Clinical & Experimental Immunology
The Journal of Translational Immunology

doi: 10.1111/cei.13295 VACCINES FOR EMERGING PATHOGENS: FROM RESEARCH TO THE CLINIC. PART 1
Clinical and Experimental Immunology REVIEW ARTICLE Series Editor: E Diane Williamson

Emerging viruses and current strategies for vaccine intervention

OTHER ARTICLES PUBLISHED IN THIS REVIEW SERIES
Vaccines for emerging pathogens: from research to the clinic. Clinical and Experimental Immunology 2019, 196: 155-156.
HLA-E: exploiting pathogen-host interactions for vaccine development. Clinical and Experimental Immunology 2019, 196: 167-177.
Novel multi-component vaccine approaches for Burkholderia pseudomallei. Clinical and Experimental Immunology 2019, 196: 178-188.
Novel approaches for the design, delivery and administration of vaccine technologies. Clinical and Experimental Immunology 2019, 196: 189-204.
Mucosal vaccines and technology. Clinical and Experimental Immunology 2019, 196: 205-214.
Vaccines for emerging pathogens: prospects for licensure. Clinical and Experimental Immunology 2019, doi: 10.1111/cei.13284

B. Afrough, S. Dowall and R. Hewson
Virology and Pathogenesis Laboratory, National Infection Service, Public Health England, Salisbury, UK.

Accepted for publication 14 March 2019
Correspondence: R. Hewson, Virology and Pathogenesis Laboratory, National Infection Service, Public Health England, Porton Down, Salisbury, Wiltshire SP4 0JG, UK.
E-mail: roger.hewson@phe.gov.uk

Summary

During the past decade several notable viruses have suddenly emerged from obscurity or anonymity to become serious global health threats, provoking concern regarding their sustained epidemic transmission in immunologically naive human populations. With each new threat comes the call for rapid vaccine development. Indeed, vaccines are considered a critical component of disease prevention for emerging viral infections because, in many cases, other medical options are limited or non-existent, or that infections result in such a rapid clinical deterioration that the effectiveness of therapeutics is limited. While classic approaches to vaccine development are still amenable to emerging viruses, the application of molecular techniques in virology has profoundly influenced our understanding of virus biology, and vaccination methods based on replicating, attenuated and non-replicating virus vector approaches have become useful vaccine platforms. Together with a growing understanding of viral disease emergence, a range of vaccine strategies and international commitment to underpin development, vaccine intervention for new and emerging viruses may become a possibility.

Keywords: molecular biology, vaccination, viral

Introduction

Immunization is arguably the most appropriate way of preventing infectious disease. The control of many important viral pathogens by vaccination is perhaps one of the outstanding achievements of medical intervention.

Vaccine-induced immunity that is established in advance of virus infection relies primarily on adaptive immune responses for protective efficacy. Critically, vaccination depends on the properties of antigen recognition, activation, expansion, memory, trafficking and the multitude of specialist functions of lymphocytes. The extent to which vaccine-induced immunity is successful also determines the spread and maintenance of a viral pathogen within a population. Viral vaccines have had profound and enduring consequences for human and animal health; the worldwide eradication of smallpox and rinderpest are testament to their outstanding contribution to modern society.

Nevertheless, infectious diseases still pose one of the greatest threats to public health, and the past three decades have brought a constant barrage of new human pathogens. More than 70% of these infections are zoonotic [1,2], entering either directly from wildlife reservoirs or indirectly via an intermediate domestic animal host [1,3]. HIV, avian influenza, Hendra (HeV) and Nipah (NiV) viruses, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome coronavirus (MERS-CoV), Ebola and Marburg filoviruses, Lassa virus (LASV), Rift Valley fever virus (RVFV) and Crimean–Congo haemorrhagic fever (CCHF) viruses are all examples of zoonoses currently emerging from wildlife. All these emerging zoonoses present a serious and increasing threat to health, biosecurity and economies worldwide. The mechanisms underlying disease transmission from animals to humans are becoming better understood [4] with the emergence of pathogens from wildlife (which represents the greatest threat to global health) occurring in a non-uniform pattern, being localized to distinct geographic 'hotspots' in Africa, Asia and South America, and with each high-threat pathogen being weighted towards a key wildlife species

Clinical and Experimental Immunology, **196**: 157–166

(e.g. bats, rodents or non-human primates (NHPs). It is clear that such diseases will continue to place a substantial burden on global health, especially in dense human populations where the pressures on environmental and economic resources are greatest. More than one billion cases of human zoonotic disease are estimated to occur annually, and emerging zoonoses result in enormous economic losses [5]. Increased urbanization, international travel, commerce and climate change increase the likelihood that emerging zoonosis will continue, if not worsen, in the future.

When a zoonotic virus spills over into a susceptible new species, it often has the advantage that the new host has little or no pre-existing immunity, enabling attachment, entry and replication of the virus in receptive cells. The amplified virus may then evade clearance by host defences for long enough to be transmitted to another susceptible host, and the lack of herd immunity will result in a rapid dissemination of the virus, leading to disease in more virulent cases of infection. Each step in the process represents an opportunity for vaccine-induced immunity and, through such intervention, transmitters and susceptible hosts are removed from a population by the pre-emptive development of protective immunity, so that the spread of infection becomes less likely. Vaccination is therefore a powerful strategy for preventing and controlling emerging zoonotic infectious disease. The development of vaccines for such emerging infections, however, needs to contend with several key challenges associated with such viruses. An emerging infection may be a recently discovered virus and the result of a rare outbreak for which basic biological information such as correlates of protection, antigenic variability or immunodominance are unknown. There may be a lack of time to develop an appropriate animal model of disease in which to study viral immunology and evaluate vaccine candidates for preclinical assessment of protective efficacy and safety. Additionally, many emerging viruses have high case fatality rates, spread easily and cannot be treated. These characteristics mandate that all experimental investigations with such infectious material be carried out at high levels of bio-safety, such as BioSafety levels 3 or 4. For such pathogens the availability of resource-heavy laboratory infrastructure is a bottleneck to basic research. Moreover, the often standard vaccine approach of using attenuated strains or inactivated viral vaccines is not always a feasible option, because of the possibility of reversion to virulence or the requirement for large-scale culture and production in high containment facilities. In addition to these significant hurdles, the economic cost of novel human vaccine development for rare pathogens, which are unlikely to provide an effective payback on investment, has been a major impediment to progress. Thus, basic research into many emerging pathogens has been neglected for years.

In 2014 the unpredicted size, speed and reach of the Ebola virus outbreak in West Africa [6] acted as a wake-up call for researchers, pharmaceutical communities and governments, emphasizing the importance of investment into the study of emerging pathogens. Spurred on by this development and at the request of its 194 Member States in May 2015, the World Health Organization (WHO) convened a broad coalition of experts to develop a research and development (R&D) Blueprint for Action to Prevent Epidemics. Focusing on severe emerging diseases with the potential to generate public health emergencies, and for which no, or insufficient, preventive and curative solutions exist, the R&D Blueprint specifies R&D needs, including vaccine research. Through international governance, the programme aims to define R&D roadmaps for prioritized pathogens and to catalyse funding strategies [7].

Current vaccine platform strategies

Key to the development of successful and effective vaccines is the design of an antigen delivery system that optimizes antigen presentation and induces broad protective immune responses. Recent advances in vector delivery technologies, immunology and basic virology have led to a deeper understanding of the molecular and cellular mechanisms by which vaccines should stimulate both arms of the adaptive immune response, thereby offering novel strategies of vaccination. Here we discuss some current vaccine approaches for safe and effective vaccines encompassing recombinant virus technology, nucleic acid vaccines and self-disseminating vaccine approaches.

Viral vector technology

Advances in recombinant virology and virus reverse genetics have provided key insights into the replication and pathogenesis of a wide range of viruses. Notably, these have facilitated the development of vectors for protein expression and vaccination. To date, several virus families have been exploited as vectors [8–11] including many for vaccination [12–15] Fig 1. A basic advantage of viral vectored vaccines is that the choice antigen is expressed in the context of an active heterologous viral infection, which stimulates the full gamut of innate immune responses required for the development of adaptive humoral and T cell-mediated immunity [9].

An important aspect of a virus-vectored vaccine for emerging viruses is that the characteristics, type and intensity of the immune response, as well as safety considerations and manufacturing techniques are determined predominantly by the vector and not the pathogen. Therefore, developing and testing a vaccine against a newly discovered virus can be significantly shortened by the use of a viral vector platform with an extensive record of safety and efficacy.

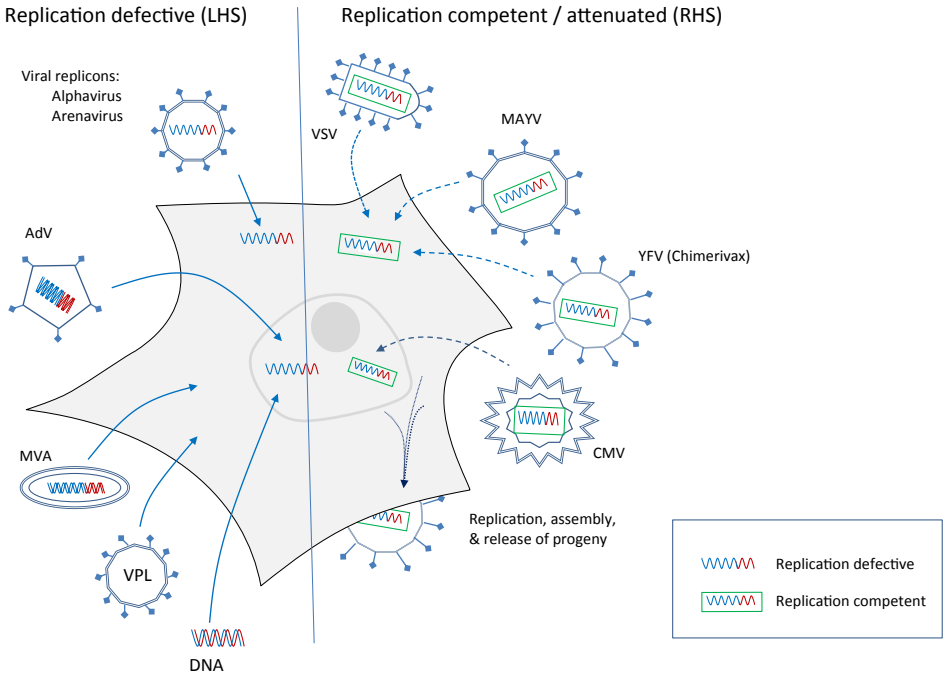

Fig. 1. Virus vector platform technologies. Replication competent but attenuated virus vectors (RHS) deliver heterologous antigen targets resulting in the induction of cellular and humoral responses. Their complement of genes enables a full round of replication and assembly of progeny virus which can amplify and spread the vaccine effect to susceptible cells. While these platforms are based on highly attenuated viral backgrounds, replication competence may lead to the development of mutations and reversion to virulence. Replication defective vectors (LHS) support effective cellular entry and a single round of expression of the target gene / antigen; they result in effective induction of cellular and humoral responses. They are unable to generate new infectious progeny and are considered safer than replication competent vectors.

Replication-competent (attenuated) viral vectors. Vesicular stomatitis virus (VSV), a negative sense RNA virus of the Rhabdoviridae family, has become a prominent replication-competent vaccine vector platform [16]. VSV is non-pathogenic in humans and has an inherent ability to elicit strong cellular and humoral immune responses. One of the most useful aspects of this virus vector platform is its almost promiscuous ability to assemble recombinant VSV (rVSV), with many different types of heterologous viral glycoproteins. The platform is designed such that the VSV G protein is replaced with a heterologous envelope glycoprotein from, for example, an emerging virus; while this arrangement renders the rVSV replication competent, the recombinant viruses are generally highly attenuated [17].

In 1937, the attenuation of a yellow fever (YF) virus via successive rounds of serial passage led to the development of the YF vaccine termed 17D. The impact of this successful vaccine was recognized by the award of a Nobel Prize in 1956 [18] and it has been widely adopted for human immunization for more than 70 years [19]. Based on the utility of the vaccine, an infectious cDNA clone of the YF17D virus [20] has enabled the development of a 17D platform that can be used to drive antigen delivery of pathogens of interest [21]. The technology (licenced as ChimeriVax™) is well suited to similarly related flaviviruses and successful recombinants have been constructed, involving a simple swap of the PrM/M-E genes of YF17D for the same membrane envelope antigens of other emerging flaviviruses, such as Japanese encephalitis, Dengue and West Nile. The resulting recombinant vaccines are efficiently delivered in a live-attenuated virus context with the safety profile afforded by the 17D non-structural genes [22]. A licenced vaccine for Japanese encephalitis (ChimeriVax™-JE) using this technology has been developed by Sanofi Pasteur (Lyon, France).

Other replication-competent platforms. The ease of direct manipulation of viral genomes together with a growing understanding of their biology has led to the development of attenuated virus vaccines with increased safety and immunogenicity. For example, a new vaccine candidate for RVFV has been developed in which a viral virulence factor has been deleted, resulting in a highly attenuated but immunogenic replicating virus [23]. A similar recombinant approach has been used to attenuate the emerging and neglected pathogen Mayaro virus (MAYV) by swapping the subgenomic promoter of this alphavirus for an internal ribosome entry site [24,25], which reduces the expression of MAYV structural proteins. While this

arrangement makes the virus unable to infect mosquito cells, replication in mammalian cells is still possible, resulting in a highly immunogenic profile. Similar studies on infectious clones of other viruses [26] have demonstrated that the genomewide de-optimization of codon usage dramatically reduces gene expression and can be used to attenuate otherwise pathogenic viruses. This strategy has been used for the prototype arenavirus lymphocytic choriomeningitis virus (LCMV) [27]; the study showed attenuation in an otherwise fatal mouse model of LCM disease and the ability to induce protective immunity. Together with a maintained and robust ability to multiply in cell culture, this live attenuated approach may be suitable for similar viruses. However, work to underpin confidence in the genetic stability of such attenuated viruses is critical before further consideration can be given to using this approach for clinical intervention, especially considering the inherent high error rates of the arenavirus polymerase.

Replication-defective approaches. For several years, recombinant adenoviruses have been adopted as promising tools for antigen delivery and vaccine efficacy [28]. Deleting the E1 gene from the adenoviral genome and supplying it in trans from a packaging cell line allows replication-deficient recombinant adenovirus to be produced, with the novel heterologous antigen gene of interest inserted at the E1 locus. Early setbacks in this platform technology relating to pre-existing anti-adenovirus vector antibodies in humans [29] have been resolved by adopting simian adenoviruses as vaccine vectors. A number of different replication-deficient vaccine vectors [30] have recently been developed from simian adenoviruses and the platform has progressed work on emerging pathogens such as Ebola virus, RVFV MERS-CoV and Zika virus.

Modified vaccinia virus Ankara (MVA) is licensed as a third-generation vaccinia type vaccine against smallpox and serves as a potent vector system for the development of new candidate vaccines against a range of infectious diseases, including those caused by emerging pathogens. Historically, MVA was developed by serial tissue culture passage in primary chicken cells of vaccinia virus strain Ankara, and clinically used to avoid the undesirable side effects of conventional vaccinia vaccines [31,32]. Adapted to growth in avian cells, MVA does not replicate in mammalian hosts and lacks many of the viral immune evasion genes [33]. The features of MVA, such as its capacity to accommodate large gene inserts [34], thermostability for application in remote regions without an established cold chain [35], ease of inexpensive manufacture to GMP standards and established regulatory package for development as an Investigational New Drug, make the recombinant MVA platform [36] highly versatile as a heterologous viral vector. In the context of emerging infections, the recombinant MVA platform has shown encouraging preclinical efficacy against Ebola, Zika, Chikungunya [37] and CCHF [38] viruses. Additionally, MVA elicits a strong immunological response against a range of other orthopoxviruses (OPXVs) (including Variola), and vaccines based on this platform can be considered as providing added value, as human immunity to OPXVs is low (after the cessation of the smallpox vaccination campaign) opening a gap for OPXV emergence, as evidenced by the recent occurrence of monkeypox virus in West Africa and onward cross boarder transmissions [39,40].

RNA replicon systems are derived from either positive- or negative-strand RNA virus genomes and embody disabled virus vectors that are non-pathogenic and unable to revert to virulence. Driven by autonomous RNA replication, RNA replicons result in high-level, cytosolic expression of recombinant heterologous antigens stimulating both the humoral and cellular arms of the immune system. Replicon vaccine approaches have closely followed technical developments to genetically manipulate viral genomes. For RNA viruses, replicons based on positive-stranded picornaviruses were some of the first [41], and these were followed by those based on alphaviruses [42] and negative-strand RNA viruses [43]. While a series of different vaccine replicon systems are available, new capabilities with negative-strand viruses have opened up opportunities against a wider range of emerging viruses. Recent developments [44] include the construction of LASV replicons packaged into LASV-like particles which allow a single round of replication and are able to confer protection against an otherwise lethal challenge of LASV in a guinea pig model of disease [44]. While recombinant replicons are devoid of the viral glycoprotein gene, its incorporation into VLPs is achieved by the use of a cell line that expresses the glycoprotein separately (in trans). This enables the scalable propagation of replicon particles in a way that aims to combine the safety of replicon-delivered LASV antigens with the convenience of simply and rapidly producing an attenuated virus. Similar replicon approaches have been used to develop promising replicon-based vaccine candidates for Ebola virus [45] and RVFV [46].

Replicon approaches have the potency of live attenuated vaccines but are inherently safer, as their design ensures a single cycle of replication in contrast to a fully replicating, live attenuated vaccine virus. For live attenuated RNA virus vaccines which incorporate error-prone polymerases, reversion to virulence is a distinct possibility after multiple rounds of replication.

Virus-like particles

Interest in the use of virus-like particles (VLPs) as vaccines candidates stems from their ability to present ordered and highly antigenic structures to the immune system. At the same time, they lack a viral genome, potentially yielding safer vaccines, as there is no viral

sequence that can revert to virulence. They induce strong B cell responses in the absence of adjuvants by efficiently cross-linking specific receptors on B cells [47], and they can also trigger T cell-mediated responses [48]. The basis of ordered viral self-assembly from protein subunits that is noted in many different virus families provides the foundation for work with VLPs, with more than 30 different viruses that infect humans or other animals being identified as able to produce VLPs. They are structurally diverse, having single or multiple capsid proteins, or a lipid envelope, although not all viruses are suitable VLP candidates. Those which are can elicit a protective response without requiring multiple booster shoots, thus significantly reducing the vaccine costs. To date, VLP-based vaccines for human papilloma virus (HPV), hepatitis B virus (HBV) and hepatitis E virus (HEV) have been licensed and are commercially available worldwide [49]. Several VLP-based vaccine candidates for human diseases are under clinical development, including those directed against Norwalk virus, Ebola and Marburg viruses and hepatitis C virus. VLP vaccines combine many of the immunogenic advantages of whole-virus vaccines with the safety advantages of recombinant subunit vaccines.

Nucleic acid vaccines

DNA vaccines have emerged as a safer alternative to standard live and inactivated vaccines for treating human and animal infections [50]. They exhibit several advantages over traditional strategies in terms of safety, stability, ease of manufacturing and immunogenicity [51]. They offer potential advantages for vaccination against emerging viruses, in that plasmids expressing a viral antigen can be produced rapidly. Furthermore, antigen is expressed *in vivo* and induces both humoral and cell-mediated immune responses. Additionally, large quantities of DNA can be produced in a short time at reduced cost, and DNA preparations are more stable than other types of vaccines, which are desirable properties for a vaccine that may be used in remote areas. Furthermore, DNA vaccines are considered safe. However, the main limitation in the development of DNA vaccines is their intrinsic low immunogenicity. Work to improve this has focused on optimizing delivery approaches with the use of gene guns, or electroporation; targeting immune effector cells; and the use of potent adjuvants. DNA vaccines are also frequently used in combination with other vaccine platforms in heterologous prime-boost strategies.

A DNA vaccine is currently licensed to immunize horses against West Nile virus [52] and has undergone Phase I clinical trials in humans [53]. DNA vaccines have also been evaluated as candidates against many emerging viruses, including EBOV [54], RVFV [55], Dengue virus [56] and CHIKV [57].

Synthetic peptides

Synthetic peptide-based epitope-vaccines (EVs) make use of short antigen-derived peptide fragments that can be presented either to T cells or B cells [58]. EVs offer several advantages over other forms of vaccines, particularly with regard to safety, ease of production, storage and distribution, without cold chain issues. They also offer the opportunity to vaccinate against several pathogens or multiple epitopes from the same pathogen. However, drawbacks include poor immunogenicity and the restriction of the approach to patients of a given tissue type [human leucocyte antigen (HLA) haplotype] [59] and, as such, they need to be tailored to accommodate the natural variation in HLA genes. Although initially this was thought to be a major impediment, new technologies have made this personalized-medicine approach feasible [60,61]. Recently, bioinformatics tools have been developed to identify putative $CD4^+$ T cell epitopes, mapped to the surface glycoproteins of the emerging viruses LASV, NipV and Hendra [62]. While these vaccine candidates still need to be experimentally tested, the approach represents an interesting and novel strategy that shows promise for vaccination and which could also address immunity in particular target populations.

Inactivated viruses

The induction of immune responses by the delivery of inactivated pathogens has been a standard and successful vaccination approach for many years, and licenced, inactivated vaccines for diseases such as poliomyelitis [63] and rabies [64] are commercially available. The long history of this approach is underpinned by a well-defined regulatory framework that can be readily applied to new disease targets [65]. The major challenge for the inactivated virus approach is that infection is not established, and therefore a full adaptive immune response is generally not achieved. However, because of the absence of living pathogens, these types of vaccines are safe and a basic capability to prepare such vaccines, especially for emergency use, might be worthwhile as a stop-gap while alternative longer-term approaches are developed. In this regard, studies of virus inactivation with X-ray radiation (as a simple and cheap alternative to gamma irradiation by the use of radioactive isotopes), which maintain the tertiary antigenic structures of virus particles while destroying infectivity, have shown useful promise for a range of applications including vaccination (B. Afrough, unpublished).

Future perspectives

Vaccines play a pivotal role in host protection against infectious diseases and have significantly reduced mortality worldwide. However, many vaccine candidates for

emerging diseases have failed to make it into clinical development. This is perhaps surprising, given the breadth of vaccine technology available and the nature of many of the diseases in question. A case in point is Lassa fever, a viral haemorrhagic illness endemic to many parts of West Africa responsible for more than 300 000 cases of serious disease and approximately 3000 deaths each year since its discovery more than 50 years ago. It is often in the headlines, being the most commonly exported VHF to other territories, including the United Kingdom, which has received a disproportionate share of traveller-related cases (each incident placing substantial burden on public health resources). During 2018, LASV caused an unusually large increase in cases in Nigeria, which led the WHO to classify it as a grade 2 public health emergency. However, despite the high burden of Lassa fever and public attention, no vaccines have so far been approved. Multiple approaches to develop a range of effective preclinical candidates have been made during the last three decades, including attenuated vaccines [66], replication competent vaccines, [67–69], non-replicating LASV vaccines [47], a rationally designed live attenuated vaccine [70] and DNA vaccines [71]. Additionally, many of these platforms have shown efficacy in animal models including NHPs [67,72,73].

These preclinical data illustrate that multiple vaccine technologies have the potential to yield protective Lassa fever vaccines. Therefore, the lack of a clinical vaccine after 50 years since the disease was first described must be due to other factors, such as economic considerations or safety issues, perhaps connected with the growing burden of regulatory thresholds for human medical interventions. Thus, bringing a LASV vaccine and, by analogy, other potential vaccines for emerging diseases to the clinic, may be very difficult – for non-technical reasons. Although there is renewed interest from multiple international agencies to develop human vaccines for certain emerging pathogens [74,75], it is prudent to consider other approaches to the control of emerging disease, including the feasibility of controlling infections at source.

The pattern of disease emergence from viral pathogens into humans from wildlife reservoirs is a clear and present threat [1–5] which will continue. This makes the task of identifying, controlling and preventing zoonoses a difficult and daunting goal, particularly when a new emerging pathogen may be completely unknown. While surveillance is an essential component of a successful control programme, effective containment of an emerging pathogen, before epizootics have the opportunity to spill over into human populations, has been achieved most effectively by large-scale culling or mass vaccination [76,77] of animals. Nevertheless, the ability to contain even known emerging viruses such as Ebola virus in wildlife is currently not possible. Furthermore, the management of diseases that involve livestock, such as RVF and CCHF, pose problems [78,79] in that conventional vaccines are not suited for use in these environments. A major limitation of conventional vaccination is the requirement for individual inoculation of each animal – a costly and impractical strategy for the target/reservoir species of those animals frequently involved in the emergence of high-risk pathogens [1].

Surveillance work focusing on epizootics that are, or may become, human pathogens is a useful goal. Currently however, predicting which animal pathogens will become established as globally significant emerging human diseases is guesswork. Nevertheless, in the early stages of a new zoonosis, pathogenic viruses are often poorly adapted to their new human host in terms of sustained human-to-human transmission [80]. This lag-phase in early zoonosis may, therefore, provide a window of opportunity to control the unrelenting zoonotic pressure of an emerging pathogen before it adapts further to humans. Vaccine targeting of the pathogen within the animal transmission species could therefore bring useful advantages.

Self-disseminating wild-life reservoir vaccines

Self-disseminating vaccines, which aim to immunologically contain emerging viruses within their non-human reservoir hosts, offer an alternative to the conventional vaccine approach. They are designed to exploit the ability of replicating virus-based vectors to spread through animal host populations, so avoiding the need for direct inoculation of every animal. In this way, vaccination of a limited number of initiator animals is used for the introduction of the vaccine into a target population. The vaccine is engineered to express target antigens from the emerging pathogen of interest, so its transmission from vaccinated to non-vaccinated animals will result in the co-ordinated spread of specific immunity for the emerging pathogen throughout the targeted animal population.

Following early work to underpin a proof of principle for a disseminating vaccine against an animal pathogen [81,82], a study targeting the human pathogen Sin Nombre orthohantavirus (SNV) in its rodent reservoir – the deer mouse (*Peromyscus maniculatus*) – proved effective. This approach used an engineered cytomegalovirus (CMV) vector (which causes a benign but transmissible infection in the host), expressing the SNV envelope glycoprotein G1 [83]. A similar approach is also being developed to interrupt zoonotic transmission of Ebola virus [84], in this case disseminating CMV vaccines specific to great apes and expressing EBOV antigens are being studied in African ape populations in the wild [85]. Interestingly, one of the goals of this work is to protect the great apes themselves from Ebola virus disease, a major threat to the survival of these animals in the wild. Also, as

Table 1. Relative advantages/disadvantages of listed vaccine platform technologies

Platform	Benefits	Disadvantages
Viral vector Replication-competent	Development of good humoral and T cell responses Several platforms available. Simple production via virus culture	Selective pressures may result in reduced expression or loss of heterologous antigens or in some case reversion to virulence
Viral vector Replication-defective	Safe. Development of good humoral and T cell responses	Production may require additional steps and cell lines
Virus-like particles	Good induction of humoral and T cell responses. Safe	Production costs may be high and require multiple steps
Naked nucleic acid	Safe. Rapid construction/production	Lower levels of inducted immune responses
Synthetic peptides	Safe. Ease of production and storage	Uncertain immunogenicity linked to target populations
Inactivated viruses	Safe. Well-defined regulatory framework	Questionable (but sometimes effective) immunogenicity
Self-disseminating wild-life vaccines	Sustained transmission within EID reservoir populations, avoiding individual inoculation	Selective pressures may lead to a possible development of virulence

approximately 30% of human Ebola virus outbreaks are known to have resulted from the direct handling of infected ape carcasses, the disseminating CMV-based strategy may be significant in protecting humans [86]. Clearly, further work needs to be conducted to assess the risks of live transmissible vaccines evolving into a pathogen with increased virulence. Engineering such wild-life reservoir vaccines to be weakly transmitting, such that their reproduction number is below 1 ($R_0 < 1$), making their transmission chains short so that they cannot be maintained, might be a way to address the justifiable safety risks. Indeed, a mathematical model has recently demonstrated the value of such an approach [87].

Conclusions

Emerging pathogens represent one of the greatest risks to global health. There is already good evidence [1,5] that zoonotic pathogens will most probably be transmitted from a few key animal species in resource-poor areas of the world. Based on recent history, it is probable that such pathogens have never been seen before. The global impact of the West African outbreak of Ebola virus in 2014 underlines how stark differences in health-care infrastructure can impact upon human-to-human transmission of emerging pathogens. Until basic health-care infrastructure in all countries can be raised to a level that enables early identification and control of high-risk pathogens at source, we will continue to respond to outbreaks of emerging disease long after epizootics have spilled over into human populations. Innovative strategies are therefore urgently required to control such pathogens, vaccination is a proven approach.

Many novel vaccination strategies that have been developed during recent years have the potential to specifically address the growing threat of new and emerging disease. The use of well-defined vaccine vector platforms, with an extensive record of safety and efficacy against similar pathogens, can expedite the process of development, validation and production (Table 1). Accordingly, the design and licensure for particular platform vaccine technologies will help to accelerate the development of new vaccines, as only the simple substitution of a new antigen gene into the vector platform is required. This allows manufacturers to move to a new target disease with minimal changes in chemistry, manufacturing and controls. Thus, new vaccine development can focus on the safety and efficacy of the inserted gene. In addition, the ability of platforms to target multiple pathogens helps to justify the investment required to build and maintain manufacturing infrastructure that specializes in one platform, because a single manufacturing facility can be ready to produce multiple vaccines at any time.

In addition, further research into, and the development of, self-disseminating vaccines to control potential pathogens in their wild-life reservoirs should be encouraged. However, the progress of new vaccines through the necessary regulatory pathways to bring them to the clinic requires long-term investment by governments and international organizations.

Disclosures

The authors confirm that they have no competing interests in this work.

References

1 Jones KE, Patel NG, Levy MA *et al*. Global trends in emerging infectious diseases. Nature 2008; **51**:990–3.

2 Woolhouse ME, Haydon DT, Antia R. Emerging pathogens: the epidemiology and evolution of species jumps. Trends Ecol Evol 2005; **20**:238–44.
3 Field HE. Bats and emerging zoonoses: henipaviruses and SARS. Zoonoses Publ Health 2009; **56**:278–84.
4 Kreuder Johnson C, Hitchens PL, Smiley Evans T *et al*. Spillover and pandemic properties of zoonotic viruses with high host plasticity. Sci Rep 2015; **5**:14830.
5 Karesh WB, Dobson A, Lloyd-Smith JO *et al*. Ecology of zoonoses: natural and unnatural histories. Lancet 2012; **380**:1936–45.
6 WHO Ebola Response Team. After Ebola in West Africa – unpredictable risks, preventable epidemics. N Engl J Med 2016; **375**:587–96.
7 A Research and Development Blueprint for Action to Prevent Epidemics. Available at: https://www.who.int/blueprint/priority-diseases/en/ (accessed 04 January 2019).
8 Hewson R. RNA viruses: emerging vectors for vaccination and gene therapy. Mol Med Today 2000; **6**:28–35.
9 Liu MA. Immunologic basis of vaccine vectors. Immunity 2010; **33**:504–15.
10 Small JC, Ertl HC. Viruses – from pathogens to vaccine carriers. Curr Opin Virol 2011; **1**:241–5.
11 Rollier CS, Reyes-Sandoval A, Cottingham MG, Ewer K, Hill AV. Viral vectors as vaccine platforms: deployment in sight. Curr Opin Immunol 2011; **23**:377–82.
12 Ljungberg K, Liljeström P. Self-replicating alphavirus RNA vaccines. Expert Rev Vaccines 2015; **14**:177–94.
13 Gilbert SC, Warimwe GM. Rapid development of vaccines against emerging pathogens: the replication-deficient simian adenovirus platform technology. Vaccine 2017; **35**:4461–4.
14 Sánchez-Sampedro L, Perdiguero B, Mejías-Pérez E, García-Arriaza J, Di Pilato M, Esteban M. The evolution of poxvirus vaccines. Viruses 2015; **4**:1726–803.
15 Clarke DK, Hendry RM, Singh V*et al*., Brighton Collaboration Viral Vector Vaccines Safety Working Group. Live virus vaccines based on a vesicular stomatitis virus (VSV) backbone: standardized template with key considerations for a risk/benefit assessment. Vaccine 2016; **34**:6597–609.
16 John K, Rose DKC. Rhabdoviruses as vaccine vectors: from initial development to clinical trials. Biology, pathogenesis of rhabdo-, filoviruses. New Jersey: World Scientific, 2015:199–122.
17 Roberts A, Buonocore L, Price R, Forman J, Rose JK. Attenuated vesicular stomatitis viruses as vaccine vectors. J Virol 1999; **73**:3723–32.
18 Norrby E. Yellow fever and Max Theiler: the only Nobel Prize for a virus vaccine. J Exp Med 2007; **204**:2779–84.
19 Monath TP, Barrett AD. Pathogenesis and pathophysiology of yellow fever. Adv Virus Res 2003; **60**:343–95.
20 Rice CM, Grakoui A, Galler R, Chambers TJ. Transcription of infectious yellow fever RNA from full-length cDNA templates produced by *in vitro* ligation. New Biol 1989; **1**:285–96.
21 Bonaldo MC, Sequeira PC, Galler R. The yellow fever 17D virus as a platform for new live attenuated vaccines. Hum Vacc Immunother 2014; **10**:1256–65.
22 Guy B, Barrere B, Malinowski C *et al*. From research to hase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine 2011; **29**:7229–41.
23 Brennan B, Welch SR, McLees A, Elliott RM. Creation of a recombinant Rift Valley fever virus with a two-segmented genome. J Virol 2011; **85**:10310–8.
24 Volkova E, Frolova E, Darwin JR *et al*. IRES dependent replication of Venezuelan equine encephalitis virus makes it highly attenuated and incapable of replicating in mosquito cells. Virology 2008; **377**:160–9.
25 Rossi SL, Guerbois M, Gorchakov R *et al*. IRES-based Venezuelan equine encephalitis vaccine candidate elicits protective immunity in mice. Virology 2013; **437**:81–8.
26 Burns CC, Shaw J, Campagnoli R *et al*. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. J Virol 2006; **80**: 3259–72.
27 Cheng BY, Ortiz-Riano E, Nogales A *et al*. Development of live attenuated arenavirus vaccines based on codon deoptimization. J Virol 2015; **89**:3523–33.
28 Tatsis N, Ertl HCJ. Adenoviruses as vaccine vectors. Mol Ther J Am Soc Gene Ther 2004; **10**:616–29.
29 Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? Hum Vaccin Immunother 2014; **10**:2875–84.
30 Dicks MD, Spencer AJ, Edwards NJ *et al*. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLOS ONE 2012; **7**:e40385.
31 Brown F, Schild GC, Ada GL. Recombinant vaccinia viruses as vaccines. Nature 1986; **319**:549–50.
32 Stickl H, Hochstein-Mintzel V, Mayr A *et al*. MVA vaccination against smallpox: clinical tests with an attenuated live vaccinia virus strain (MVA) [author's translation]. Dtsch Med Wochenschr 1974; **99**:2386–92.
33 Goossens M, Pauwels K, Willemarck N, Breyer D. Environmental risk assessment of clinical trials involving modified vaccinia virus Ankara (MVA)-based vectors. Curr Gene Ther 2013; **13**:413–20.
34 Smith GL, Moss B. Infectious poxvirus vectors have capacity for at least 25000 base pairs of foreign DNA. Gene 1983; **25**:21–8.
35 Alcock R, Cottingham MG, Rollier CS *et al*. Long-term thermostabilization of live poxviral and adenoviral vaccine vectors at supraphysiological temperatures in carbohydrate glass. Sci Transl Med 2010; **2**:19ra12.
36 Volz A, Sutter G. Modified vaccinia virus Ankara: history, value in basic research, and current perspectives for vaccine development. Adv Virus Res 2017; **97**:187–243.
37 Nagata LP, Irwin CR, Hu WG, Evans DH. Vaccinia-based vaccines to biothreat and emerging viruses. Biotechnol Genet Eng Rev 2018; **34**:107–21.

38 Buttigieg KR, Dowall SD, Findlay-Wilson S *et al*. A novel vaccine against Crimean–Congo haemorrhagic fever protects 100% of animals against lethal challenge in a mouse model. PLOS ONE 2014; **9**:e91516.
39 Reynolds MG, Guagliardo SAJ, Nakazawa YJ, Doty JB, Mauldin MR. Understanding orthopoxvirus host range and evolution: from the enigmatic to the usual suspects. Curr Opin Virol 2018; **28**:108–15.
40 Sklenovská N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. Front Public Health 2018; **4**:241.
41 Andino R, Silvera D, Suggett SD *et al*. Engineering poliovirus as a vaccine vector for the expression of diverse antigens. Science 1994; **265**:1448–51.
42 Frolov I, Agapov E, Hoffman TA *et al*. Selection of RNA replicons capable of persistent noncytopathic replication in mammalian cells. J Virol 1999; **73**:3854–65.
43 Lundstrom K. Replicon RNA viral vectors as vaccines. Vaccines (Basel) 2016; **4**:39.
44 Kainulainen MH, Spengler JR, Welch SR *et al*. Use of a scalable replicon-particle vaccine to protect against lethal lassa virus infection in the guinea pig model. J Infect Dis 2018; **217**:1957–66.
45 Halfmann P, Ebihara H, Marzi A *et al*. Replication-deficient ebolavirus as a vaccine candidate. J Virol 2009; **83**:3810–5.
46 Dodd KA, Bird BH, Metcalfe MG, Nichol ST, Albariño CG. Single-dose immunization with virus replicon particles confers rapid robust protection against Rift Valley fever virus challenge. J Virol 2012; **86**:4204–12.
47 Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines 2010; **9**:1149–76.
48 Buonaguro L, Tornesello ML, Buonaguro FM. Virus-like particles as particulate vaccines. Curr HIV Res 2010; **8**:299–309.
49 Jain NK, Sahni N, Kumru OS, Joshi SB, Volkin DB, Russell Middaugh C. Formulation and stabilization of recombinant protein based virus-like particle vaccines. Adv Drug Deliv Rev 2015; **93**:42–55.
50 Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. EMBO Mol Med 2014; **6**:708–20.
51 Liu MA, Wahren B, Karlsson Hedestam GB. DNA vaccines: recent developments and future possibilities. Hum Gene Ther 2006; **17**:1051–61.
52 Powell K. DNA vaccines – back in the saddle again? Nat Biotechnol 2004; **22**:799–801.
53 Ledgerwood JE, Pierson TC, Hubka SA *et al*. A West Nile virus DNA vaccine utilizing a modified promoter induces neutralizing antibody in younger and older healthy adults in a Phase I clinical trial. J Infect Dis 2011; **203**:1396–404.
54 Martin JE, Sullivan NJ, Enama ME *et al*. A DNA vaccine for Ebola virus is safe and immunogenic in a Phase I clinical trial. Clin Vaccine Immunol 2006; **13**:1267–77.
55 Boshra H, Lorenzo G, Rodriguez F, Brun A. A DNA vaccine encoding ubiquitinated Rift Valley fever virus nucleoprotein provides consistent immunity and protects IFNAR(S/S) mice upon lethal virus challenge. Vaccine 2011; **29**: 4469–75.
56 Porter KR, Ewing D, Chen L *et al*. Immunogenicity and protective efficacy of a vaxfectin-adjuvanted tetravalent dengue DNA vaccine. Vaccine 2012; **30**:336–41.
57 Mallilankaraman K, Shedlock DJ, Bao H *et al*. A DNA vaccine against chikungunya virus is protective in mice and induces neutralizing antibodies in mice and nonhuman primates. PLOS Negl Trop Dis 2011; **5**:e928.
58 Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 2007; **6**:404–14.
59 Paris R, Bejrachandra S, Thongcharoen P *et al*. HLA class II restriction of HIV-1 clade-specific neutralizing antibody responses in ethnic Thai recipients of the RV144 prime-boost vaccine combination of ALVAC-HIV and AIDSVAX((R)) B/E. Vaccine 2012; **30**:832–6.
60 Singh-Jasuja H, Emmerich NP, Rammensee HG. The Tubingen approach: identification, selection, and validation of tumor-associated HLA peptides for cancer therapy. Cancer Immunol Immunother 2004; **53**:187–95.
61 Sirskyj D, Diaz-Mitoma F, Golshani A, Kumar A, Azizi A. Innovative bioinformatic approaches for developing peptide-based vaccines against hypervariable viruses. Immunol Cell Biol 2011; **89**:81–9.
62 Oyarzun P, Ellis JJ, Gonzalez-Galarza FF *et al*. A bioinformatics tool for epitope-based vaccine design that accounts for human ethnic diversity: application to emerging infectious diseases. Vaccine 2015; **33**:1267–73.
63 World Health Organization (WHO). Polio vaccines: WHO position paper. Wkly Epidemiol Rec 2016; **91**: 145–68.
64 Denis M, Knezevic I, Wilde H, Hemachudha T, Briggs D, Knopf L. An overview of the immunogenicity and effectiveness of current human rabies vaccines administered by intradermal route. Vaccine 2018; pii: S0264-410X(18)31635-9. https://doi.org/10.1016/j.vaccine.2018.11.072.
65 Elmgren L, Li X, Wilson C *et al*. A global regulatory science agenda for vaccines. Vaccine 2013; **31**:B163–75.
66 Lukashevich IS, Patterson J, Carrion R *et al*. A live attenuated vaccine for Lassa fever made by reassortment of Lassa and Mopeia viruses. J Virol 2005; **79**:13934–42.
67 Geisbert T, Jones S, Fritz E *et al*. Development of a new vaccine for the prevention of Lassa fever. PLOS Med 2005; **2**:e183.
68 Jiang X, Dalebout TJ, Bredenbeek PJ *et al*. Yellow fever 17D-vectored vaccines expressing Lassa virus GP1 and GP2 glycoproteins provide protection against fatal disease in guinea pigs. Vaccine 2011; **29**:1248–57.
69 Clegg JC, Lloyd G. Vaccinia recombinant expressing Lassa-virus internal nucleocapsid protein protects guinea pigs against Lassafever. Lancet 1987; **2**:186–8.
70 Cheng BYH, Nogales A, de la Torre JC, Martínez-Sobrido L. Development of live-attenuated arenavirus vaccines based on

codon deoptimization of the viral glycoprotein. Virology 2017; **501**:35–46.

71 Cashman KA, Broderick KE, Wilkinson ER *et al*. Enhanced efficacy of a codon-optimized DNA vaccine encoding the glycoprotein precursor gene of Lassa virus in a guinea pig disease model when delivered by dermal electroporation. Vaccines (Basel) 2013; **1**:262–77.

72 Lukashevich IS, Carrion R Jr, Salvato MS *et al*. Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates. Vaccine 2008; **26**:5246–54.

73 Cashman KA, Wilkinson ER, Shaia CI *et al*. A DNA vaccine delivered by dermal electroporation fully protects cynomolgus macaques against Lassa fever. Hum Vaccine Immunother 2017; **13**:2902–11.

74 World Health Organization (WHO). WHO publishes list of top emerging diseases likely to cause major epidemics. 2015. Available at: http://www.who.int/medicines/ebola-treatment/WHO-listof-top-emerging-diseases/en/ (accessed 04 January 2019).

75 Coalition for Epidemic Preparedness Innovations (CEPI). Global partnership launched to prevent epidemics with new vaccines. 2017. Available at: http://cepi.net/cepi-officially-launched (accessed 04 January 2019).

76 Jones BA, Grace D, Kock R *et al*. Zoonosis emergence linked to agricultural intensification and environmental change. Proc Nat Acad Sci USA 2013; **110**:8399–404.

77 Swayne DE. Impact of vaccines and vaccination on global control of avian influenza. Avian Dis 2013; **56**(4Suppl):818–28.

78 Indran SV, Ikegami T. Novel approaches to develop Rift Valley fever vaccines. Front Cell Infect Microbiol 2012; **2**:131.

79 Bukbuk DN, Dowall SD, Lewandowski K *et al*. Serological and virological evidence of Crimean–Congo haemorrhagic fever virus circulation in the human population of Borno State, Northeastern Nigeria. PLOS Negl Trop Dis 2016; **10**:e0005126.

80 Parrish CR, Holmes EC, Morens DM *et al*. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev 2008; **72**:457–70.

81 Barcena J, Pages-Mante A, March R *et al*. Isolation of an attenuated myxoma virus field strain that can confer protection against myxomatosis on contacts of vaccinates. Arch Virol 2000; **145**:759–71.

82 Spiesschaert B, McFadden G, Hermans K *et al*. The current status and future directions of myxoma virus, a master in immune evasion. Vet Res 2011; **42**:76.

83 Rizvanov AA, Khaiboullina SF, Van Geelen AG *et al*. Replication and immunoactivity of the recombinant *Peromyscus maniculatus* cytomegalovirus expressing hantavirus G1 glycoprotein *in vivo* and *in vitro*. Vaccine 2006; **24**:327–34.

84 Tsuda Y, Parkins CJ, Caposio P *et al*. A cytomegalovirus-based vaccine provides long-lasting protection against lethal Ebola virus challenge after a single dose. Vaccine 2015; **33**:2261–6.

85 Ghai R. Ebola: outbreaks cause crisis for great apes and humans. Toronto: The Jane Goodall Institute of Canada, 2014.

86 Murphy AA, Redwood AJ, Jarvis MA. Self-disseminating vaccines for emerging infectious diseases. Expert Rev Vaccines 2016; **15**:31–9.

87 Nuismer SL, Althouse BM, May R *et al*. Eradicating infectious disease using weakly transmissible vaccines. Proc R Soc B 2016; **283**:20161903.