



Regulatory workshop on standardisation of clinical procedures, endpoints and data robustness of human challenge studies – A stakeholder meeting report

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ABSTRACT

Inno4Vac, a public-private partnership funded by the IMI2/EU/EPPIA Joint Undertaking (IMI2 JU), brings together academic institutions, SMEs, and pharmaceutical companies to accelerate and de-risk vaccine development. The project has made significant strides in the selection and production of challenge agents for

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Controlled human infection studies
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influenza, respiratory syncytial virus (RSV), and toxigenic *Clostridioides difficile* for controlled human infection model studies (CHIMs). A regulatory workshop held on March 20, 2024, addressed the standardisation of clinical procedures, ethical considerations, endpoints, and data integrity, highlighting the ongoing initiatives related to these CHIMs. Key discussions focused on refining trial protocols to balance statistical power with participant burden, overseen by a data safety monitoring board. The meeting emphasised the importance of harmonizing CHIM protocols to ensure robust, reproducible, and transparent research. Mandatory trial registration and adherence to the Findable, Accessible, Interoperable, and Reusable (FAIR) data principles were recommended to enhance data reuse and scientific value. This report consolidates efforts to standardise CHIM protocols, essential for accelerating therapeutic innovations and advancing global health research.

Table of abbreviations

ADCC	Antibody-Dependent Cellular Cytotoxicity	ICP	Immune Correlate of Protection
AE	Adverse Events	IgA	Immunoglobulin A
CDI	<i>Clostridioides difficile</i> Infection	IgG	Immunoglobulin G
CHDR	Centre for Human Drug Research	IMI2	Innovative Medicines Initiative 2
CHIM	Controlled Human Infection Model	IMI2 JU	IMI2/EU/EFPIA Joint Undertaking
CHIVIM	Controlled Human Influenza Virus Infection Model	MERB	Medical Ethics Review Board
EFPIA	European Federation of Pharmaceutical Industries and Associations	MN	MicroNeutralisation
ELISA	Enzyme-Linked ImmunoSorbent Assay	NCTD	Non-toxigenic <i>Clostridioides difficile</i>
ELISPOT	Enzyme-Linked ImmunoSpot assay	PCR	Polymerase Chain Reaction
ELLA	Enzyme-Linked Lectin Assay	PPE	Personal Protective Equipment
ESCMID	European Society of Clinical Microbiology and Infectious Disease EU The European Union	PPIE	Patient and Participant Involvement and Engagement
FAIR	'Findable, Accessible, Interoperable, and Reusable'	qPCR	Quantitative Polymerase Chain Reaction
FCA	Flow Cytometry Assay	REC	Research Ethics Committee
GMP	Good Manufacturing Practice	RSV	Respiratory Syncytial Virus
HA(I)	HemAgglutination (Inhibition)	SMEs	Small and Medium-sized Enterprises
		SOP	Standard Operating Procedure
		TCD	Toxigenic <i>Clostridioides difficile</i>
		TCID ₅₀	50 % Tissue Culture Infectious Dose

1. Introduction

Controlled Human Infection Model (CHIM) studies (also known as human infection studies, human challenge studies, human challenge trials and, in the case of influenza, CHIVIM [controlled human influenza virus infection model] studies) are clinical studies that provide a unique platform for studying the pathophysiology of infectious diseases and for accelerated testing and early derisking of potential new vaccines and drugs in controlled clinical settings.

The Innovative Medicines Initiative 2 Joint Undertaking (IMI2 JU)-funded Inno4Vac project is dedicated to the selection and manufacture of the three challenge strains namely, influenza virus, respiratory syncytial virus (RSV), and *Clostridioides difficile*, and to the conduct of CHIM studies. Previous Inno4Vac workshops within the IMI2 project have addressed regulatory aspects of challenge strain selection and Good Manufacturing Practice (GMP) [1–3], ethics considerations for approval of CHIMs [4], and regulatory framework of CHIMs [5].

On March 20, 2024, a CHIM workshop focusing on the standardisation of clinical procedures, endpoints, and data robustness was held at the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands. This one-day event brought together a broad range of international stakeholders, including representatives from academia, industry, regulatory bodies, and Medical Ethics Review Boards (MERBs)/Research Ethics Committees (RECs), as well as a CHIM volunteers participation group (1DaySooner). The primary agenda was to discuss standardising of clinical procedures, ethics aspects, endpoints, and data integrity, including updates on ongoing initiatives with respect to the three CHIMs. To ensure the reproducibility and representativeness of those models it is critical to select and/or develop relevant endpoints and appropriate strain selection; the studies under development should

be designed in an adaptive setting which permits to explore different challenge doses, several potential endpoints (clinical, microbiological) and a fitting population selection that enables an efficient execution. This report provides a summary of the discussions held during the workshop.

2. Influenza

2.1. Introduction

Influenza virus causes considerable morbidity and mortality through annual outbreaks/epidemics and occasional pandemics. Currently licensed seasonal influenza vaccines require annual updating due to continuous antigenic drift and limited protective efficacy. Many of these vaccines were successfully tested in human challenge models to demonstrate protective immunity [6,7]. Current challenge strains are often old with low infectivity rates and induce little symptomatic disease limiting their use. There is an urgent need for next generation influenza vaccines which induce broader and more durable protection, which require updated human challenge models for demonstrating vaccine efficacy and immune correlates of protection (ICP).

2.2. Refining immune correlates of protection for influenza CHIM

An ICP is an immune response statistically associated with protection, which can be used in epidemiological studies and vaccine licensure. Whereas ICP are important tools to facilitate vaccine development, they are difficult to identify in perhaps the general population is better than a general population because of the uncertainty around the exposure of individuals to the virus. As such, protected and exposed individuals and unprotected and unexposed individuals cannot be identified in natural exposure trials. This ambiguity is diminished in

CHIM trials, where all participants are exposed to the challenge virus, thereby allowing for the controlled examination of protection status. Regarding correlates, the questions of who, what, when, and how must be answered [8]. It is necessary to define which individuals of the target population will be selected and to determine the specific endpoints and ICP that will be measured. The protective response will differ, e.g., by age group and previous influenza exposure. ICP derived from CHIM studies can be influenced by the type of sample collected (i.e., nasal swabbing, nasal mucosal lining, bronchoalveolar lavage), the target immune assessment (antibodies, T-cell response, interleukins, PBMCs), the compartment assessed (blood, mucosa), the timing of the evaluation (pre-infection, post-infection in the acute or convalescent phase), considering the epidemiology relative to the influenza season. It is critical to start by defining the clinical endpoints to be selected in the CHIM, such as infection prevention, reduction in viral load or disease severity.

For the CHIM model to be standardised it is important to have standardised immunological assays to assess the immune response. Without standardised assays, results obtained in different clinical studies cannot be directly compared necessitating large randomised controlled efficacy trials with the exposure of a larger number of participants, while a standardised assay would facilitate meta-analysis of the results of all studies, leading to earlier insight into the value of the vaccines under study. Earlier studies have shown that standardisation and introduction of biological standards can reduce the interlaboratory variation [9]. The Inno4vac consortium will capitalise on standardised assays developed during the IMI-supported FLUCOP project. FLUCOP was a broad collaboration across academia, public health and vaccine companies aimed at the standardisation and development of assays for assessing influenza vaccine ICP [9–16], addressing how human clinical samples should be tested in immunological assays and resulting in standardised immunological assays. The primary aim was to standardise the haemagglutination inhibition (HAI) and virus neutralisation (VN) assays. The secondary aims were to advance the understanding and application of cell-mediated immunity (CMI) and neuraminidase (NA) assays (see Table 1). The Inno4vac project has developed a prioritised list of immunological assays for comprehensively studying serological and mucosal as well as cellular immune responses, using the FLUCOP assays and other assays to assess the multifaceted immune response (Table 1).

2.3. Influenza clinical trial design

The Inno4Vac consortium aims to develop a novel symptomatic influenza A/H3N2 CHIM to advance the field, as this subtype undergoes more rapid antigenic variation, and the consortium considered the need was greatest for an Influenza A/H3N2 challenge model, as the most relevant for compounds in development. Relevant influenza A/H3N2

Table 1
List of prioritised immunological assays.

Serological assays	Mucosal response assays	Cellular assays
HA-inhibition assay	IgA ELISA	Interferon-gamma ELISPOT
Microneutralisation assay	Mucosal cytokines	CD4 and CD8 intracellular cytokine staining FCA
Enzyme-linked lectin assay (ELLA)		Memory B cell ELISPOT
ELISA for HA Stalk antibodies		Antibody Secreting cell ELISPOT
		ADCC assay for HA Stalk antibodies

Bold – standardised assay protocols available from FLUCOP. ADCC – antibody-dependent cellular cytotoxicity, ELISA – enzyme-linked immunosorbent assay, ELISPOT - Enzyme-Linked ImmunoSpot assay, FCA – flow cytometry assay, HA – Hemagglutinin.

viruses are characterised *in vitro* for viral growth and for pre-existing immunity in the target CHIM population. The best candidates are then down selected by *in vivo* testing for influenza disease development in ferrets. After the characterisation studies a novel CHIM virus will be produced. Using a qualified cell line, a contracted vendor will produce the challenge virus under Good Manufacturing Practice (GMP). Once the GMP-produced challenge virus is available, we will conduct a dose escalation clinical study.

The primary objectives of the study are to determine the inoculation dose needed to induce influenza symptoms with the new challenge strain in 50–70 % of exposed healthy volunteers, and to investigate the safety and tolerability of controlled infection with a GMP-produced wild-type influenza A/H3N2 strain and, ultimately, to establish a standardised CHIM for influenza for future drug and vaccine testing. A safe starting dose will be determined after the results of ferret studies become available. The starting dose will also be compared to prior studies using influenza viruses.

The primary endpoints encompass the infection rate based on two positive PCRs between days 2–8, and the symptomatic infection rate (as per modified Jackson criteria [17], symptoms plus lab-confirmed infection), expected symptoms (via the FLU-PRO questionnaire [18]), persistent symptoms (lasting over 14 days), and both unsolicited adverse events (AEs) and severe solicited AEs.

Secondary objectives are to assess influenza virus kinetics in nasal wash and swabs, to characterise influenza disease, and to evaluate the systemic and mucosal immune response. The secondary endpoints include viral load, determined by virus-specific qPCR and, if applicable, virus quantitative titre in cell culture (TCID50). Other endpoints involve self-reported symptoms (Modified Jackson score and FLU-PRO), local nasal mucosal IgG and/or IgA responses, and serum hemagglutination inhibition titres (HAI). Additionally, microneutralisation (MN) antibody titres and cellular immune responses (e.g., influenza-specific T and B cell frequencies) in blood and nasal samples will be measured for further analysis. In mucosal samples specifically, the target cells to be assessed are Tissue Resident Mucosal cells, mainly T-cells.

The clinical study design includes an initial pilot group of three participants (sentinel group) who will receive the starting dose of the challenge virus (Fig. 1). If two or more participants exhibit clinical signs of infection, the cohort will be expanded to seven additional subjects. As



Fig. 1. Proposed dose-escalation scheme.

is conventional in phase 1 trials, three participants in the sentinel cohort will be enrolled at the same time, after careful assessment based on preclinical safety data. The seven additional participants will, in principle, also be enrolled simultaneously, although operational limitations may force a split into two groups. A total of at least seven participants should meet the clinical signs of influenza disease (including sore throat, nasal congestion, rhinorrhoea, sneezing, coughing, headache, malaise, and chilliness; and laboratory confirmation) to confirm the target dose. If the initial dose does not meet this threshold, an escalation to the next dose level (ten-fold dose increase) will be implemented for three new subjects. If this dose does not result in disease in two out of three, the dose will be increased ten-fold once more, representing the maximum dose. If the final disease rate at the highest dose is between 50 and 70 %, it is considered adequate. If 50 % is not reached, the virus strain will be considered not suitable as a challenge strain.

The minimal quarantine period agreed was nine days. However, to reduce unnecessary in-patient stay, individuals who have no evidence of infection by PCR (two consecutive negative tests) and exhibit no clinical symptoms could be discharged on day 7 or 8 post-inoculation.

The main inclusion criteria for the CHIM are healthy volunteers, between 18 and 45 years old, who are seronegative (HAI <10) for the challenge influenza virus at screening. These volunteers must be willing to stay in the inpatient isolation unit for up to 14 days after the inoculation (to clinically monitor symptomatic volunteers after day 8 or 9) and tolerate the study procedures at screening.

In addition to standard exclusion criteria (e.g., drug abuse, smoking, clinical abnormalities, etc.), exclusion criteria comprise prior inoculation with a virus from the same virus family, participation in a CHIM in the past 6 months, and having received any vaccine four weeks prior to study start. Individuals with a history of pulmonary or immunological disease and those living with or being in close contact with vulnerable individuals (pulmonary disease or immune-compromised) will also be excluded. Finally, participants who show positive results for a respiratory virus at the baseline visit, as indicated by a nasopharyngeal swab or exhibit symptoms of a respiratory virus will also be excluded.

The eligibility screening will occur in two rounds. At the first visit, blood will be collected to assess the strain-specific HAI serostatus. A second screening will take place for seronegative participants in which a general health assessment, medical history, a test nasal wash, and an anterior rhinoscopy will be performed.

2.4. Discussion

2.4.1. Extension of the number of participants in the dose-escalation schedule

In the existing protocol, dose escalation ceases when the dose yields a 70 % or higher disease rate among ten subjects in a dose group with a total of 30 subjects planned in the study. If influenza infection and disease occur within one of the lower infectious doses the sample size may be increased to allow evaluation of additional secondary endpoints.

2.4.2. Endpoints: infection vs. clinical disease or both?

From a regulatory perspective, clinical endpoints are preferred next to virological ones in evaluating the efficacy of a new vaccine/drug. Regulators are primarily interested in the prevention of clinical disease, not just prevention of an asymptomatic infection. This may be less applicable to vaccine candidates that prevent virus transmission, but most vaccine candidates' development focuses on disease prevention and the regulatory pathway to licensure of transmission-blocking vaccines remains uncertain. Novel vaccines that target disease severity have a higher likelihood of regulatory approval.

If a 70 % infection rate is achieved based on PCR with a relatively low disease rate (symptomatology), exploring a higher dose group with at least three individuals might be beneficial to find symptomatic subjects. This could potentially reveal more consistent symptomatology or validate observations in a larger population. However, once the

infection rate with relevant clinical symptoms is met, further dose escalation should be done with caution.

2.4.3. Use of IgA as an immunological marker

Mucosal IgA may potentially prevent not only infection but also transmission [19]. Incorporating baseline nasal IgA into the influenza CHIM study would allow correlating IgA to viral load. Furthermore, influenza strain specific IgA could be utilised as a marker for seronegativity for subject selection out of season. However, its utility may be limited during the influenza season. Implementing this approach requires the development of standardised assays, which are currently unavailable. In another respiratory virus CHIM, IgA correlates more strongly with protection against RSV infection than serum neutralising antibodies [20]. In SARS-CoV-2 seronegative adults IgA accelerated viral clearance [19].

2.4.4. Would a TCID₅₀ assay have added value over qPCR?

Detection of infectious virus by TCID₅₀ is especially important as an endpoint measure for infection if the 70 % rate for clinical influenza disease is not reached. Influenza PCR data can fluctuate, and subjects can test positive for extended periods without bearing infectious virus, while TCID₅₀ values may be a surrogate for contagiousness. However, as TCID₅₀ is expensive and time-consuming, testing could be confined to participants exhibiting clinical symptoms (to confirm influenza disease) and limited to two or three time points at which PCR is positive rather than at all sampling points.

2.4.5. How to increase the infection rate and occurrence of symptoms?

One approach could be more extensive immune screening, followed by the inclusion of individuals with low influenza-strain specific or cross-reactive T-cell frequencies, as this could likely result in more symptomatic illness.

An alternative could be to escalate the dose until a virological endpoint is reached and then expand the study with subjects who may be more susceptible, such as seronegative subjects with a low influenza-specific T cellular response. The inclusion of individuals with comorbidities may not be an option during the model development phase due to safety concerns. For instance, while rhinovirus has been administered in CHIMs to individuals with asthma and chronic obstructive pulmonary disease, influenza presents more serious safety concerns. On the other hand, the current influenza models induce mild symptoms and may not represent clinically significant disease. Our aim is an improved model that better mimics natural infection and more accurately predicts vaccine efficacy. For example, a more elaborate immunological screening (e.g., cellular immunity or mucosal IgA) of participants may result in a higher disease rate. Ultimately, this would lead to a range of study designs tailored for the research question with a simple, less costly model or a more complex but perhaps more meaningful one.

2.4.6. Undiagnosed co-infections in participants

Around 20 % of the population carries viral RNA in their nasal passages. At baseline, those diagnosed with concurrent respiratory viral infection can be excluded, but the impact of the virome in those who are included could also be factored into subsequent analyses. Screening for respiratory viruses by PCRs can be performed to identify individuals with an active, concurrent respiratory viral infection. This is the preferred strategy for safety reasons as individuals with co-infections exhibit more severe symptoms. Even if they would exhibit comparable or milder symptoms, it is crucial to avoid confounding the controlled nature of the CHIM. Likely, group sizes of CHIM studies are not sufficiently large to meaningfully draw conclusions on co-infections. However, if carriage of all viruses needs to be excluded, metagenomics of nasal samples would be needed.

Overall, the consortium reflected on the importance of developing a more robust influenza CHIM using a well-designed dose escalating study with thorough immunological screening of participants, monitoring of

infection by PCR and culturing of infectious virus, disease symptomatology as well as using standardised assays to evaluate the multifaceted immune response after challenge.

3. RSV

3.1. Introduction

Despite the recent licensure of novel vaccines and monoclonal antibodies, RSV represents a major continuing global public health challenge. Infection with RSV typically causes a mild upper respiratory tract infection in healthy adults, but severe illness is very commonly seen amongst infants, younger children, and older adults. The presence of pre-existing medical conditions pre-dispose to severe RSV infection in adults and this is increasingly important in an aging population. RSV thus exerts a major burden on health resources around the world, particularly during winter seasons.

Two vaccines are now licensed for older adults that effectively reduce the risk of severe disease and hospitalisation, while prevention of severe RSV in infants is being addressed by maternal vaccination or passive immunisation. However, major limitations still exist. RSV does not induce long-lasting immunity following infection and it remains unclear how durable vaccine-induced protection will be. Correlates of protection have not been clearly defined as no genuinely immune individuals exist, so measurements of vaccine immunogenicity by serum neutralising antibodies remain poorly predictive of subsequent vaccine efficacy, particularly for clinical endpoints other than severe disease caused by lower respiratory tract infection. Importantly, field trials have shown that the licensed intramuscular RSV vaccines are substantially less efficacious in preventing milder disease and infection itself. Interventions that target the entry and exit of RSV, thereby preventing infection, interrupting transmission and conferring broad population immunity remain a priority.

3.2. Outpatient RSV CHIM study approach

Recently, an outpatient CHIM has been performed by the University Medical Centre Utrecht (UMCU), The Netherlands. The outpatient model for RSV CHIM studies offers several benefits. Primarily, it reduces vaccine development costs in the early clinical development phase as CHIM costs are primarily driven by the costs of participant quarantine and compensation. Additionally, it provides a more participant-friendly approach as the burden on subjects is reduced, potentially making recruitment easier and faster. Lastly, the more widespread availability of the RSV CHIM when in-patient quarantine capacity is not a limiting factor could allow these studies to become a routine part of the early clinical development pipeline. As more studies are conducted and the predictive accuracy of these early phase efficacy trials is established, down-selection of vaccine candidates with no efficacy signal could be achieved at a much earlier stage, avoiding costly phase 2/3 field trials. Conversely, evidence of clinical efficacy in the RSV CHIM could accelerate the next developmental phase of interventions by increasing confidence and allowing prioritisation of limited downstream resources. The overall cost reduction of programmes that include CHIM would therefore lead to more sustainable drug development, more affordable products, and, ultimately, vaccine equity.

As a first step towards outpatient RSV CHIMs, the UMCU team performed a systematic review of all outpatient CHIMs conducted for respiratory pathogens to identify key safety, logistical, and ethical risks (Siegal, personal communication). Information about the standard operating procedures (SOPs) and the overall trial experience was gathered through questionnaires or structured interviews with all principal investigators.

Safety concerns (both volunteer and third party) in an outpatient CHIM revolve around participant safety, transmission of pathogens, transportation precautions, and access to emergency care. The risk of

transmission of pathogens can be reduced by limiting enrolment to subjects in households that do not have children, elderly, or patients with morbidities and subjects with occupations that do not involve exposure to children, elderly, or patients with morbidities.

Logistical challenges in an outpatient setting include home sampling. Additional costs may arise from sample transportation or home visits by study staff. However, outpatient studies are invariably less costly than inpatient studies. From an ethics standpoint, the risk of third-party transmission can be a barrier to ethics approval. However, for commonly circulating pathogens, the increase in risk caused by conducting an outpatient CHIM is negligible compared to the background risk of being exposed through natural infection. Population immunity for such pathogens also decreases the risk of a potential outbreak.

Based on this framework, an outpatient RSV CHIM was conducted in Utrecht, the Netherlands, from October to December 2023. The Department of Hygiene and Infection Control helped to develop a phase-based approach to ensure safety in the ethical approval process in which the risks of transmission were demonstrated in the inpatient setting before transitioning to the outpatient setting.

3.3. RSV clinical trial design

As in the influenza CHIM, the RSV CHIM will be a dose-escalation study, aiming for an infection rate of 50–70 % (Fig. 1), although due to the intrinsically less pathogenic nature of RSV infection, meeting a threshold for clinical symptoms is less feasible and will not be required. The adaptive design expedites dose escalation. An infection rate of 50–70 % at the optimal dose will be the primary objective. The need to dose all participants in a particular dose group of 10 volunteers can be eliminated (adaptive dose design) if two out of three pilot subjects remain uninfected, thus allowing escalation to the next higher dose. Unlike the influenza CHIM, the RSV CHIM will be outpatient, reducing participant and clinical research unit burdens and costs. No formal statistics were performed to calculate sample size.

The primary objectives are to establish a CHIM for RSV for future drug and vaccine testing, to identify the inoculation dose needed to induce RSV infection with a new strain in 50–70 % of exposed healthy volunteers, and to investigate the safety and tolerability of the novel GMP-produced wild-type RSV B challenge strain. Endpoints include infection rate (2 positive PCRs on consecutive days between days 2 and 8), RSV disease rate, and unsolicited AEs and severe solicited AEs.

Secondary objectives are to assess RSV kinetics in nasal washes and swabs, to characterise RSV disease, and to evaluate the systemic and mucosal immune responses. Endpoints include viral load (by quantitative PCR (qPCR) and virus quantitative culture (TCID₅₀)), onset, peak, time-to-peak, duration and area under the curve, correlation of viral load with symptom scores, antibody response in nasal mucosal lining fluid and blood, and proportion of subjects with seroconversion (>4-fold) in blood.

Finally, the exploratory objectives aim to evaluate the systemic and mucosal immune response by measuring cytokines in blood and nasal samples, performing transcriptomics in nasal samples and whole blood, and determining cellular responses (e.g. antigen-specific T cell frequencies) in nasal scrapings/swabs and blood.

Healthy male and female volunteers aged 18–45, serosuitable for pre-existing neutralising RSV antibodies at screening, will be included. Subjects must tolerate daily nasal washes and swabs. Two nasal sampling devices, the FLOQSwab and the nasal scrape, will be considered. The swab yields twice the cellular content as the scrape but may be less well tolerated than the scrape. This could be alleviated by reducing the frequency and number of rotations of FLOQSwabs per sample.

3.4. Discussion

3.4.1. RSV inoculation in the home setting

As the RSV CHIM will be performed in an outpatient setting, study

visits could possibly be performed in the homes of participants. Inoculation with the RSV Memphis B 37 strain must, however, be performed within 15 min of the inoculum being prepared, due to rapid decline in viral titre. Home-based inoculation is therefore challenging. In addition, while immediate serious adverse reactions to RSV challenge have never been reported, emergency medical care must be available at the time of inoculation. As a consequence, RSV virus inoculation will need to happen in a clinical trial centre well equipped for the purpose to prevent spread to any clinical trial staff. For the inoculation, surgical face masks, gowns and goggles were used in the Utrecht study. Lung function testing, an aerosol producing procedure, was done daily. For this procedure, staff wore FFP2 face masks and gowns. For all other sampling, normal face masks were used.

3.4.2. Variation in nasal sampling

Interindividual variability in anatomy of the nose will influence samples taken from the upper respiratory tract. In addition, the sample collection method (scrape vs swab) and inter-operator variability will influence the quality of samples collected. During infections, patchiness, or areas with varying virus concentrations, are theoretically possible. The immune response also varies across the respiratory tract.

As a precaution, potential participants will undergo anterior rhinoscopy screening to identify severe septum deviations or other anatomical abnormalities that could hinder correct sampling.

Pilot experiments with nasal scrapes have shown considerable inter-individual and inter-sample variation, ranging from no cells to a few thousand cells recovered, with relatively few immune cells [21], suggesting that the FLOQSwab may be the preferred option [22].

3.4.3. Optimising the infection rate

Pre-screening for neutralising antibody titres against RSV, while potentially helpful, is both costly and burdensome, yielding a marginal increase of infection rate of about 10–20% [20,23]. The associated costs and efforts may not justify this increase, which also biases the study population with regard to the level of pre-existing immunity.

Saliva testing presents a viable alternative to serum testing for detecting mucosal neutralising antibodies. It could potentially be a more straightforward and less intrusive sampling method for participants but has not been sufficiently validated. It should be paired with total protein content determination as a normalisation standard to ensure accuracy.

Interestingly, without pre-screening, the infection rate among healthy older adults (65–75 years) was notably higher (70%–75%) in a newly established RSV CHIM in older participants than in younger participants [24]. Despite exhibiting symptoms similar to those of younger adults, these older individuals also shed significantly more virus. Inclusion of older participants would necessitate dose escalation in this age group.

3.4.4. Feasibility of a 70% infection rate

The Memphis B 37 strain, the most extensively studied RSV challenge agent, yields an infection rate of approximately 55% without serum pre-screening. While there may be variability across studies, populations, and timing, a 70% infection rate may represent the upper limit in a pre-screened population. Setting a more realistic target infection rate can mitigate the risk of prematurely terminating the programme due to not meeting an arbitrary cut-off. Yet, at the same time, it is important to set ambitious goals for the programme as a >70% infection rate achieved with the newly developed RSV strain would be highly preferred from a vaccine development perspective.

3.4.5. RSV CHIM in outpatient settings – the risk of transmission to the public

Public transmission constitutes the primary concern when conducting RSV CHIM studies in outpatient settings. A systematic study of the risk of transmission to the public is needed.

The instructions to the participants are straightforward: maintain a

social distancing of 1.5 m and wear a face mask. However, investing time in discussing potential risks with participants is crucial. Understanding the rationale behind these measures increases the likelihood of adherence. Generally, participants of the Utrecht RSV study thought it was quite simple to follow the procedures.

The outpatient RSV CHIM approach presents an opportunity to generate additional data on outpatient vs inpatient studies, enhancing the researcher's comfort level in conducting these studies in outpatient settings.

3.4.6. Securing consent from household members

The stance of MERBs and RECs on obtaining consent from household members due to the increased risk of RSV transmission is a topic of particular interest. In one study retrieved in the systematic literature review, consent was sought from household members to ensure their understanding of the inherent risks. The Utrecht study, however, only opted to exclude participants with household members at an increased risk, as the risk of otherwise low-risk household contacts becoming infected by a study participant was considered no greater than infection in the community.

RECs often encounter studies that necessitate obtaining consent or disseminating information to families or individuals in contact with study participants. The information provided must be tailored to the reader's involvement (risk). While written informed consent of contacts may not always be necessary, keeping people informed and raising awareness is crucial. This also prompts them to notify the study team depending on the situation and the infection.

4. *C. difficile*

4.1. Introduction

Healthcare costs associated with *C. difficile* infection (CDI) are substantial [25] and the high relapse rates as well as the rise of antibiotic resistance [26] has made the development of novel products to combat this mostly nosocomial disease urgent. Infections with *C. difficile*, the leading cause of healthcare-associated diarrhoea, are typically treated with fidaxomicin, vancomycin, or metronidazole. However, the relapse rates are high (20–60%) and repeated use of antibiotics can lead to increased antibiotic resistance [27,28], necessitating new treatment strategies. With the advent of live bacterial therapeutic products and a better understanding of mucosal immunity, there is a promise of novel therapeutic and preventive avenues in the near future. A toxigenic *C. difficile* CHIM would be a gamechanger for the rapid down selection of such novel products. However, toxigenic *C. difficile* CHIM studies have not been performed before and raise questions regarding the safety for participants and bystanders (including relatives and household members) and regarding the optimal study design. Ethical aspects with regards to *C. difficile* CHIMs have been discussed in a previous workshop [4], concluding that there is no absolute ethical objection to such studies given the availability of rescue treatment options. In the current meeting, all stakeholders agreed that a careful design according to the principles of “go low, go slow” would be needed to safeguard the health of volunteers whilst exploring the establishment of this novel CHIM. The design of such a novel CHIM study, particularly with regards to dose, attack rates and susceptibility markers was discussed.

4.2. Preliminary results of a non-toxigenic *C. difficile* trial

Non-toxigenic *C. difficile* (NTCD), which lack the genetic loci for toxin production (PaLoc and CdLoc), cannot cause overt disease [29]. However, it occupies the same gut microbiota niche as toxigenic *C. difficile*. This makes the NTCD model a safe platform for investigating microbiota susceptibility markers and provides valuable insights for the dose, potential attack rate and design considerations for a subsequent toxigenic *C. difficile* CHIM. At the Leiden University Medical Centre, a

clinical trial is currently ongoing with non-toxicogenic *C. difficile* (NCT05693077). The primary objective for the NTCD study is to evaluate the safety and the colonisation rates after oral administration of different doses of NTCD spores with or without vancomycin pretreatment. Primary endpoints are number and grade of related AEs during the first month after NTCD/placebo ingestion and positive *gluD* PCR or culture on two or more time points between 3 and 14 days after the last exposure day. A secondary endpoint is the identification of microbiota markers associated with successful colonisation. An exclusion criteria list is designed to minimise the risk to trial subjects. The adaptive dose design is based on safety data and aims to achieve at least 60 % colonisation in one study dose cohort. Escalation is performed by adding pre-treatment with vancomycin to disrupt microbiota and facilitate colonisation by NTCD [29–31]. The results of this clinical trial will be used to inform the subsequent design of the toxicogenic *C. difficile* clinical trial.

4.3. Toxicogenic *C. difficile* (TCD) clinical trial

The first step in the development of a TCD CHIM was the appropriate selection of challenge material. This material will consist of spores to be administered orally, to resemble as closely as possible the natural infection route. A TCD challenge strain was selected which is representative of circulating strains, has a common toxin profile, and is susceptible to rescue treatment antibiotics [32]. Ideally, the challenge material should be produced under full GMP for clinical use, including adventitious agent testing. However, it is very challenging, if not impossible under current regulations, to maintain full GMP for the spore forming organisms, especially *C. difficile*, with no manufacturing sites with prior experience able to do so. Thus, the closest option is to adhere to GMP principles in the production process as much as possible, which aligns with current requirements for Auxiliary Medicinal Products according to EMA guidelines.

The primary objectives of the TCD CHIM are safety, tolerability and achieving microbiological and clinical endpoints in at least 70 % of the volunteers at one dose level. Safety is assessed by recording AEs during the first month post-TCD spore ingestion. Clinical endpoints are based on the European Society of Clinical Microbiology and Infectious Disease (ESCMID) guidelines [33]. Microbiological endpoints similarly are aligned as much as possible with ESCMID guidelines and require a positive toxin gene real-time PCR or a positive toxin enzyme immunoassay (EIA) for the presence of free toxins in faecal samples on two or more occasions between days 3 and 14 post-challenge, confirmed by at least one positive culture [33,34].

Secondary objectives include assessment of the quantitative microbiological load of *C. difficile* in infections, baseline host microbiota factors influencing infection, and changes in host gut microbiota post-infection. Exploratory objectives are the immune response following successful colonisation and/or infection, bile acid metabolism, and the evolution and adaptation of *C. difficile* *in vivo*.

The trial will begin with a dose-finding study, similar to the influenza CHIM, aiming for a clinical infection rate of >70 % (Fig. 1). The current protocol draft design has five subjects in the sentinel group and 15 in the expansion group as larger group sizes are expected to be necessary, given the strong colonisation resistance in healthy volunteers. For *C. difficile* it was proposed to limit the number of different doses of spores to be tested as a dose-response relationship is not expected [30]. E.g., given the fact that the *C. difficile* spores can germinate and replicate, the initial dose provided in the challenge capsules is likely unrelated to the microbiological or clinical endpoint frequency in a given dose group. Rather, it was suggested to escalate by introducing antibiotic pretreatment, thereby increasing susceptibility of trial subjects to the TCD challenge.

The TCD trial differs from the NTCD trial in its stricter follow-up protocol. From day zero (challenge), daily stool and AE collection, as well as bi-weekly blood samples, are proposed. After day 14, follow-up

will be reduced to thrice-weekly stool collection, weekly AE collection and blood samples. Any participant diagnosed with *C. difficile* infection during follow-up will undergo a same-day physical check-up with collection of a blood and stool sample. A final follow-up visit on day 84 will be done to check colonisation status, with follow-up continuing until decolonisation is confirmed (a negative *C. difficile* PCR test on at least two time points).

Treatment of symptomatic *C. difficile* infection (CDI) will follow standard of care. In the first episode of CDI, the first option proposed according to the IDSA recommendations is fidaxomicin (2 times daily 200 mg, for 10 days), and the second option is vancomycin (4 times daily 125 mg, for 10 days) [33]. Recurrences will be treated with allogenic faecal microbiota transplantation.

The TCD CHIM presents more ethical considerations than the NTCD CHIM, with no direct benefits for the volunteers. The most significant risk for the participants in the TCD trial is developing severe infection or multiple recurrent infections. Development of severe CDI is mitigated by the inclusion of low-risk volunteers and strain selection based on low-risk features, i.e., the strain is susceptible to rescue treatment and is not associated with outbreaks or epidemics. Participants are closely monitored to prevent diagnostic delays and ensure prompt treatment. Biosafety risks, such as environmental TCD spore spread, are minimised through strict hygiene measures and symptomatic volunteer treatment, by an experienced phase 1 unit with access to appropriate medical care. Given the presence of TCD in the environment, the additional risk posed by the CHIM is deemed limited.

4.4. Discussion

4.4.1. Agreement to reach both endpoints (microbiological and clinical)

There is considerable discussion around the use of microbiological and clinical outcomes. Although clinical outcomes are preferred from a product development perspective, clinical outcomes are also generally perceived more “risky” as compared to microbiological outcomes. For *C. difficile* it is unclear if and in how many individuals microbiological and clinical outcomes may overlap. If subjects reach the microbiological endpoint but not the clinical one, they are deemed colonised. Conversely, a positive result for both endpoints indicates an infection. Testing of future vaccines based on toxin immunity, achieving mere colonisation in a CHIM might not suffice. While valuable insights can be gleaned from colonisation, particularly regarding microbiological susceptibility markers, the ultimate goal is to observe clinical disease before testing new preventive and/or therapeutic interventions.

The aspiration to reach a clinical disease endpoint is driven by its potential to provide more comprehensive data. However, aiming for a clinical endpoint in 70 % of challenged subjects at a dose level might not be attainable. Nonetheless, the CHIM may be useful even at a lower attack rate, as it will still be valuable for product development albeit at increased costs given the necessity of a larger trial population. Moreover, and most importantly, the knowledge acquired from the process, irrespective of the outcome, contributes to the broader understanding of the disease and aids in future research endeavours.

4.4.2. The escalation schedule

The proposal to use only the lower dose of *C. difficile* spores was accepted under the assumption that the lack of dose-response relationship is confirmed in the NTCD trial. As an alternative to dose escalation, adjustments in dose and duration of antibiotic pre-treatment could be a viable strategy to increase the attack rate in the CHIM. The initial dose group should target healthy volunteers without any antibiotic pre-treatment to remain with a cautious stepwise approach. Next dose groups can then target increased colonisation rates or symptom occurrence. For every escalation step, results are awaited, evaluated, and then used to inform subsequent steps.

4.4.3. Optimal vancomycin pre-treatment timing and duration

Due to widespread use in microbiome intervention studies, vancomycin is used as a primary method of inciting *C. difficile* susceptibility. However, if colonisation endpoints are not met using vancomycin, transitioning to an alternative antimicrobial like an oral cephalosporin, fluoroquinolone, or clindamycin could be considered. In that case, expert advice for antibiotic selection and duration should be solicited.

4.4.4. Treatment considerations for chronic carriers

Individuals who do not clear *C. difficile* three months after exposure, and do not have any clinical symptoms of a *C. difficile* infection, may be deemed chronic carriers. There are arguments to not treat these asymptomatic colonised subjects, as asymptomatic colonisation is common in the general population [34] and colonisation is often short-term and of a transient nature [35]. However, not treating asymptotically colonised subjects at the end of the study could lead to debate on the psychological impact of chronic carrier status and potential health risks to others. Experience with a previous hookworm CHIM study shows that most of the chronically infected subjects do not experience psychological impact of their carrier status. Therefore, in case of asymptomatic *C. difficile* colonisation at the end of the study, shared decision making between the research physician and trial subject can be a good solution to guide treatment choices. The risk of becoming a chronic carrier should be described in the informed consent form.

4.4.5. When to give rescue treatment?

Rescue treatment will be offered when a subject has symptoms of CDI, based on ESCMID guidelines [33]. But what would one do with someone who has symptoms, is PCR positive, but free toxin negative? Or has symptoms but diagnosis is pending? In such cases, the provision of rescue treatment of individuals should always be guided by safety considerations.

5. Harmonisation and standardisation

5.1. Ethics of patient involvement in research design and conduct

Some 15 years ago, Chalmers and Glasziou highlighted that approximately 85 % of research is wasted due to poor design, irrelevant questions, or inadequate reporting [36]. To address this problem, better ethics, governance and integrity processes need to be developed [37]. One practical action is to encourage greater involvement from clinicians and patients when formulating research questions and setting priorities should involve clinicians, scientists, and the public. Working together, they can collectively identify critical issues within a specific research area, conduct systematic reviews to gather existing evidence, and agree on research priorities.

In the current research landscape, grant applications increasingly require evidence of patient and participant involvement and engagement (PPIE – although a variety of other, similar acronyms are often used). Lessons can be learned from community advisory boards in human immunodeficiency virus research and treatment [38–40]. In CHIM studies, engaging potential participants can optimise the design of studies, and limit the burden to the participants. Care needs to be taken to choose the right people to involve, the size of the sample, and the manner/design of questions asked. There is a need for continuous improvement in the methodology used to encourage patient and participant involvement so as to ensure robustness and high-quality evidence that can contribute to high quality study design.

5.2. Involving the right people in PPIE

As discussed above, identifying the right people for involvement in PPIE can be challenging. While well-organised patient and advocacy groups exist for some diseases, such as renal or rheumatoid, this is not the case for all infectious diseases. Although there are organisations like

the *C. difficile* Trust in the UK and previously the U.S.-based *C. diff* Foundation, similar advocacy groups for many other infectious diseases would be beneficial. The presence of 1DaySooner at this meeting, representing volunteers participating in CHIM, was acknowledged. It is crucial to consider input from volunteers, and effective strategies should be developed to ensure their involvement, as well as the engagement of the general public.

For RSV, a patient advisory board exists [41], comprising parents of children who have had lengthy admissions or have been admitted to the intensive care unit. This advisory board played a significant role in the grant application, study design, feedback provision, recruitment procedures, and study execution for the Utrecht RSV outpatient CHIM study. This exemplifies the importance of involving the right people in PPIE.

5.3. Promoting harmonisation and standardisation in CHIM study reporting

The importance of accurate reporting cannot be overstated. Aggregating data allows us to ask and answer larger questions and maximise the contribution of each volunteer. A review was conducted to examine the number of volunteers who experienced severe AEs related to the challenge, as documented in peer-reviewed publications. Among 94 studies that classified AEs by severity, severe challenge-related AEs were reported by 5.6 %–15.8 % of participants [42]. This variability was primarily due to ambiguous reporting, which raised questions about what constitutes an AE and when it should be reported.

To determine whether reporting in clinical trial registries was less ambiguous, data from these registries, specifically from clinicaltrials.gov, were collected and compared with corresponding data reported in peer-reviewed publications describing the same studies. It was found that approximately 25 % of CHIM studies, which had been completed for a minimum of one year, had reported their results in peer-reviewed publications.

Clinicaltrials.gov was chosen to review the data for the data analysis as it is the largest registry and provides machine-readable results. Overall, 46 clinicaltrials.gov records were reported in 195 publications. Notably, 94 % of the clinicaltrials.gov records described AEs at the individual level, compared to 61 % of the publications. In 23 instances, the results were reported differently in the clinicaltrials.gov record and the publication. [Clinicaltrials.gov](https://clinicaltrials.gov) records were more complete in about 80 % of those instances. As a general trend, publicly funded, medium-sized trials were more likely to have discrepancies in reporting [Danny Toomey, personal communication]. Most importantly, five serious AEs were reported in clinicaltrials.gov records and could not be found in any related publications.

For optimal reuse of CHIM data, a full participant flow needs to be reported in a public, machine-readable registry. This includes the number of volunteers challenged, infected, and with (serious) AEs by trial arm. Ensuring the maximum reuse of the scientific data is an ethical imperative. However, this is not without challenges. Legal obstacles, such as the General Data Protection Regulation, International data exchange, and arrangements for pseudonymisation and anonymisation, can complicate data sharing. In the realm of genetic data, the issue is even more pronounced. Such data in particular may be impossible to de-identify participants. Given the relative complexity and participant sacrifice required for CHIM trials, it is recommended, as a matter of best practice, that as much information is obtained from data as possible. This requires consideration as to how CHIM trial data can be stored in a way that makes it accessible and likely to be reused.

5.4. Mandatory trial registration

The interconnection between registries and regulators, including MERBs/RECs, is becoming more prevalent. In the UK, for instance, trial information from studies approved by National Health Service (NHS)

RECs are automatically uploaded to the ISRCTN registry. The same is true for drug studies under the European Clinical Trial Regulation. Scientific journals are instrumental in driving researchers to register studies, thereby enhancing compliance and augmenting research transparency² The increase in initial trial registrations has represented a significant success from a transparency perspective, but now the emphasis has moved towards providing more comprehensive trial data, a change that can again be pushed by pressure from scientific journals.

6. Conclusion

The Inno4Vac project continues to make significant strides in the development of challenge agents, with preparations underway for the commencement of the first controlled human infection studies. This meeting served as a platform to deliberate on the protocols that will govern these studies.

For each new or significantly modified challenge agent, the initial phase should involve a dose-escalation adaptive design. The features of those designs incorporating a limited-sized sentinel group enables safe escalation if there is no response at the lower doses. Once the primary objective is achieved and a dose strain is identified, MERBs/RECs can be approached for a population expansion if a new secondary objective requires further powering.

It becomes critical to ensure that testing procedures are standardised to validate the robustness and reliability of the models. Once protocols have been standardised, CHIM trials can be planned in multi-centre, multi-country settings.

Similarly, additional efforts are deemed necessary for study registration and final reporting, keeping adherence to a standard format that could facilitate comparison between studies and to promote the reuse and aggregation of data, thereby enhancing the overall value and impact of CHIM research.

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² RECs should also consider the potential risk to the participant of informing household members; this can put the participant at risk of retaliation or undue influence of the parents or other household members.

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Declaration of competing interest

Dileep Dasyam, Okba Haj-Ali Saflo, Sandra Morel, and Juan Pablo Yarzabal are employed by GSK and hold financial equities in GSK. These authors declare no other financial and non-financial relationships and activities. All other authors declare no competing interests.

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