What Is the Predictive Value of Animal Models for Vaccine Efficacy in Humans?

The Importance of Bridging Studies and Species-Independent Correlates of Protection

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Animal models have played a pivotal role in all stages of vaccine development. Their predictive value for vaccine effectiveness depends on the pathogen, the robustness of the animal challenge model, and the correlates of protection (if known). This article will cover key questions regarding bridging animal studies to efficacy trials in humans. Examples include human papillomavirus (HPV) vaccine in which animal protection after vaccination with heterologous prototype virus-like particles (VLPs) predicted successful efficacy trials in humans, and a recent approval of anthrax vaccine in accordance with the “Animal Rule.” The establishment of animal models predictive of vaccine effectiveness in humans has been fraught with difficulties with low success rate to date. Challenges facing the use of animal models for vaccine development against Ebola and HIV will be discussed.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed

Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

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H. Golding et al.

The development of vaccines against human pathogens commonly includes animal studies at early stages of vaccine evaluation. Proof-of-concept (POC) studies provide justification for further vaccine development and down-selection of vaccine constructs and adjuvants. Entry into clinical phase 1 studies (to evaluate vaccine safety and obtain early immunological measurements) requires preclinical toxicity studies in animals conducted under “good laboratory practices” (GLPs) using the intended human vaccine. Phase 2 trials are conducted to optimize vaccine dose and vaccination schedule and to identify the most relevant immunological assays. Phase 1 and phase 2 trials are conducted in populations at low risk for infection and the number of participants is low (≤ 300). Phase 3 trials are large-scale (in the thousands), conducted in high-risk populations (or geographical regions), and are powered to show reduction in infection rates or protection from severe disease in vaccinated compared with unvaccinated subjects. They are costly and may be conducted over several years in multiple locations.

In cases in which human efficacy studies are not ethical and field trials are not feasible, such as vaccines against diseases with sporadic or low incidence (e.g., Ebola), or agents that can be weaponized (e.g., anthrax), data from a well-established animal challenge model could complement the human safety and immunogenicity trials and provide a basis for vaccine approval under the Food and Drug Administration (FDA) “Animal Rule” (FDA 21CFR601.90) (Burns 2012).

The design and usage of animal studies to predict vaccine effectiveness in humans have been challenging. This review will focus on animal vaccine studies that include homologous or heterologous challenge strains with outcomes that closely mimic human infections and are likely to be predictive of vaccine effectiveness.

PROOF-OF-CONCEPT STUDIES

POC studies are often conducted in small animal species (i.e., inbred mice) that allow the use of a large number of animals and multiple iterations

of vaccine products aimed at identifying the best vaccine candidate and early evaluation of immunological end points. However, there are several important considerations that preclude direct extrapolation of the data from small animal studies to humans. Inbred strains of mice that render data more reproducible do not reflect immune response variability in the human outbred populations. Differences in pathogen-recognition receptors may account for differences between humans and rodents in response to microbial vaccines and some adjuvants (Schroder et al. 2012; Lai et al. 2014); dose and regimen of immunization and route of injections are often different between small animal species and humans. There are also species-specific B-cell and T-cell repertoires and HLA presentation of dominant epitopes to CD4 and CD8 T cells. Because of these limitations, some POC studies are conducted in larger animal species in which dose selection and vaccination protocols are expected to be more translatable to humans.

EVALUATION OF VACCINE EFFECTIVENESS IN ANIMAL CHALLENGE MODELS

There are two main scenarios of vaccine evaluation in animal challenge studies:

1. Using a well-characterized animal challenge model for a final selection of vaccine components and prime-boost protocols to proceed into clinical trials. In specific cases, these studies can be used to support a request for “Fast Track” designation by the FDA for vaccines with an “Unmet Need” designation. The qualifying criteria for Fast Track include a drug/vaccine that is intended to treat a serious condition and nonclinical or clinical data showing the potential to address unmet medical needs (see Guidance for Industry: Expedited Programs for Serious Conditions—Drugs and Biologics at www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm358301.pdf)
2. Using a relevant animal challenge model (i.e., similar pathology to human disease)

in support of licensure in accordance with the FDA Guidance for Industry: “Product Development under the Animal Rule” (Snoy 2010; Burns 2012) (see www.fda.gov/downloads/drugs/guidancecompliance/regulatoryinformation/guidances/ucm399217.pdf).

Key attributes of relevant animal challenge models for evaluation of vaccine effectiveness:

1. Animal species should show key characteristics of the human disease following exposure to the challenge pathogen (time from exposure to onset of disease, time course/progression of disease, clinical manifestations, morbidity, and lethality).
2. The challenge agent used in the animal study should be relevant to the human disease.
3. The immune marker(s) selected should reflect the protective immune responses generated by humans.
4. The vaccine dose and vaccination schedule chosen for adequate and well-controlled studies in animals should elicit an immune response in animals reflective of that in humans.
5. Ideally, the immunological assays should be species-independent.
6. There should be a robust statistical plan.

In some cases, in the absence of an animal model that directly recapitulates human infection and disease (i.e., human pathogen does not infect or shows different disease patterns in animals), the experimental parameters of the animal challenge study may need to be modified to resemble human disease (i.e., smallpox, papillomavirus [PV], HIV). In other cases, the pathogen needs to be adapted for replication in the animal host. However, host adaptation might lead to changes in pathogen fitness and/or disease course, or to altered antigenic characteristics compared with the unadapted pathogen.

Importantly, even with these limitations, vaccine evaluation in animal challenge studies (not necessarily for approval under the “Animal

Rule”) provides an opportunity to conduct in-depth immunological measurements and to explore multiple potential surrogate markers of protection or clinical end points. If a primary immunological end point is identified, it may lead to adjustment of the vaccine dose in animal pivotal studies to achieve immune responses at levels comparable to humans (“humanized dose”).

SPECIFIC EXAMPLES

Human Papillomavirus (HPV) Vaccines

Because of the species-specificity of the papilloma viruses, investigators relied on the biological effects of nonhuman PV in animal models. The bovine virus-like particle ([VLP]/L1) vaccine was protective in cattle against bovine PV infection. Subsequent POC studies in dogs and rabbits with prototype VLP/L1 vaccines showed $\geq 90\%$ effectiveness in preventing warts following exposure to PV (Breitburd et al. 1995; Suzich et al. 1995; Kirnbauer et al. 1996). These encouraging studies were followed by large clinical vaccine trials in women with HPV VLP/L1, showing high titers of anti-HPV L1 antibodies and protection against type-specific HPV infection and precancerous cervical changes (Harper et al. 2004; Villa et al. 2005). The first HPV vaccine (Gardasil) was approved in the United States in 2006.

Anthrax Vaccines

Anthrax vaccine adsorbed in combination with a course of antibiotics was approved under the FDA “Animal Rule.” Postexposure prophylaxis (PEP) of disease following suspected or confirmed *Bacillus anthracis* exposure, when administered in conjunction with recommended antibacterial drugs.

In the studies used to support approval of anthrax vaccine, rabbits and nonhuman primates (NHPs) were vaccinated twice (day 0 and 28), and were challenged on day 70 with an aerosolized form of *B. anthracis* spores. Survival was vaccine-dose-dependent and correlated with prechallenge titers of toxin-neutralizing

H. Golding et al.

antibody (TNA). The prechallenge TNA titers in protected NHPs and rabbits were similar. Statistical analyses confirmed that TNA responses correlated with animal survival after aerosol challenge with *B. anthracis* spores (Burns 2012).

Bridging Animal Protection Data to Humans

Three assumptions were made:

1. TNA titers in animals could be used to link protection in animals to humans.
2. The distribution of antibody titers obtained in human subjects in an appropriately powered clinical trial, in conjunction with the protective antibody levels in pivotal animal protection studies, could be used to predict that the vaccine is “reasonably likely to provide clinical benefit in humans.”
3. The methodology used to measure TNA antibody levels in both the animal studies and humans was species-independent. TNA $NF_{50} \geq 0.56$ (on day 69–70) corresponded with 70% probability of survival in both animal species. Importantly, the protective TNA titers in animals could be bridged to TNA titers in humans at day 63, that is, a time point shortly after the recommended 60-day antibiotic regimen is discontinued and the risk of infection resulting from germination of residual spores is increased. Ultimately, the lower bound of the 95% CI of the proportion achieving TNA $NF_{50} \geq 0.56$ at day 63 was used for approval of BioThrax under the “Animal Rule” (Ionin et al. 2013).

EBOLA VACCINES

Ebola virus (EBOV) and Marburg virus (MARV), members of the Filoviridae family, cause an acute and rapidly progressing hemorrhagic fever (HF) with mortality rates up to 90% (Miranda et al. 1999; Peters and Khan 1999; Roels et al. 1999; Sadek et al. 1999). Several animal species including guinea pigs and NHPs were evaluated for pathogenesis, therapeutic, and vaccine studies (Hevey et al. 1998; Schou and Hansen 2000; Geisbert et al. 2002).

The unprecedented large outbreak of EBOV in 2014–2015 in West Africa showed the urgent need for an EBOV vaccine. Several vaccine candidates moved rapidly from preclinical studies in NHPs into phase 1/2 clinical studies in the United States and several countries in West Africa. However, because most phase 3 clinical trials took place toward the tail end of the EBOV outbreak, they are unlikely to fulfill the criteria of traditional pathways to licensure, based on randomized controlled trials (RCTs) with clinical end points (Chowell and Viboud 2015; Chowell et al. 2015; Cleaton et al. 2016).

Currently, the two most likely options for approval of EBOV vaccines are (Krause et al. 2015a,b):

1. Accelerated approval based on a surrogate immunological end point that is “reasonably likely” to predict clinical benefit. The immunological end point could be defined based on animal studies, and/or from naturally infected or exposed/protected humans.
2. Licensure based on the “Animal Rule.”

Both types of vaccine approval will require postlicensure studies (probably during future EBOV outbreaks) to show clinical benefit in humans.

MAIN CHALLENGES TO ESTABLISHMENT OF VALIDATED ANIMAL MODEL FOR EBOV VACCINES

Pathogen (Virus Strain, Dose, Route-of-Exposure)

NHPs including rhesus macaques and cynomolgus macaques infected with EBOV and MARV show similar pathology and lethality rates as observed in humans and therefore have been used extensively (Geisbert et al. 2003a,b). However, the susceptibility to infection, disease manifestation, and infection outcome differ between NHP species depending on the virus strain, route of administration, and challenge dose. An additional complication to studying EBOV vaccines in NHPs is that the natural exposure in humans may differ from the route of infection used in animal studies: intramuscular

infection with 10^3 pfu (plaque-forming units) (Marzi et al. 2015a,b,c) or aerosol administration in the case of studies of bioterrorism agents (Geisbert et al. 2008; Twenhafel et al. 2013).

Surrogate Markers in Animal Studies and Assays

The predominant immune response and surrogate markers may vary among vaccine platforms. The replicating vesicular stomatitis virus (VSV)–EBOV–Zaire glycoprotein (GP) vaccine is likely to protect by antibody-dependent mechanisms: protection of cynomolgus macaques vaccinated with VSV–EBOV–Zaire (2×10^7 pfu) from lethal challenge with an EBOV–Makona strain (10^3 pfu) correlated with high titers of anti-EBOV–GP IgGs in surviving versus nonsurviving animals (1:12,805 and 1:64, respectively) (Wong et al. 2012). However, in other studies that used the same vaccine platform, titers of anti-GP antibodies that correlated with protection varied significantly (between 10^2 and $\geq 10^4$), possibly reflecting differences in the ELISA-binding assays (Marzi et al. 2015c). Measurements of neutralizing antibodies are more likely to be species-independent, but so far they showed high variability among laboratories. There is also a clear need to replace BSL-4-restricted assays using live wild-type EBOV with reporter-gene-based neutralization assays that can be conducted in BSL-2 facilities. However, these assays did not correlate with protection any better than the EBOV-specific ELISA in NHPs.

In contrast with replicating VSV–EBOV vaccine, protection of NHPs against EBOV challenge after vaccination with nonreplicating adenovirus-based vaccines appears to be mediated primarily by $CD8^+$ T cells (Geisbert et al. 2011; Sullivan et al. 2011; Stanley et al. 2014). Cell-mediated immunity (CMI) assays are particularly difficult to validate, and data from CMI assays in animals is difficult to bridge to humans because of differences in MHC–HLA restrictions.

Bridging Animal Protection Data to Humans

Interim reports on safety and immunogenicity from several ongoing clinical trials of EBOV

vaccine have been recently published, including IgG ELISA data. ChAd3–EBOV–Zaire vaccine administered at a dose range of 1×10^{10} – 1×10^{11} particles induced good IgG titers mainly in the highest dose group (≥ 1000 and ≥ 1500 in 90% and 60% of subjects, respectively) (Tapia et al. 2016). In the case of VSV–EBOV–Zaire vaccine, the IgG GMTs were 4079 and 1300 for the groups receiving 20×10^6 and 3×10^6 pfu, respectively (Regules et al. 2015).

In addition, one report of vaccine effectiveness ($\geq 80\%$) was published from a study that used ring vaccination with the VSV–ZEBOV (one dose of 20×10^6 pfu) during the Ebola outbreak in Guinea (Henao-Restrepo et al. 2015). Unfortunately, only limited numbers of blood samples were collected, and no correlates of protection were reported.

There are several important observations regarding available EBOV IgG data that may complicate bridging NHP data to humans:

1. IgG titers reported from human vaccine trials were significantly lower (up to 10-fold) compared with IgG titers measured in most NHP studies (Ewer et al. 2016).
2. ELISA assays used in NHP and human studies do not use identical sets of reagents.
3. Data on passive immunity: cocktails of MAbs (including ZMapp)-protected NHPs, both pre- and postinfection prophylaxis (Murin et al. 2014), but did not reach statistical significance in a randomized controlled trial in Ebola-confirmed subjects (The PREVAIL II Writing Group 2016). Post-exposure treatment of NHPs with polyclonal EBOV (or MARV)-specific IgGs provided protection from lethality at various levels (Dye et al. 2012). In contrast, transfusion of up to 500 ml of convalescent plasma in 84 patients with confirmed EBOV infection did not result in significant improvement in survival (van Griensven et al. 2016a,b).

In summary, several parameters need to be improved to make NHP models predictive of effectiveness of EBOV vaccines and for licensure under the “Animal Rule.” These include further validation of the animal challenge models,

H. Golding et al.

development of species-independent validated immunological assays, and identification of antibody titers (and/or CMI levels) reasonably likely to predict clinical benefit in humans.

HIV-1 VACCINES

Evaluation of HIV vaccine effectiveness in animal challenge models is difficult because of the fact that the virus infects only humans (with the exception of chimpanzees, which are protected from challenge studies under a moratorium). NHPs of different species infected with simian immunodeficiency viruses (SIVs) or with recombinant SIVs containing envelopes from HIV strains (SHIVs) have been used in the past as a surrogate of HIV infections. In these studies, NHPs were infected via systemic (intravenous) or mucosal (intravaginal, intrarectal) routes with either a single high-dose challenge or by repeat administration of small doses (for mucosal challenge). The level of virus replication and disease outcome varied significantly (Sharma et al. 2015; Del Prete et al. 2016; Lopker et al. 2016).

The data from early protection studies in NHPs were not predictive of vaccine efficacy trials in humans. Rhesus macaques immunized with adenovirus serotype 5 (Ad5) vectored SIV gag protein alone or as a booster inoculation after priming with DNA showed strong antigag immune response, reduction of peak viremia, and control of viremia after intravenous challenge with pathogenic SHIV 89.6P virus (Shiver 2002). In other studies, Indian rhesus macaques immunized with an Ad5-based vaccine expressing SIV gag, tat, rev, and nef proteins as a booster to DNA prime showed significant reduction in viremia after intrarectal challenge with SIVmac239 (Wilson 2006). Yet, clinical trials that used Ad5-based vaccines failed to show efficacy. Most revealing was the failure of the Merck phase 3 vaccine trial of Ad5-based vaccine expressing several internal HIV genes (MRKAd5) (STEP) in 3000 volunteers, which resulted in early termination and evidence of increased number of infections in the vaccine arm compared with the placebo arm (Buchbinder et al. 2008; McElrath et al. 2008).

A subsequent phase 2B trial (HVTN 505) of a DNA-prime followed by rAd5 vaccine regimen (containing both Gag-Pol and Env genes from three clades) in a high-risk population was also halted for futility with no vaccine efficacy (but no enhanced infection rate) (Hammer et al. 2013). There are a number of possible reasons why an Ad5 vectored vaccine that conferred protection in NHPs failed to show efficacy in humans: NHPs developed higher levels of cell-mediated immune response following Ad5/HIV and DNA/Ad5/HIV vaccination compared with humans (Bett et al. 2010); many people have been exposed to Ad5 and therefore have immunity to this viral vector; and mismatch between the vaccine used in NHPs and infecting HIV strains in vaccine recipients.

In a subsequent National Institute of Allergy and Infectious Diseases (NIAID) mini-summit, evidence was presented that the Ad5/HIV vaccine increased the frequency of Ad5-specific CD4⁺ T cells in the gastrointestinal mucosa of NHPs after rAd5-SIV vaccination, and increased SIV acquisition (Bukh et al. 2014). Furthermore, human gut biopsies of individuals vaccinated with DNA prime and rAd5/HIV boost (in HVTN 204 and HVTN 505) revealed a high number of activated adenovirus-specific CD4⁺ CCR5⁺ T cells (but not HIV-specific T cells) (Fauci et al. 2014). It was concluded that any vaccine strategy that activates CD4⁺ T cells at the mucosal site could increase risk of HIV infection (Fauci et al. 2014).

Thus far, only one HIV vaccine trial (RV144) that used a pox vector (ALVAC/HIV) prime followed by gp120 bivalent envelope (B/E) proteins boost achieved a modest 31% efficacy in a cohort of low-risk individuals (16,000) in Thailand (Rerks-Ngarm et al. 2009). Extensive posttrial studies identified a set of immunological end points that either positively (anti-V1-V2 antibodies, IgG3 subclass) or negatively (IgA antibodies) correlated with protection. No strong HIV-neutralizing antibodies or cell-mediated immunity were detected in uninfected vaccine recipients (Haynes et al. 2012; Yates et al. 2014; Zolla-Pazner et al. 2014). No preclinical studies predicted the outcome of RV144. However, recently, an NHP study using

an SIV vaccine prototype (ALVAC-SIV+SIV gp120/alum or MF59) showed delayed acquisition of SIV_{mac251} via the rectal route. No clear-cut correlate of protection was identified. However, delayed virus acquisition correlated to some degree with increased numbers of mucosal innate lymphoid cells (ILCs) that produce interleukin (IL)-17 as well as with mucosal IgG to cyclic V2. Transcriptome analysis revealed increase in RAS pathway genes that are likely to up-regulate ILC activity and natural killer (NK) cell target genes (Vaccari et al. 2016). It will be very difficult to confirm these findings in an HIV vaccine trial.

The field of HIV vaccines has recently shifted toward vaccine designs that include “native” forms of envelope trimers and sequential envelope immunogens predicted to bind the unmutated germline B-cell receptor (BCR) in naïve B cells and drive somatic mutations and selections of broadly neutralizing antibodies against conserved targets in the HIV spike. It is hoped that the new immunogens will provide broad coverage against diverse circulating strains, likely to be transmitted through the mucosal routes (Xiao et al. 2009a,b; Fera et al. 2014; Mann and Ndung’u 2015; Mascola 2015). Therefore, the ability of NHP challenge studies to predict HIV vaccine efficacy will need to be reevaluated.

VACCINE DESIGN AND MEASUREMENT OF IMMUNE RESPONSES IN NHPs: CONFOUNDING FACTORS

The vaccine candidate to be tested in NHPs should be very similar to the intended human vaccine. The use of an SIV version of the human vaccine is highly questionable, especially for envelope-based vaccines, because the gp120 envelope is significantly different between SIV and HIV. Furthermore, the putative immunoglobulin germline predecessors of highly mutated broadly neutralizing antibodies, found in some HIV patients, are different between rhesus macaques and humans (Yuan et al. 2011).

What antibody functions are most relevant for in vivo protection if no neutralization of tier 2 viruses can be shown? Antibody-dependent cell-mediated cytotoxicity (ADCC) and anti-

body-dependent cellular phagocytosis (ADCP) have been implicated in RV144. Both depend on engagement of host effector cells expressing FcRs and possibly activation of the complement system. These mechanisms may vary between NHPs and humans because of diversified FcRs and their cell-specific distribution/expression (Chan et al. 2016). They are also affected by glycosylation patterns and antibody isotypes (Chung et al. 2014, 2015; Santra et al. 2015; Tay et al. 2016). Furthermore, passively transferred polyclonal antibodies enriched for ADCC activity (from an elite controller) failed to protect macaques from mucosal challenge (Dugast et al. 2014). This and other studies illustrate the difficulties in bridging in vitro assays with in vivo protective activity (Sholukh et al. 2014; Hessel and Haigwood 2015).

CMI against internal genes are likely to be determined by species-specific antigen processing/presentation in the context of diverse HLA proteins. The internal gene sequences are not identical between SIV and HIV. NHP CMI may not predict human responses. To date, most humoral and CMI assays have not been standardized and were not shown to be species-independent. So, bridging NHP data to humans could be a major obstacle.

WHICH NHP CHALLENGE VIRUSES ARE SUITABLE FOR PREDICTING HIV VACCINE EFFECTIVENESS?

SIV Challenge Models

Indian-origin rhesus macaques are most often used for AIDS research, showing high viral loads and disease progression similar to HIV infections in humans. Although these SIV infection models could be of value in POC studies for limited vaccine prototypes, they are unlikely to be predictive of HIV vaccine effectiveness in humans, especially for the next-generation vaccines as discussed above.

SHIV Challenge Models

Most of the early constructed SHIVs contained envelopes derived from chronically infected pa-

H. Golding et al.

tients representing a limited number of clades, and did not include envelopes of transmitted viruses. Furthermore, they required multiple animal-to-animal passages in rhesus macaques to reach high-level replication and disease patterns similar to human HIV. This adaptation often resulted in changes to the envelope configurations (more open trimers), altered antigenic profile and susceptibility to neutralization by MABs, and sCD4 (Boyd et al. 2015; Sharma et al. 2015).

More recently derived SHIVs are better adapted to mucosal infection of NHPs without multiple passages in vivo. Importantly, they represent transmitted/founder (T/F) strains from diverse clades (A, B, C, D, CRF01_AE). Some new SHIVs contain specific mutations designed to improve interaction with the rhesus macaque CD4 receptor (Del Prete et al. 2014; Asmal et al. 2015; Li et al. 2016). Large stocks will need to be prepared from the new SHIVs and subjected to careful titration and evaluation of genetic homogeneity and replication rates in vivo after in vitro expansion.

Protection against repeat low-dose mucosal challenge will depend on the size of the challenge dose and the number of repeat inoculations. The most significant hurdle in predicting

vaccine effectiveness against HIV is the need to protect against heterologous viral swarms that undergo continuous evolution in HIV patients. Therefore, protection against more than one SHIV challenge in NHPs (mismatched clade/strain compared with vaccine envelope) may be required to predict vaccine effectiveness in humans.

Finally, evidence of protection in NHPs should be correlated with in vitro standardized assays, easily transferrable to human trials. Bridging data from challenge studies in NHPs to humans will rely on identifying the most reliable immunological end point and its post-vaccination levels predictive of protection from infection/disease in vivo (as was established for the anthrax vaccine). Currently, it is not expected that HIV vaccines will be approved based on the “Animal Rule.”

CONCLUDING REMARKS

The establishment of animal models predictive of vaccine effectiveness in humans has been fraught with difficulties with low success rate to date. Table 1 summarizes the current status of the animal models for the four pathogens discussed above. Each pathogen presents

Table 1. Summary of animal models for predicting vaccine effectiveness

Pathogen	Key characteristics of animal challenge model		Immunological end points/assays			Animal studies predictive of vaccine protection against disease in humans
	Pathogen challenge stock (human; animal prototype)	Disease pattern similar to humans	Correlate of protection identified	Species-independent assay available	Bridging animal data to human	
HPV	Animal pathogen prototypes (bovine, dog)	Partially	Yes	No	Yes	Yes
Anthrax ^a	Human pathogen/validated	Yes	Yes	Yes/validated	Yes	Yes
Ebola	Human pathogen	Yes	Partially	Not finalized; not validated	No	Partially
HIV	Animal pathogen prototypes (SIV, SHIV)	Partially	No	No	No	No

HPV, Human papillomavirus; SIV, simian immunodeficiency virus; SHIV, HIV strain.

^aAnthrax vaccine was approved under the FDA “Animal Rule.”

unique hurdles related to the robustness of the animal challenge model, availability of well-characterized challenge stocks, and selection of optimal dose, route, and schedule of infection. The comparability of the immune responses to vaccine immunogens in animals versus humans and establishment of “species-independent” surrogate of protection beneficial for bridging data from the animal challenge studies to human clinical trials. This is a more stringent requirement for vaccines that are likely to be approved under the “Animal Rule.” For traditional approval, the correlate/surrogate of protection could be identified/confirmed subsequent to a successful phase 3 vaccine trial.

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