches for MERS-CoV. *Nature communications* 2015;6:7712.

68. Muthumani K, Falzarano D, Reuschel EL, Tingey C, Flengai S, Villarreal DO, et al. A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. *Sci Transl Med* 2015;7(301):301ra132.