Designing animal models for evaluation of therapeutic and vaccine candidates for emerging and biodefence-related pathogens

While a number of promising drug therapies and vaccines have been identified for safeguarding bioterrorism organisms and diseases, further development requires well characterised animal models for testing these candidates prior to regulatory approvals for use in humans.

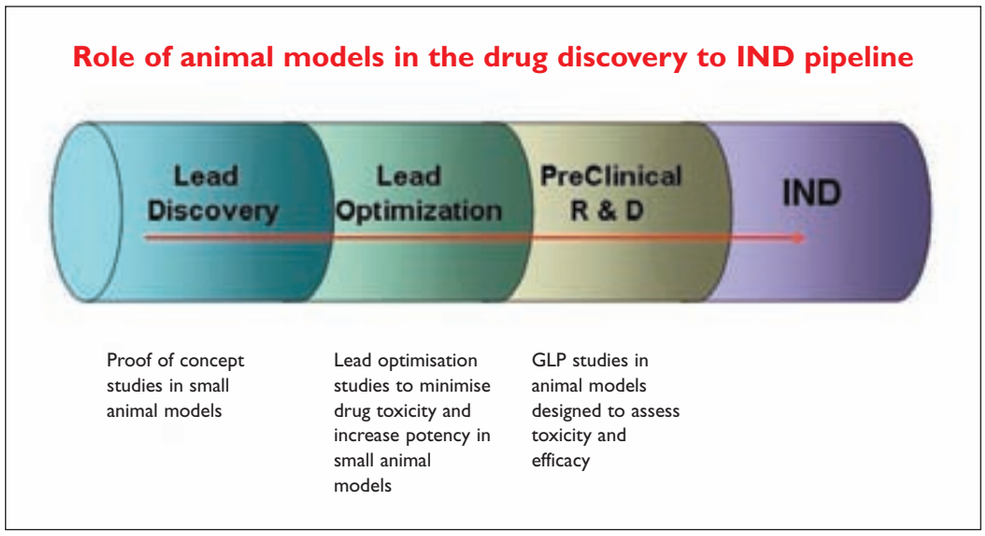
By Dr Colleen Jonsson and Dr Peter Silvera

The National Institute of Allergy and Infectious Diseases (NIAID) programme – the In Vitro and Animal Models for Emerging Infectious Diseases and Biodefense Program – provides targeted screening and evaluation of potential therapeutic and prevention modalities for emerging infectious agents and bioterrorism pathogens using *in vitro*, small animal and non-human primate models to test safety and efficacy prior to their approval for use by the US Food and Drug Administration (FDA).

The safety of biodefence-related drugs and vaccines must be evaluated in human subjects. However, human efficacy testing of such products may not be feasible or ethical, and therefore, in such instances, the 'Animal Rule' issued by the FDA in 2002 may be applied. The FDA may approve a product if human safety data has been established, and the requirements of the Animal Rule are met based on adequate and well-controlled animal efficacy studies that establish that the product is reasonably likely to provide clinical benefit in human beings. The rule can be applied if the following four criteria are met:

- There is a reasonably well understood pathophysiological mechanism of toxicity of the product and its prevention or substantial reduction by the product;
- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterised animal model for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans, generally, the enhancement of survival or prevention of major morbidity;
- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in humans and animals, allows selection of an effective dose in humans.

Figure 1
This figure represents the utility of animal models at each stage leading to an IND (Investigational New Drug)



Examples of biodefence-related pathogens for which the Animal Rule is applicable include: Smallpox, Anthrax, Botulism, Plague, Tularemia, Viral Hemorrhagic Fevers (eg Ebola) and Alphaviruses (eg Venezuelan Equine Encephalomyelitis). To highlight a few, current animal models for the development of rPA vaccines for anthrax include the use of New Zealand White rabbits and non-human primates, both rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques.

To date, the murine model appears to be most appropriate for efficacy testing of F1 and V based plague vaccines, although both cynomolgus macaques and African Green Monkeys (*Cercopithecus aethiops*) have also been used. Vaccine development efforts for Smallpox have, to date, used mice, rabbits and non-human primates where the challenge virus used for each model is different. Major efforts are under way to develop Modified Vaccinia Ankara (MVA) as a safe alternative vaccine for Smallpox using the non-human primate model. The murine model is most commonly utilised for evaluation of the Tularemia LVS (Live Vaccine Strain) and next generation tularemia vaccines, although non-human primates are also used.

In vitro and animal models promote development and testing of vaccines, therapeutics and diagnostics, while preclinical safety testing speeds the development of new generation products. In the past few years unprecedented opportunities have opened the door for innovation, and challenges, in development of these models in BSL3 containment.

Several major issues must be considered when establishing animal models for discovery and development of new therapeutics that require BSL3 laboratories, regulatory issues (eg Select Agent Rule, GLP compliance, CDC and USDA permits); personal protective equipment; and containment of animals, animal tissues and fluids (eg BioBubbles, biosafety cabinets, necropsy work stations). Additionally, the processes for work flow among the various laboratories must be thoroughly reviewed to ensure high quality results and biosafety.

As one moves through the various milestones, it is important to understand the utility of animal models at each stage leading to an IND (Investigational New Drug). One can break the process into three major phases, (1) lead discovery, (2) lead optimisation and (3) preclinical research and development (**Figure 1**).

Lead discovery

Even in the very early stages of therapeutic discovery, validation of the antiviral activity of lead candidates relies on robust, small animal models that have a well characterised natural history of the infection process of the pathogen and other key elements (**Table 1**). These types of animal models, usually mice or hamsters, are used for general screening of antiviral activity of lead candidates.

Unfortunately, for most emerging pathogens and many biodefence-related pathogens, well-characterised models are not usually available. Minimally, it is important that such models are characterised with respect to the temporal dynamics of the levels of the pathogen in the animal in the major target organs. In early screening,

additional clinical and pathology information from the animal model of the infectious disease can be informative; however, evaluation of efficacy often follows selection of the most potent therapeutic or vaccine candidates.

Regardless of the purpose of the model, screening or efficacy, working with these animals in BSL3 requires active and on-going risk assessment for containment of the pathogen. Numerous options have become available in recent years; however, each system has advantages and disadvantages. The greatest challenges lie in finding a flexible unit with appropriate airflow, one that can be used with more than one animal model, and is easy to access for daily clinical evaluations and final decontamination.

In addition to the animal screening model, there are two additional complementary activities for antiviral testing that require animal models: (1) dose range and (2) optimal route of administration of the therapeutic. The maximum tolerated dose is determined for candidate compounds using the screening model, but not infected with the pathogen. The basic premise is to extrapolate from any known *in vitro* data for the candidate and administer various concentrations of drug by at least two routes at two concentrations to determine the effects of treatment.

Table 1: Key parameters in development of an animal model for an infectious disease

1	Infectious does
2	Route of infection
3	Virus adaptation
4	Animal age
5	Sex
6	Natural history of infection
7	Animal rule

Lead optimisation

With the recent two-animal ruling by the FDA for the licensing of drugs or vaccines directed against

Table 2: Evaluatory end points for screening and efficacy animal model

1	**CLINICAL**	
		Symptoms
		Chemistry
		Haematology
		Morbidity and mortality
2	**IMMUNOLOGICAL**	
		Humoral response
		Neutralisation response
		T-Cell response
3	**HISTOPATHOLOGICAL**	
4	**VIROLOGICAL**	
		TCID50
		QRTPCR

diseases of low or no incidence, the ferret represents an inexpensive small, non-rodent animal model for evaluating efficacy. The ferret is attractive for pulmonary research studies because of the long trachea, large lung capacity and bronchiolar branching. This has been particularly evident in influenza and SARS CoV research in our laboratories. The two-animal rule states that a therapeutic may be licensed if they meet two criteria. First, the therapeutic should show adequate protection in a challenge of infection in two species of animals. Secondly, the agent must be shown to be safe in humans.

To evaluate efficacy, one desires a comprehensive set of analyses with well defined end points (**Table 2**). Considerations for development and standardisation of animal efficacy models include: (i) species selection, (ii) challenge strain and dose, (iii) route of exposure, ie parenteral, eg subcutaneous, intravenous versus respiratory, ie intranasal, intratracheal or aerosol, (iv) clinical endpoints, ie mimic human disease, (v) challenge strain and dose affects, (vi) protection against multiple challenge strains, (vii) statistical issues – this is particularly challenging when using non-human primates since cost and available often preclude large study group sizes, and (viii) label indication, ie one needs to decide if the product is to be used for pre or post-exposure prophylaxis. At this stage it is important that standard operating protocols are in place for each end point and each of the assays are qualified. Further, technical personnel are expected to qualify on the assays.

As mentioned earlier, one of the major challenges in conducting the efficacy studies often lies in the sheer number of animals required to obtain statistically significant data for each of these end points. In effect, the containment of the animals in the BSL3, and the number of samples taken at each necropsy will often limit the number of animals one can employ. The transition of the efficacy model to GLP for preclinical research stage will require the involvement of study coordination, quality assurance and quality control personnel. Often, these persons are new to working in biocontainment and so adequate consideration needs to be made in biosafety training.

Validation of appropriate animal model systems are key to the provision of efficacy data that will support licensure of the product under investigation. As stated previously, the requirements of the Animal Rule are met based on adequate and well-controlled animal efficacy studies. Therefore, studies must yield reproducible endpoints, i.e. outcome of challenge in naïve animals with a predetermined dose, route and infectious strain should be predictable. The preparation and route of administration of the infectious agent should be standardised and consistent to allow for data comparisons between studies. In order to bridge animal efficacy data with human data, validated assays must be used. Furthermore, the model should be transferable between testing laboratories allowing for comparable results to be generated using identical protocols.

Early phase efficacy studies are crucial for evaluation of proof of concepts and humoral- and cell-mediated immunogenicity data of candidate vaccines. Promising vaccine candidates are further evaluated in animal models to provide additional immunogenicity data, defining dose range and vaccine regimen including efficacy studies. Passive immunisation studies would also be performed in attempts to elucidate immune mechanisms of protection. Animal immunogenicity data is generated with validated immunological assays, and used to bridge human immunogenicity. Together, with animal efficacy data, including data generated from passive immunisation studies, correlates of vaccine-elicited immune protection can be identified. Finally, definitive or pivotal vaccine efficacy studies are required to be conducted under GLP to provide supporting data for licensure of vaccines.

Preclinical research and development

Before a new drug or vaccine entity can be approved by the FDA for administration to humans, the developer must demonstrate not only

that it is efficacious for its intended use, but also that it exhibits an acceptable margin of safety within the dose range, the route and pattern of administration, and the target population expected in the clinical setting. Such demonstration of safety includes testing of the drug in appropriate *in vitro* and animal models to show that the drug has no unacceptable genetic, reproductive, immunologic, cardiovascular, neurologic, or general toxicity, as well as demonstrating the kinetics of absorption, distribution, metabolism and excretion of the drug and its metabolites.

Safety testing is performed using standard animal models and study designs that have been accepted by the FDA and other international regulatory agencies. Initial testing will include single-dose range-finding (RF) studies in rodents (eg, rats or mice) and large animals (eg, dogs or primates) to determine the maximum dose that can be administered by the intended clinical route without producing serious and irreversible toxicity. These studies evaluate a minimum number of animals and endpoints, and the results are used to select dose levels for subsequent safety studies.

If the intended clinical dosing regimen is expected to involve administration of multiple doses, the singe-dose RF studies will be followed by multiple dose RF studies using the anticipated clinical dosing regimen. Finally, the doses that are selected based on the results of the RF studies will be used to conduct definitive safety studies for each drug in each species. The latter studies include a larger number of animals and evaluation of multiple parameters of toxicity, and are performed in strict compliance with the FDA Good Laboratory Practice regulations.

Phase I, II and III clinical trials – providing safety, dose ranging, immunogenicity and efficacy data in support of a Biologics License Application – lead to Phase IV, manufacturing and eventual lot release. The Pre-IND Phase I studies include model development, proof-of-concept and early immunogenicity studies. Immunogenicity studies would continue into Phase II developing bridging data, performing dose-ranging studies, developing vaccination regimens and conducting preliminary challenge studies. Challenge studies should continue through Phase II including the development and validation of assays and associated equipment. During Phase III, using the final formulation, definitive or pivotal efficacy studies would be conducted under 21 CFR Part 58 GLP regulations utilising fully validate assays and associated equipment allowing for bridging of animal and human immunogenicity data.

Summary

Conventional human vaccines are subject to multiple stages of review and regulation required for an IND application. Animal models are needed to ensure development and testing of vaccines, therapeutics, and diagnostics for emerging and biodefence-related pathogens, and should be conducted alongside clinical studies at each stage of investigation. Discovering and developing novel therapeutics to thwart disease and outbreaks is of paramount concern. In designing well-constructed animal models to test safety and efficacy, we take a thorough approach prior to regulatory approvals for use in humans. DDW

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