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Meeting Report

Controlled Human Infection Studies: Proposals for guidance on how to design, develop and produce a challenge strain

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ABSTRACT

There is an increasing need to establish quality principles for designing, developing and manufacturing challenge agents as currently these agents are classified differently by various jurisdictions. Indeed, considerations for challenge agent manufacturing vary between countries due to differences in regulatory oversight, the categorization of the challenge agent and incorporation into medicinal/vaccine development processes.

To this end, a whitepaper on the guidance has been produced and disseminated for consultation to researchers, regulatory experts and regulatory or advisory bodies. This document is intended to discuss fundamental principles of selection, characterization, manufacture, quality control and storage of challenge agents for international reference.

In the development phase, CMC documentation is needed for a candidate challenge agent, while standard operating procedure documentation is needed to monitor and control the manufacturing process, followed by use of qualified methods to test critical steps in the manufacturing process, or the final product itself. These activities are complementary: GMP rules, which intervene only at the time of the routine manufacturing of batches, do not contribute to the proper development and qualification of the candidate product. Some considerations regarding suitability of premises for challenge manufacturing was discussed in the presentation dedicated to "routine manufacturing".

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1. Introduction

A meeting was organized at the Paul-Ehrlich-Institute (PEI) in Langen, Germany, in 2019 to discuss quality requirements for challenge agents used in Controlled Human Infection Studies (CHIS), which are important prerequisites for safety and relevance of these studies [1]. The importance of CHIS for COVID-19, including the role in speeding up vaccine development has been discussed previously [2,3]. The Wellcome Trust and HIC-Vac, an international network of researchers who are involved in CHIS to accelerate the development of vaccines against pathogens, set up a consortium in 2020 to develop international standards pertaining to challenge agent manufacture and storage, extending the current general WHO guidelines relating to challenge studies to ensure safety, quality and consistency in manufacturing [4]. A draft whitepaper on the guidance has been produced and disseminated, and is now available for consultation to researchers, regulatory experts and regulatory or advisory bodies. The meeting reported here was organized to discuss the whitepaper and its applications. The meeting was chaired by Jean-Hugues Trouvin, IABS, and Isabelle Bekeredjian-Ding, Paul-Ehrlich Institute, Langen, Germany. Note that the whitepaper has evolved considerably since the webinar was held and is now seen as a considerations document.

2. Manufacturing guidance project

Carine La, Associate Director of Clinical Sciences, hVIVO, provided an overview of the manufacturing guidance project. CHIS are used to evaluate vaccines, prophylactics and therapeutics but also to gain a better understanding of different diseases. CHIS are aimed to expose a small number of individuals to pathogens, referred to as challenge agents. How can these challenge agents be produced in such a way that they are safe and of high quality? There is an increasing need to establish quality principles for designing, developing and manufacturing challenge agents considering that currently these agents are classified differently by various jurisdictions. Hence, the whitepaper should include low- and medium-income countries' considerations, but also reflect whether challenge agents could be produced outside GMP-accredited environment without jeopardizing safety of the study participants. A final version of the whitepaper should be available by mid-August 2021.

3. General considerations

Hilde Depraetere, Director of Operations at the European Vaccine Initiative, provided some general consideration on quality development. Firstly, where available, applicable local, national and regional guidelines on the manufacture of challenge agents should always be considered. Secondly, the focus should be on the intended use of the challenge agent and the safety of the volunteers participating in the trials. Thirdly, the ICH guidelines [5] should be followed, where applicable, considering challenge agents are biological products. More specifically, ICHQ5a, 5c, 5d, 6b, 7, 8, 9, 10 and 11 contain articles that could be applicable for the development of these agents.

The proposed process to follow starts with the development of a target product profile (TPP), including non-clinical, clinical, regulatory and CMC aspects. Next, the quality considerations of the challenge agent need to be worked out in a quality target product profile (QTPP), translating the characteristics from the TPP into quality considerations to ensure the safety, potency, consistency and stability of the challenge agent. The aspects included are the dosage form, route of administration, stability, quality attributes and the container closure system. In the next step, based on the quality target profile, critical quality attributes (CQA) are defined, related to the safety and pathogenicity of the challenge agent, including the identity of the challenge agent, potency, purity, stability, dosage form, sterility and transmission. For instance, sterility of the challenge agent should be controlled appropriately for any parenteral route of administration, whereas for topical or oral routes a controlled bioburden (absence of specified pathogens) is suitable. Finally, a quality control strategy is developed, which is a set of monitoring processes and final product tests, to ensure the manufacturing process is followed and documented.

In conclusion, the design and manufacturing of a human challenge agent is a stepwise process, from establishing a TPP, identifying key quality attributes and release criteria to developing a process for routine manufacturing. This in turn leads to the development of standard operating procedure documentation to monitor and control the manufacturing process, followed by use of qualified and validated methods to test critical steps in the manufacturing process, or the final product itself. In terms of suitable premises for the routine manufacturing, a case-by-case approach should be adopted to take into account specificities (essentially growth capacities) of the challenge agent, while maintaining the suitable quality system level, adapted to the actual situation.

4. Challenge agent selection and characterization

Akamol Eddie Suvarnapunya, Deputy Chief of the Department of Diarrheal Disease Research at the Walter Reed Army Institute of Research, presented a challenge agent for diarrheal disease research. There is a high risk of diarrhea that can impact military operations globally. *Shigella sonnei* and *Shigella flexneri* are the two most prevalent serotypes causing diarrhea in the world, with *S. sonnei* most common in developed countries.

In the early days, the *Shigella* challenge agent was prepared by making bacterial lawns on agar, which were then harvested and diluted in skimmed milk, with attack rates of around 50%. The use of bicarbonate buffer as a vehicle for delivering the agent resulted in a higher and more consistent attack rate. In 1998, frozen glycerol master stocks and working cell banks of the challenge agent were made. Dose finding studies reported the target rather than the actual colony-forming units, making results difficult to reproduce, particularly for a pathogen with a very low infectious dose such as *Shigella*. Inconsistency between studies was also potentially due to different lots of challenge agent used, naïve versus endemic populations, variability introduced due to requirement of multiple passages and/or a lack of standardized administration procedures. Therefore, a lyophilized stock was produced that just simply needs to be hydrated before administration, thus eliminating the need for multiple bacterial passages and simplifying the entire process [6]. From a single research seed vial of *S. sonnei* strain 53G, a master cell bank and a working cell bank were created, which were identical except for one passage. From one ampoule of the working cell bank, a 30-L fermentation seed culture was started, vials were filled and lyophilized.

Lot release testing was relatively basic, focusing on purity. This testing included a Gram stain and visual inspection of the type and appearance of the colonies from samples taken at various points of the fermentation, fill, and lyophilization process. A small number of vials from the lyophilized lot were also tested for pH and moisture content. Each individual vial of the lot was subjected to visual examination of the lyophilized product for expected color, consistency, and appearance. Finally, the concentration of viable bacteria in CFUs per mL was determined.

Post lot release testing was broken down into two categories: purity and pathogenesis. Tests for purity included biochemical identification, antibiotic susceptibility, and whole genome sequencing. In particular, the microbial limits test (MLT) was done for specific objectionable agents as defined in the USPs. Tests for pathogenesis of the challenge strain included surface expression of Invasion Plasmid Antigen B (IpaB), invasion into cultured epithelial cells, plaque formation in cultured epithelial monolayers, and virulence in guinea pigs.

Forced degradation studies of the lyophilized *S. sonnei* 53G showed the effect of storage at a range of temperatures, indicating that the product was stable at 4 °C or lower, whereas after 10 weeks at 37 °C a

four-log loss in CFU/mL was observed. Finally, routine testing over 8 years showed that over 80% of the CFU remains virulent, similar to the date of manufacture.

S. flexneri 2a is also available as glycerol stocks, whereas lyophilized stocks of *S. flexneri* 3a will become available in 2021. The production of these *Shigella* challenge strains and other bacterial, viral, or parasitic challenge strains may benefit from the structured formal framework as discussed in this white paper.

5. Manufacturing and control

Alan Bell, Director of Clinical Sciences at hVIVO, gave a brief overview of the manufacturing and control section of the document. The manufacturing control process encompasses the definition of challenge agent characteristics, definition of the quality considerations to ensure safety, potency and stability of the challenge agent, and definition of a quality control strategy, and the routine manufacturing itself. The control strategy for a challenge agent includes elements which impact both the process as well as the product, and its purpose is to show that acceptance criteria are met during the manufacturing process. Key elements to consider are the acceptance criteria for QC release, the manufacturing process, recommended test controls, considerations for formulation, stability and labeling.

Special attention should be given to biodegradable materials and environmental changes (e.g., change in pH), but also to the description of procedures used in the transport of the material during the manufacturing process, as well as the storage conditions. The respective manufacturing area (from the starting material up to the final product) should be physically separated, to try and reduce the risk of cross-contamination. The processes around the destruction of the challenge agent (i.e., cleaning procedures after production) also need to be considered.

The test controls are used to verify that the process and procedural controls are performed as expected. In-process testing is usually some form of analytical or functional test to ensure that selected manufacturing operations perform appropriately to achieve the intended quality. The result of release testing should be documented, e.g., a certificate of analysis. Characterization testing of certain attributes can be done outside of lot release testing for the purposes of demonstration of consistency and where necessary comparability: whenever and wherever relevant, genomic and phenotypic stability as well as other quality attributes such as viability, motility or morphology should be assessed during the manufacturing and storage phases. Process monitoring should be implemented when there are several batches of the same challenge agent being produced on a regular basis.

Labelling serves two purposes; the pharmaceutical purpose is to instruct the end user on its use. Therefore, the label itself, or the leaflet that accompanies the vial, should, as a minimum, include the name and expiry date of the challenge agent, the concentration as well as the number of doses, if the product is being issued in a multi-dose container. Temperature recommendations for storage as well as during transport should be clearly provided. If necessary, packages should contain cold chain monitors and the packaging process for shipment should conform to the IATA standards. The label or leaflet should also include a statement indicating the volume and nature of the diluent used for the reconstitution, as well as post reconstitution storage and expiry information based on the stability studies performed. The second, regulatory purpose is to inform the end user that it is not a “medicinal product” but a challenge agent (including a discussion on its pathogenicity). Where appropriate, labelling recommendations provided in the WHO good manufacturing practices for biological products should be followed.

6. Routine production: which standard to use?

Jean-Hugues Trouvin, IABS, clarified what GMP is and more importantly, what it is not, using the medicinal product as a model for the challenge agent. Two activities in the life cycle of the product are important: first, the CMC development for description and qualification of the production process and its quality control strategy and second, the routine phase after approval for production and release of product batches. Documenting the manufacturing process and the quality control strategy is called the CMC documentation, which is reviewed by the competent authorities. Tech transfer and deployment of the manufacturing process, if done, should be based on this documentation.

These activities are included into the pharmaceutical quality systems contributing to the delivery of a product of quality; well-characterized, with a defined purity profile, stable during storage and administration, with well-controlled intra- and inter-batch reproducibility. All these quality attributes essentially ensure the reliability and relevance of CHIS outcomes.

As described in ICHQ10, GMP defines the organization and framework for the production of a medicinal product batches either for clinical trials (investigational medicinal product) or for commercialization post-authorization. However GMP does not apply to the CMC activities (development phase), aimed at establishing the relevant quality profile, manufacturing process and quality control strategy for the considered product under development. GMP consists of a series of principles and recommendations to contribute to the proper execution of the declared production process, to guarantee that the material and equipment as well as the personnel are validated and qualified for the intended activities. Every working condition should be well specified, to contribute to a consistent and reliable production and quality control operations before release of the final product.

The viewpoint of WHO, as described in the technical reports on human challenge trials for vaccine development in 2014 and 2016, is that the quality of the challenge agent should be comparable to a candidate vaccine, and that the regulation for these trials needs to be well defined by the national regulatory authorities. The COVID-19 blueprint document, issued in 2020, insists on the need that the selected strains are sent to a GMP manufacturer, to prepare batches of the challenge agent in appropriate formulation, for use in the SARS-CoV-2 model. What level of requirements for the manufacturing conditions should be envisaged? GMP or ISO are good tools to identify the key elements and procedures to be put in place for production control and release of challenge agent batches under reliable conditions. In the whitepaper, section 5C proposes the key principles to be followed. The level of requirements will be at the discretion of the regulatory authorities. The regulatory authorities could use a case-by-case approach to adapt their final requirements, but the GMP principles are expected to be the basis for discussion. Challenge trials have an important added value in the development of therapeutic and prophylactic strategies against pathogens, and as such, challenge agent batches should be of reliable and consistent quality. All efforts have to be made using a formalized approach. Based on the Critical Quality Attributes and Critical Process Parameters identified during the CMC development phase, GMP rules are a good basis for reflecting upon the key points to be monitored in the production environment, using a proportionate approach, with the help of decision-making tools such as risk analysis and risk mitigation. He concluded that GMP, on its own, is not sufficient to ensure quality and safety of a challenge agent. After a relevant CMC development phase, GMP contributes to the right execution of a process duly designed, developed and declared in the CMC documentation.

7. COVID-19 as relevant example

Pauline Meij, head of the cell and gene therapy facility and qualified person for the production of advanced therapy medicinal products (ATMPs) at the Leiden University Medical Center shared the experience of the manufacturing of SARS-CoV-2, for use in a controlled human infection study. Three different product categories are produced at the facility: chemical synthesized products, ATMPs and challenge agents for controlled infection studies. The facility contains clean rooms class A/B

and laboratories for chemical synthesis and one clean room for aseptic filling and lyophilization of products. The challenge agents produced are Schistosoma, hookworm and SARS-CoV-2. It was decided to investigate whether SARS-CoV-2 could be manufactured, to be ready in case a controlled human infection model was developed. To guide the manufacturing, a core team was set up, including a clinical expert, a preclinical expert or virologist, a process or product development expert, a regulatory and GMP expert, and finally, a project manager. Given the strict timelines, it was decided to manufacture the SARS-CoV-2 as a medicinal product, with all regulations enforced using the existing quality system, including product release by a qualified person. A GMP facility was used, with the possibility of production under biosafety level 3 (BSL3) conditions. For the CMC development aspect, the excipients, the formulation and the dosing of the product was discussed, as were the storage and stability of the product. As well, the in-process controls and QC testing of the product were defined, and a product dossier was written according to the format of a medicinal product (IMPD), to implement everything in the existing system. Finally, batch documentation was necessary for the production.

As regards the production activities, the facility had two biosafety cabinets available, to separate work on the cell lines and work on the viruses, which were stored in different incubators. However, many adjustments were needed to be able to work according to GMP and BSL3 guidelines: adjusted disinfection strategies, adjusted gowning procedures, but also the microbiological monitoring, definition of decontamination procedures after the production and autoclavable batch documentation. Most adjustments were specific for the situation. As an example, it is not allowed to bring plates for microbiological monitoring outside of the BSL-3 area of the cleanroom facility. So, an incubator for culturing these plates was needed and people that were allowed in the BSL -3 area had to be trained to read out these plates. Secondly, cleaning requirements for BSL-3 were different from cleaning requirements for GMP. So, a cleaning strategy had to be developed meeting both (BSL-3 and GMP).

It is recommended to start with the generation of a manufacturing flow chart, based on the CMC documentation. Some adjustments in the manufacturing protocol were necessary to have a GMP-compliant protocol. A production workflow schedule was drafted to include all steps, from revival of the cells, production of a working viral stock, the drug substance and the drug product at 10E5/0.5 ml. Both manufacturing and quality control could be performed in a very short time frame. The products passed the QC testing and stability data are generated.

8. Hookworm as second relevant example

Jeffrey M Bethony, Director of the *Necator americanus* Third Stage Larvae (NaL3) Production Unit at The George Washington University described the manufacturing process used for infective hookworm third stage larvae. Hookworms (*Necator americanus*) are endemic in many tropical areas, where millions of people are infected. A controlled human hookworm infection (CHHI) model, where you evaluate the dose of infection, is important for both an understanding of the pathophysiology of this parasite, as well as a CHIM platform to accelerate testing of candidate hookworm vaccines that have reached their final stage of clinical development. The CHHI model is also useful for the testing of other anthelminthic drugs, anthelmintic drug combinations, and evaluate new diagnostics.

A technology transfer of the manufacturing process for this CHHI to Brazil is intended, where hookworm is endemic, by establishing several steps that were put in place at the current production facility at GW. First, a human donor program will be established in Brazil. Secondly, a dose escalation study will be done, and finally the CHIM will be used to test the efficacy of two hookworm vaccines that are in their final stages of development: *Na*-GST-1 and *Na*-APR-1, both of which have been found to be safe and immunogenic in Brazilian resident in hookworm endemic areas.

At George Washington University, the NaL3 infectious challenge agents must be produced under an Investigational New Drug (IND) application of the US FDA. Pursuant to this, the GW team undertook a dose-ranging study, to determine the number of larvae that would result in a stable, reproducible, and measurable eggs count (clinical trials endpoint), but not impact on the health of the participants. Infecting participants with 25 or 50 NaL3 can achieve 100% infection. The time from inoculum to the first evidence of patent (established) infection is on average 84 days. Initially, a peak of infection can be observed, which evolves into chronic established infections, with the number of eggs per gram feces remaining stable over a number of years. The mean number of eggs per gram of feces for donors is around 100.

Given that the challenge agent is isolated from human feces, collected on a weekly basis, a BSL2+ facility and not a BSL3 is used for the isolation of larvae. The release criteria used are viability/motility (90%), species confirmation (by PCR), and microbial quality (bioburden) conforming to USP 61 and 62. When fit for use, the NaL3 challenge agent is placed on the arm of a participant and a slight skin rash appears 1 h post application, which becomes more pronounced after 7–14 days.

Volunteers who are experimentally infected with NaL3 may develop patent infections and shed hookworm eggs in their feces. It is extremely unlikely that a bystander individual – for example, someone living in the same household as the study subject – could become infected with *N. americanus* due to exposure to these hookworm eggs that are shed, for several reasons. First, to become infectious to humans, hookworm eggs must hatch to release larvae into the environment. Hatching and development into infective larvae require a moist and warm environment (optimally between 23 and 33 °C) and a certain period of time, generally estimated to be at least a week. Second, infectious NaL3 can infect humans only by coming into contact with skin; they are not infectious by oral ingestion. Since almost all residents of the Washington, DC area have access to flush toilets, secondary infection is an extremely unlikely event. Nevertheless, all study subjects are counseled to practice good hygiene, to always defecate in a flush toilet, and to dispose of all fecally-contaminated matter immediately into that toilet.

Linda Schellhaas, consultant in quality assurance and GMP, GLP and GCP regulations and associated documentation requirements, presented the quality systems for the cGMP compliant production of hookworms at George Washington University. A quality program, meeting the US FDA requirements, must include the statutory requirement that anything administered to humans should meet the relevant efficacy, quality and safety standards. The cGMP regulations for full-scale manufacturing of drugs and finished pharmaceuticals and the industry guidance for cGMP for phase 1 investigational drugs, which provides for certain leniencies from the full cGMP regulations, both apply. The objectives were to assure that quality was built into the process through the proper design, monitoring and control of manufacturing, in order to produce a product that is consistent according to quality standards, appropriate for its intended use under the IND, that the product is safe for human subjects, and that the identity, strength, quality and purity characteristics of the product are assured through adequate control.

In order to achieve these objectives, critical areas of compliance for the phase 1 product were identified: the need for robust, approved standard operating procedures (SOPs) that would address both the general quality system, as well as production-specific SOPs, and product release SOPs, covering validation of critical processes and test methods to assure appropriate transparency and traceability of the whole production process and confirms that the product lot meets the established specifications prior to its release.

One of the first steps in achieving the objectives was implementing a quality manual in order to define the overarching policies and procedures within the facility to assure that quality processes were in place. A general quality system was implemented to address subjects such as approval and management processes, training programs, documentation requirements, how to handle deviations, corrective action and

preventive action plans, equipment use, calibration and maintenance, QA monitoring, as well as record retention and recording. Simultaneously, production specific SOPs were implemented that included raw material management, reagent and solution preparation, labeling, testing methods and lot release procedures. All SOPs included approved worksheets and forms in order to assure that the necessary documentation remained available after the production process was complete.

Concerning the lot release process, every lot to be used in a clinical trial undergoes formal release process: the production records, the test results, as well as all supporting records and the certificate of analysis are audited. The final approval in product release is completed by QA. Good documentation practices are critical to the whole process, to show adherence to the procedures and regulatory requirements. It allows for reconstruction of the manufacturing process and demonstrates that required quality processes are in place.

9. Round table discussion

Moderators: Isabelle Bekeredjian-Ding; Pieter Neels.

Panelists: Winfred Badanga Nazziwa, UNCST Uganda; Hilde Depraetere, EVI; Eric Karikari-Boateng – FDA Ghana; Alex Mann, hVIVO; Pauline Meij, LUMC; Jetsumon Sattabongkot Prachumsri, Mahidol University; Paula Salmikangas NDA; Dean Smith, Health Canada; Scott Stibitz, FDA; Peter Stjärnkvist, MPA; Jean-Hugues Trouvin, IABS; Wim Van Molle, Sciensano.

Isabelle Bekeredjian-Ding asked to what degree the quote "you cannot choose how you manufacture" – actually applies to a human challenge agent? **Hilde Depraetere** responded that as the type of human challenge agents can be quite variable, not all considerations in the whitepaper are applicable to all types of them. However, there are some general principles that need to be followed. Everything described in the whitepaper with regard to the selection, characterization, and proposed testing, like testing for adventitious agents etc., are things that have to be done, regardless of the type of human challenge agent being developed. This was illustrated for both examples, SARS-CoV-2 and hookworms, where the recommendations in the whitepaper were followed. **Jean-Hugues Trouvin** added that the model and the formalization of all these checklists of points to be considered in the development as well as in the implementation of the routine manufacturing process should be used as a basis. We may have to consider some adaptations, but we should have in mind the basic principles and after that, how and to what extent we can adapt these principles to the situation. **Isabelle Bekeredjian-Ding** added that the most important aspect to keep in mind is "what are we going to use this for?". If we are going to use the data for generating evidence in a clinical trial, we need the trial results (and the challenge agent) to be robust and reproducible.

Jetsumon Sattabongkot Prachumsri commented that she is very glad to see the whitepaper because it is the basis for a guideline and outlines the categories that need to be considered step by step. It is challenging to go into the details with it, especially for malaria, but the basis of the paper is good enough. However, when it comes to specific things, like e.g., testing the quality of your batch, it may not be that simple, because in the case of malaria, it is specific for humans. If you want to check the challenge agent - the efficacy of the parasite after you produce many lots, in general, the current state of research, especially in the case of *Plasmodium vivax*, does not allow culture of the parasite *in vitro* to check the viability and infectivity, which is a limitation. The quality from batch to batch – how can that be checked? Because you don't want to have to inoculate two volunteers every time to prove that the dose from this lot will give the same level of infection in your volunteers. So, this remains challenging. But the whitepaper is very useful.

Eric Karikari-Boateng thought that the whitepaper is a good document but there are some things that are missing. Especially if it is a virus and you are producing it in a cell line. You should be able to show that your source has maintained the integrity from the beginning to the end. The integrity at the end of production has to be maintained. If the integrity is not maintained, the host cell protein profile can change the quality of your drug substance so that has to be checked. A second issue is comparability i.e., scaling, because there might be a difference between the batch size for your clinical challenge and the batch size of your commercial batch. If they are different, you have to do comparability in accordance with ICHQ5. Moreover, for a regulator, the definition of a "non-GMP environment" is unclear.

Scott Stibitz supported the importance of stressing the principles, because the challenge agent manufacture is so varied and each one is so unique. It will not be possible to have a prescriptive approach to this. The whitepaper can provide examples and frameworks, and today, we are much more in harmony than we were in Langen in 2019. We are using completely different languages, but we are in the same place. GMP for challenge agents is not necessarily full scale GMP, but it can depend on the challenge agent itself. For a COVID challenge agent, maybe that is much more important. The principles are really the message in our Phase 1 GMP guidance document.

Wim Van Molle stressed that the intention of this CHIM – this challenge agent – is to be used First-In-Human. When talking about the manufacturing process, we should not forget the scale we are looking at. The challenge agent is for use in a clinical trial with no need to produce hundreds of millions of doses, as is the case for COVID vaccines, for example. The two examples (hookworm and COVID) can be considered good models because all the testing, including for adventitious agents, have been performed, and if manufacturers and developers are familiar with different agents or different types of organisms, this should always be looked at case by case. We are focusing on the First-In-Human trial, hence, the first principle is the safety of the agent, whether it is a candidate vaccine or a challenge agent. We also know that at least for First-In-Human studies, the general requirements for quality are less stringent from what is required for a manufacturing authorization. It is important to bear in mind that it is not the intention to license the challenge agent but to license the vaccine used in the challenge study.

Robert Sauerwein pointed out that regulatory approval will be required for batch release. Experts are qualified according to categories for medical devices, therapeutics, medical diagnostics etc. These all have different criteria. How do we present a challenge agent in the regulatory environment? **Wim Van Molle** replied that a challenge agent is not a medicinal product. In the Netherlands, products or agents are categorized for their assessment, whereas in Belgium, people involved in clinical trials can also be involved in the assessment of dossiers for marketing authorization. The example of the hookworm showed that the manufacturers are characterizing and testing their challenge agent as if it would be a medicinal product, so they are putting the bar at the same level. So, we should in the best possibilities assess this as close as possible to a medicinal product, bearing in mind that it will be used in clinical trials, not needing the same requirements as would be needed for the marketing authorization of a medicinal product. **Dean Smith** added that in Canada, there is not much experience with challenge agents as such, but there have been many discussions internally and observation of discussions elsewhere, like in Germany and the US. The most relevant group would deal with it, and the expertise required to deal with this would be recruited, especially if it would be a particular pathogen. This is a challenge to regulators as well as a challenge for manufacturers of these agents – to be very open-minded. The document and its structure around the principles is very positive, rather than trying to be too detailed and too prescriptive – that is a very dangerous territory. The examples chosen today cover everything from an example that is closer to routine manufacturing processes for a virus (the COVID example) to hookworm and others, including malaria agents, which are out of the typical range but certainly can be managed, provided a regulator is sufficiently open-minded about the principles and not the specifics of any one regulation. For clinical trials in Canada, materials are required to be comparable to the stage of development, so Phase 1, Phase 2 etc., where full validation of all of the processes is not required. But the regulator should certainly ensure that the assays are fit for purpose and,

where possible, be fully qualified at least, but perhaps not fully validated in the routine sense. And all of the conditions around GMP (maintenance, SOPs, document control …) must be in place to ensure integrity, but it must be realistic to the stage of development – that is the key piece. We are not going into large scale manufacturing, but to the extent you scale up, comparability would be essential.

Martin Schutten asked whether regulatory authorities have different criteria in judging aspects when challenge agents are concerned, compared to attenuated or live vaccines? And where is the border between a challenge agent and a live vaccine, e.g., the example of live *Shigella*? This border should be looked into in the whitepaper. **Wim Van Molle** responded that this depends on the stage of development: whether you are going for the First-In-Human or into Phase 2 or Phase 3. When it comes to comparing your agent to what you would require for an attenuated strain, it is difficult to say, but if you are going for a First-In-Human, what you will require for an attenuated vaccine will also "grow" in the further phases. And again, for the First-In-Human, emphasis is always put on the safety of the product, so if your attenuated vaccine strain should answer to a minimal set of safety requirements, then your challenge agent should also respond to this. An example are the COVID-vaccines: what was required in the clinical trials may have been less than what would be required in the normal situation, except for the safety requirements. **Peter Stjärnkvist** explained that there have not been any clinical trials involving challenge agents in Sweden, so it is unclear what would be required. But it should be the same as for an investigational medicinal product to be used in clinical trials, including GMP. It is important for this type of agent, where safety is extremely important. Everybody seems to agree on having the same quality requirements as for a medicinal product, even if it is not a medicinal product.

Eric Karikari-Boateng remarked, that when looking at the scenarios, if the challenge agent is linked to a vaccine and is in a clinical stage it should be manufactured according to GMP, but what type of GMP? Because for Phase 1, the product should have clear release specifications, and the manufacturing process should be well-defined but without process validation or analytical method validation. But the basic GMP requirements would have to be met, at least the starting material and the manufacturing process should be well-defined to assure the quality and safety of the products.

Isabelle Bekeredjian-Ding summarized that this discussion highlights the importance of these trials. If high requirements are set, it forces you to think and plan long-term; these models and trials are not going to nor should be a quick shot. **Peter Stjärnkvist** clarified that process validation is not a requirement for an investigational medicinal product in a clinical trial, so that is not included. **Paula Salmikangas** added that even for medicinal products, the requirements are not the same for every medicinal product – they vary depending on the risks. Here, the risks of the challenge agent should dictate the level of information needed. With regard to GMP requirements, an important risk is the release of the infectious challenge agent into the environment. For every medicinal product, an environmental risk assessment is necessary, so the GMP requirements should also take into consideration this environmental aspect. **Scott Stibitz** added that challenge agents are considered drugs by the FDA, not so much because they are intended to diagnose, treat, or prevent disease, but because they affect the structural function of the body. With regard to the environmental issues: with outpatients, you have little control, but with inpatient studies, e.g., in a diarrheal disease challenge, patients are not released until they have negative stool cultures. This indicates that the environmental aspect can be approached. Perhaps this can be included in the whitepaper.

Dean Smith commented on the stability of challenge agents: a key element is characterization of the material you are making. An understanding of the material and its characteristics is central to designing a stability program. Stability considerations will be different for stable material, versus material required to be kept under stringent conditions (e.g., −80 °C) *prior to* use and with short intervals *in* use. In the latter case, stability testing will likely be most challenging for the in-use conditions, with sufficient ICH-type of guidance around general stability assessment. In Health Canada, there is a focus on the use of forced-degradation-studies to assess relative stability, including through scale-up and process changes, in addition the collection of real-time and temperature data. The use of forced degradation to assess stability pre- and post-change allows quicker insights into potential changes in stability through manufacturing and scale-up, and can permit earlier decisions, while real-time and temperature data is collected. The specific timing of stability test intervals should be a function of the product characteristics and the questions being asked. Regarding quality control of malaria challenge material, which reportedly does not currently have good *in vitro* means of characterization, this is something a developer should discuss with the regulator: i.e., the limitations with the culture process. Options to consider could involve indirect measures that would correlate with a human infection model, such as measures of viability and infectivity as determined by gene expression in the parasite required to establish an infection. Robust *in vitro* characterization allows better control within the system. In the end, a human is by definition an animal model, and animal models are quite time consuming and variable. If there are limitations in terms of timing in the development of your process, a regulator should not prevent a developer from using animal model assays at hand, but it is always in the interest of a developer to find those non-animal-model-based potency/viability assays as quickly as possible. This will aid the product development, be more robust and may assist with ethical issues as well. **Alex Mann** confirmed that those comments can apply to other organisms besides malaria. For instance, norovirus is not the easiest to confirm potency and viability, except by quantifying in a human (as a human infectious dose). If you are not performing regular challenge studies, you may not know that your batch is consistently giving you the same results; if you have 3-5-year gap between your studies, you may need to do a small characterization study on a small number of subjects, to make sure it still has its potency and viability.

Isabelle Bekeredjian-Ding asked the panel about the special challenges with COVID-19 in view of the issues that have been discussed. **Pauline Meij** replied that the whole process was started as if a medicinal product was manufactured, with a focus on the consistency, the safety, the quality of the production process. Expertise was brought in, e.g., experts were hired with expertise in the production of viruses as medicinal products. Decisions were made on the manufacturing, the release testing, the transfer from research to GMP, etc. As this was an agent which could be produced in the GMP facility it was easy to use the whole quality system around it. If you have an agent like hookworm, which cannot be produced in a GMP facility adapted to the production of a medicinal product, it is a different story, how to assess the whole process. **Isabelle Bekeredjian-Ding** concluded that it is important to have some time and experience for development of the challenge agent, to learn how to work with these pathogens. This is important in the context of pandemic preparedness and the next "Pathogen X". **Pauline Meij** strongly agreed, the coronavirus experts involved were very important to set up this whole process.

Winfred Badanga Nazziwa (Uganda) pointed out that currently, the first challenge study ever is being implemented in Uganda: on schistosomiasis. With regard to COVID-19 challenge studies, there would be a number of challenges and ethical issues. And so many questions would be raised, not only from the stakeholders and regulators, but also from the general public. With regard to the white paper being discussed, it is a good document which will provide guidance, not only to the regulators but also to the national agencies that regulate research. For example, in Uganda, some challenge studies may not be regulated by the National Drug Authority, if the challenge agent is not classified or does not fall into the category of a 'drug'. Such challenge studies would instead receive oversight from the UNCST which is another national regulatory agency, that oversees all research activities in the country. So, this guidance document will be handy and helpful and provide guidance to

the national agency. A recommendation would be to make the document more inclusive and adaptable to all the agencies, including applicable national bodies or agencies that oversee research.

Dean Smith pointed out that while the idea of human challenge studies on COVID-19 has been difficult for many authorities to contemplate, we are moving into a phase with the variants, where leading agencies are talking more openly about the use of human challenge studies. As such, the ability of manufacturers to pivot and make new challenge viruses is important to consider in jurisdictions that support CHIM. On a related manufacturing point, the WHO animal models subgroup, has been very clear that cell line and cell substrates selection is critical, because mutations do develop during production in these strains, and it is essential to control and verify consistency of product. **Pauline Meij** responded that the Leiden group looked into the mutations which were introduced during the whole culture of the viruses. Careful selection of strain, cell line and culture medium were performed. Every step needs to be assessed very carefully.

Isabelle Bekeredjian-Ding asked whether one can speed up the process of manufacturing of a new strain once you have experience with the agent itself. This is important because we are looking out for possibilities on how to license vaccines against variants. Basically, the question is: given the dynamics of virus mutation, can the manufacturing of new strains keep up with these dynamics?

Joris Vandeputte enquired in how far *in vitro* can keep up with this issue. **Dean Smith** responded that the *in vitro* studies in terms of neutralization and binding studies with variants are very insightful. Immuno bridging studies could potentially evolve out of that. WHO has indicated clearly, with agreement from agencies like EMA, FDA and Health Canada, that at this point, several of the authorized vaccines still offer good protection against hospitalization and serious disease, so currently there is no need to change. However, there is absolutely a need to explore alternatives. It would be a very valuable if constructs were available to evaluate variants in, for example, a CHIM, as a small-scale quick study. That means that manufacturers would have to quickly adapt to other strains as a challenge material. While funding issues are clearly off the table of regulators, if regulators are more willing to consider those types of studies that would potentially reassure funders dealing with the groups capable of making those materials.

Alex Mann went back to the manufacturing process of challenge agents. Once you have done all the groundwork for an earlier isolate, with all the documentation and all the principles in place, as described in the whitepaper, it can make it easier to reproduce the process for a variant, partially shortening the manufacturing timeframe. So, this exemplifies the need for some of those principles to be applied even if you are working in a non-GMP facility – if you are working in a GLP facility or an academic laboratory – regardless of the challenge agent, if you are trying to do another run with a new variant or a new batch lot, having that consistency and the process in place is obviously beneficial. **Jean-Hugues Trouvin** said that this is a very good example of where we could take benefit of the development work previously done on a given strain, and after that to apply this development work and the experiences gained for a new variant of the strain. **Dean Smith** pointed out that this is essentially the use of platform manufacturing technology experience of manufactures. In general, regulators during the pandemic have been relying more heavily on the platform technology experience of manufacturers to expedite decisions where possible. This has always been the case to some extent but is now more universally so and would certainly be applicable to evaluation of challenge materials as well.

Jean-Marie Préaud asked whether a GMP mock inspection could help and advance the GMP requirements or GMP-like requirements. **Isabelle Bekeredjian-Ding** responded that inspection is an interesting point, which also requires expertise. Personally, she is not sure a mock inspection makes sense. **Joris Vandeputte** added that it is something you do, e.g., at an institute that will produce a challenge agent, to assure in advance that everything is all right. **Alex Mann** expanded that if you are going to subcontract to do your manufacturing, you want to know that the subcontractor will do a good job. Many manufacturers are not used to using viruses and infectious agents, so you need to work with them, to work out how you implement your process and quality control tests within a GMP facility. However, if using a GMP facility, you want to make sure that their processes are adherent to the GMP criteria, and you want to be reassured before you go into the actual manufacturing process. **Jetsumon Sattabongkot Prachumsri** did not agree since her challenge agent (*Plasmodium vivax* malaria) is very far from GMP. The idea of practice following the guideline is preferred as it is impossible to be GMP – for now – until they can do the *in vitro* culture of the parasite. **Alex Mann** agreed, however clarified that even under non-GMP conditions, it is about reassurance that you *are* doing what you claim you can do. At the end of the day, you are still responsible for your agent and the subcontractors. However, if your challenge agent cannot be produced under GMP, you would not perform a GMP audit. **Dean Smith** added that in most agencies' guidance, it is very clear that early engagement with the regulatory agency you are going to be dealing with is highly desirable. This permits an explanation of the objectives, and the conditions under which the manufacturing will take place. Whether that would entail an inspection is an area of its own, but early engagement is helpful. **Peter Stjärnkvist** pointed out that if you are going to produce according to GMP, the facilities must be inspected either by the regulatory authority or have an audit ("mock inspection") by the qualified person responsible for the release of the products used in the clinical trial. So, some sort of audit or inspection is mandatory to call it GMP.

Jetsumon Sattabongkot Prachumsri asked whether it should be called "non-GMP" or "GMP-like". **Peter Stjärnkvist** clarified that sometimes "GMP-like" is said that in order to have a GMP facility, it must be inspected by the regulatory authority. However, it could also be inspected by a qualified person. That means that the facility does not necessarily have to have a manufacturing license, which is usually required for manufacturing a drug. The most important point is to consider how the manufacturing site is organised and whether or not it follows the GMP principles.

Isabelle Bekeredjian-Ding inquired whether the person who does this inspection would need specific expertise. **Peter Stjärnkvist** responded that an audit must be carried out by a qualified person or a person under his/her command. It is called "audit" if done by a qualified person and "inspection" if done by an authority. Therefore, the term "mock inspection" refers to some sort of audit.

Isabelle Bekeredjian-Ding asked **Winfred Badanga** how this is implemented in Uganda. She responded that this is something new, the trial is approved, but has not been anything like that. So, the document will be a good guidance. National ethics committees will have an oversight of challenge studies because they do not fall under the mandate of the drug agency. Therefore, the guideline will be really handy.

Martin Schutten asked whether it would be prudent to discuss bioburden issues in the whitepaper. **Alex Mann** clarified that the intent was to pull out conceptual principles that can be considered for the manufacturing of a given challenge agent, that can be applied on a case-by-case basis. Each pathogen has its own nuances that may need to be addressed during manufacturing, as we found when we engaged the researchers that are manufacturing these agents. Examples are planned be added to the document to exemplify the concepts, but without going into all potential scenarios.

Summarizing, the main take-home messages from the round table discussion were:

- Before considering the GMP conditions for the routine production of a challenge agent, one has to consider also the development and qualification phase of that challenge agent, i.e., the CMC aspects which are key for the safety of the volunteers and reliability of the clinical trial results
- All of the conditions around GMP must be in place to ensure integrity, but in line with the stage of development

- The utility of an audit or "mock inspection" to be prepared for an inspection by an authority
- The variability of regulatory environments in different countries, where an agency or regulatory authority other than the national drug authority may be responsible for review and approval of clinical trials involving human challenge agents
- Platform manufacturing technology experience may be applicable to evaluation of challenge materials, to speed up production of variants of established challenge agents

10. Conclusion

For the design, development and manufacture of challenge agents, a stepwise approach must be considered. This starts with the development and CMC documentation for a candidate challenge agent, followed by the implementation of the "routine" manufacturing process. These activities are complementary: CMC activities are guided by technical guidelines mentioned above whereas GMP rules, applicable for the routine production, do not guarantee the proper development and qualification of a candidate product, whether challenge agent or otherwise.

A GMP manufacturing site is understood as a facility which has received a manufacturing authorization after a formal GMP inspection by regulatory authorities. However, if a challenge agent is not considered as a medicinal product, it cannot be claimed that the manufacturing will be carried out in a GMP facility. In these cases, the term "GMP-like" could be useful, indicating that GMP principles were followed as a structuring document to help elaborate a valid and reliable manufacturing plant.

Disclaimer

The opinions, findings and conclusions contained in this report are those of the authors and do not necessarily reflect positions or policies of their employers.

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References

[1] Bekeredjian-Ding I, Van Molle W, Baay M, Neels P, Conrad C, van Diepen A, et al. Human challenge trial workshop: focus on quality requirements for challenge agents, Langen, Germany. Biologicals 2020;66:53–61. October 22, 2019.
[2] Baay M, Neels P. SARS-CoV-2 controlled human infection models: ethics, challenge agent production and regulatory issues. Biologicals 2020;67:69–74.
[3] Baay M, Neels P. Controlled human infection to speed up SARS-CoV-2 vaccine development. Front Immunol 2021;12:658783.
[4] WHO Expert Committee on Biological Standardization. Human challenge trials for vaccine development: regulatory considerations (annex 10). Available from: http://apps.who.int/medicinedocs/documents/s23329en/s23329en.pdf.
[5] International council for harmonisation of technical requirements for pharmaceuticals for human use (ICH). Harmonisation for better health. Available from: https://www.ich.org. Last accessed: June 7, 2021.
[6] Talaat KR, Bourgeois AL, Frenck RW, Chen WH, MacLennan CA, Riddle MS, et al. Consensus report on Shigella controlled human infection model: conduct of studies. Clin Infect Dis 2019;69:S580. s90.