Options of innovative vaccine approval without human efficacy data

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Dr. Nhung Do

aus Hanoi

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Betreuerin und 1. Referentin: Dr. Bettina Klug

Zweite Referentin: Dr. Birka Lehmann

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Abbreviations

9vHPV vaccine	Nine-valent Human Papillomavirus Vaccine
Art.	Article
API	Active Pharmaceutical Ingredient
AUC	Area Under the Curve
B. anthracis	*Bacillus anthracis*
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHMI	Controlled Human Malaria infection
CHMP	Committee for Medicinal Products for Human Use
CIN2+	Cervical Intraepithelial Neoplasia grade 2+
C_{max}	maximum serum Concentration
CP	Centralized Procedure
CTD	Common Technical Document
CBER	Center of Biologics Evaluation and Research
DCP	Decentralized Procedure
e.g.	“exempli gratia”, for example
et al.	“et alii”, and others
EMA	European Medicines Agency
EUND	Extraordinary Use New Drugs
FDA	Food and Drug Administration
FD&C Act	Federal Food, Drug, and Cosmetic Act
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
HCT	Human Challenge Trial
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonisation
ICP	Immunological Correlate of Protection
i.e.	“id est”, that is
MAA	Marketing Authorisation Application
MenB	Meningococcal disease group B
MRP	Mutual Recognition Procedure
N. meningitidis	*Neisseria meningitidis*
PEI	Paul-Ehrlich-Institut
P. falciparum	*Plasmodium falciparum*

PK	Pharmacokinetic
POC	Proof-of-Concept
qHPV vaccine	Quadrivalent Human Papillomavirus vaccine
RCT	Randomized Clinical Trial
RR	Relative Risk
SBA	Serum Bactericidal Antibody
SmPC	Summary of Product Characteristic
TNA NF_{50}	Toxin-neutralizing antibody at 50 % level of neutralization
USA	United States of America
VE	Vaccine Efficacy
VRBPAC	Vaccines and Related Biological Product Advisory Committee
WHO	World Health Organization

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1. Challenges in the development of vaccines

Vaccines are an important part of the healthcare systems worldwide. Their benefit to public health lies in the elimination or reduction of infectious diseases, which are often severe and lead to death, obstruction or chronic illness. The general principle of vaccination is to induce the human's immune system by application of alive, attenuated microorganisms, inactivated pathogens or conjugates with relevant factors, antigens or sequences of the pathogen's genome. This allows building of an immunological memory (i.e. antibody against a specific disease). In case of a real infection, the organism is prepared and can handle with the pathogen faster and more effectively.[1]

Vaccination and vaccine development is well established. Exposure of naïve persons with secrets from smallpox-infected persons was the first vaccination attempt performed in Asia long time before Edward Jenner systematically investigated the vaccination process in 1796. He treated an eight-year-old boy with smallpox secrets from a human infected by cow smallpox and observed the reaction. As expected, after initial outbreak of the disease, the boy was protected against any other smallpox infection.[2] Due to the dangerous and lethal property, the WHO has established an eradication programme to systematically identify and eradicate smallpox by vaccination and information of the population. Since 1980, smallpox is declared as eradicated by the WHO after the last case was observed in 1977.[3]

Long experience in vaccine development combined with established adjuvants and similar route of administration (mostly i.m.) render pharmacokinetic (PK) studies in most cases unnecessary and reduce efforts and costs in vaccine development.[4] The use of adjuvants can increase vaccine efficacy by enhancing, accelerating and/or prolonging immune responses.[5] Nonetheless, interaction between adjuvant and the vaccines' active ingredient must be carefully investigated as this can cause unpredictable changes in the effect or adverse events.

Pharmacodynamic investigations are mainly focused on the understanding of the vaccine's induced immunogenicity. These studies evaluate the immune response caused by the vaccine such as the evaluation of antibody class, amount and function, specificity and cross-reactivity of the immune reaction.[6] Data collection of immunogenicity is performed within all stages of vaccine development, from the first initial trials to large protective efficacy trials, up to the constant surveillance in the post-authorisation phase. The identification of a relationship between immunogenicity and efficacy is crucial and allows the establishment of the laboratory parameter Immunological Correlate of Protection (ICP). ICP is "a type and amount of immunological response that correlates with vaccine-induced protection against a clinically

apparent infectious disease and that is considered predictive of clinical efficacy".[6] An ICP can be identified from investigations in clinical efficacy trials. The development of specific antibodies after vaccination can be quantified and correlated with the protection against the disease. Hereby, differences between population and immune status of the trial subjects (naïve or not) need to be considered. ICPs may also be derived from animal studies. In this case, the correlation to human data needs to carefully consider the possible differences between both species to avoid misleading results.[6] Overall, the availability of ICPs facilitate the analysis of efficacy and the immune response caused by a vaccine. An established ICP can correlate antibody development with immune response (e.g. hepatitis B, diphtheria, tetanus) so that the performance of efficacy trials is not essentially required.[6] Otherwise, protective efficacy needs to be determined in human efficacy trials.

The difference between vaccines and other standard development drugs is that clinical trial subjects are in most cases healthy. The prime aim is not to cure an existing disease but to protect the subjects from it (preventative vaccination). There are also attempts in the development of therapeutic vaccines (e.g. cancer vaccines[7]), which are not in the scope of this thesis. Similar to other drugs, clinical trials for vaccines can be categorized in three phases:[6]

Phase 1 trials evaluate safety, possible adverse effects and immune response of the vaccine candidate in a small number of volunteers (< 50 trial subjects, healthy adults). These studies First dose-ranging studies are conducted to identify the correct dose or dose range. Furthermore, the effect of the adjuvant component is tested.

In **phase 2** trials safety and immunogenicity are investigated on the target population with hundreds of children or adults. In dose/formulation finding studies the final formulation for the next phase 3 investigations is further defined by the identification of the correct dose of the active substance as well as type and dose of the adjuvant. Additionally, safety, adverse events and probably first efficacy are evaluated in phase 2 trials. If possible, the trial design should be a randomized clinical trial (RCT). Essential information for the phase 3 trials can be achieved at this stage: endpoints and case definition, control of study centers, study design and potential need for improvement.

Phase 3 clinical trials are performed in 1,000 up to more than 10,000 trial subjects in the RCT trial design. Large pivotal trials on the target population should provide evidence of efficacy and safety and are essential for licensure. A validated manufacturing and release of the investigational medicinal product, similar to the later commercial vaccine, is required. The innovative vaccine is tested on the healthy target population acknowledging that not all subjects will be infected since disease incidence can only be predicted by public health data. Therefore, clinical trials need to be performed on a larger number of subjects to achieve statistical robust

results. The number of trial subjects is chosen according to the estimated attack rate (incidence of disease). High attack rate means that the generated efficacy data of the study population are robust and less study subjects are required, while for diseases with low attack rate increase in study subjects is probably required.[8]

Performance of protective efficacy trials allows the calculation of the vaccine efficacy (VE), which is determined by the observation of incidences between the vaccine group and control group (placebo):[9]

$$VE = (1 - RR) \cdot 100\%$$

$$RR = \frac{Relative\ risk\ Group_{vaccinated}}{Relative\ risk\ Group_{unvaccinated}}$$

A result of 0% is obtained for vaccines with no protective efficacy, while 100% means complete protection. Established and approved vaccines demonstrate high efficacy rates (e.g. >90% after 2 doses for varicella, rubella, mumps, measles).[10] However, no concrete threshold of the required minimum vaccine efficacy is given in any guideline. The final regulatory outcome depends on the results provided by the sponsor (vaccine efficacy, safety and quality), the overall situation of public health and disease severity or alternative treatment options.

Clinical trials represent an important part in the development of vaccines (Figure 1) and their results are essential for the evaluation of the benefit-risk ratio for licensure. Regional genetic differences, microorganism specialty and frequency of disease (rare vs. epidemic outbreaks) require flexibility from the pharmaceutical industry and regulatory agency.

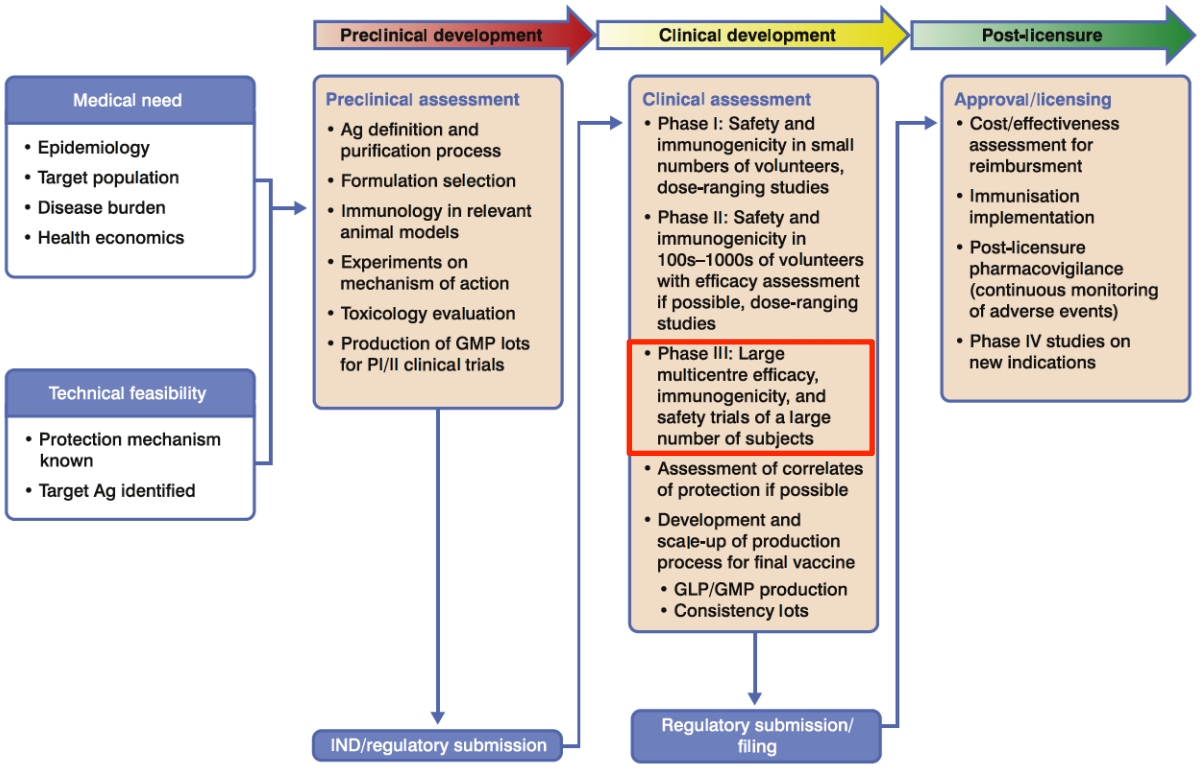

Figure 1: Steps of vaccine development modified after Leroux et al.[11]

2. Alternative ways to obtain evidence in support of vaccine efficacy and/or to an Immunological Correlate of Protection

The evidence of efficacy is not always feasible in cases the disease does not occur, occurs too unpredictable and short for the performance of an adequate efficacy trial. The WHO has recognized this challenge for sponsors and provides alternative options for the estimation of efficacy with little or no human data in the "Guideline on clinical evaluation of vaccines: regulatory expectations".[6]

The choice of these methods depends on the nature of disease and vaccine. Scientific advice with Regulatory Authorities is recommended. The collection of all relevant and available data and a combination of methods often provides the basis of vaccine approval. In the following, possible methods for the replacement or reduction of efficacy trials will be evaluated more closely.

2.1. Nonclinical efficacy studies

Nonclinical studies may be conducted *in vitro* (e.g. cell lines, cell structures, serum) or *in vivo* (i.e. animal studies) and are generally performed prior to the first use in man. They are used to characterise the vaccine and verify the manufacturing process (e.g. by determination of the vaccine's lot-to-lot consistency and potency). *In vivo* studies on animals support the understanding about the vaccine's immunogenicity. They are essential for the proof-of-concept (POC) and for the identification of the optimal dose and dose schedules in clinical trials. Furthermore, determination of toxicity and safety of the vaccine is evaluated by repeated dose toxicity studies on animals.[12]

Several guidelines agree with the licensure of vaccines based on nonclinical efficacy studies, in cases where human efficacy trials are not possible or feasible.[4,6,13,14] The replacement of human efficacy trials with animal data is critical since the differences in both species play a major role and can influence data outcome to a high extent. Deep understanding about the vaccine's immunogenicity and the physiological difference between human and animal species are prerequisites. The choice of an animal model is most important. An adequate animal model should reflect a human immune response as similar as possible. Identification of immune markers for the bridging of animal to human data is essential. Both should be discussed with the regulatory agency in advance.[13]

The Food and Drug Administration (FDA) offers a program for the qualification of animal models, which is exclusively meant for the Animal Rule. The Animal Rule is an approval pathway for drugs (vaccines included) in the USA, which enables marketing authorisation on basis of animal efficacy data (refer to section 3.1). A qualified animal model is "a specific combination of an animal species, challenge agent and route of exposure that produces a disease process or pathological condition that in multiple important aspects corresponds to the human disease or condition of interest".[15] Since a qualified animal model is not dependent on a specific developmental vaccine, it allows comparability of results from independent investigations of different institutions and can be used in multi drug (vaccines included) development programs. Investigations for other active substances can refer to the qualified animal model without the need for a repeated assessment by the FDA. However, the FDA has not published any qualified animal model up to now.

Since animal efficacy trials are exceptions for the evidence of efficacy, there is no standardized guideline for the performance. In regions, where Good Laboratory Practice (GLP) is applicable, the studies should be performed following GLP requirements to allow reproducible data of high quality and integrity. Pilot studies and POC studies support understanding and design for the intended animal efficacy studies. In the USA, guidance on animal efficacy studies is given by the FDA in the Guidance for the submission on basis of the Animal Rule. Here, the following conditions are requested:[13]

- The route of administration should be the same as intended for the use in human.
- The evidence of efficacy should not only be based on surrogate endpoints, but have a connection to clinical benefit.
- The correlation between challenged animal model and the targeted human disease should be significant.
- Dose selection is always a critical parameter and should be closely evaluated. For vaccine development, it is important that the chosen dose for animal studies causes the same immune response to human.
- A relationship to the intended human population group should be reflected in the choice of animal sex, immune status and age.
- In case supportive care is required, this needs to be conducted with the placebo arm.
- The study design of animal efficacy studies should be randomized and blinded. As there is normally no non-inferiority study possible, the studies can be placebo-controlled. Hereby, the placebo group should be also compared to natural history studies to inhibit false data from artificial situations.

Similar to human clinical trials, a study plan needs to be established, in which an overview about the aim and performance of the study is given and special points to consider are addressed in advance (e.g. inclusion and exclusion criteria, controls, endpoints, male/female subjects). Table 1 gives an overview about further relevant elements to consider for animal efficacy trials.

Overall, animal efficacy trials may represent possible alternatives in cases human clinical trials are not feasible and when the comparability between both species is possible. The challenge lies in the transfer (bridge) of immune response from animal to human for the correct interpretation of results. Therefore, immune response data should collected whenever possible. The existence of a qualified animal model can support comparability between different vaccines or studies from independent sponsors and increases data integrity. Additional requirements or conditions depend on the investigational vaccine and need to be discussed with the regulatory authority.

Animal efficacy studies have been used to study protective efficacy of vaccines against anthrax disease caused by *Bacillus anthracis* for licensure in the USA. Anthrax is spread worldwide, but does not occur very often in industrial regions. The risk is increased with close contact to animal products such as bones and skins. *B. anthracis* produces highly toxic and very persistent spores, which often results in death and hence was applied as feared and dangerous bioweapon in recent times (e.g. in 2001 as contaminated letters in the USA).[16,17] There is currently no established ICP for anthrax disease in place. Due to the rarity of disease and the toxic nature of the bacterial spores human efficacy studies are not feasible and human challenge trials would be unethical. Hence, two pivotal animal studies from rabbit and monkey have been performed. The animals have been vaccinated and then challenged with aerosolized spores from *B. anthracis.* A 70% probability of survival for rabbits and monkeys was observed, which was sufficient for approval of the application.[18]

The complexity for the determination of efficacy with animal studies is increased for vaccines, which are used in combination with other drugs or vaccines. The influence of the combination on immune response and the superiority of the combination need to be investigated. As for anthrax disease, superiority of a combined treatment from vaccines and antibiotics could be shown in rabbits. The combined treatment results in a survival rate of 70-100% (compared to 44% and 23% from two studies with rabbits with single antibiotic treatment).[18] An additional randomized, open-label, multi-center phase 2 study with 154 human study subjects investigated the combination of anthrax vaccine with ciprofloxacin. The influence on PK was evaluated by the determination of the ciprofloxacin plasma concentration in human serum. The PK specific parameters outlining the maximum plasma concentration (C_{max}) and area under the curve

(AUC) within 12 hours were comparable to the ciprofloxacin treatment prior to vaccination. The immune response induced by vaccination was not modulated by co-treatment with ciprofloxacin.[19]

Table 1: Checklist of elements of an adequate and well-controlled animal efficacy study protocol.[13]

PROTOCOL CONSIDERATIONS		
• Indication to Be Studied		
• Agency Concurrence on the Details of the Animal Model		
• Comparability of the Study Design to the Clinical Scenario		
STUDY DESIGN ELEMENTS	**Described**	**Justified**
• Controls		
• Size of Study Groups and Male/Female Composition of Groups		
• Animal Characteristics (†) (e.g., species, age, weight, source of animals)		
• Inclusion and Exclusion Criteria for Acceptance Into Study		
• Dose, Route of Exposure, and Preparation of the Challenge Agent		
• Trigger for Intervention		
• Dose, Regimen, and Route of Administration of the Investigational Drug		
• Randomization		
• Blinding		
• Statistical Plan		
• Endpoints		
• Euthanasia Criteria		
• Observation Frequency and Schedule		
• Animal Care Interventions		
• Plan for Ensuring the Quality and Integrity of the Data		
(†) See section IV.D for further description.		

Although smallpox has been eradicated since 1977, the availability of vaccines for the preparedness of an unexpected incidence is especially essential for people with high risk (attenuated immune system). Due to the eradication, no wild type virus causing smallpox is available and protective efficacy trials are not possible. Smallpox is caused by variola viruses, which belong to the family of orthopoxviruses.[3] Protective efficacy of smallpox must therefore be estimated from appropriate studies in animals. Therefore, a specific test design needs to be established to surrogate the infection with smallpox in animals.[20] For a marketing authorisation in Europe, mice and monkeys have been challenged by viruses from the orthopoxfamily with or without vaccination, which allowed a correlation of immune response in animals by the use of animal efficacy studies.[21]

2.2. Human Challenge Trials

In Human challenge trials (HCTs), vaccinated study "subjects are deliberately exposed to an infectious agent in a controlled setting".[6] HCTs are only permitted if a successful treatment is available and when they are ethically justified.[4]

Challenge trials are not required in the dossier of a marketing authorisation application (MAA) for a new vaccine. However, they may be useful to support approval, when standard efficacy trials are not possible or feasible. HCTs give direct evidence to human reaction. With only little subjects involved, data for vaccine protective efficacy can be directly estimated and in some cases a relevant ICP can be detected. For the conduct of HCTs, the relevant regional requirements should be followed. In the USA, challenge trials fall under Investigational New Drug (IND) application while in Europe, the challenge stock is considered to be a medicinal product and needs to fulfill quality requirements and the requirements set forth in the Clinical Trial Directive.[22,23] Further ethical issues need to be clarified with the respective ethics committee. Performance under good clinical practice (GCP) is favorable and especially essential for studies, which should be used for a MAA.[24]

Beside their value for situations where efficacy trials are not possible or feasible, HCTs can support many areas of vaccine development:

- In the initial stages of vaccine development, HCTs play a major role in the understanding of the vaccine's mode of action (POC) and its behavior in man. These investigations allow to identify unexpected adverse events or to confirm expected immunological reactions. Especially in cases where animal data do not offer sufficient and reliable results for an extrapolation to human, or when there is no human like clinical response

from animal investigations, HCTs provide direct measurable effects without the need for bridging data from animal investigations.

- The optimal dosage, dose range or immunization schedule for further clinical trials can be identified with the help of HCTs.
- HCTs may be used as bridging trials between different populations (e.g. age groups, nationality) and reduce the need for additional clinical trials.
- Selection of adjuvants and other modifications in the formulation.
- Pre-selection between different developmental vaccines: HCTs can belong to novel approaches in the reduction of pivotal efficacy trials. Prior to the investigation of large and cost intensive efficacy trials, HCTs may be used to screen between different investigational vaccines for the selection of the most promising candidates, avoiding unnecessary cost intensive investigations in advance.[24]

In some situations, HCTs may also be a first-in-man clinical trial. Investigations at this stage of development are very challenging and should follow strict requirements as set out in the relevant guidelines or authority advices. The Committee for Medicinal Products for Human Use (CHMP) has just revised a guideline on "strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products".[25] This guideline will come into force in February 2018 and is valid for small molecules as well as biotechnological substances. It provides special points to consider when first studies in human and early clinical trials are planned. Especially the choice of the starting dose, order and sequence of subject involvement and strategies on risk identification and risk management need to be carefully studied. As mentioned earlier, vaccine development differs from the development of standard investigational drugs. Vaccine-specific parameters such as antigen, adjuvant, provoked immune response, target population, initial situation of the immunity of the trial subject (naïve or not) and manufacturing process should be considered to detect and handle possible expected and non-expected adverse events (Figure 2).

In the planning phase for HCTs, caution should be set on the probability of severe volunteer's infection with the agent, which cannot be completely excluded. Volunteers must be aware of the risks and give informed consent to be included in HCTs. Especially investigations of first-in-man studies are not predictable and bear a high risk for unexpected events. In some cases, quick action upon emergency cases is required. The need and availability of special clinical facilities that can provide continuous monitoring, suitable medical care and collection and decontamination of waste should be evaluated on a case-by-case basis.[24]

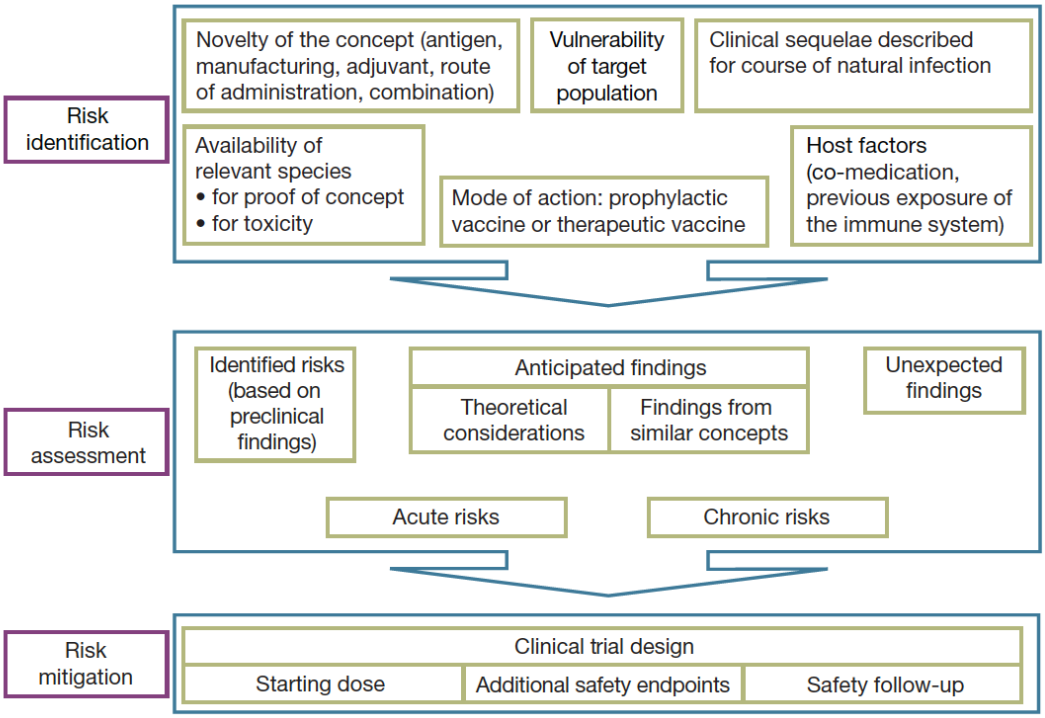

Figure 2: Risk assessment for a vaccine intended for first-in-human administration.[26]

The choice of the challenge agent for HCTs is driven by ethical and safety considerations and may require a modification if the wild type is too dangerous. This allows most possible safety measures for the volunteers and a controlled study design. On the other hand, modification of pathogens comes along with reduced comparability to the real disease, making HCTs less reliable. Since the use of an adequate pathogen model is most important for the generation and interpretation of correct and reproducible data, well-established challenge models are required to allow comparability between studies and vaccines/vaccine formulations.

The Controlled Human Malaria infection (CHMI) model has been established as a suitable standard with proven reproducibility and safety, recognized by regulatory authorities for the investigations on malaria. Malaria is an infection caused by parasitic protozoans through different live cycles and two hosts. In short, the live cycle of the *Plasmodium* can be described as follows[27]: The female Anopheles mosquito hosts sporozoites, which is the infective form of *Plasmodium* (mosquito stage, A). By the bite of the malaria-infected mosquito, sporozoites are transmitted into the human, the second host. They arrive at the liver cells by transport through blood vessels. Here, they develop to merozoites, which are released by the rupture of the liver cell (liver stage, B). Merozoites invade human red blood cells and multiply or some merozoites develop to gametocytes (blood stage, C). Gametocytes can be taken up by the mosquito, in

which they fertilize and transform to sporozoites (mosquito stage, A) and can infect other hosts. For the development of a malaria vaccine these stages may be individually targeted (Figure 3), which is discussed elsewhere in more detail.[28] With help of the CHMI model safety and efficacy of malaria vaccines can be investigated. Vaccinated and unvaccinated volunteers are challenged with sporozoites of *Plasmodium falciparum* by the bite of infected female Anopheles mosquitos in a controlled study setting.[29] After the controlled challenge of the trial subjects, adverse events of the vaccine or symptoms of the disease (e.g. fever, headaches, nausea) can be directly investigated. Having an established challenge model at hand, the generated data are reproducible and comparability between different research centers and different innovative vaccines is possible.

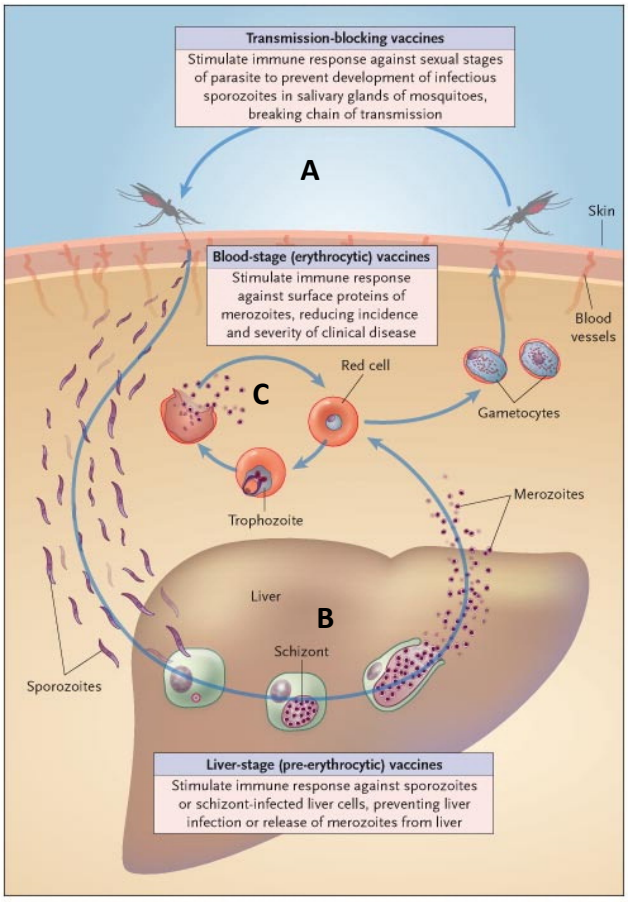

Figure 3: Life cycle stages of malaria and potential malaria vaccine targets.[30]

Data from HCTs are generally accepted for the POC, but in general not for the evidence of efficacy by regulatory authorities as the generated results are based on limited subjects (mostly healthy adults) without reflection of the diversity in population, age groups and/or nationalities. In addition, the challenge models are often modified to reduce severity of symptoms.

2.3. Passive Protection Studies

Following the exposure to a pathogen, the healthy immune system produces antibodies (i.e. immunoglobulin with unique protein surfaces) to protect the human or animal for the next infection. Passive protection is the protection of the individual by the administration of antibodies against the pathogen. Protection of newborns by antibodies from the mother's placenta or by breast feeding represents the natural form of passive protection.[6] Studies on passive protection aims to identify and investigate the level of protection from antibodies (i.e. specific immunoglobulins), which are produced as immune response against a specific disease. After administration of serum of immune humans or animals or biotechnologically manufactured specific antibodies to naïve animals, the animals are challenged with the infective agent and the protection level of the antibody can be determined.[6,31] Passive protection studies therefore support understanding of the mechanism of action by identification of specific immune factors relevant for the disease.

Especially in cases when protective efficacy trials are not feasible, passive protection studies may be a possible alternative to achieve an immunological marker of protection.[6] In fact, they allow quantification of the level of protection i.e. identification of the required amount of antibody for a minimum level of protection against a defined amount of infectious agent. Thus, they can support the preparation of clinical trials in finding of the correct dose and identification of the required time for the start and duration of protection after application of the vaccine.[31]

The Institute of Medicine recommended the use of passive protection studies in the US Research program of the Centers for Disease Control and Prevention (CDC) for the investigations on efficacy of anthrax vaccine. Passive protection should be performed on nonhuman primates with different challenge doses of anthrax spores to identify the optimal antibody level for anthrax's vaccine. The use of passive protection studies should increase the knowledge about data correlation between animal and human for a correct interpretation of animal results. This is especially important for anthrax disease, as animal efficacy studies are the basis for the evidence of protective efficacy.[31]

A passive protection model has been used in a marketing authorisation application of a vaccine against meningococcal disease group B (MenB). Meningococcal disease is caused by *Neisseria meningitidis*, from which 12 serogroups are known. The incidence of meningococcal disease is decreasing and mainly *N. meningitidis* from serogroup B and C are predominant in the EU and USA.[32] For the description of primary pharmacodynamics, antisera from immune mouse have been applied to animals, which have been challenged with *N. meningitidis* demonstrating protection of the animals against development of the disease.[33]

2.4. Comparison of Immune Response

The immune response after vaccination is an important parameter for the understanding of the vaccine's immunogenicity and the description of its efficacy. Similar vaccines can stimulate comparable immune responses of one or more antigens. This instance can be used for the correlation and interpretation of data between two vaccines. The immune response from similar approved vaccines, where efficacy have been demonstrated, can be compared and a protective efficacy can be estimated for the innovative vaccine.[6]

This strategy has been used for the evidence of efficacy of the active immunization against MenB. Instead of protective efficacy trials, which have been considered to be not feasible, antibody formation against bactericidal structures (immune response) has been measured as surrogate parameter. Serum bactericidal antibody (SBA) titre has been determined in trial subjects after vaccination and correlated to those of validated reference SBA strains of MenB. A threshold of SBA titre ≥ 1:4 was defined on basis of literature data and former investigations on meningococcal disease. If the correlated titre precedes the threshold, clinical efficacy can be suggested.[33,34]

Comparison of immune response is also used for the interpretation of clinical bridging studies. With the help of bridging studies, new age or population groups can be introduced. Furthermore, these studies support changes in manufacturing, formulation or composition of adjuvants/excipients in the development stage or during lifecycle of a vaccine, if analytical comparability is not sufficient. Sometimes additional information in dosing or dose schedule is required, which can be addressed by the comparison of the immune responses in special trial settings. The basis for bridging of data is the availability of at least one efficacy clinical trial. The efficacy from this trial is bridged by comparison of the immune response to investigations in the new setting (non-inferiority trial). In non-inferiority trials, the new aspects of interest (e.g. population, age group) are directly compared with already established aspects from previous trials and evaluated following their relevant endpoints or other parameters of the immune response (e.g. ICP, antibody concentration).[6]

Successful immunobridging has been performed for vaccines against human papilloma virus (HPV). Infection with human papilloma virus (HPV) can be sexually transmitted and is recognised as the main cause for the development of cervical cancer. Different genotypes of HPV exist, which have distinct impact on cancer development. HPV-16 and HPV-18 are highly oncogenic and often detected in cervical cancer. About 70% of cervical cancer are caused by either HPV-16 or HPV-18 in Germany.[35] The activity of a vaccine containing both genotypes as biotechnical produced antigen bulks (Cervarix®) was determined in one main efficacy trial with

19,000 females aged 15-25 years.[36] Cervical leasions, especially of type Cervical Intraepithelial Neoplasia (CIN) grade 2+ (moderate to high-grade dysplasia), are associated with HPV-16 or HPV-18 infection. The detection of CIN2+ after vaccination was used for the determination of protective efficacy resulting in 100% efficacy against CIN2+ associated HPV-16 and HPV-18 For the evidence of efficacy in another age group, two bridging studies with 158 and 2,067 clinical subjects of the age groups 9-14 years have been included and compared with the 15-25 years group. This clinical bridge confirmed non-inferiority to the already proven efficacy in 15-25 years old females. The geometric mean titer (GMT) antibody was used as surrogate parameter for demonstration of the immune response, which could be confirmed with a more than 2-fold increased GMT in the group 9-14 years compared to 15-25 years, so that the final indication could include females from 9 years of age.[36] A 9-valent human papillomavirus vaccine (9vHPV, Gardasil®9) protects against HPV subtypes 6, 11, 16, 18, 31, 33, 45, 52 and 58. One protective efficacy trial was performed with 12,033 clinical subjects against subtypes 31, 33, 45, 52 and 58 (96.7% protective efficacy). Thereafter, additional studies have been performed to bridge between different age groups, between males and females and between another approved quadrivalent HPV (qHPV) vaccine and 9vHPV vaccine to provide proof-of-efficacy by non-inferiority of the results.[37] Both cases for HPV vaccination demonstrated that the use of immunological bridging could reduce clinical trials and the amount of trial subjects.

Comparison of the immune responses is an important tool for the interpretation and correlation of data. New marketing authorisations as well as extensions can be achieved with no or a limited clinical trial setting.

3. Pathways for vaccine approval without efficacy trials

In the European Union, medicinal products (vaccines included) can be approved centrally or nationally. The European Medicines Agency (EMA) is responsible for the centralized procedure (CP), which is a single marketing authorisation, valid in all member states of the EU. Specifically, the CHMP as part of the EMA reviews the application and provides an opinion, on which the decision of the European Commission is based.[38] National licenses can follow the procedures Mutual Recognition Procedure (MRP), Decentralized Procedure (DCP) or purely national marketing authorisation.[39] Here, country-specific differences may be possible due to implementation of the directive into national law of the respective countries where country specific requirements and differences may exist.

In the USA, the FDA grants approval for new medicinal products. The CBER (Center of Biologics Evaluation and Research), which is a complex within the FDA, is responsible for biological products and reviews applications for new vaccines with the involvement of the external committee, Vaccines and Related Biological Product Advisory Committee (VRBPAC), for independent advice.[40,41]

The following sections focuses on procedural approval options for vaccines in the EU (centralized procedure) and USA with no or only limited human efficacy data. Hereby, the situations of emergency use and the available standard approval procedures will be mentioned only briefly as they are beyond the scope of this thesis.

3.1. Europe

In Europe, the CHMP Guideline on clinical evaluation of new vaccines proposes the use of animal data, use of ICP and measurement of functional immune responses as examples for possible alternative approaches when human efficacy trials are not feasible.[4] As published in a concept paper on 15th May 2017, this guideline is currently under revision and should be updated with experience from already assessed vaccines and their outcomes in scientific advice procedures. Herein, more details to the evidence of efficacy with the use of alternative methods can be expected.[42] A technical dossier may be filed on the legal basis of Art. 8(3) of Directive 2001/83/EC for a mixed application. Herein, new data can be combined with literature data including correlation of data from other vaccines, or surrogate parameters.[39]

The CP is mandatory for orphan medicines, advanced therapy medicines and new active substances for the treatment of specific diseases as described in detail in Art. 3(1) and point 1

of Annex of Regulation (EC) No 726/2004.[43] Optionally, medicinal products with new active substances or "significant therapeutic, scientific or technical innovation or (…) in the interests of patients or animal health at Community level" (Art. 3(2), (EC) No 726/2004) can be approved via CP.[43] Thus, new vaccines, vaccines of community interest such as influenza vaccines and vaccines containing recombinant antigens are applicable for CP.

The Conditional Approval and the Approval under exceptional circumstances are the current possible options to grant marketing authorisation for vaccines with limited human clinical efficacy data in the EU.

The **Conditional Approval** as per Article 14(7) of Regulation (EC) No 726/2004 applies to drugs that address an unmet medical need (i.e. treatment of a life-threatening or serious disease, emergency situations or orphan drug) and the product must fulfill all of the following requirements:[43]

- Positive benefit-risk balance
- Address unmet medical need
- The sponsor may be able to provide comprehensive data post-approval
- The immediate availability of the drug gives a benefit for public health

The marketing authorisation is valid for one year and can be based on limited clinical data. Annual renewal and the possibility to transform to a standard marketing authorisation, in case all comprehensive data are available, are the characteristics of a conditional approval.[43,44] The conditional approval may be additionally combined with Accelerated assessment, in which the time for review of a MAA is reduced to 150 days compared to the standard review time of 210 days. This applies to therapeutic innovations that address an unmet medical need and hence are of interest to public health (Art. 14.9, Regulation (EC) 726/2004).[38]

If there is no possibility to complete missing safety or efficacy data post-approval, the submission pathway of **Approval under exceptional circumstances** can be followed (Art. 14(8) of Regulation (EC) No 726/2004).[38] Reasons may be the rarity of disease or that the needed investigations are not possible or ethical. Due to this fact, transformation to a standard marketing authorisation is not likely possible. The granted marketing authorisation is to be renewed after 5 years and thereafter remains valid. Nonetheless, the sponsor needs to assess the benefit-risk-ratio annually.

The vaccine Imvanex® has been approved on 31st July, 2013 by the EMA. It is indicated for the "Active immunisation against smallpox infection and disease in persons 18 years of age and

older".[21] The applicant was unable to provide all efficacy data and applied for approval under exceptional circumstances with the following reasons:[21]

- Rarity of disease because smallpox is eradicated since 1977.
- The conduct of human challenge trials is considered to be unethical and not feasible.

This vaccine is especially important for patients with a weakened immune system (e.g. HIV infection, Atopic Dermatitis) because the modified vaccine virus is not able to replicate in human. Animal efficacy studies have been performed to support the MAA. However, protection could not be predicted based on the measurement of human antibody response alone. Although human efficacy data are not available and even nonclinical investigations do not completely provide all comprehensive data, a good safety profile investigated in several clinical trials in a total of 3,066 human subjects combined with the need for persons at high risk were sufficient reasons for the CHMP to grant marketing authorisation approval under exceptional circumstances. Nonetheless, the CHMP requested additional post-approval Phase 3 studies to confirm immunogenicity in comparison with placebo or comparator products.[21]

A standard MAA as per Art. 3(2) of Regulation (EC) No 726/2004 without conditions is possible in cases complete comprehensive information are available and presented[38]. Although no protective efficacy trials have been performed the evidence of protective efficacy could be sufficiently proven without any obligations. This was done for the vaccines Bexsero® and Trumenba®. Protective efficacy was based on the surrogate parameter SBA, which was correlated to the immune response (as described in section 2.4). Both vaccines received marketing authorisation in the EU via CP, in 2013 and 2017, respectively.[33,45]

3.2. USA

Recognizing the need for guidance and encouraging innovation for the treatment of life-threatening diseases, the FDA provides an extra approval pathway on basis of nonclinical data (i.e. Animal Rule). The **Animal Rule,** which has been added to the Federal Food, Drug, and Cosmetic Act (FD&C Act) in May 2002 as a subpart I to part 314 (21CFR314) for drugs and a subpart H to part 601 (21CFR 601) for biological products, enables drug approval on basis of nonclinical data.[46] With this approval pathway, the FDA encourages pharmaceutical industries in the development of drugs and vaccines when human efficacy studies are not feasible. The basis of drug approval is well-controlled efficacy studies on animals following strict regulations, when the following conditions are fulfilled (21CFR314, 21CFR601):

- The drug prevents or reduces a serious or life threatening disease.
- Human efficacy trials are not feasible or ethical.

This rule is not applicable if the innovative substance can be approved on standard pathways (traditional or accelerated approval).

A complete waiver of human efficacy trials is critical and requires detailed knowledge about the vaccine's immunogenic properties. However, the FDA agrees that substantial evidence of effectiveness is achieved, when:[13]

- the positive effect is shown in more than one animal species or in an animal species that is well-characterised to predict a similar reaction to human.
- there is a correlation between endpoint of an animal study and the benefit to human (enhanced survival, reduction or prevention of morbidity).
- the effective dose for the use in human can be selected by available pharmacokinetics and pharmacodynamics data.

Besides, the FDA will review all other available data such as data from humans or *in vitro* studies, too. Importantly, human safety studies are excluded from this exception and need to fulfill the standard requirements for drug development. According to the Animal Rule, the FDA can oblige the sponsor to perform additional post-marketing studies to prove safety, clinical benefit and further provisions for a restricted use or distribution of the drug or vaccine. In any cases, the patient needs to be informed (package leaflet) that the drug or vaccine is approved on basis of animal efficacy trials (21CFR314, 21CFR601).[13]

BioThrax® is the first vaccine, which has been approved by the FDA via the Animal Rule. The initial approval in 1970 was traditional supported by randomized field trials performed in 1962 for the evidence of efficacy against *B. anthracis* indicated as "Pre-exposure prophylaxis of disease in persons at high risk of exposure".[19] On 23rd November 2015, the second indication was approved via Animal Rule for "Post-exposure prophylaxis of disease following suspected or confirmed *Bacillus anthracis* exposure, when administered in conjunction with recommended antibacterial drugs".[19] As mentioned earlier, the lethal disease does only occur in seldom circumstances or as happened in earlier times, in form of bioweapon in letters. Hence, the availability of this vaccine is of public health's interest to react in emergency cases and the conditions for marketing authorisation on basis of the Animal Rule were fulfilled. As per Animal Rule, animal studies replace human efficacy trials (see section 2.1). The FDA waived the need for additional use restrictions and exempted the need for pediatric investigations as anthrax has an orphan drug designation. However, patient information about the approval way is requested and additional post-marketing studies are demanded, which should be performed in case of event occurrence and should prove clinical benefit and safety.[47] The evidence of safety needs to be confirmed on human subjects. Hence, two human studies have been performed to

investigate immunogenicity and safety of the vaccine, demonstrating comparable antibody responses as seen in animal studies and similar adverse reactions as for the treatment for pre-exposure prophylaxis.[18] Bridging of animal data to human is a critical point for the approval process following the Animal Rule. Here, the level at 50 % neutralization of Toxin-neutralizing antibody (TNA NF_{50}) and animal survival rate have been correlated and extrapolated to human.[19]

Having successfully applied for the status of submission on basis of the Animal Rule, the sponsor may wish to check whether other benefits in approval procedures may apply. The following FDA programs may be combined depending on the vaccine's property:[48]

- **Fast track**: facilitate development and expedite review for products targeting serious conditions and unmet medical need
- **Priority review**: shorter review time (6 instead of 10 months) for drugs providing significant improvement against current available standard therapies

Another interesting pathway in the US is the **Accelerated Approval**, which was introduced in 1992 by the FDA (21 CFR 601 subpart E for biologics; 21 CFR 314 subpart H for drugs). Although Accelerated Approval does not exempt the need for clinical trials, approval can be based on surrogate or intermediate endpoints for the demonstration of clinical benefit (for vaccines: for the demonstration of protective efficacy). This allows a reduction or reduced duration of clinical trials and vaccine approval may be granted earlier. Hereby, close collaboration with the FDA is favored and post-marketing investigations for completion of missing data are needed.[48,49] The vaccines Bexsero® and Trumenba® have been approved by the FDA according to the accelerated pathway in 2015 and 2014, respectively. Both vaccines target MenB in adolescence 10 to 25 years of age by the active immunization with the use of a combination of three cell surface antigens and one outer membrane vesicle (Bexsero®) or a bivalent lipoprotein (Trumenba®) of *N. meningitidis*. For both vaccines protective efficacy was investigated by the use of the surrogate parameter SBA, which was correlated to the immune response (see section 2). On basis of accelerated approval, the FDA requested both sponsor to verify their results in investigations on effectiveness.[50,51]

4. Special considerations for dossier preparation

The marketing authorisation application should be designed in accordance with the harmonised Common Technical Document (CTD) format as described in ICH Topic M4 “Common Technical Document for the Registration of Pharmaceuticals for Human Use – Organisation CTD“.[52]

In general, the content in each section depends on the individual vaccine and the legal basis for submission (e.g. full application, conditional approval, Animal Rule). The relevant guidelines valid for the submission country and agreements from scientific advice with the competent authority should be followed. In the following, considerations in dossier preparation for the special cases, in which efficacy data are limited or not available, are presented.

4.1. Administrative and prescribing information

Administrative and prescribing are typically region specific and not part of the ICH harmonised CTD dossier. In this section, country specific requirements such as labeling information (e.g. product information in the individual language(s)) are placed.

A black triangle with the statement “This medicinal product is subject to additional monitoring” should appear on the product information in the EU. This is true for all medicinal products with new active substances approved after 1st January 2011, for products approved by conditional or exceptional circumstances pathway, for biological medicines (e.g. vaccines, blood or plasma product) or for medicinal products which the obligation of additional studies for the completeness of comprehensive data.[53] If a conditional approval is desired, the applicant should inform the EMA in advance by a “letter of intent” prior to the submission of the application dossier. Concurrent to application submission the applicant needs to include a request on conditional marketing authorisation in which a justification and fulfilment of conditions to this approval procedure is presented.[44] In the Summary of Product Characteristics (SmPC) and the Patient Leaflet the granted approval under exceptional circumstances or conditional approval should be clearly stated (Art. 8, (EC) No. 507/2006).[54] The Animal Rule requests an explicit explanation of the reasons for approval on basis of the Animal Rule i.e. why efficacy studies could only be performed on animals and not on human subjects. The product information should also inform about the approval basis according to the Animal Rule.[13]

Vaccination of people within a population additionally protects individual non-vaccinated persons ('herd immunity'). This demonstrates the importance that vaccination does not only affect one individual but the whole population and is described as vaccine effectiveness (combination of vaccine-induced and population protection). Determination of vaccine effectiveness is not always possible and requested in an application dossier. The information is, however, useful and should be provided at latest in the post-authorisation period. Vaccine effectiveness is difficult to determine especially for rare diseases, in which limited efficacy data already is a problem. The application dossier should consist of a plan for data collection post-authorisation in close collaboration with public health authorities to further evaluate vaccine efficacy and effectiveness.[4,13]

4.2. Quality aspects

Complete and robust quality information is essential and a prerequisite for approval. Consistency and reproducibility of quality criteria are important for further investigations on nonclinical and clinical aspects. Manufacturing of the medicinal product should follow GMP for pharmaceutical preparations.[55] For vaccines, additional requirements for the control of biological products need to be recognised, since biological sources and production always go along with variability, which needs to be controlled more strictly to guarantee sufficient and consistent quality.[56]

In the quality part of the dossier a detailed description and characterisation of the active substances (e.g. viruses, proteins, toxoids or their conjugates) needs to be presented. The manufacturing process of the Active Pharmaceutical Ingredient (API) should be characterised, validated and controlled. Information about the API should contain raw materials, specification and source for the production of the API (e.g. cell banks, Master Seed Lot and Working Seed Lot) as well as information about its manufacture, stability and used reference standards are essential. Validation of the manufacturing process for the finished product is also required to achieve the same potency and lot-to-lot consistency, which is to be demonstrated in batch analysis. The quality information should include the product's specification and analytical methods, description of the formulation and the container closure system, used reference standards and adjuvants, sterility and stability for the drug product.[56,57]

Since use of an adjuvant has a major impact on the vaccine, it should be described and characterised in detail. The manufacture and the source for manufacturing of the adjuvant have

to be stated. Due to the fact that the adjuvant may adsorb or bind the antigen, whether indented or not, the combination should be characterised and described. If appropriate, the combination or co-administration of vaccines should also be analysed with regard to their compatibility to each other and the adjuvant (e.g. influence on stability, immune reaction, adsorption).[5,58,59]

4.3. Nonclinical aspects

The requirements on nonclinical investigations in the USA and EU are well in line with the WHO Guideline on nonclinical evaluation of vaccines, which motivated the CHMP to withdraw the Note for guidance on preclinical pharmacological and toxicological testing of vaccines).[60,61] Standard nonclinical studies as described in the WHO Guideline on nonclinical evaluation of vaccines are required, which include[12]

- Repeat dose toxicity on one animal species (no single dose toxicity required, local tolerance can be included or need to be performed separately)
- Reproductive toxicity: not required, reproductive organs can be assessed from histopathology out of other toxicity studies
- Embryo/foetal and perinatal toxicity are only required if the vaccine is intended for the use in women or pregnancy, not necessary for children vaccination.

The POC can be confirmed with challenge studies in animal models. Further investigations, to determine the need and type adjuvants are to be performed in accordance to the EMA guideline on adjuvants in vaccines.[5,62] Nonclinical immunogenicity data on animals are required for estimation of dose consistency in manufacturing and control of manufacture and should be estimated by data from three batches. Especially in cases of limited clinical data, complete and robust nonclinical information is essential. Applicants aiming at approval via Animal Rule need to provide results from appropriate animal efficacy studies supporting evidence of efficacy. Additional nonclinical or any available supportive data (*in vitro, in vivo*) can be included.[13]

4.4. Clinical aspects

A justification why protective efficacy studies are not feasible should be included in the clinical overview.[4] Although protective efficacy trials may not be feasible, human safety clinical trials are

needed to confirm the harmlessness of the developmental vaccine. This is explicitly requested for approval following Animal Rule.[13] The performance of clinical trials should follow GCP. The ICH GCP Guideline represents a harmonised guidance for the EU and USA.[63] National regulatory requirements on the conduct and design of clinical trials should be respected. The requirements for clinical trials in the EU are described in the clinical trials directive 2001/20/EC and ethical principles should follow the declaration of Helsinki, amended versions.[22,64] In the USA, prior to the performance of an clinical trial on humans, a submission of an IND application to the FDA is needed for the evaluation of safety. Therefore, data from nonclinical pharmacology and toxicology studies, manufacturing and information on the clinical protocol and investigator needs to be provided (21 CFR part 312).[23,65]

In addition, the regulatory requirements as described in the CHMP "Guideline on clinical evaluation of new vaccines"[4] and the WHO "Guidelines on clinical evaluation of vaccines: regulatory expectations"[8] should be respected. Accordingly, pharmacokinetic studies are not routinely required. Only in cases of new delivery systems, adjuvants or excipients these investigations are needed. Studies for the determination of lot-to-lot consistency have to verify the constant manufacturing process. Ideally, these investigations should be included in immunogenicity studies or other studies on protective efficacy, if feasible.[4] For vaccines that should be co-administered with other vaccines, the combination has to be evaluated with regard to possible immune interference causing a change in immune response.[58,59]

The reason for limited efficacy data (rarity or ethical reason) exclude in most cases the need for investigations on children, except this rare disease occurs in children only. Investigations on children are obligatory for the marketing authorisation in the USA as well as in the EU, if no waiver or deferral has been granted.[66,67] Hence, the applicant has to check the regulatory databases for applicability of class waivers or apply for a waiver for paediatric investigation, if applicable. The application dossier should contain information about paediatric investigations (e.g. waiver or deferrals).

A collection of any available comprehensive clinical trial information or relevant data should be provided to enable the best possible overview for the evaluation by the competent authority. In some cases, the use of HCTs is preferable to achieve more comparable data on the efficacy and immune response in human, especially if no appropriate animal model is available. Of course, questions on ethics need to be addressed and agreed with the ethics committee. In cases where data from clinical efficacy studies are available, additional non-inferiority immunogenicity trials may be performed to allow a bridge to e.g. additional populations or age groups.

5. Discussion

The evidence of protective efficacy is essential for a successful marketing authorisation of vaccines and traditionally determined by the performance of human clinical trials.[6] The presented alternative options for the demonstration of efficacy allow a reduction or replacement of human trial subjects. They are essential for the demonstration of protective efficacy, in cases human clinical trials are is not possible or feasible. Table 2 gives a brief overview about the discussed alternative methods.

Animal efficacy studies are well recognized and encouraged by the FDA with the separate approval pathway Animal Rule. A detailed guidance describes the prerequisite and applicability for the Animal Rule and facilitates the preparation and communication between sponsor and regulatory authority for a new MAA.[13] This measure allowed the approval of BioThrax®'s second indication. The approval of the vaccine for Imvanex® confirmed that, animal efficacy studies are also accepted in the EU, although no individual approval pathway exists. The procedures conditional approval and approval under exceptional circumstances are possible options.[43,44] In addition, individual countries within the EU can approve national marketing authorisations on basis of animal efficacy studies within the frame of their national regulations. A national approval was granted to BioThrax® in Germany in 2013. It was approved by the national authority Paul-Ehrlich-Institut (PEI) for the pre-exposure prophylaxis with the indication "Active immunization for the prevention of disease caused by *Bacillus anthracis*, in individuals 18 through 65 years of age, whose occupation or other activities place them at risk of exposure, regardless of the route of exposure".[68,69] This indication is based on the clinical investigations performed in the US and data collected from the CDC. The applicant announced its intention for further MAA in other countries of the EU[70], but up to now, no additional license are available yet. Certainly, strategic approaches on the marketing situation may have an impact on the decision of MAA and countries. Possible routes for the licensure in other countries of the EU may be to follow a MRP procedure by the use of Germany as reference member state. Alternatively, purely national approval pathways in the chosen countries may also be applicable. BioThrax®'s second indication in the USA, which was based on the Animal Rule, is not yet approved in Germany. The regulatory system in Europe does not exclude the possibility for approval on basis of nonclinical data. However, except for the brief description in the guidelines on clinical evaluation of vaccines provided by the WHO[6] and CHMP,[4] there is no detailed guidance for animal efficacy trials in the EU. For the approval of this second the applicant needs to request scientific advice with the national competent authority.

Table 2: Overview of alternative methods for the demonstration of efficacy.

	Nonclinical efficacy studies	Human Challenge Trials	Passive Protection Studies	Comparison of immune response
Study setting	protective efficacy studies performed on animals	small number of human subjects are vaccinated and challenged with the infective agent in a controlled setting	administration of antibodies to naïve animals, which are thereafter challenged with the infective agent	1. correlation of immune response from clinical trials of similar vaccines 2. bridge between established data from pivotal trials and new setting (evidence of non-inferiority)
Study subjects	animals, 2 species	human	animals	human
Conditions	appropriate animal model for available	ethically justified, controlled trial setting to guarantee most possible safety measures	knowledge and availability (manufacture and/or isolation) of antibody	availability of comparable trials and ICP or other immunological parameters for comparison
Pros	reduced risk for human subjects	direct immune response from human	identification of required antibody level	reduction in trial subjects, duration and costs; human data available
Cons	human immune response may still differ from animal	small extract of population; comparability of challenge models to wild type questionable	immune response may be caused from other unknown antibodies or antibody interaction	
MAA: USA	Animal Rule	POC; supportive data; MA under special conditions of small indication	POC; supportive data	Accelerated approval; supportive data
MAA: EU	standard, Exceptional Circumstances, Conditional	POC supportive data	POC supportive data	standard, Exceptional Circumstances Conditional
Examples	Anthrax (BioThrax®) Smallpox (Imvanex®)	Malaria (Mosquirix™) Cholera (Vaxchora™)	Anthrax research programe MenB (Bexsero®)	HPV (Cervarix®, Gardasil®) MenB (Bexsero®, Trumenba®)

Similar to the Animal Rule, the national authority of Canada, Health Canada, has amended Canada's Food and Drug Regulations in 2011 to create the separate submission pathway "Extraordinary Use New Drugs" (EUND), aiming at preparedness for emergency cases. This approval pathway allows drug submission with limited clinical data where human efficacy trials are not possible or feasible and hence the evidence of safety and efficacy could not be completed (C.08.002.01 (1) Food and Drug Regulations).[14,71] It is applicable to drugs (vaccines included) indicated for emergency use and for the prevention of exposure of persons to dangerous substances with lethal potency and in which, human data cannot be provided.[71] The applicant is requested to file a robust and complete package of quality and nonclinical data. Additional nonclinical investigations may be needed to support the evaluation of safety and efficacy as they partly replace clinical data. Further post-marketing studies to collect missing data for efficacy and safety are requested. Importantly, the use of the drug after approval is restricted to the Canadian government and the product information should contain the following statement in capital letters "HEALTH CANADA HAS AUTHORIZED THE SALE OF THIS EXTRAORDINARY USE NEW DRUG FOR [naming purpose] BASED ON LIMITED CLINICAL TESTING IN HUMANS".[14]

Passive protection trials and HCTs are generally considered and accepted for POC studies but efficacy data by these methods alone are currently not sufficient for a marketing authorisation in Europe and USA (Table 2). Passive protection studies are important and useful tools in the development of vaccines. They are used for a better understanding of the mode of efficacy (e.g. in special research programs for anthrax disease) or they may be part of MAAs for the overall collection of comprehensive data (e.g. Bexsero®).[31,33]

During one special meeting in May 1998, the VRBPAC has agreed, that HCTs may be sufficient to prove efficacy in the USA only for infrequent vaccines and a controlled small indication. The requirements were well-controlled studies, which have been performed under GCP and availability of adequate safety data. The basis for discussion was the evaluation of an application for a cholera vaccine used for US travelers into endemic areas.[72,73] Based on this agreement combined with experience and increased knowledge on the cholera vaccine, Vaxchora™ was approved in 2016 in the US. The applicant could demonstrate vaccine tolerability in four clinical trials with altogether 3,235 adults receiving one dose of the vaccine. Vaccine efficacy was demonstrated in HCT with 134 volunteers, who have been vaccinated and after 10 days or 3 months challenged with cholera resulting in 90% or 80% protective efficacy, respectively.[74] The application was approved in June 2016.[75]

In the EU, HCTs have been included as initial studies in the application dossier for the malaria vaccine, Mosquirix™. Malaria naïve adults have been treated with the vaccine and challenged according to the CHMI model with *P. falciparum*. Different adjuvant systems,

dosing and dose schedules have been considered and evaluated with the use of HTC, which was the rationale for the performance in further clinical trials. Nonetheless, investigations evaluating vaccine efficacy (e.g. in different age groups, dosing schedules) needed to be presented to demonstrate a positive benefit-risk balance. The applicant obtained a positive opinion by the CHMP according to Art. 58 of Regulation (EC) No 726/2004.[76] The Art. 58 procedure is based on the collaboration between the WHO and CHMP. Hereby, the CHMP evaluates the application dossier for a vaccine, which is later to be used outside of the EU. Following assessment of the vaccine dossier, the CHMP gives an opinion, which is the basis for a marketing authorisation in countries outside of the EU, but not applicable for a marketing authorisation inside of the EU since no commission decision is made.[38] This is especially relevant to developmental countries, in which the regulatory agency does not have enough capacity.

The comparison of immune responses for a complete waiver of protective efficacy trial in a new marketing authorisation requires profound knowledge of the immunologic properties and availability of literature data or comparable vaccines (Table 2). There is no detailed guideline on how and when this method is used and which expectations the regulatory authorities have. Still, by a scientific and strategic planning in close collaboration with regulatory authorities this method can successfully support a marketing authorisation without the need for an extra approval pathway (e.g. Bexsero®). The use of bridging studies to extrapolate data on basis of one existing efficacy trial is widely used in many application dossiers in the USA and EU already (e.g. HPV vaccines Cervarix® and Gardasil®9).[36,37,77,78] This strategic instrument can reduce efforts, costs and involvement of humans in clinical trials, which is not only favored by the sponsor, but also regulatory agencies. The published concept pater for the revision of the guideline on clinical development of vaccines by the CHMP reflects the need for an update as a result from current knowledge gained from regulatory experience during assessment of now approved vaccines. The revised guideline may provide more details in the prediction of efficacy with alternative methods and considerations in the conduct of bridging and immunogenicity studies.[42]

Harmonization and standardization from regulatory health authorities between USA and the EU as well as within the countries of the EU are endorsed for the applicant by means of reduction of costs and efforts to respect different conditions between different countries. In addition, a close collaboration of the regulatory authorities benefit from the transparency, reduction of replicated assessments and can ensure similarity of assessments as well as provide important exchanges about state of the art of scientific knowledge. The FDA and health authorities within the EU have well-established regulatory conditions for the approval of MAAs of vaccines. Some harmonized guidelines for both countries exist already (e.g. ICH,

WHO guidelines) but there is still need for improvement. Especially innovative approaches, for which no guideline currently exists, need advice from the regulatory authority. Importantly, the individual situations such population differences and vaccine specialty should be respected.

6. Conclusion

Nonclinical efficacy trials and the comparison of immune response are two currently well-accepted alternatives for the evidence of efficacy. For Europe, a more detailed guidance about the applicability and performance of animal efficacy trials, favorably in consideration of the requirements for the Animal Rule, may support understanding and motivate sponsors for the development of vaccines with challenging properties in the evidence of efficacy. The WHO's proposed alternatives are well recognized by regulatory authorities and sponsors but primarily intended for exceptional situations. With increased knowledge and experience in the performance and assessment of the proposed alternative options, the development of novel vaccines may be adapted to reduce risk on human trial subjects and fasten the time for vaccine development.

In conclusion, the regulatory environment in the EU and USA offer different options to support marketing authorisation of vaccines and respect the circumstances when efficacy trials are not possible. A close collaboration and scientific advice with the regulatory authority is essential for a successful MAA, as the special situations need to be recognized and assessed individually.

7. Summary

The development of vaccines is crucial for the protection against severe infectious diseases. Applicants aiming at approval of a new marketing authorization need to confirm efficacy, safety and harmlessness of the innovative medicinal product. Different from standard drug products, vaccines need to demonstrate protection against a disease (protective efficacy). Sometimes, low, unpredictable or no occurrence of disease outbreak makes human efficacy trials not possible or feasible. The regulatory agencies have recognized these limitations and propose alternative approaches for the determination of vaccine efficacy.

Animal efficacy studies can be used to extrapolate to human efficacy. Importantly, an adequate animal model with similar immune response to human is required. With introduction of the Animal Rule in 2002, the FDA has established an extra approval pathway, which explicitly accepts animal efficacy data. The first vaccine (against anthrax) received approval following the Animal Rule already. The EU legislation accepts animal efficacy data, depending on the individual vaccine and targeted disease. A vaccine against the eradicated disease smallpox received marketing authorization under exceptional circumstances with the use of animal efficacy studies.

Deliberate exposure of infectious agents to human volunteers is another option for the evidence of efficacy. These studies have to be ethically justified and performed in a controlled setting. Modification of the challenge agent may influence the comparability to the wild type pathogen. Established human challenge models are currently used for the proof of concept in vaccine development in support of marketing authorization applications. Similar to human challenge trials, passive protection studies are mainly used for the proof of concept or as supportive data in the dossier of a marketing authorization application. Hereby, antibodies against a disease are applied to naïve animals, which thereafter are challenged with the pathogen. This method allows a quantification of the protection level and is mainly used in research programs.

Development of an immune response after vaccination is the essential mechanism of action for vaccines. The production of antibodies can be used to correlate and extrapolate data from literature information or comparable vaccines. This instance is used for marketing authorizations based on the comparison of the immune response. In addition, demonstrating non-inferiority in immune response enables bridging of data to new populations, age group or manufacturing conditions for a change of the vaccine's specification or indication.

From the presented methods, especially nonclinical efficacy trials and the comparison of immune response are the most well-known and accepted strategies. The severity of disease, availability of alternative medical care and other supportive data as well as the individual safety profile of the innovative vaccine influences the decision on marketing authorization and should be discussed individually in scientific advice procedures with the regulatory authority.

8. References

1. Vaupel P, Mutschler E, Schaible HG. Anatomie, Physiologie, Pathophysiologie des Menschen: WVG; 2015.
2. DAZ. Medizingeschichte: Geschichte der Schutzimpfung. 2003. http://www.deutsche-apotheker-zeitung.de/daz-az/2003/daz-17-2003/uid-9640 (accessed 19 August 2017).
3. WHO. Emergencies preparedness, response. Smallpox. 2017. http://www.who.int/csr/disease/smallpox/en/ (accessed 29 September 2017).
4. CHMP. Guidelines on Clinical Evaluation of new Vaccines. 2006. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003870.pdf (accessed 21 July 2017).
5. EMA. Guideline on Adjuvants in Vaccines for Human Use. 2005. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003809.pdf (accessed 17 August 2017).
6. WHO. Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations. 2016. http://www.who.int/biologicals/BS2287_Clinical_guidelines_final_LINE_NOs_20_July_2016.pdf (accessed 16 March 2017).
7. Banchereau J, Palucka K. Immunotherapy: Cancer vaccines on the move. *Nat Rev Clin Oncol* 2017; **advance online publication**.
8. WHO. Guidelines on clinical evaluation of vaccines: regulatory expectations. Annex 1. 2004. http://www.who.int/biologicals/publications/clinical_guidelines_ecbs_2001.pdf?ua=1 (accessed 15 July 2017).
9. Halloran ME, Struchiner CJ, Longini IM, Jr. Study designs for evaluating different efficacy and effectiveness aspects of vaccines. *Am J Epidemiol* 1997; **146**(10): 789-803.
10. EMA. Assessment report: ProQuad. 2006. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000622/WC500044067.pdf (accessed 12 September 2017).
11. Leroux-Roels G, Bonanni P, Tantawichien T, Zepp F. Vaccine development. *Perspectives in Vaccinology* 2011; **1**(1): 115-50.
12. WHO. Nonclinical evaluation of vaccines. 2005. http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/en/ (accessed 23 July 2017).
13. FDA. Guidance for the industry: Product Development Under the Animal Rule. 2015. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM399217.pdf (accessed 11 April 2017).
14. Health_Canada. Guidance Document - Submission and Information Requirements for Extraordinary Use New Drugs (EUNDs). 2014. http://www.hc-sc.gc.ca/dhp-mps/brgtherap/applic-demande/guides/eund-dnue-eng.php (accessed 11 April 2017).
15. FDA. Guidance for Industry and FDA staff: Qualification Process for Drug Development Tools. 2014. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597.pdf (accessed 3 August 2017).
16. RKI. Anthrax (Milzbrand, Bacillus anthracis). 03.08.2017. http://www.rki.de/DE/Content/InfAZ/A/Anthrax/Anthrax.html?nn=2386228 (accessed 10 August 2017).
17. D'Amelio E, Gentile B, Lista F, D'Amelio R. Historical evolution of human anthrax from occupational disease to potentially global threat as bioweapon. *Environ Int* 2015; **85**: 133-46.
18. FDA. FDA approves vaccine for use after known or suspected anthrax exposure. 2015. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm474027.htm (accessed 3 August 2017).
19. FDA. Biothrax - Summary Basis for Regulatory Action Template. 2015. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM474886.pdf (accessed 6 August 2017).

20. CPMP. Note for Guidance on the development of vaccinia virus based vaccines against smallpox. 2002. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003900.pdf (accessed 2 October 2017).
21. EMA. Assessment report: Imvanex. 30.05.3013 2013. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002596/WC500147898.pdf (accessed 18 August 2017).
22. European Parliament CotEU. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. 2001.
23. FDA. Investigational New Drug (IND) Application. 2016. http://www.fda.gov/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/approvalapplications/investigationalnewdrugindapplication/default.htm (accessed 20 September 2017).
24. WHO. Human Challenge Trials for Vaccine Development: regulatory considerations. 2016. http://www.who.int/biologicals/expert_committee/Human_challenge_Trials_IK_final.pdf?ua=1 (accessed 11 May 2017).
25. CHMP. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. 2017. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2017/07/WC500232186.pdf (accessed 20 August 2017).
26. Goetz KB, Pfleiderer M, Schneider CK. First-in-human clinical trials with vaccines - what regulators want. *Nat Biotech* 2010; **28**(9): 910-6.
27. CDC. Malaria - Biology. 2016. http://www.cdc.gov/malaria/about/biology/index.html (accessed 17 August 2017).
28. Burrack S. Status, challenges and regulatory strategies to develop a malaria vaccine. DGRA webpage: Rheinischen Friedrich-Wilhelms-Universität Bonn; 2013.
29. Laurens MB, Duncan CJ, Epstein JE, et al. A consultation on the optimization of controlled human malaria infection by mosquito bite for evaluation of candidate malaria vaccines. *Vaccine* 2012; **30**(36): 5302-4.
30. Okie S. Betting on a malaria vaccine. *N Engl J Med* 2005; **353**(18): 1877-81.
31. Medicine I, Agency MFU, Program CRCDCAVSER. An Assessment of the CDC Anthrax Vaccine Safety and Efficacy Research Program: NAP; 2002.
32. RKI. Meningokokken-Erkrankungen. RKI-Ratgeber für Ärzte. 2016. http://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Meningokokken.html (accessed 29 September 2017).
33. EMA. Assessment report: Bexsero. 2012. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002333/WC500137883.pdf (accessed 25 September 2017).
34. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* 1969; **129**(6): 1307-26.
35. RKI GdeKiDeV. 3.16 Gebärmutterhals. Krebs in Deutschland 2011/2012; 2015.
36. EMA. Assessment report: Cervarix. 2007. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000721/WC500024636.pdf (accessed 15 August 2017).
37. EMA. Assessment report: Gardasil 9. 2017. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003852/WC500189113.pdf (accessed 15 August 2017).
38. European Parliament CotEU. Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. 2004.

39. European Parliament CotEU. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use 2001.
40. FDA. Vaccines and Related Biological Products Advisory Committee. 2017. http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/default.htm (accessed 28 September 2017).
41. FDA. About CBER. 2016. http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CBER/ucm123340.htm (accessed 28 September 2017).
42. CHMP. Concept paper on revision of the guideline on clinical development of vaccines. 2017. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2017/06/WC500229930.pdf (accessed 25 August 2017).
43. Parliament E. Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. 2004 (accessed 10. August 2017).
44. EMA. Guideline on the scientific application and the practical arrangements necessary to implement Commission Regulation (EC) No 507/2006 on the conditional marketing authorisation for medicinal products for human use falling within the scope of Regulation (EC) No 726/2004. 2016. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/03/WC500202774.pdf (accessed 10 August 2017).
45. EMA. Assessment report: Trumenba. 2017. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004051/WC500228997.pdf (accessed 29 September 2017).
46. FDA. New drug and biological drug products; evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. Final rule. *Fed Regist* 2002; **67**(105): 37988-98.
47. FDA. Supplement Approval for for Anthrax Vaccine to include post-exposure prophylaxis. 2015. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM474299.pdf (accessed 6 August 2017).
48. FDA. Fast Track, Breakthrough Therapy, Accelerated Approval, Priority Review. 14.09.2015 2015. http://www.fda.gov/forpatients/approvals/fast/ucm20041766.htm (accessed 10 August 2017).
49. Beasley DWC, Brasel TL, Comer JE. First vaccine approval under the FDA Animal Rule. *Npj Vaccines* 2016; **1**: 16013.
50. FDA. Approval Letter - BEXSERO. 2015. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm431446.htm (accessed 29 September 2017).
51. FDA. Approval Letter - TRUMENBA. 2014. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm421034.htm (accessed 29 September 2017).
52. ICH. Organisation of the Common Technical Document for the Registration of Pharmaceuticals for Human Use. 2016. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/CTD/M4_R4_Organisation/M4_R4__Granularity_Document.pdf (accessed 19 August 2017).
53. EMA. Medicines under additional monitoring. 2013. http://www.ema.europa.eu/docs/en_GB/document_library/Other/2013/04/WC500142430.pdf (accessed 18 August 2017).
54. Commission E. Commission Regulation (EC) No 507/2006 of 29 March 2006 on the conditional marketing authorisation for medicinal products for human use falling within the scope of Regulation (EC) No 726/2004 of the European Parliament and of the Council 2006.

55. WHO. WHO good manufacturing practices for pharmaceutical products: main principles. 2014. http://apps.who.int/medicinedocs/documents/s21467en/s21467en.pdf (accessed 17 August 2017).
56. WHO. Good manufacturing practices for biological products. 1992. http://www.who.int/biologicals/publications/trs/areas/biological_products/WHO_TRS_822_A1.pdf?ua=1 (accessed 18 August 2017).
57. FDA. Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product. 1999. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm092272.pdf (accessed 10 July 2017).
58. FDA. Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Diseases: Production, Testing and Clinical Studies http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM175909.pdf (accessed 19 August 2017).
59. CPMP. Pharmaceutical and biological aspects of combined vaccines. 1998. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003939.pdf (accessed 12 August 2017).
60. EMA. Questions and answers on the withdrawal of the CPMP Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465). 2016. http://www.ema.europa.eu/docs/en_GB/document_library/Other/2016/07/WC500210581.pdf (accessed 21 August 2017).
61. FDA. Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications 2006. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm092170.pdf (accessed 20 June 2017).
62. WHO. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. 2013. http://www.who.int/biologicals/areas/vaccines/ADJUVANTS_Post_ECBS_edited_clean_Guidelines_NCE_Adjuvant_Final_17122013_WEB.pdf (accessed 23 July 2017).
63. ICH. ICH E6(R2) Good Clinical Practice (GCP). 2016. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf (accessed 17 August 2017).
64. Association WM. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013; **310**(20): 2191-4.
65. FDA. Guidance for Clinical Investigators, Sponsors, and IRBs: Investigational New Drug Applications (INDs) — Determining Whether Human Research Studies Can Be Conducted Without an IND. 2013. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM229175.pdf (accessed 13 August 2017).
66. European Parliament CotEU. Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004. 2006.
67. FDA. Pediatric Study Plans: Content of and Process for Submitting Initial Pediatric Study Plans and Amended Initial Pediatric Study Plans - Guidance for the Industry (draft). 2016. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm360507.pdf (accessed 30 September 2017).
68. PEI. Impfstoffe gegen Milzbrand (Anthrax). 2013. http://www.pei.de/DE/arzneimittel/impfstoff-impfstoffe-fuer-den-menschen/milzbrand/milzbrand-node.html (accessed 18 April 2017).
69. Emergent. SmPC BioThrax. 2017. https://portal.dimdi.de/websearch/servlet/Gate;jsessionid=3B4CA9BD8767E7594D633B250

E3D7D34?accessid=pharmnet_par_freeinfo&query=enr=2604178 (accessed 1 October 2017).
70. Zeitung P. Emergent BioSolutions erhält vom Paul-Ehrlich-Institut Zulassung zur Vermarktung von BioThrax in Deutschland. 2013. http://www.pharma-zeitung.de/emergent-biosolutions-erhalt-vom-paul-ehrlich-inst.5798.php (accessed 1 October 2017).
71. Government_of_Canada. Food and Drug Regulations (C.R.C., c. 870). http://laws-lois.justice.gc.ca: Minister of Justice.
72. Sheets RL, Fritzell B, Aguado de Ros MT. Human challenge trials in vaccine development: Strasbourg, September 29 - October 1, 2014. *Biologicals* 2016; **44**(1): 37-50.
73. FDA. Guidance for Industry: General Principles for the Development of Vaccines to Protect Against Global Infectious Diseases. 2011. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM282995.pdf. (accessed 17 August 2017).
74. Levine MM, Chen WH, Kaper JB, Lock M, Danzig L, Gurwith M. PaxVax CVD 103-HgR single-dose live oral cholera vaccine. *Expert Rev Vaccines* 2017; **16**(3): 197-213.
75. FDA. Approval letter: VAXCHORA. 2016. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM506321.pdf (accessed 5 September 2017).
76. EMA. Assessment report: Mosquirix. 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Medicine_for_use_outside_EU/2015/10/WC500194577.pdf (accessed 2 October 2017).
77. FDA. Approved products - Gardasil 9. 30.08.2017 2017. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm426445.htm (accessed 30 September 2017).
78. FDA. Approved products: Cervarix. 31.07.2017 2017. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm186957.htm (accessed 30 September 2017).

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.