

MANUFACTURING VIRUS FOR HUMAN CHALLENGE STUDIES

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The Viral or Human Challenge Model (HCM) is increasingly being considered by those wishing to accelerate the development of novel agents in the treatment of infectious diseases. The challenge of infectious agents with vaccine or drug *in vivo* offers early access to mode of action (MOA) and proof of concept (POC) data prior to designing later, larger phase field trials.^{1, 2}

Although safety data may be limited due to low cohort numbers, the effect of metabolites and ADME parameters on drug efficacy, subject symptomology and viral dynamics can add value to pharmacokinetic and pharmacodynamic data (PK/PD). Such data may be crucial in early screening and the up / down selection of therapeutic candidates.

ADDRESSING MARKET NEEDS

Dose response may be characterised through SAD / MAD studies in the presence of the target organism in healthy volunteers emulating complex PK-biomarker-clinical endpoint models in patients. Where patient models may give rise to ethical or access issues, human challenge testing in HV populations can provide high-quality longitudinal data with known inoculation and recovery time-points.

The HCM offers a new paradigm to those requiring sequential sample analyses to investigate the differential role of biomarkers in disease progression and can be used to identify new therapeutic targets in both subjects and virus.³

A wide range of organisms have been used as challenge agents in academic studies: viruses (Dengue), bacteria (*Salmonella typhi*) and malarial parasites (*Plasmodium falciparum*). However, to be approved for use in clinical trials, organisms are generally accepted to have to

conform to current Good Manufacturing Practice (cGMP) and be manufactured accordingly.⁴

Market pressures for novel therapeutic agents stem from clinical need (symptomology) versus comparative risk (epidemiology). Those infectious conditions with a high index for both severity of disease and a potential for rapid distribution may become targets for governments and industry alike. Within this context recently emergent threats such as ebola-Zaire and reassorted influenza viruses (e.g. H5N1) have commanded intense interest and attracted specific funding.⁵

Antigenic drift and shift in influenza may rapidly give rise to global health issues owing to its mode of infection. The role of accelerated research programs using influenza virus challenge agents may thus prove attractive where viral escape requires industry to bring flu drugs and vaccines rapidly to market.

CHOOSING THE RIGHT AGENT

The choice of any specific influenza strain as a challenge agent may be based on a number of factors: prevalence, symptomology, attack rate, availability of isolates and cost of manufacture. Since 2009 the main circulating influenza viruses have been stable at a ratio of 60 / 20 / 20 percent (H3N2 / H1N1 / Flu B):

- H3N2 (Influenza A/Texas/50/2012 (H3N2)-like virus)
- H1N1 (Influenza A/California/7/2009 (H1N1)pdm09-like virus)
- Influenza B (B/Phuket/3073/2013 and B/Brisbane/60/2008-like virus)

Choosing the optimal viral challenge agent requires a long-view as to likely longevity of pathogens in society relative to their effects on health. Recently a new circulating H3N2(v) A/Switzerland/9715293/201-like virus, has emerged and may become the predominant H3N2 flu virus in the Northern Hemisphere for a prolonged period.

As mentioned, the manufacture of virus e.g. influenza, for use as a challenge agent in clinical trials should conform to cGMP. This advice is based upon previous experience that suggests that both the dose ranging and the POC studies (in which the virus and a vaccine or antiviral are administered) are likely to be considered as a 'clinical study' (Directive 2001/20/EC) and that according to Eudralex Volume 10 (guidance on IMPs and NIMPs, revision 1, March 2011), the inoculating virus may be classified as NIMP in the European Union. Therefore to ensure a clinical trial utilising a challenge agent is approved in the EU and reciprocally in the US, the appropriate cGMP requirements should be applied during manufacture and reflected in the IMPD or IND item 7.

OPTIONS FOR MANUFACTURE

The manufacturing process for viruses may vary between species according to their target, host cells or receptors. Respiratory Syncytial Viruses (RSV) may be cultured only in cell lines (BEAS, HEp2, HELA, CAP, AMKC). However, influenza viruses may be grown both in embryonated eggs from specific pathogen-free (SPF) flocks or in AGE1.CR or Madin Darby canine kidney (MDCK) cell-lines. There are advantages and disadvantages to both approaches – embryonated eggs may select for lower-lung epitopes in influenza virus⁶ and aspirates may contain high levels of antigenic ovalbumin. Cell lines may become infected with Mycoplasma or other adventitious agents and have been associated with low yields. However, regulations governing cGMP stipulate the same overall approach to production with standards based upon proofs of the purity and safety of the final product.

Typically, seed-virus may be acquired from the community or from institutes holding stocks for research and manufacture. Virus from the community has the advantage of known origin and thus possible symptoms and sequelae but approved, institutional stock may be well characterised through passage in cell lines e.g. for vaccine manufacture,

and have antiserum available for use in adventitious agent testing. Live virus usually requires specific import and export licensing to ship across borders e.g. from the supplier to the client or manufacturer. Licensing may take several weeks to progress (e.g. USDA permits) and consideration should be given to including antiserum for the target virus on the permit as well as other products that may be required for future use.

POST-MANUFACTURE ANALYSES AND CHARACTERISATION

Once received at the manufacturing site there may be a requirement for pilot studies on limited numbers of eggs or in the target cell line to investigate infective titres and subsequent harvest counts. Infectivity may vary widely between isolates and may be associated with attenuation, due to extended passage in communities or previous amplification and purification rounds, the presence of defective infective particles (DIP)⁷ or contamination with other organisms e.g. *Mycoplasma* spp.

Following identification of the optimal infective titre and completion of the Master Batch Records, the main manufacturing round may take place in a designated clean room. Inoculation of up to 300 eggs may produce ~2.5 to 3.0 L of pooled harvest over 3-4 weeks. The seed virus for inoculation is usually taken from the passage pool generated during the pilot studies. Harvest of the challenge stock (e.g. allantoic fluid) is followed by clarification using low speed centrifugation. Cell line harvests (e.g. two-stage bioreactor) may take place continuously or as a final extract at 17 days post-infection.⁸ In both cases, the viral harvest will be formulated (TCID₅₀, viral titre, HAI), filtered, aliquoted and flash frozen in liquid nitrogen for storage at ≤-70°C prior to further analysis.

The detrimental effect of freeze-thaw cycles on viral titres is well documented⁹, therefore aliquots of the harvested virus should be created early to allow for dissemination of samples to sites for

adventitious testing without recourse to thawing the bulk product. Assessing the purity of harvested virus is essential prior to use in healthy volunteers for both ethical and scientific reasons. Impure, contaminated or co-infected inoculum may produce adverse effects in subjects and may lead to unplanned illness and adversely affect the safety and proof of concept (POC) data.

Adventitious testing should reflect the likelihood of a specific pathogen to have contaminated the seed stock or clinical sample and the types of organism that may be associated with the source subject or amplification medium. Testing may encompass a wide range of agents, from human pathogens (e.g. Human Parainfluenza Virus Type I and II) to organisms associated with the culture medium (e.g. Avian Sarcoma Leukosis Virus (ASLV)). Adventitious testing usually takes several months owing to complexity of the assays (co-culture, RT-PCT, PERT) and prolonged incubation periods (e.g. *Mycobacterium* spp.).

Once the concentration and purity of virus has been established as part of the Release Testing program, the relative infectivity and pathogenicity of the challenge stock must be established in an animal model. Traditionally ferrets (*Mustela putorius furo*) have provided the best approximation to human disease.¹⁰ Ferrets make good mammalian models owing to their small size, lung physiology, emulation of human symptomology following infection and the presence of sialic acid receptors in the respiratory tract. Pre-clinical studies in ferrets provides *in vivo* safety data to the challenge stock Investigators Brochure (IB), typically consisting of symptomology scores, viral load counts, cytokine analyses and autopsy observations (tissue damage, compartmentalisation of virus, immune-activation).

Although the viral inoculum may be approved for use by the relevant authority following review of the IB, IMPD / IND CMC data, use of cGMP challenge stock should not be considered until a charac-

terisation study has been performed in healthy volunteers.

Characterisation studies typically involve low numbers of subjects (36-48) inoculated with a range of viral titres (10⁵-10⁷) to assess optimal infectivity. Attack rates may be defined as: detecting virus in nasopharyngeal swabs; defined symptomatology (fever, cough) and/or seroconversion >14days following challenge. Characterisation trials also allow for the identification of virally as opposed to drug or vaccine associated adverse events. Such

studies require specialised premises and staffing to prevent cross-contamination between subjects and staff and subjects / staff and the community.

In summary – the Human Challenge Model is recognised as a useful early phase tool for assessing safety, proof of concept and mode of action in drug and vaccine trials. Challenge agents may comprise a number of pathogens previously proven in academic studies. Authorisation for use of specific viral challenge agents in clinical trials is

associated with a high burden of proof. Such agents may be classified loosely as non-IMP. Where virus is to be used in dose escalation or proof of concept studies, the regulatory authorities advise that the challenge stock be manufactured to cGMP standards. cGMP manufacture is associated with high complexity, high cost and may take 12 to 18 months from identification of source virus to application in a challenge trial.

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