Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology
doi: 10.1111/cei.13284
VACCINES FOR EMERGING PATHOGENS: FROM RESEARCH TO THE CLINIC. PART 2
Clinical and Experimental Immunology
REVIEW ARTICLE
Series Editor: E Diane Williamson

Vaccines for emerging pathogens: prospects for licensure

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Summary

Globally, there are a number of emerging pathogens. For most, there are no licensed vaccines available for human use, although there is ongoing research and development. However, given the extensive and increasing list of emerging pathogens and the investment required to bring vaccines into clinical use, the task is huge. Overlaid on this task is the risk of anti-microbial resistance (AMR) acquisition by micro-organisms which can endow a relatively harmless organism with pathogenic potential. Furthermore, climate change also introduces a challenge by causing some of the insect vectors and environmental conditions prevalent in tropical regions to begin to spread out from these traditional areas, thus increasing the risk of migration of zoonotic disease.

Vaccination provides a defence against these emerging pathogens. However, vaccines for pathogens which cause severe, but occasional, disease outbreaks in endemic pockets have suffered from a lack of commercial incentive for development to a clinical standard, encompassing Phase III clinical trials for efficacy. An alternative is to develop such vaccines to request US Emergency Use Authorization (EUA), or equivalent status in the United States, Canada and the European Union, making use of a considerable number of regulatory mechanisms that are available prior to licensing. This review covers the status of vaccine development for some of the emerging pathogens, the hurdles that need to be overcome to achieve EUA or an equivalent regional or national status and how these considerations may impact vaccine development for the future, such that a more comprehensive stockpile of promising vaccines can be achieved.

Keywords: bacterial, human, vaccination

Accepted for publication 26 February 2019
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Introduction

Globally, there are a number of emerging and re-emerging pathogens. Some of these cause endemic disease in regions of the globe, where they are maintained in zoonotic reservoirs and transmitted to man either by direct or indirect contact. For most of the emerging pathogens there are no licensed vaccines available for human use, although there is ongoing research and development. However, given the extensive and increasing list of emerging pathogens and the time and investment required to bring vaccines into clinical use, the task is huge. Overlaid on this task is the risk of anti-microbial resistance (AMR) acquisition by micro-organisms which can endow a relatively harmless organism with pathogenic potential. Furthermore, climate change also introduces a challenge by causing some of the insect vectors and environmental conditions prevalent in tropical regions to begin to spread out from these traditional areas, thus increasing the risk of migration of zoonotic disease.

Vaccination provides a defence against these emerging pathogens. However, to date, vaccines for pathogens which cause severe, but occasional, disease outbreaks in endemic pockets have suffered from a lack of commercial incentive for development to a clinical standard. While approval of vaccines for diseases caused by such pathogens would

make a significant impact on disease outbreaks, taking niche vaccines into clinical development, including Phase III clinical trials for efficacy, requires a large investment in time and money.

An alternative is to develop such vaccines to request US Emergency Use Authorization (EUA), or an alternative status in the United States, Canada and European Union (EU) making use of a considerable number of alternative regulatory mechanisms that are available prior to licensing, so that the products are deployable at the first indications of a disease outbreak.

This review covers the status of vaccine development for some of the emerging pathogens, the hurdles that need to be overcome to achieve EUA or an equivalent regional or national status and how these considerations may impact vaccine development for the future, such that a more comprehensive stockpile of promising vaccines can be achieved.

Emerging and re-emerging pathogens

Pathogens which are classed as emerging or re-emerging are identified through global surveillance programmes and organizations such as the World Health Organization (WHO). Although labelled 'emerging', most of these pathogens are not new and have either been quiescent in the environment until the conditions are opportune to emerge or have evolved from a parent organism to adapt to the prevailing conditions. Thus, there is an intricate relationship between the environment, the climate, wildlife and human existence and lifestyle. The coronaviruses exemplify this point: the ancestral virus possibly existed approximately 10 000 years ago [1]. Coronaviruses have a wide species range infecting birds, bats, chickens, pigs, dogs, cats and rodents [2]. However, the first human coronavirus was described only in the 1960s [3,4], and the coronavirus causing severe acute respiratory syndrome (SARS) was discovered only in 2003 [5–7], while that causing Middle East Respiratory Syndrome (MERS) first emerged in 2012 [8]. It is likely that warm-blooded flying birds and bats have co-evolved with the coronaviruses to aid dissemination [9]. For example, SARS is thought to have first infected Old World bats, then spreading to horseshoe bats [10], civets and finally to man [11]. In the 2003 outbreak of SARS in China and adjacent countries, phylogenetic analysis suggested that the virus spread from bats to humans, possibly through the intermediary civet species. Neither bats or civets showed any clinical signs of infection, and it is thought that bats are the main zoonotic reservoirs for the virus [12].

Organizations such as the WHO, National Institutes for Allergy and Infectious Diseases (NIAID) and the US Centers for Disease Control (CDC) publish lists of emerging pathogens which may be viral, bacterial or rickettsial in nature. The WHO priority list contains viruses which have been prioritized as the most likely to cause epidemics and for which the WHO will establish a Blueprint programme for accelerated research and development (R&D) [13]. The list published in February 2018 is shown in Table 1.

NIAID [14] and CDC [15] also publish lists of pathogens of priority which comprise bacteria and viruses, but categorize these into three groups depending on pathogenicity, accessibility and the availability of vaccines and therapies. All the viruses listed by the WHO also occur on these lists, alongside bacterial pathogens of concern. One of these is *Yersinia pestis*, causative of bubonic and pneumonic plague, which is recognized by all three bodies (WHO, CDC, NIAID) as a current priority following the exceptionally large and serious outbreak between September 2017 to April 2018 in Madagascar [16], where the disease is endemic. In addition, NIAID recognizes the added threat to human health posed by the acquisition of AMR by pathogens and WHO has also published a priority list [17] of bacterial species for which R&D is required to develop new antibiotics (Table 2).

Table 1. Prioritization of research and development effort by the World Health Organization (WHO) for pathogens, February 2018 [13]

Pathogen	Additional pathogens of concern
Congo Crimean haemorrhagic fever (CCHF) virus	
Ebola virus	
Marburg virus	
Lassa fever virus	Other arenaviruses, e.g. LCMV, Junin, Machupo viruses
MERS	Other highly pathogenic coronaviruses
SARS	
Nipah	Henipaviruses
Rift Valley Fever virus (RVFV)	
Zika	
	Emergent non-polio enteroviruses (including EV71, D68); Severe Fever with Thrombocytopenia Syndrome (SFTS)

LCMV = lymphocytic choriomeningitis virus; MERS = Middle East respiratory syndrome; SARS = severe acute respiratory syndrome.

Table 2. Priority list of bacterial species with anti-microbial resistance (AMR) (WHO, February 2017) [17]

Bacterial species
Priority 1: Critical
Acinetobacter baumannii, carbapenem-resistant
Pseudomonas aeruginosa, carbapenem-resistant
Enterobacteriaceae, carbapenem-resistant, extended spectrum beta-lactamase-*producing*
Priority 2: High
Enterococcus faecium, vancomycin-resistant
Staphylococcus aureus, methicillin-resistant, vancomycin-intermediate and resistant
Helicobacter pylori, clarithromycin-resistant
Campylobacter spp., fluoroquinolone-resistant
Salmonella spp., fluoroquinolone-resistant
Neisseria gonorrhoeae, cephalosporin-resistant, fluoroquinolone-resistant
Priority 3: Medium
Streptococcus pneumoniae, penicillin-non-susceptible
Haemophilus influenzae, ampicillin-resistant
Shigella spp., fluoroquinolone-resistant

Endemic disease context

Globally, there are many regions of endemic disease which are maintained by reservoirs of zoonotic pathogens in the local wild animal species. These pathogens comprise viral, bacterial and rickettsial species and some have complex lifecycles, infecting an environmental (e.g. standing water) or animal reservoir and then being transmitted either by direct contact with man or indirectly, via an insect (e.g. mosquito) or mammalian (e.g. bat, civet, camel) vector, to man, from which they may be spread by human-to-human transmission. (Fig. 1). An example of a serious and widespread bacterial infection is mosquito-transmitted *Plasmodium falciparum*, causing malaria in many tropical regions. Malaria causes significant morbidity and co-morbidity in these regions in which it is endemic. Due to the complex life cycle of the causative bacterium, it has been challenging to achieve a vaccine for malaria. The most advanced candidate is the RTS,S/AS01 vaccine [18], which is undergoing a pilot implementation in three countries in sub-Saharan Africa, with a view to determining its impact on disease prior to a wider implementation [19]. Despite the availability of approved vaccines [20,21], typhoid fever and cholera remain significantly debilitating enteric diseases in regions of the world where hygienic living conditions are poor and there is little access to health care. Both infections are caused by bacteria (*Salmonella typhi* and *Vibrio cholera*, respectively) which exist in contaminated water and food. Tuberculosis (TB) is a respiratory infection which is widespread globally. Caused by *Mycobacterium tuberculosis*, and transmitted to man from zoonotic reservoirs (badgers, cattle) with a high potential for subsequent human-to-human transmission, TB is prevalent in susceptible individuals living in overcrowded conditions [22]. Although the BCG vaccine has been in routine use for many years, variable efficacy has been reported, depending on region of use [23,24] with 60–80% reported in the United Kingdom but lower levels in equatorial countries. The emergence of multi-drug-resistant TB (MDR TB) in recent years raises the bar for treatment of this disease and makes a high level of vaccine efficacy even more important [25]. Examples of zoonotic reservoirs which maintain endemic viral diseases are numerous and are summarized in Table 3. In particular, viral endemic disease is caused by the coronaviruses (MERS and SARS) in Saudi Arabia and the Eastern Mediterranean countries; and by the Lassa arenavirus, which is endemic in West African countries and caused a serious outbreak of Lassa fever in Nigeria in 2018 [26]; other viruses which are endemic and cause viral haemorrhagic fever include dengue, which is widespread in tropical and subtropical regions worldwide [27], the filoviruses (Ebola and Marburg) which have a zoonotic reservoir in bats in sub-Saharan Africa [28]; and yellow fever virus, which is prevalent in Africa, Central and South America and the Caribbean [29]. Interestingly, the same mosquito (*Aedes aegypti)* which spreads yellow fever virus also transmits the dengue, chikungunya and zika viruses [30]. Chikungunya virus has a widespread distribution in Africa, Asia, India and South America and with occasional cases in Europe, and causes a debilitating, but rarely fatal, disease [31]. Zika virus emerged in Brazil in 2015 [32], although it was first detected in monkeys in Uganda as early as 1947 [33], and the first documented human case occurred in Nigeria in 1954 [34]. The large Brazilian outbreak of Zika viral disease culminated in 2017, with thousands of cases reported [35]. Rift Valley Fever Virus (RVFV) is another zoonotic virus which is endemic in sub-Saharan Africa and primarily infects cattle, sheep and goats, but can be transmitted to man by mosquito bite to cause an acute fever [36].

An example of a bacterium which has been classed as a re-emerging pathogen and which is endemic in global regions is *Y. pestis*, causative of plague. Bubonic

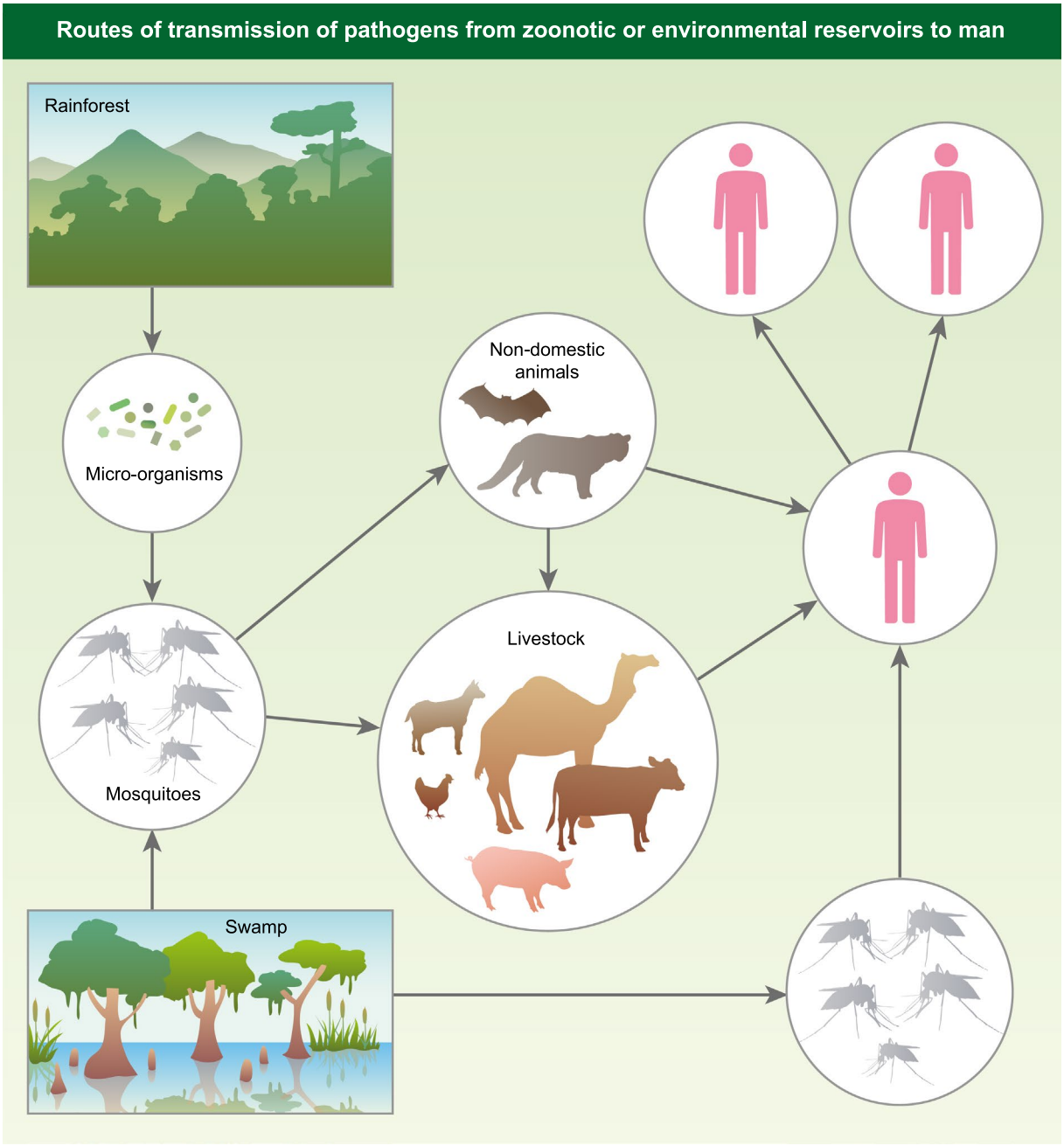

Fig. 1. Routes of transmission of pathogens from zoonotic or environmental reservoirs to man.

plague is endemic in regions such as Madagascar, the Democratic Republic of the Congo (DRC), India and China [37–39]. Over thousands of years, *Y. pestis* has evolved away from the enteric yersinia species to become a lethal flea-transmitted bacterium [40]. It is transmitted to man, typically from a zoonotic reservoir in infected rats or other rodents (e.g. ground squirrels or prairie dogs), by flea-bite to cause bubonic plague [41] (Fig. 2). If not detected and treated, this can develop into either septicemic plague or the most serious form of all, a secondary pneumonic plague. In turn, individuals with pneumonic plague can transmit this by aerosol droplet to others, to establish a primary pneumonic plague infection. Each year, a few cases of plague are also reported in the Southwestern United States, where the disease has been endemic in the rodent population since the late 1800s [42]. As well as infecting the rodent population, infected fleas can spread *Y. pestis* to other wildlife species (e.g. rabbits/hares or, rarely, domesticated animals [43]) which, in turn, raises the potential of

Table 3. Viral zoonoses

Virus	Zoonotic reservoir	Transmission to man by	Endemic in	Recent outbreaks (worst case figures given)
MERS	Bats, camels	Direct contact with camels	Saudi Arabia and Eastern Mediterranean countries	Worldwide 2018 2260 cases (CFR 36%)
SARS	Bats, civets	Contact with civets	China, Hong Kong, Singapore, Taiwan	2004: >8000 cases (CFR 8·5%)
Lassa fever virus	Rats	Contact with rat urine, faeces	Benin, Ghana, Guinea, Liberia, Mali, Sierra Leone, and Nigeria	Nigeria 2018 413 confirmed cases (25% CFR)
Dengue		Mosquitoes	Tropical and subtropical regions worldwide	Worldwide: 390 M cases per year (25% severe) Brazil 2016:1.5M (<1% CFR)
Ebola	Bats	Contact/bats as food source; human–human transmission in respiratory droplets	West Africa, sub-Saharan Africa	Guinea, Liberia, Sierra Leone 2014/15: 28,616 (CFR 40%)
Marburg	Bats	Contact, human-human transmission	West Africa, sub-Saharan Africa	Uganda 2017: 3 cases (CFR 100%)
Yellow fever virus		Mosquitoes	Africa, Central and South America and the Caribbean	Brazil 2018: 723 cases (33% CFR) Africa 2013: 170 000 cases (CFR 35%)
RVFV	Sheep, goats, cattle	Mosquito bite	Sub-Saharan Africa	Niger 2016: 348 cases (CFR 9·5%)
Chikungunya		Mosquito bite	Africa, Asia, India	Central Africa 2018: 13,978 cases (0% CFR)
Zika virus		Mosquito bite	Africa, South America	Brazil 2017: 170 535 cases

MERS = Middle East respiratory syndrome; SARS = severe acute respiratory syndrome; CFR = Code of Federal Regulations; RVFV = Rift Valley Fever Virus.

transmission to man by inhalation to cause a primary pneumonic plague. In endemic areas, outbreaks of plague are often associated with seasonal environmental changes, causing rodents to stray closer to human habitation. The plague outbreak in Madagascar during 2017/18 was particularly serious, with an estimated 2671 cases and 239 deaths (8·9% fatality rate) [44]. This outbreak was approximately sixfold greater than usual, with an unusual predominance of pneumonic, rather than bubonic plague.

Although *Y. pestis* is susceptible to antibiotics, such as the aminoglycosides (gentamycin, streptomycin), the fluoroquinolones (e.g. ciprofloxacin) or tretracyclines (e.g. doxycycline) [45], these need to be administered very early to a suspected plague case, and ideally before symptoms emerge. Additionally, there have been reported instances of antibiotic resistance including to multiple antibiotics [46,47]. Hence, there is a clear and increasingly urgent need for an efficacious vaccine.

Other bacterial endemic diseases include melioidosis and glanders which, although not caused by zoonotic pathogens, are endemic in Southeast Asia where the bacteria reside in soil and are transmitted to humans through occupational exposure, e.g. working in paddy fields [48]. Another bacterial disease which is endemic in the northern hemisphere is tularaemia, caused by the bacterium *Francisella tularensis*, which has zoonotic reservoirs in the rabbit, hare and rodent populations in the South, Central and western United States and is transmitted to man by ticks and biting flies [49].

Rickettsial species, such as *Coxiella burnetii*, causative of Q-fever, comprise bacteria which exist within another cell, and as such Q-fever is contracted when humans are exposed to aerosolized droplets from the urine, milk, faeces or birth detritus from infected sheep, goats and cattle [50].

Availability of vaccines

For all these pathogens, there is a requirement for efficacious approved vaccines to curtail or prevent regular disease outbreaks. For some of these pathogens (e.g. RVFV, CCHF)

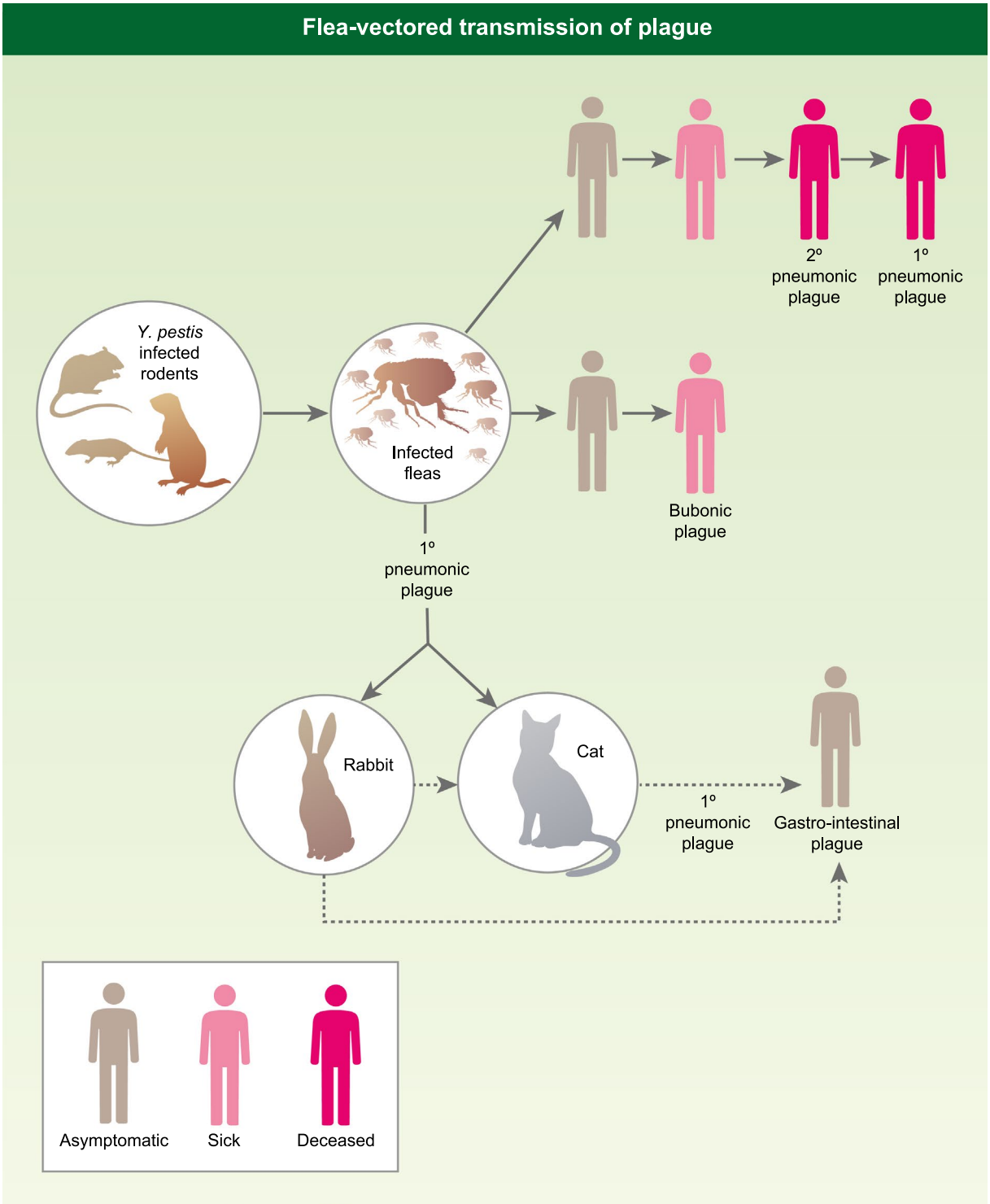

Fig. 2. Flea-vectored transmission of plague.

there are vaccines [51], but these are not widely available [52] or have been used and withdrawn for safety or regulatory reasons (e.g. the live vaccine strain for tularaemia) [53] or they are not universally suitable, requiring a screening test prior to administration due to the potential for a hypersensitivity response (as for the vaccine for Q-fever) [54].

There is no readily available licensed plague vaccine, although a series of killed whole cell vaccines (KWCV)

has been produced and used in the past, mainly in the biodefence context (reviewed in [55]). Additionally, live attenuated plague vaccines, derived from an attenuated mutant strain as the EV series, have been used in the former USSR (fUSSR) and are still used in Asia, notably in Russia and China [56,57]. EV76, the most commonly cited of these, provides protective efficacy against plague, but is licensed for human use only in countries of the fUSSR and is documented to cause serious adverse effects in non-human primates and malaise and adverse effects in human vaccinees [57].

Vaccine requirements

Whatever the context, all these diseases would be positively impacted by the availability of efficacious and approved vaccines. However, to a greater or lesser extent they are all niche diseases with no major commercial incentives to drive vaccine development programmes. This is a space that non-governmental organizations (NGOs) such as the Coalition of Epidemic Preparedness Innovations (CEPI) [58] and Global Vaccine Alliance (GAVI) [59] have entered and they are supporting vaccine efforts for some of the diseases listed above. Additionally, philanthropic funders such as the Gates Foundation are supporting vaccine R&D efforts [60]. In the United Kingdom, and subsequent to the Ebola outbreak in West Africa, vaccine networks for human and veterinary vaccines have formed to prioritize vaccine efforts in these respective contexts [61], while the Department of Health, together with Innovate UK, has supported R&D of vaccine candidates for the prioritized pathogens [62]. As a result of these global initiatives, a number of candidate vaccines are being developed for emerging bacterial and viral pathogens, examples of which, although by no means exhaustive, are cited here [63–74]. WHO reports ongoing global vaccine R&D efforts [75] and also tracks the progress of clinical trials for emerging pathogens [76].

Vaccine indications

Here it may be worth drawing a distinction between prophylactic vaccination, i.e. general use prophylaxis (GUP), given routinely and not necessarily in the face of specific, predicted outbreaks, in contrast to post-exposure prophylactic (PEP) vaccination, to be given after a suspected exposure to a pathogen, or to ring-fence an outbreak or, indeed, post-exposure therapeutic (PET) vaccination, to be given after an actual exposure. In the PEP and PET contexts, the benefit of vaccination vastly outweighs the risk of disease (i.e. the risk : benefit ratio is low), while in the prophylactic context the risk : benefit ratio may be greater than, or equal to, 1·0). For infections with short incubation times, e.g. less than 72 h, as for pneumonic plague, PEP or PET vaccination may only be useful if administered under antibiotic cover. In the United States, once a vaccine candidate has been thoroughly tested for safety in non-clinical models, and in an escalating-dose, statistically powered, Phase I design in the clinic, from then on it may be possible to pursue approval for an EUA, rather than pursue the full-length pathway to biological licensing authorization (BLA). This can enable the earlier availability of vaccines for use in endemic regions. However, it should be noted that EUA is only available through the Food and Drug Agency (FDA) in the United States. Some alternative regulatory mechanisms that may be considered prior to licensing are outlined below.

Vaccine development process

From the discovery phase to the clinic, vaccine development requires the completion of a series of steps represented as a generic outline in Fig. 3. The regulatory agencies lay down specific guidance for these steps [77,78] and this review presumes no authority in this regard, but seeks to give a generalized overview of a generic vaccine development process. Exit from the discovery and preclinical phases requires substantive data demonstrating immunogenicity and efficacy in at least one suitable animal model(s). Where possible, efficacy in the animal model should be demonstrated by direct exposure to the pathogen concerned. Technology transfer for manufacture under good manufacturing practice (GMP) will require a demonstration of known provenance of all essential materials required in the manufacture of the vaccine candidate. This includes seed stocks of cell lines from which recombinant proteins may be expressed, e.g. *Escherichia coli*, human embryo kidney cells, baculovirus, tobacco mosaic virus; or seed stocks of attenuated vaccine vectors, e.g. viral vectors such as adenovirus, vesicular stomatitis virus, modified Vaccinia Ankara; or bacterial vaccine vectors, e.g. salmonella, listeria; the genetic constructs cloned into the cell line in question; all culture media and components and all formulations and excipients.

Transfer of the manufacturing process to GMP may include scale-up and conversion to, for example, fermentation conditions, or to plant-based, mammalian cell line or insect cell line expression on an expanded scale. This transfer will probably require the demonstration of consistency between consecutive batches at GMP, which will also enable the development of scaled-up downstream processing methodology. Vaccine components (the drug substance) from these batches may be formulated (the drug product) as required and used in safety/toxicology studies and for immunogenicity/efficacy in an appropriate second animal model, which will be as representative as possible of the human response. The second animal model

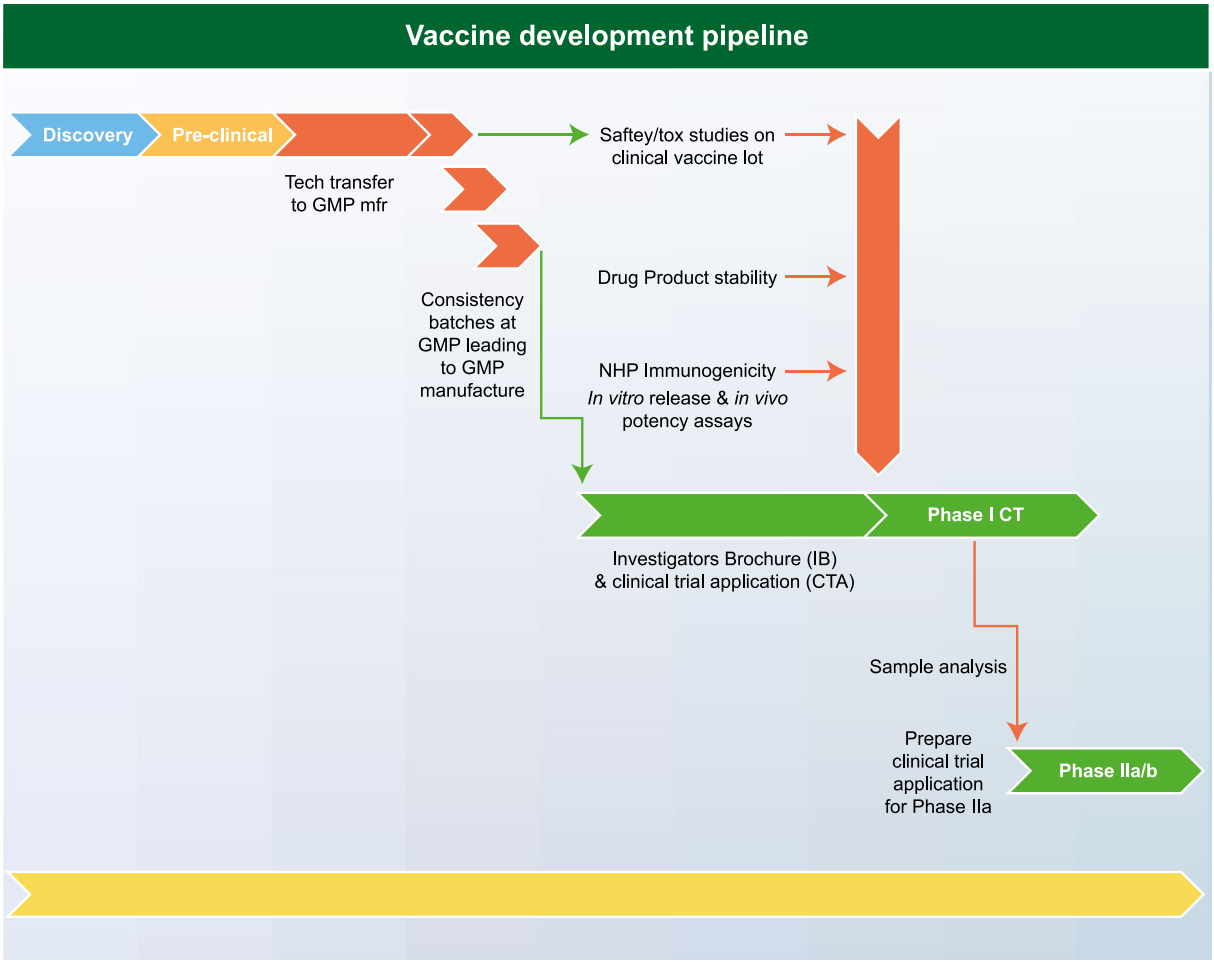

Fig. 3. Vaccine development pipeline.

is often, but not necessarily, a non-human primate, and the selection of this second model will depend entirely on the vaccine indication.

There will also be a requirement to generate sufficient stability data on both the drug substance and the drug product and to demonstrate that the drug product is stable for at least the duration of the intended Phase I clinical trial under prevalent conditions in that location. Clearly, extended stability testing under a range of conditions, including accelerated conditions (high ambient temperature and relative humidity), will also be required to progress the vaccine through development. As well as determining its stability, both the drug substance and product will require characterization for properties such as identity, purity, isoelectric point, osmolality, endotoxin levels and potency, to demonstrate consistency between batches and to allow for release of these for clinical trials.

It is essential to ensure safety of the drug product before entering a clinical trial, and the drug product may be tested in suitable small animal models for the absence of adverse effects under conditions of repeated dosing at the anticipated human dose level, as well as in the intended human schedule, but at multiples of the anticipated human dose-level. The use of rodent models (mouse or rat) for this testing allows for sufficient statistical powering of such safety/toxicological testing. If the clinical trial is to be conducted in women of child-bearing age, it may be necessary to carry out reproductive toxicology testing of the drug product; in this case it may be necessary also to use a sensitive rabbit model and to administer the vaccine to pregnant rabbits to screen for adverse effects in the mother and potential teratogenic effects in the F_1 generation.

Regulatory review

The national regulatory authority will expect to review the existing safety data and outlines of further protocols to be used. A review of all the data pertaining to the candidate vaccine will be required by the regulators in order to proceed to a clinical trial. Depending on the specific requirements of the regulatory authorities in the country of origin/manufacture of the vaccine and also

the intended location of the clinical trial, this may take the form of an investigator's brochure, protocol and summaries of manufacturing, non-clinical and clinical data in a clinical trial application (for the European Medicines Agency, EMA) or an investigational new drug (IND) application (e.g. US FDA). Other international regulators (e.g. in Canada, Japan and China) will have variations on the documentation required.

Regulatory mechanisms

In the United States, the alternative approaches to full marketing approval that are available for vaccines under development and which may be considered through the FDA are to either request an EUA or to request expanded access (EA), sometimes called compassionate use. The EUA is issued in support of potential and actual public health, military and domestic emergencies involving chemical, biological, radiological and nuclear agents (CBRN), including emerging infectious diseases, e.g. pandemic influenza. Such FDA-approved medical products which may be stockpiled for use in emergencies are referred to as medical countermeasures (MCM) and include biological products, e.g. vaccines, drugs and devices. Specifically, the EUA authority is separate and distinct from use of an investigational medical product held under an IND and must be able to treat serious or life-threatening diseases or conditions.

In contrast, EA submissions are for products used under an IND or a protocol (treatment plan), or submitted as a protocol amendment to an existing or new IND. The EA categories cover use under either an individual patient IND or protocol, individual emergency EA use, or EA for an intermediate-size population or for widespread use (large populations). Other US regulatory programmes for biologicals and drugs to treat serious or life-threatening conditions include fast-track designation, breakthrough therapy designation, accelerated approval pathway and priority review designation.

In Canada, access to drugs through special access programmes (SAPs) exists for serious and life-threatening conditions, when marketed alternative products are not available or unsuitable and evidence supports the intended use. Priority review of marketing submissions and 'notice of compliance with conditions' for such products may also be considered and granted by Health Canada.

In the European Union, the EMA supports early patient access to new medicines and which are eligible for a marketing authorization application under the centralized procedure and either target unmet medical needs or those which have a major public health interest. The EMA has launched a priority medicines (PRIME) scheme to facilitate early dialogue and support the development of medicines that target unmet medical needs and which promotes an accelerated regulatory assessment. In addition, the PRIME scheme is intended for seriously debilitating or life-threatening diseases, compassionate use of unauthorized medicines for patients with an unmet medical need (when no satisfactory treatment is available in the European Union) and also provides conditional marketing authorization.

The regulation and rules of access to compassionate use programmes varies between National government authorities across EU Member States. In the United Kingdom, the early access to medicines scheme (EAMS) gives patients with life-threatening or debilitating conditions access to innovative medicines without marketing authorization, but for which there is a clear medical need. A two-stage evaluation process initially considers promising innovative medicine (PIM) designation before an EAMS scientific opinion. Also in the United Kingdom, the potential supply of unlicensed medicinal products (Specials) may be considered by the Medicines and Healthcare Products Regulatory Agency (MHRA) for the importation of medicines that have marketing authorization status from countries outside the United Kingdom and European Union.

Prequalification by the WHO

Where there is an unmet need of priority to the WHO for a new vaccine, the WHO may establish a Blueprint programme [79]. As vaccine candidates for the specific Blueprint programme advance through the development process, and particularly when they have attached clinical data, the WHO may run a prequalification check to determine whether the candidate meets their requirements on behalf of UN agencies that will ultimately purchase vaccines [80]. To do this, WHO will engage with subject matter experts to draft and publish a target product profile (TPP) for a vaccine requirement. WHO will use its Scientific Advisory Group of Experts (SAGE) to review vaccine candidate performance against the TPP and to prequalify a vaccine candidate(s) which fulfils the TPP.

Clinical trial requirements for vaccine licensure

Phase I clinical trial

Assuming that the preclinical safety and toxicology testing has been satisfactory, a protocol for Phase I testing of the vaccine candidate may be submitted to the regulatory authorities for approval. The primary objective of the Phase I clinical trial is to test the vaccine candidate for safety in informed and consenting adult volunteers who will be selected according to pre-agreed inclusion/exclusion criteria. As this is the first time the candidate is being used in man, the Phase I trial is typically small and cautious. If a range of dose-levels is being tested, it may be necessary to dose a sentinel group of single subjects from each arm of the study to assess safety through

week 1 of immunization and starting at the lowest level before proceeding to the next level. The data from the sentinel group would be reviewed before proceeding with the main study. Then the first cohort would be dosed at the lowest level before proceeding to the next level, and so on. Volunteers will be closely monitored for adverse effects in the clinic at each dosing time-point and will typically maintain a diary at home to record any symptoms arising between time-points. An independent safety monitoring panel, including medically qualified personnel, will be required to monitor the reporting of adverse effects. Volunteers may also be blood-sampled to assess vaccine immunogenicity from baseline and serial samples, and depending on vaccine type may be required to supply additional samples which can be collected non-invasively, e.g. saliva/stool samples. All volunteers may be followed up for a pre-agreed period on completion of the study, to check for latent adverse events and to monitor, e.g. for maintenance of circulating antibody titres or memory response to the vaccine.

Phase II clinical trials

The second phase of clinical trials typically allows for an enlarged study to assess vaccine safety and to monitor immunogenicity. During this phase, significantly expanded cohorts of volunteers may be dosed with vaccine at dose-level(s) selected as optimum from the Phase I trial and can be monitored for safety and a more detailed immunogenicity assessment, which may include assays of both serological and cellular memory responses. Once again, volunteers may be blood-sampled for baseline and serial serum/plasma samples, and depending on vaccine type may be required to supply additional samples which can be collected non-invasively, e.g. whole blood/saliva/stool. In the event that a Phase III trial for efficacy is not feasible, for ethical or practical reasons, it may be necessary to plan a blood-sampling regimen in the volunteers which will enable sufficient sample volumes to be available for Animal Rule studies (discussed below).

If sufficient safety data are gained from a Phase II trial, the regulatory authorities may be able to consider the vaccine for EUA.

Phase III clinical trials

The third phase of clinical trials is typically designed to assess efficacy. However, where this is not feasible for either practical/logistical or ethical reasons, Phase III could be regarded as a further, significantly enlarged trial for vaccine safety with adequate follow-up of vaccinated volunteers. A Phase III field trial of vaccine efficacy may be feasible in the event of an anticipated seasonal outbreak, in which case vaccination could be given prophylactically and well in advance. As long as effective biosurveillance programmes are in place to instigate a rapid response to new infections in an unvaccinated placebo cohort, the latter group may be included in a prophylactic efficacy trial. Conversely, reactive mode vaccination in the context of an actual outbreak is likely to be more complicated, as it would be unethical to omit anyone at risk of infection from the vaccination programme. Hence, sufficient safety data on the candidate vaccine would be required in case of need to administer it to pregnant/nursing mothers, children and elderly people as well as to the general adult population. Additionally, it may be necessary to administer an adjunct to the vaccine, such as an appropriate antibiotic, to both cohorts (vaccinated and placebo). Whatever the context, careful consideration of the trial design needs to be made and approved by the regulators to achieve adequate statistical powering and to determine the need to provide supplementary antimicrobial therapy, and also the need for a placebo cohort.

The impact of the FDA's Animal Rule

After the anthrax letters incident in the United States in 2001 in which anthrax was released through the postal system, resulting in five fatalities and widespread anxiety [81], the FDA issued the Animal Rule (FDA 21 CFR) [82] to expedite the development of vaccines and therapies for biothreat agents for which it is neither feasible nor ethical to carry out Phase III efficacy studies in man. In this situation, the Animal Rule makes provision for the substitution of animal efficacy data for human efficacy data. The animal efficacy data should demonstrate a reasonable likelihood that the candidate vaccine or therapy would be efficacious in man [82]. The animal efficacy data should be provided ideally from more than one animal model, or from one model only if that model is well-characterized and authentically represents the human disease syndrome [82]. In addition, the FDA may require supporting data to include pharmacokinetic/pharmacodynamic (PK/PD data) and a determination of the pathophysiological mechanism of action of the biothreat agent and a 'reasonable understanding' of the protective mechanism of the proposed vaccine or therapy. Thus, if the candidate is a vaccine, a reasonable understanding of the protective mechanism(s) being invoked by it requires an identification of the immune correlates of protection.

Immune correlates of protection and surrogate markers of efficacy

Immune parameters which correlate with protection in the selected animal models may include the total titre of circulating antibody induced to the vaccine and the determination of a minimum cut-off titre required to confer

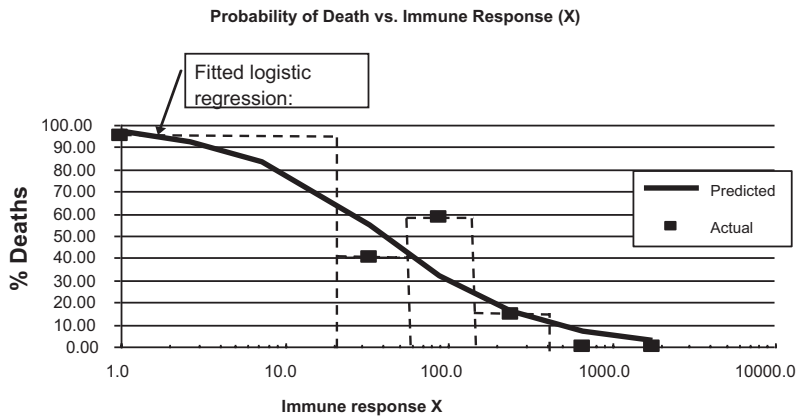

Fig. 4. Use of surrogate markers of efficacy to predict vaccine efficacy.

protection. Alternatively, the titre of functional or neutralizing antibody within that total may provide the correlate, particularly where the neutralization of specific virulence factors produced by the biothreat agent is identified and quantifiable [83]. For some vaccines, the induction of a cellular memory response instead of, or in addition to, circulating antibody will provide the correlate. This is measurable by the *ex-vivo* recall response of peripheral blood mononuclear cells (PBMCs) on stimulation with the vaccine antigen(s) [83].

In the absence of direct efficacy testing in the human vaccinee, the immune correlate identified above provides a surrogate marker of efficacy. If this is a functional antibody, the titre induced in man needs to be compared with the titre required in the animal model(s) to provide protection. This can be achieved, for example, by the *in-vitro* neutralization of a specific virulence factor, or by a competitive enzyme-linked immunosorbent assay (ELISA), where the test antibody is competed with a known neutralizing antibody for binding to the target antigen or by the passive transfer of antibody from a human vaccinee into a naive animal, followed by pathogen challenge [83].

Whatever the immune correlate may be, its effect would be expected to follow the pattern shown in Fig. 4, where as the value increases, the likelihood of death in the vaccinee decreases [83]. Thus, vaccine efficacy = relative reduction in risk of death $= 1 - \frac{(\%\text{ of vaccinated subjects at} < \text{protective level})}{(\%\text{ of unvaccinated subjects at} < \text{protective level})}$.

Although a number of immunotherapies and pretreatments have been licensed under the Animal Rule, the first vaccine to be licensed in these circumstances is Biothrax for therapeutic vaccination in suspected exposure to anthrax [84].

Conclusions

Much progress has been made on the collective understanding of emerging biothreats, but much more needs to be done to bring vaccines into clinical use to protect vulnerable people in endemic areas, and to prevent the global spread of emerging pathogens. The scale of the task is large, but with new understanding of the hazards that these emerging pathogens present and the co-operation of the scientific community in global partnerships, together with manufacturing, governmental and NGO support and the engagement of the regulators, much can be achieved.

Disclosures

The authors declare no competing interests.

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