

REGULATORY REQUIREMENTS FOR VIRAL-CHALLENGE STUDIES: INFLUENZA CASE STUDY

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In virus challenge studies, healthy volunteers are administered a pathogenic or virulent strain of virus. Such strains can be attenuated viruses that produce a much milder set of symptoms compared to the naturally occurring or fully active virus. If the volunteers are administered an investigational drug (e.g., antiviral, vaccine, immunomodulatory drug) besides inoculation with the virus, the studies are called viral-challenge studies.

In a historical context, the concept of challenge studies is not new. The experiments conducted by Louis Pasteur in the 19th century, where chickens were challenged with a weakened bacteria causing chicken cholera and immunized from further chicken cholera infection, can be seen as a type of challenge study. In the early 20th century, scientists used a self-challenge approach when developing vaccines and drugs

Viral inoculation studies have been performed in the United Kingdom since 1946 when the Medical Research Council established the Common Cold Unit (CCU) (also known as the Common Cold Research Unit [CCRU]) at Salisbury, Wiltshire^{1,2}. The aim was to undertake laboratory and epidemiological research on common colds in view of reducing human and economic costs. Common colds account for a third of all acute respiratory infections and the economic costs are substantial in terms of days off work. The volunteers were infected with preparations of corona- and rhinoviruses and were housed in small groups of two or three, with each group strictly isolated from the others during the course of the stay. In 1989, the CCU closed down after failing to find a cure.

In current clinical research practice, the use of viral-challenge studies as proof-of-concept (POC) studies is gaining wider acceptance. Healthy volunteers are inoculated with a challenge strain of a virus, usually influenza, and administered a vaccine or antiviral before or after the inoculation. Although viral inoculation studies can be performed with a wide range of viruses, this article will focus on respiratory viruses and influenza in particular.

VIRAL-CHALLENGE STUDIES

The vaccine or antiviral first goes through a complete non-clinical development program to assess its safety and efficacy. Afterwards, it goes through a full Phase I, first-in-man, pharmacokinetic (PK) and tolerability study.

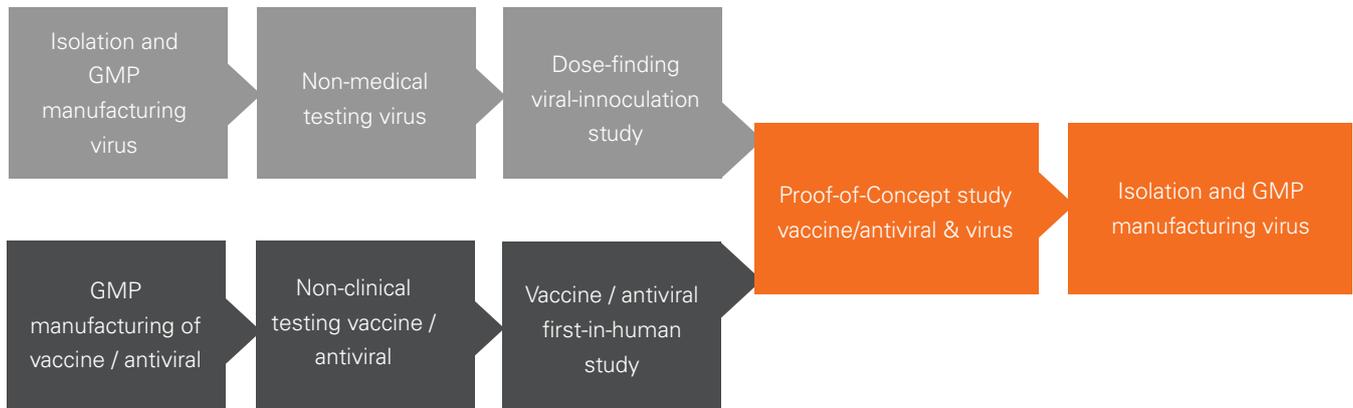
The influenza virus is isolated by a combined nasal/throat swab from an ill patient. An aliquot of this clinical sample is then used to inoculate specific pathogen-free eggs (SPF), which are grown through sequential passages. Another option is to grow the virus through a cellbank. The virus is then manufactured under GMP standards and ensured that

it is free of adventitial agents and other pathogens. A non-clinical program with the virus is then initiated (see Figure 1).

To establish the correct viral dose for the viral-challenge POC study, in which the vaccine or antiviral drug will be administered, a dose-finding study in humans must first be conducted. The virus is administered to the study subjects using intranasal drops. The viral dose to be selected for the viral-challenge POC studies is the dose at which 80% of the inoculated volunteers show clinical symptoms. After the phase I study with the vaccine or antiviral and the viral-inoculation dose-finding study, sufficient information is available to start the POC studies.

Two possible study designs are used for the POC studies—treatment and prophylaxis. In treatment studies, the volunteers are screened and randomized. They are first inoculated with the challenge strain and after a given incubation period, the investigational vaccine/antiviral or placebo treatment will be initiated (either to both symptomatic/asymptomatic or to symptomatic volunteers alone). Patients are quarantined during the study if indicated, depending on the type of inoculum³⁻⁵.

FIGURE 1: DEVELOPMENT FLOW OF VIRUS AND VACCINE OR ANTIVIRAL UP TO VIRAL-CHALLENGE PROOF OF CONCEPT STUDY



In prophylactic studies, the volunteers are screened and randomized. They first receive the investigational vaccine/antiviral, or placebo treatment, followed by inoculation with the challenge strain. Patients are quarantined during the study if indicated, depending on the type of inoculum. Depending on the indication, study considerations, and objectives, one of the two designs, or a combination of both, may apply.

Pharmacodynamic (PD) endpoints in challenge studies usually include measurements, such as clinical respiratory symptoms, nasal discharge weight, and quantitative measurements of viral shedding, and/or cytokines in nasal washes.

Challenge studies can provide useful exposure-response and safety information and the opportunity to demonstrate pharmacological activity in humans under controlled conditions. Data from challenge studies contribute to dose selection for Phase IIb and Phase III studies, and provide the opportunity to explore the effects of different times of drug initiation relative to virus exposure. Challenge studies can be used to assess a first efficacy.

REQUIRED QUALITY INFORMATION ON THE VIRUS

The influenza virus to be used first needs to be isolated from a patient showing clinical signs of infection with

the virus. The virus is usually isolated by nasal/throat swab. Given that virulence decreases with age, the virus is usually isolated from young patients. The reduced risk of co-infection and a better-defined medical history also make young patients the preferred host to isolate the virus from. In addition to evaluating acute respiratory illnesses of the patient and his family members, information on current and past medical history, travel history, and social history should be systematically recorded. The sample should be tested by reverse transcription-polymerase chain reaction (RT-PCR) for the presence of the desired virus.

To avoid co-infection of the sample, the sample needs to be screened for the presence of other viruses. In the case of isolation of an influenza virus, the sample should also be tested by PCR for human rhinovirus, respiratory syncytial virus (RSV), parainfluenza virus types 1, 2, and 3, human metapneumovirus, and adenovirus, and the results should be negative. Plasma samples from the patient should also be negative for human T-cell leukemia virus 1 and 2, human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), HIV ribonucleic acid (RNA), hepatitis B surface antigen (HBsAg), hepatitis B deoxyribonucleic acid (DNA), anti-hepatitis C virus (HCV) antibody, HCV RNA and Hepatitis A virus. The patient, from whom the influenza virus has been isolated, will be followed to assess that he remains in good health.

An aliquot of this clinical sample will then be used to inoculate SPF eggs that are then grown through sequential passages or using mammalian cells. The number of passages will depend on the required infectious virus titre for preclinical (i.e., ferret model) and human testing. The virus should be manufactured according to GMP requirements.

NON-CLINICAL TESTING

Non-clinical testing of a virus requires both in-vitro and in-vivo pharmacology studies. In-vitro pharmacology in cellular-based assays includes in-vitro infectivity, antigen characterization, and susceptibility to antiviral agents.

The in-vivo non-clinical testing is usually performed in ferrets. Ferret models (*Mustela putorius furo*) have been established for numerous viruses that cause respiratory infections, including human and avian influenza viruses, coronavirus, nipah virus, and morbillivirus among others. Ferrets are an appropriate mammalian model for these studies because they show numerous clinical features associated with human disease, such as fever, lethargy, and sneezing. In addition, sick ferrets have the ability to infect healthy ferrets. Ferrets and humans share similar lung physiology, and human and avian influenza viruses exhibit similar patterns of binding to sialic acids (i.e., the receptor for influenza viruses), which are distributed throughout the respiratory tract in both species. Furthermore, their

small size eases the logistic burden.⁷⁻⁹

During the in-vivo pharmacology studies, infectivity and safety are tested. Temperature and body weight changes, clinical observations (e.g., sneezing), and infectious viral load are monitored. Safety pharmacology studies are not needed and no formal toxicology is needed if the profile of the virus corresponds with the characteristics described in the literature. No reproduction toxicology studies are needed if appropriate contraceptive measures (i.e., double barrier method: chemical and physical contraception) are used in the clinical studies.

The non-clinical testing of the vaccine or antiviral to be used in the viral-inoculation study should be performed according to standard non-clinical testing requirements described in the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.¹⁰⁻¹²

REGULATORY REQUIREMENTS FOR STUDIES

The regulatory requirements for viral-challenge studies in humans can be divided in two parts, the requirements for the studies and the requirements for the infrastructure (Phase I units) where these studies are performed. In general, given that the concept is rather new, very little guidance exists around this type of viral-challenge/inoculation studies. There is no guidance on this subject provided by the European Medicines Agency (EMA) or any national European authority. An FDA guideline on the development of influenza drugs briefly mentions this type of study¹³.

In the viral dose-finding study, healthy volunteers are inoculated with a virus to establish the dose for the POC study. The dose-finding study is usually an open-label study in which several cohorts of volunteers receive an ascending dose of the virus until the optimal dose is found. The optimal dose is the dose that has the appropriate safety and illness/

infectivity profile to be used as an influenza virus challenge strain in future challenge studies. For each strain, only one dose-finding study needs to be done. The strain (in the optimal dose) can then be used in multiple challenge studies.

Whether or not this study can be considered a clinical study in the strict sense remains a question for discussion. Because the objectives of the stand-alone experiment are not in line with the definition of a clinical study as described in Directive 2001/20/EC article 2 (a)¹⁴, the classification as a clinical study can be challenged, and the study is not considered a clinical study as per Directive 2001/20/EC.

Viral-challenge studies, in this case, would be regarded as an "experiment," which will only require approval from the ethics committee and not from health authorities. Such studies, however, should be approached with care because although the European directive does not consider viral-challenge studies to be a clinical study, national legislations may not agree and may consider them as clinical studies.

Furthermore, there is the risk that the dose-finding study may have to be repeated if performed without health-authority approval, especially if the chemistry, manufacturing, and controls (CMC) data of the virus are considered insufficient by the health authority at the time of submission of the POC study. It is, therefore, highly recommended that dose-finding studies are considered as clinical studies.

The POC study (in which the virus and a vaccine or antiviral is administered) will in any case be considered as a clinical study according to Directive 2001/20/EC. The virus, however, does not need to be considered an investigational medicinal product (IMP) because it does not match the definition of an IMP given in Directive 2001/20/EC, Article 2 (d) (14) and the Guidance on Investigational Medicinal Products (IMPs) and Non Investigational Medicinal Products (NIMPs) (15)

If an inoculating virus is used to evaluate the efficacy of an investigational product, the inoculating virus is classified as a "condition" and not as an "intervention" (i.e., the disease or the health issue worth studying in a clinical study according to FDA classification at ClinicalTrials.gov¹⁶ According to Eudralex Volume 10, guidance on IMPs and NIMPs, revision 1, March 2011¹⁵, the inoculating virus could be classified as NIMP in the European Union. In this case, the inoculating virus is a "challenge agent." A challenge agent by definition is usually given to study subjects to produce a physiological response that is necessary before the pharmacological action of the IMP can be assessed. A challenge agent may be a substance without marketing authorization, but could have a long tradition of clinical use.

Information regarding the quality of the inoculating virus should be provided in the non-investigational medicinal product dossier (NIMPD). There is no standard format available for presenting the information regarding an inoculating virus; however, guidelines on the requirements for quality documentation concerning biological investigational medicinal products in clinical studies, EMA/CHMP/BWP/534898/2008¹⁷ could be used as a reference.

THE QUALITY INFORMATION

regarding an inoculating virus can be presented in the sections 2.1.S, 2.1.P, and 2.1.A of the NIMPD (18) similar to the quality documentation requirement for IMPs¹⁴ reference?). It is not necessary to provide extensive information, similar to a biological drug used for marketing authorization. The NIMPD should mainly focus on the quality attributes related to safety aspects, considering the state of development or clinical phase. Appropriate GMP requirements should be applied¹⁹⁻²¹

The quality part of the NIMPD should include information related to the quality,

manufacture, and control of the NIMP. It is preferable to present data in tabular form accompanied by a brief narrative highlighting the main points. The information that should be provided include virus isolation, manufacturing process, control of materials, including master cell bank and working cell bank systems, control of inoculating virus (i.e., quantity, identity, and purity), analysis, and stability, among others. In section 2.1.A.2 of the NIMPD (adventitious agents safety evaluation²², information assessing the risk with respect to potential adventitious agents and other human pathogens contaminations should be provided²³.

Another important issue is the regulatory and operational aspect of running the viral-challenge study itself. It is extremely important to avoid cross-contamination between the patients infected with the virus on one side and the study staff on the other side. Furthermore, a back-up plan to treat a patient with an antiviral agent or other drugs should be available if he or she becomes too ill after the challenge. This issue is equally true for the virus dose-finding study as for the challenge study together with the vaccine. The aim is to avoid the virus from being spread to the "outside world" and to avoid infected study staff to infect the patients and jeopardize the study results by infecting placebo patients.

Patients will need to be isolated in a

specifically designed quarantine unit and will be treated according to the principle of "reversed-barrier nursing." This method is comparable to barrier nursing used in an intensive care unit (ICU) setting, where the aim is to keep pathogens away from the ICU patients by creating a barrier between the outside world and the inside of a patient room by using gloves, masks, gowns, and disinfectants. With reversed-barrier nursing the aim is to keep the challenging agents confined in the facility using the same principles.

CONCLUSION

Recently, viral-challenge studies have become widely accepted as POC studies to demonstrate the efficacy of antiviral and vaccine therapeutics for RSV, influenza, and other common cold viruses. The regulatory framework for this type of studies has not been fully developed. The exact regulatory requirements for viral-challenge studies need to be discussed with the health authorities of the country where the study will be performed.

Due to their nature, viral-challenge studies can only be performed in specialized clinical pharmacology units. Conducting these studies in a controlled quarantine environment allows for a superior study design, which is more cost-effective. This approach critically accelerates the selection of a safe and effective dose and dosing regimen for a new antiviral drug or

vaccine because it allows for early detection of efficacy. It, therefore, lowers the risk of a negative outcome when performing a large field-based Phase III study.

Viral-challenge studies are performed under tightly controlled circumstances, and such studies do not seem appropriate to replace large field-based trials in "real-life" circumstances. In this respect, the use of viral-challenge studies in the framework of vaccine or antiviral drug development needs to be discussed further with regulatory authorities.

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