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Regulatory workshop on challenge strain development and GMP manufacture – A stakeholder meeting report

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ABSTRACT

Within the Innovative Health Initiative (IHI) Inno4Vac CHIMICHURRI project, a regulatory workshop was organised on the development and manufacture of challenge agent strains for Controlled Human Infection Model (CHIM) studies. Developers are often uncertain about which GMP requirements or regulatory guidelines apply but should be guided by the 2022 technical white paper "Considerations on the Principles of Development and Manufacturing Qualities of Challenge Agents for Use in Human Infection Models" (published by hVIVO, Wellcome Trust, HIC-Vac consortium members). Where those recommendations cannot be met, regulators advise following the "Principles of GMP" until definitive guidelines are available. Sourcing wild-type virus isolates is a significant challenge for developers. Still, it is preferred over reverse genetics challenge strains for several reasons, including implications and regulations around genetically modified organisms (GMOs). Official informed consent guidelines for collecting isolates are needed, and the characterisation of these isolates still presents risks and uncertainty. Workshop topics included ethics, liability, standardised clinical endpoints, selection criteria, sharing of challenge agents, and addressing population heterogeneity concerning vaccine response and clinical course. The organisers are confident that the workshop discussions will contribute to advancing ethical, safe, and high-quality CHIM studies of influenza, RSV and C. difficile, including adequate regulatory frameworks.

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Table of abbreviations

ABS	Access and Benefit Sharing
API	Active Pharmaceutical Ingredient
CDMO	Contract Development & Manufacturing Organization
CHIM	Controlled Human Infection Model
CMC	Chemistry, Manufacturing, and Controls
CRO	Contract Research Organisation
EC	Ethics Committee
EVI	European Vaccine Initiative
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HCT	Human Challenge Trial
HRA	Health Research Authority
HTS	High Throughput Sequencing
IABS	International Alliance for Biological Standardization
ICF	Informed Consent Form
IHI	Innovative Health Initiative
LBV	Live Bacterial Vaccines
LUMC	Leiden University Medical Centre
MCB	Master Cell Bank
MVS	Master virus seed
NGS	Next-Generation Sequencing
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PCR	Polymerase Chain Reaction
QC	Quality Control
QP	Qualified Person
(R)EC	Research Ethics Committee
RG	Reverse Genetics
RSV	Respiratory Syncytial Virus
(S)AE	(Serious) Adverse Events
WCB	Working Cell Bank
WHO	World Health Organization
WGS	Whole Genome Sequencing

1. Introduction

Controlled Human Infection Model (CHIM) studies can help study disease pathogenesis and immune responses [1] and contribute to the development of approved vaccines, such as the RTS,S vaccine against Malaria [2,3]. CHIMs have become relevant for numerous pathogens, including SARS-CoV-2 [4,5], influenza, respiratory syncytial virus, and others [6], and are crucial components in developing preventive and therapeutic approaches, especially for vaccines. CHIM studies use pathogens (challenge agents) for infecting healthy volunteers in a controlled fashion; therefore, it is paramount that care is taken to minimise the risk to volunteers through careful selection, isolation, development, stability assurance, and production of the challenge agent [7], as well as the availability of rescue treatments [8,9]. Good Manufacturing Practice (GMP) guidelines are designed to ensure that biological products are manufactured to a minimum set of standards. However, as challenge agents are not classified as medicinal products, it is currently unclear which and whether GMP requirements or guidelines apply. Thus, standardised regulations are urgently required.

The European Vaccine Initiative (EVI), in collaboration with partners Sciensano and the International Alliance for Biological Standardization (IABS), organised a stakeholder meeting within the framework of the Inno4Vac sub-project CHIMICHURRI entitled: “Regulatory Workshop on Challenge Strain Development and GMP Manufacture”. The event was held on 4 October 2022 in Florence, Italy, and brought together Inno4Vac members and scientific and regulatory experts to discuss the progress made and regulatory challenges encountered in selecting, developing, and manufacturing challenge agents for influenza virus, Respiratory Syncytial Virus (RSV), and *Clostridioides difficile* CHIM studies. These discussions will allow CHIMs to be positioned in a well-defined regulatory framework and contribute to a roadmap for integrating CHIMs into vaccine development.

2. CHIM development white paper

Wim Van Molle (Sciensano) presented a summary of the technical white paper independently developed and published in January 2022 by hVIVO, the Wellcome Trust, and the HIC-Vac consortium members on the quality considerations for challenge agent production for use in CHIM studies [10]. The white paper presents basic principles for the selection, characterisation, manufacture, quality control and storage of challenge agents. This paper is of great value to developers of CHIM challenge agents, especially as the 2017 World Health Organization (WHO) paper outlining regulatory considerations for human challenge trials does not provide guidance on the manufacture or quality control of the challenge stock. The WHO document only states that “its quality should be comparable to a candidate vaccine at the same clinical trial phase” [11].

According to the white paper, developers must consider various aspects of challenge agent selection, characterisation, and production when establishing a CHIM study. The challenge agent chosen should be representative of the current circulating pathogen, should aim to elicit manageable symptoms, and should have a rescue treatment in place. Preclinical, clinical, regulatory, and Chemistry, Manufacturing, and Controls (CMC) characteristics are also critical components when choosing an appropriate challenge strain and are well described in the publication. Once isolated, quality considerations (including identification and monitoring of Critical Quality Attributes) must be applied to manufacture the challenge agent. A banking system should be established to store all challenge agents and be used as the basis for new batches for future production. The agent must be well characterised, including the full genome, potential resistance genes, stability (including genomic stability), purity, potency, and benefit and risk. Manufacturing processes require a quality control strategy to cover all steps in the process. Other considerations include technology transfer, a quality management system, qualified personnel, equipment, facilities, distribution, transport, and documentation.

The most significant concern to most developers is the question of the applicability of GMP guidelines to the production of challenge agents and what needs to be done if specific recommendations of the white paper cannot be met. Questions are also raised about whether a challenge agent can be manufactured under GMP at all, given that GMP certification applies only to medicinal products. Additionally, from a regulatory, safety and quality point of view, the question arises as to what the risk would be if a challenge agent was “non-GMP”. For example, if the candidate isolation, the banking system, and drug substance manufacture were all done in a non-GMP environment, but the product manufacture was carried out under GMP conditions, this approach would be similar to first-in-human trials or clinical trials in general, where the GMP qualification is adapted according to the advancement of the phase. The risks and what would be acceptable to regulators need to be defined. All stakeholders should have a shared understanding and must devise a better general system.

2.1. Clostridioides difficile (C. difficile): Introduction and GMP production challenges

Wiep Klaas Smits (Leiden University Medical Center (LUMC)) presented the current approach and challenges encountered for *C. difficile* challenge strain selection, characterisation and GMP manufacturing.

C. difficile is a bacterial pathogen transmitted by spores via the faecal-oral route. The spores are crucial for transmission because, as an anaerobic organism, it does not survive otherwise outside the hosts. Many *C. difficile* strains are resistant to antimicrobials. Currently, three antimicrobials are indicated for treatment (metronidazole, vancomycin, and fidaxomicin), but relapses are still common (30 %–60 %), and epidemic lineages are known to cause more severe disease [12–14].

The manufacturing approach starts with using clinical material with carefully documented provenance and adhering to good clinical practice (GCP) standards. However, one of the first challenges encountered in challenge strain production is growth media selection for *C. difficile* isolation. In research laboratories, *C. difficile* is traditionally isolated on blood plates because they give robust growth. However, according to the principles of GMP, animal-based products present a risk, e.g., of bovine spongiform encephalopathy (BSE) transmission, and should be avoided as much as possible.

For the strain selection process, as a qualified reference laboratory, the LUMC has banked over 2000 strains, and this collection was used as a starting point to perform strain selection. Together with a group of experts, criteria for the most appropriate strain were established, and the bank was screened for strains with profiles complying with these criteria. Criteria included the ability to cause disease but reduce the risk of outbreaks, robust growth, sporulation under laboratory conditions, and full genomic characterisation. The purification of spores poses a CMC challenge as spores can persist in production facilities, and commonly used density gradients give a risk of chemical contamination being carried downstream into the product; thus, there is a need for alternative methods.

Regarding administration, there are no established methods for toxigenic *C. difficile* strains. However, previous studies administered non-toxigenic strains as a spore suspension in a beverage [15,16]. Although a natural route of administration, it does not allow tight control over the number of viable cells reaching the gut. Capsules offer a more controlled option.

The search is ongoing for a CRO willing and able to undertake GMP production from master cell bank to spore-filled capsules. The proposals received are costly, with current offers at about 1.7 million Euros. Concerning production timelines, it is also important to realise that these companies operate on a first-come, first-served basis; this could impact decision-making and the production timeline for challenge agents within the project.

2.2. C. difficile: GMP release standards and quality control (QC) release criteria

Oleg Krut (Paul-Ehrlich-Institut (PEI)) presented the *C. difficile* GMP release standards and QC release criteria. There is no fixed regulatory framework for CHIMs or challenge agent production. However, the existing framework for Phase I clinical trials of live bacterial vaccines (LBV) can be a sound basis for a CHIM regulatory framework. LBVs and challenge agents share common attributes: live bacteria/spores are a key component, the route of administration (e.g., oral, inhalation, uro-mucosal), and the mode of action is to induce infection in both cases. The risk profile is also comparable since small batches are produced, and only a small cohort will receive the challenge agent. In short, CHIMs resemble Phase I clinical trials, and if these similarities are accepted, existing regulatory assessment logic can be used. Both potency and safety attributes for challenge agents can be derived from the already established LBVs (e.g., typhoid or cholera vaccines), and the European Pharmacopoeia (Ph. Eur.) regulatory framework can be used as guidelines.

It is essential that challenge agents are representative of wild-type clinical strains, well-characterised, susceptible to treatment, and do not induce highly aggressive infections. These attributes should also be controlled at the production stage so that the production process does not influence these critical parameters. The implementation of GMP for LBV is accomplished later in LBV development, in Phase II or III clinical trials. In comparison, the corresponding roadmap for challenge agents remains unclear. Will there be one preparation, with no further development? Or will there be a chance to implement the missing GMP features at a later stage? The risk profile will differ as more individuals become exposed to the challenge agent. It is necessary to ensure proper production; this is where GMP is instrumental.

Some quality attributes essential for release criteria should also be checked: stability, potency, identity, and microbial purity of the drug substance. Whole-genome sequencing (WGS) using next-generation sequencing (NGS) is a prominent identification method suitable for strain and MCB characterisation. However, since it is not a pharmacopoeia method, it requires qualification or validation; this process may present a challenge. In both early and later stages (including the drug substance/drug product stage), more robust and compatible methods like PCR ribotyping, PCR testing, etc., could be implemented as guidance exists and validation of these methods is quite clear. The absence of phages in the strain candidate is critical for genomic stability. Genomic instability harms production and production facility and can also impact virulence. Hence, caution is advised in the strain selection process.

Bacterial viable counts can be used for potency, but the dose variation and the acceptable limit of spores for the drug substance must be defined. Toxin secretion is a critical feature that needs to be confirmed if it is present and controlled. Toxin secretion is variable depending on, for example, the medium and culture conditions. However, ensuring it has not changed during the scale-up process is feasible. It should be defined at strain selection and reconfirmed at the later stages.

Regarding safety, microbial purity is critical. Since the administration route for *C. difficile* is oral, the accepted Ph. Eur. method guidelines (Ph. Eur. 2.6.12 and Ph. Eur. 2.6.13) can be used [17]. Encouragingly, many of the attributes are already there, and although some points still need to be discussed, challenge agent production is on the right track.

2.3. C. difficile: Microbiological endpoints

Maria Vehreschild (University of Cologne (UHC)) presented a key point of discussion related to the development of a *C. difficile* CHIM. Currently, the model aims to show colonisation, but the problem is establishing a definition of *C. difficile* colonisation in healthy volunteers based on microbiological criteria only. The literature on colonisation is limited, and for study design, it is essential to have a proper definition from the start, which can be taken along from one study to the next, thus making study comparisons easier.

The suggestion was that there should be independent tests indicating the presence of the *C. difficile* strain. Possible options include a glutamate dehydrogenase (GDH) positive test, which generally covers non-toxigenic strains, a specific in-house PCR, or another PCR test for non-toxigenic strains. These tests should be performed on samples taken at least 72 h apart to rule out transient colonisation. The faecal samples should be taken from different bowel movements, and there should be a positive culture to determine the identity of the isolate. These aspects would constitute the minimum criteria for colonisation to be present.

Feedback from attendees showed that the criteria used in different studies vary considerably. However, the overall perception is that multiple time points over a more extended period are necessary. One suggestion was to perform PCR testing on samples from multiple time points and identity confirmation on the first and last samples. An intermediate solution would be to develop a PCR test specific exclusively to the challenge strain, allowing for PCR testing and identity confirmation in a single step. That would, however, depend on whether it is

possible to develop a PCR specific enough for the challenge strain, which cannot be known until the full genome sequence of a representative panel of similar strains is available.

It was agreed that a step-by-step, i.e., staged approach to developing the model is best, and looking at a microbiological endpoint is a good start because the microbiological versus clinical endpoint is critical from a regulatory perspective, a vaccine manufacturer perspective, and an ethics perspective. The initial study cohorts will provide some information about this colonisation endpoint. Then, re-evaluation will help determine the next steps to obtaining symptomatic disease, including dose escalation.

2.4. Feedback from regulators on the C. difficile CHIM

Workshop participants, presenters and regulators further discussed key issues raised in the *C. difficile* presentations. These discussions are summarised below.

2.4.1. Strain selection: Screening for virulent strains without the capability to produce fulminant disease

There are two qualifying points to consider. First, the lineage of the strains is a key feature. Generally, the hypervirulent strains fall into specific clades and clade 1 strains, such as RT014, usually cause mild disease. The second qualifying point would be the presence or absence of binary toxin, where presence is associated with more severe disease. Eliminating hypervirulent clades and expression of binary toxin carriage in challenge strain selection minimises the chances of fulminant disease.

The opposite problem is ensuring the strains are sufficiently virulent to cause disease. The current challenge strain contains toxins A and B, necessary for disease, and was recently isolated from a hospitalised patient with *C. difficile* disease. It is unknown whether it can induce disease in a (young) healthy population. However, given that the disease is predominantly present in the elderly population, the age of study volunteers needs consideration. The CHIMs must be capable of managing the level of disease.

2.4.2. The study population and clinical vs. microbiological endpoints

The desired study population needs clarification. A considerable percentage of the population (varies by country and study, approximately 5–10 %) is commonly colonised without disease. The question arises whether the population should include susceptible individuals and/or whether participants would need to be made susceptible by pre-treating with antibiotics, for example, and the associated risks and benefits. It is also critical to establish if the target is a clinical endpoint that may occur only in a specific population or if microbiological endpoints are acceptable. Ideally, the endpoint would depend on the model's intended use (e.g., therapeutic, prophylactic, or identifying toxigenicity in the population). In Phase II trials, microbiological endpoints are desirable. In-depth discussions with stakeholders are required to develop a meaningful clinical trial protocol and endpoints, taking a cautious stepwise approach going from colonisation to symptomatic disease and using this as a guide for potential dose escalation or consideration of further procedures, such as pre-treatment.

2.4.3. How should the developing disease be managed in volunteers?

Notably, CHIM studies will not replicate full-blown *C. difficile* colitis; nevertheless, rescue treatments such as antibiotics are necessary, underscoring the need for challenge strains to be susceptible to treatment modalities. Faecal transplant is also an authorised treatment method for the treatment of recurrent *C. difficile* and needs to be considered. In most cases, *C. difficile* infection causes self-limiting diarrhoea; in the context of a CHIM, investigators will not wait for volunteers to develop severe symptoms before giving treatment, as this impacts the perceived safety risks of such a CHIM. Halting criteria and rescue treatments are to be clearly defined.

2.4.4. The role of CHIM studies: Can they replace field trials?

The current issue for debate with regulators is whether it is still ethical to conduct a field trial if a CHIM study provides convincing efficacy data. Unless conducting a field trial is not feasible or extremely difficult, it is unlikely that CHIM trials will be the pivotal clinical trials leading to the licensure of a product. It would also be difficult to convince regulators to use CHIM data to make the final licensure decision for a disease that is reasonably common in certain populations. The intention is to develop CHIMs that are useful for various applications at an earlier stage, such as selecting potential treatments and learning more about the biology of the host-pathogen interaction to develop or select products. For treatment testing, symptomatic infection that can be safely achieved is more likely to be representative of the natural disease. However, there are still many uncertainties, and the advice is to remain receptive to all possibilities. Then, as more data are gathered about the model characteristics and the selected strains, the goal for the CHIM can be refined while keeping all safety considerations at the forefront.

The early stages of CHIMs could also be used to understand why some individuals get infected, and others do not; is there an immune or microbiome signature? This information may not directly apply to testing a future product but is valuable in understanding disease pathogenesis. A non-toxigenic challenge study is underway at LUMC to collect preliminary data profiles on susceptibility, etc., to provide a starting point for toxigenic models. The message is clear: New approaches and possibilities must be considered to develop better vaccines. It is also important to note that the scope can impact CMC expectations. The above-described preliminary data will influence the filing and the GMP vs. non-GMP expectations.

2.4.5. Principles of GMP: Regulators' expectations

Regulators would expect GMP to be applied for starting materials (e.g., MCBs and working cell banks (WCB)). However, because no active pharmaceutical ingredient (API) is being manufactured, there is no possibility of a GMP license in many jurisdictions. In this case, it is best to use the "principles of GMP", i.e., to apply all the rules that would be used for GMP. For *C. difficile*, a recent isolate can be used as the starting material and worked into an MCB. Further, if only a limited number of challenge agent batches will be produced, it will be sufficient to have only an MCB; this is also accepted for clinical trial materials.

2.4.6. Contract development & manufacturing organization (CDMO) experience and manufacturing costs

Researchers expressed concern that most CDMOs lack experience in culturing anaerobic bacteria, and the high costs of the service are often prohibitive. Therefore, are CDMOs appropriate for the task, are the costs justifiable, and how critical is GMP vs "principles of GMP"? A quality system and qualified personnel are undoubtedly needed, and while a CDMO may have the dedicated facilities and GMP licence, their lack of experience means the product may not be better than if agents were made in-house by experienced and qualified persons, according to "principles of GMP". For example, the non-toxigenic challenge study underway at LUMC has material released by a qualified person (QP), and production is not done in a GMP facility but follows GMP principles.

From a regulatory perspective, assigning a responsible person (the QP) and ensuring that the manufacturing process is well-documented and adheres to GMP principles can offer additional assurance for the quality of the material being released. Thus, regulators may be more willing to accept these conditions even if the challenge agent cannot be manufactured under official GMP as it is not defined as a medicinal product/API. However, this should be discussed beforehand with the competent regulatory authorities so as not to jeopardise the acceptability of the final product.

Concerning the CDMO costs, researchers need to consider how development costs relate to the total cost of the trial. Manufacturing costs can typically be negotiated. If a GMP facility is required by regulatory agencies, with minimum GMP requirements clearly defined, this

can influence the costs.

2.4.7. Acceptability of next-generation Sequencing (NGS) for validation and safety

Concerning NGS as a means for WGS, there is currently no corresponding chapter in the Ph. Eur., but this may change in the future as the Ph. Eur. Commission has created a new Working Party at the European Directorate for the Quality of Medicines & HealthCare (EDQM) level for the elaboration of a new general chapter for the detection of extraneous agents (chapter 2.6.41) [18]. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q5A guideline for evaluating viral safety will also be updated to include NGS [19]. Therefore, in the future, NGS will likely be accepted in regulatory files documenting challenge agent validation and safety. Whole-genome sequencing (WGS), including short- and long-read sequencing, may be required to determine genome stability and gene order.

2.5. Influenza & RSV update on sourcing of virus swabs/isolates

Othmar Engelhardt (Medicines and Healthcare products Regulatory Agency (MHRA)) summarised the outcomes of two previous workshops on influenza strain selection where it was determined that for this project, an H3N2 virus would be used, and discussions centred on the choice of H3N2 virus based on antigenic and genetic characteristics. Using a past rather than a current virus was decided because its natural history would be known. However, viruses should not be older than 5–10 years to reduce potential biosafety risks associated with using a very old strain. Viruses should be chosen from non-predominant clades or antigenic groups with less than 50 % population immunity. Population immunity is important for the safety and feasibility of a CHIM. Three virus groups were chosen as candidates: 3C.3b, 3C.3a, and 3C.2a1b.2a.1. Other criteria for the final choice of challenge strain were that viruses should induce influenza-like illness, different from some previous challenge viruses that induced disease only weakly, and that the virus had to be susceptible to rescue medication (antivirals). Cell culture is generally preferred for producing the challenge agent because egg production, though possibly easier, often leads to adaptive changes that can change virus antigenicity. The experts favoured wild-type viruses over viruses generated by reverse genetics (RG) for several reasons, notably the stringent regulatory hurdles in some countries. At present, obtaining viruses that can be taken forward into production of a CHIM challenge agent is a big issue. One year into the project, no definite candidates had been identified that could be moved into production. The main challenges seemed to be ethics, liabilities, other legal issues and potentially access and benefit sharing legislation. Assessing the growth characteristics of candidate viruses in various cell lines was the next step, but it would be best to do this on viruses that have a chance of being chosen for production.

Christopher Chiu (Imperial College London (ICL)) summarised the same issues around RSV. In many ways, RSV is more straightforward. Unlike influenza viruses, RSV has limited strain diversity. The two RSV subtypes (A and B) are distinguished primarily by their differences in the F and G surface proteins. The F protein is largely conserved, but contemporary strains (ON1 and BA) contain G protein duplications.

Previous RSV workshops determined that a B strain was preferred over an A strain, provided it could grow *in vitro* to sufficiently high titres (10^5–10^6 pfu/ml) before moving on to GMP manufacture. However, it was decided that both A and B isolates should be collected and characterised to give more options. Any strain since 2015 was more likely to be acceptable since no significant differences have been observed since then. It was also agreed that the GMP challenge agent should be a wild-type virus, i.e., grown in culture from a clinical swab of a primary isolate, because of uncertainties about RG virus performance in other studies. However, RG molecular clones will be generated as laboratory tools and could be a backup if the wild-type virus strategy fails.

Regarding the current state, there has been minimal RSV circulation for the last two years, making it challenging to collect fresh isolates. A collaboration was formed with the University of Utrecht, which has a collection of RSV swabs from several years. A panel of challenge agent candidates was identified from this collection, full characterisation is ongoing, and legal contracts on sharing the virus were initiated. However, obtaining local ethics approvals has caused significant delays.

The plan for strain characterisation for clinical safety and performance includes many steps: firstly, *in vitro* growth characteristics and cytopathic effects must be studied to weed out strains that would not perform well in GMP manufacture and to prevent unexpected downstream differences in clinical characteristics. Observing the replication kinetics is particularly important for RSV B strains. A selection of the best-performing strains will then proceed to sequence analysis to ensure no mutations are introduced by laboratory amplification. These sequence data will be used to ensure that the strains are representative of circulating strains for continued relevance. A selected number of strains will be studied in primary human bronchial epithelial cells, as they are the most representative *in vitro* model of human airways. Lastly, it is essential to check the quantity of defective interfering particles. These particles with viral RNA cannot replicate but can induce immunity and block infections if they occur in too high a quantity.

2.6. Influenza and RSV challenge agent selection and manufacture

Adrian Wildfire (CHIMunomics) elaborated on the influenza and RSV challenge agent selection and manufacture.

A previously produced influenza H3N2 challenge strain (A/Belgium/4217/2015 (H3N2)) was based on a paediatric nasal sample. The chosen strain belonged to clade 3C.3b, had relevance to other circulating strains or strains used in vaccines at the time, and showed sensitivity to neuraminidase inhibitors (oseltamivir). In the pilot study, the virus could be cultured on MDCK cells. Further selection of the optimal seed stock was based on patient clinical information, phylogenetic relatedness to the Northern Hemisphere H3N2 vaccine candidate strain (clade 3C.3; A/Switzerland/9715293/2013), and the replicative capacity of the cultured material in embryonated eggs. The virus seed stock obtained after two MDCK passages was further passaged once through embryonated eggs. Both the cell culture-propagated seed stock and the egg passage were inoculated into embryonated eggs from two different sources and infectious virus titres after harvest informed the GMP manufacture. Ferrets were inoculated intranasally with 6.76x10^6 TCID50/mL (dose1) or 2x10^7 TCID50/mL (dose2), and all became infected with high viral loads in the nose and throat. Pathology findings were typical of mild to moderate respiratory tract inflammation with minimal lung lesions.

Various adventitious agents were tested for with sequence testing. However, as there is no specific regulatory guidance for adventitious agent testing, it is imperative to discuss with regulatory authorities their expectations. Performing a risk-benefit analysis and adapting or designing the testing regimen to tackle the risks of contaminants is also necessary. The screening of the manufactured product was done similarly to what is described in the guidance for vaccines. However, as the product was not a vaccine, adapting the adventitious agent testing was necessary. Vaccine guidance is good, but it is not precisely what is needed.

For RSV, suitable isolates from hospitalised infants were collected and screened. Seven RSV isolates were identified, and three with the best clinical profiles progressed to the WCB level. In deciding on the most suitable recovery cell lines, three were identified as likely candidates based on previous studies and tissues of origin: MRC-5, HuH-7 and A549. The A549 cell line proved most effective for recovering the candidate RSV isolates. MRC5 was not advanced due to poor performance characteristics with the chosen RSV candidate primary samples, but it was later used for the MCB/WCB.

The GMP RSV stock later passed the complete range of testing

tailored to MRC-5. Testing was based on US-FDA and industry guidelines, scientific advice, and pilot studies [20–23]. In the biosafety testing scheme, NGS was deemed acceptable for many tests, including mycobacteria. The virulence assay also showed that the chosen isolate was highly virulent, and genomics confirmed the amino acid duplication typical of recent ON1 strains. For the characterisation study, cotton rats were infected at three different doses. Macroscopic findings were relatively minor, and the degree of shedding was very high. Regarding the characterisation trial, the study was designed and consolidated in a protocol and ICF. The outcomes of the animal studies guided the study design, and it was also premised on the J. DeVincenzo challenge trial model [24]. Unfortunately, the characterisation trial never materialised as it was deemed too expensive to carry out. One batch failed due to a contamination event, which showed that it is essential to be very careful working with CDMOs with insufficient experience in the field. Characterisation of a second batch by a commercial partner was planned but has yet to happen. All the animal experimentation, sequencing, and other testing show it is a very effective virus.

2.7. Influenza & RSV challenge virus and legal aspects, ABS (access and benefit sharing)

Christopher Chiu (ICL) presented the following questions on legal aspects surrounding challenge agents for discussion.

2.7.1. What are the legal requirements for obtaining informed consent for using the original swabs or isolates for challenge agent manufacture?

In the context of just the EU, multiple jurisdictions have varying requirements and practices. In other locations, for example, in some institutes in the UK, the Informed Consent Form (ICF) for a challenge study may be required to include language to obtain informed consent for using these viruses for challenge agent manufacture, including commercialisation. However, many viruses are collected without this specific intention. It is unclear in which instances generic consent may be sufficient and in which instances a special separate ICF is required, also stating it has potential commercial purposes. If the patient withdraws their consent, it should also be made clear that their original sample can be destroyed. However, once it is made into a challenge virus, it becomes a new product, and patients no longer have a claim over it. Official guidelines guided by legal expertise are necessary to make the process easier for future researchers.

2.7.2. Institutional liability: Are there any risks for potential donors?

Institutions asked to supply original viruses for sponsors to culture and eventually use in clinical studies may have concerns about possible liability. In such instances, the potential donors should be covered in their contract, and all liability falls on the sponsor. The problem is that some donors decline because they still feel some liability and are concerned that their names are associated with the virus should anything go wrong. Another point is that the challenge agent is currently not seen as a medicinal product; thus, minimal official regulatory information exists. However, involving the relevant (national) competent authorities is highly recommended before running a study. Local approvals may be required to import a challenge strain, and, in most instances, the regional authorities will lack expertise in CMC or challenge models. In Belgium, for example, it is advisable to have the federal agency give scientific advice on how the challenge agent was produced (be it either fully GMP or adhering to "GMP principles"). The sponsor then presents that report to the regional authorities. Ultimately, guidelines must indicate the steps to be considered, depending on the specific country, before importing or using a challenge agent.

2.8. Influenza & RSV regulatory aspect of RG challenge virus

Christopher Chiu (ICL) presented the following question on the regulatory aspects of RG viruses for discussion.

2.8.1. Would an RG approach to influenza be acceptable across Europe? What criteria would justify the use of an RG molecular clone?

One option to bypass the problems associated with obtaining influenza samples is to use RG platforms to manufacture what is essentially a wild-type virus using a genetic sequence. RG has been used extensively in United States (US) challenge studies, but acceptance in the EU may be problematic given the different rules in different jurisdictions. For example, in the Netherlands, GMO applications must address safety and the potential risks of releasing a GMO into the environment. If the GMO is identical to a circulating virus, this is a good argument to support acceptance. In the UK, applications are managed and assessed by local GMO committees. Typically, they are for contained-use studies where participants will be quarantined, and the GMO material is not expected to be released. Risk mitigations remain necessary. In Belgium, assessments are also done at a regional level, and previous experience has shown that much discussion is needed to inform about GMO safety. In general, if it can be demonstrated that the GMO product is not more severe or dangerous than what is already circulating, then it could be accepted.

As an alternative to taking older viruses from other parts of the world or using RG, it was suggested that waiting for the coming flu season to collect swabs, with the appropriate informed consent, may be worth considering. GMP virus characterisation and production is a significant investment. While collecting new isolates during a flu season might be easier, characterising them will require significant effort and present additional risks and uncertainty about which isolate to take forward into production. Other groups are taking the approach of having a continuous pipeline of collecting contemporary strains, e.g., hVIVO, and it may be worthwhile to try to encourage sharing.

2.9. Feedback from regulators on Influenza & RSV CHIM models

Regulators addressed the following questions:

2.9.1. Is it necessary to have both an MCB and a WCB?

Whether both are necessary depends on the amount of material that will be produced. It is up to the CHIM developer to decide, but so far, regulators have been satisfied with trials having only an MCB.

2.9.2. What substrate should be used for influenza isolation?

Cell culture is preferable to eggs. Although egg production may be easier, it usually leads to adaptive changes that can change virus antigenicity.

2.9.3. Is it necessary to use a validated cell line for virus isolation and production?

Regulators accept that older viruses taken from various laboratories would not likely have been grown on validated cell lines but instead on lines used for diagnostics or amplifying viruses. For screening candidates for isolation, a non-validated line could also work. However, once production of the challenge agent to be used in patients begins, a validated cell line is recommended. The benefit is that all the requisite safety testing is already done, including adventitious agent screening, and there is documentation to support that. Alternatively, a cell line could be developed, screened (for adventitious agents) and validated, but this requires much time, effort, and money.

2.10. Influenza & RSV challenge agent manufacture – NIH approach

Chelsea Lane (National Institutes of Health (NIH)/the National Institute of Allergy and Infectious Diseases (NIAID)) shared the NIH approach to the influenza virus strain selection.

The antigenic cartography method was used to inform strain selection [25]. Strains had to represent current strains, not historic strains that are antigenically different from circulating strains. Viral isolates were also pre-screened for hemagglutination inhibition (HAI) assay

titres of less than 1:40 for serum from recently infected or vaccinated individuals. The RG-A/Texas/71/2017 (H3N2; clade 3C.3a) strain was selected and manufactured for the first dose-finding study. RG-A/Arkansas/08/2020 (H1N1pdm09; clade 6B.1A.5a.2 N156K, K209 M) is the next strain, for which manufacturing is planned, and the goal is to release this GMP material by March 2024.

Regarding challenges and future considerations, the 3C.3a strain selection process up to the release of GMP material took several years to complete. The cell-based approach was employed to circumvent egg-based adaptations, and the lack of MCBs available for virus propagation also proved challenging. At the time, cell lines were limited to sHEK-293 or Vero cells; consequently, NIH is now supporting development of an hCK line (a modified MDCK cell line) [26] and MDCK MCBs for future manufacturing needs. The RG approach was also unsuccessful for some H1N1 viruses, and plaque purification of viral isolates is being considered as an alternative.

Daniel Stoughton (NIH/NIAID) continued with an overview of the manufacturing process for human challenge material for the Phase I-Phase II GMP grade.

The proposed plan is to manufacture one lot of influenza virus challenge strains to Phase I-Phase II GMP grade. For these phases, validation requirements are minimal; only safety assays and no performance runs are done. The goal is to make material specifically for Phase I and II clinical studies. The aspirational goal is to take clinical isolates and, if possible, expand them only in well-characterised and fully validated MCBs and WCBs. However, clinical isolates may be expanded in other cell lines and then transitioned into MCBs and WCBs by limiting dilution or plaque purification to manufacture a master virus seed (MVS). When MVS plaque purification is performed, NGS is used to check sequences for adventitious agents and to identify variants that may or may not appear during the passaging process. The NIAID/DMID team is trying to identify other cell lines and understands that high-titre doses may require very high viral production levels. If high production levels cannot be attained, a concentration step with tangential flow filtration is needed. A pilot run is usually done at half or quarter-scale. The pilot or engineering lot is used for non-clinical evaluations of pathotypes, various animal models, and antiviral susceptibility testing.

For the H3N2 process, RG was used instead of a clinical isolate. Plasmids were transfected into a HEK-293 MCB, and the virus isolate was expanded. Then, a pre-MVS was made and processed with all the requisite testing. The first GMP manufacturing run was completed in 2021, and the second in June 2022. Having substantial material also made it possible to carry out stability and dilution studies to support use in the clinic.

Catherine Luke (NIH/NIAID) shared the status of the dose-finding challenge study for the reverse genetics-derived A/Texas/71/2017 (H3N2) clade 3c.3a influenza virus study (NCT04978454) [27]. This study was conducted under the Collaborative Influenza Vaccine Innovation Centres (CIVICs) program at two sites in the United States. It began in September 2021 and was completed in September 2022. Data analysis is ongoing.

The study was an adaptive dose-escalating design conducted in healthy adults aged 18–45 years. Participants were pre-screened for and selected based on HAI titres of 1:40 or lower against the challenge virus. Three virus dose levels were evaluated: 10^4, 10^5, and 10^6 Median Tissue Culture Infectious Dose (TCID50). The initial challenge virus material stock titre was approximately 2 x 10^6 TCID50/ml, thus limiting the maximum dose level that could be evaluated. The target attack rate was 55–80 %. There were no serious adverse events. However, an increase in the frequency and severity of systemic and respiratory symptoms was observed at increasing dose levels. The most commonly reported symptoms were fever, severe headache, malaise, stuffy nose, sore throat, and sneezing.

The primary objectives centred around defining the optimal infectious dose. The attack rate was defined based on a combination of qualitative PCR positivity using a commercially available respiratory virus panel, along with symptoms score, which was a clinician-administered symptom survey. The secondary objectives included safety through day fifty-seven and observing serum antibody levels at baseline and post-challenge. Results from primary and secondary endpoints will be posted on clinicaltrials.gov in late 2023 [27].

3. Influenza & RSV CHIM models – round table discussion

The NIH team received questions from the panel about their future strategy for generating new challenge strains and sharing strains outside the US. Together with the consortium, the NIH hopes to harmonise which strains are available to avoid being redundant and, where possible, to share these strains across the groups. Currently, the NIH is committed to making new strains as needed; however, this will largely depend on the availability of funds. Funding will be assessed regularly, but sharing strains, if possible, will certainly help from a funding perspective.

Concerning sharing challenge strains, the process is still in its infancy. How a mutual exchange will be evaluated and initiated has not yet been decided. From the NIH perspective, it is vital that the sites using these challenge strains have a unit up and running and are familiar with running human challenge studies. As mentioned in previous discussions, issues around liability still need to be adequately addressed. Nonetheless, the goal is still to share strains, cell banks, or cell lines for growth and propagation with the extramural community. The NIH group is also considering sharing GMP protocols, and the idea is to have a generic set of protocols be made available or published.

Chris Chiu shared the strategy used by another consortium for the SARS-CoV-2 challenge model. An alpha strain and a delta strain were manufactured to GMP. The group committed to the Wellcome Trust to make the delta virus freely available to global investigators if applicants could show that they could safely conduct a study, had the local regulatory approvals in place, and that the study adds to global knowledge. To assess these criteria, they set up an international access management group of experts with experience in human challenge studies and an understanding of the research environment in their localities. The committee will review applications and decide whether to release the virus. Regarding matters of liabilities and intellectual property, the only requirement is that the sponsor of the clinical study fully takes on the liability. The consortium is still looking into establishing a similar process for the challenge strains discussed in this workshop. The NIH team also agreed that this strategy aligned with their thinking.

The NIH team addressed technical questions about using plaque purification in manufacturing. It was clarified that plaque purification is only performed if clinical isolates are used rather than RG systems. The isolates typically received may have been expanded once in a non-GMP cell line. Once the clinical isolates enter the GMP process, NGS for adventitious agents is performed. However, if that is not possible, the isolate is expanded in an MCB or WCB, followed by three rounds of plaque purification as the first step to bringing the material into the GMP world. In short, plaque purification bridges the non-GMP to the GMP. Other researchers have encountered difficulties acquiring clinical isolates with the right antigenic characteristics. From the NIH experience with coronavirus challenge strains, one issue is that the cell lines used by the manufacturer of the viral strains are not very good or are not GMP-compliant. The NIH is trying to develop a panel of cell lines for original testing and to see if they could eventually be used to produce enough material to work out production in a GMP cell line. This process would probably rely on limited dilution or plaque purification to narrow the gap between non-GMP and GMP.

The final issue pertained to ethics reviews and ethics committees' (EC) attitudes toward CHIM studies. Across Europe, there are wildly divergent attitudes towards this type of research. As a potential solution, the consortium plans to bring together international ECs to discuss issues, share expertise, and ultimately try to harmonise the assessment of challenge studies across different jurisdictions [28]. The workshop

participants agreed that, in their experience, many ECs have little to no experience with CHIMs; consequently, there are very divergent opinions. There should be a dialogue between the ECs and the scientists to inform and discuss what challenge studies involve and the related risks. Education and exchange are essential steps to understanding the methodology and different ethics review processes. This will allow ECs to make informed recommendations and ultimately harmonise processes.

In the UK, the Health Research Authority's approach to the SARS-CoV-2 challenge was based on the WHO Working Group recommendation [29] to set up a specific ethics committee with specific training and a diversity of members, including challenge study experts and non-experts. The UK had the advantage of having a central coordinating organisation to help facilitate the strategy, but promoting a similar engagement across the different European jurisdictions might prove more challenging. The regulators present offered to put the consortium in contact with the relevant competent authorities in their countries. Soon, the consortium hopes to bring the most qualified committees together in a workshop to discuss the UK experience and ethics aspects and ultimately facilitate harmonising the review processes across Europe [30].

4. Conclusions

The discussions during the meeting led to several recommendations with respect to GMP manufacturing and CMC requirements for *C. difficile*, influenza and RSV challenge agents. For all challenge agent manufacturers, the hVIVO, Wellcome, and HIC-Vac white paper "Considerations on the Principles of Development and Manufacturing Qualities of Challenge Agents for Use in Human Infection Models" is an invaluable resource, providing guidance on the minimum requirements for high-quality, safe manufacture of challenge agents.

Discussions around potential GMP vs non-GMP manufacturing approaches and the acceptability to regulators were critical points. Given that there is no fixed regulatory framework for challenge agent production, the advice is to follow the principles of GMP until definitive guidelines for CHIM are available. It is important to address all issues pertaining to safety with the current testing regimes. This includes ensuring that the pathogen is manufactured in a process where it is unlikely that changes occur in the pathogen or pathological elements are introduced, which may affect the study participants. The product can be released to GMP quality by a QP, who can assure that the principles of GMP were followed. Acceptance criteria for the product should be discussed with the competent regulatory authorities before development and manufacture.

Participants concluded that wild-type viruses were preferred to those produced via RG platforms for influenza and RSV strain selection. However, researchers face multiple issues in obtaining virus samples. Ethics, liabilities, and legal concerns around virus sharing are the main challenges. Participants expressed some uncertainty around the informed consent language requirements when collecting virus isolates and indicated that official guidelines informed by legal and ethics experts are necessary. Such guidance would make it easier for researchers to understand and fulfil the requirements for obtaining informed consent for swab collection for challenge agent manufacture.

If RG is used in development, having the GMO products accepted poses another hurdle. There is greater reluctance across Europe than in the US, and even if the RG virus is identical to a circulating one, it is still treated as a GMO and implications and environmental regulations apply for contained use and deliberate release. The multiple jurisdictions and rules within Europe are problematic for a harmonised approach. In general, if it can be demonstrated that the GMO product is not more severe or dangerous than circulating strains, then gaining regulatory acceptance may be realistic.

The workshop concluded that sharing virus isolates would be necessary, but addressing concerns around donor liability when sharing isolates is another challenge to overcome. Consideration should also be given at the international level for import requirements. Ultimately, generic guidelines should be written indicating the steps to be considered, depending on the specific country, before bringing in or using a challenge agent. The NIH hopes to harmonise with the consortium on what virus strains are available and, where possible, to share these strains and cell banks and cell lines across the groups. From the NIH perspective, precisely how a mutual exchange will be evaluated and initiated has not yet been decided, and liability aspects remain to be adequately addressed. Sharing of the GMP protocols is being considered by the NIH group, with the idea being to ultimately create a generic set of protocols that can be made available or published. As was done for the SARS-CoV-2 challenge, the consortium is still looking into establishing an international committee of experts who will manage the sharing process by setting criteria, reviewing applications, and deciding whether to use a shared virus.

The consortium will continue to seek ethics committee guidance on CHIM studies. Participants agreed that providing education and guidance on CHIM studies and their potential risks is a necessary first step toward improving regulation, applicability, and acceptance. The consortium hopes to facilitate ethical thinking and harmonisation of the review processes across Europe and has activities planned to drive this forward.

The workshop participants concluded that the discussions and reflections were constructive and that several challenges must be addressed. However, they remain confident that this step-by-step approach of openly discussing matters with all stakeholders will ultimately improve CHIM studies, their position in disease prevention and treatment, and the respective regulatory frameworks, not just within Europe but globally.

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