



Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Meeting report

Third human challenge trial conference, Oxford, United Kingdom, February 6–7, 2020, a meeting report

A B S T R A C T

The third Human Challenge Trial Meeting brought together a broad range of international stakeholders, including academia, regulators, funders and industry, with a considerable delegation from Low- and Middle-Income Countries.

Controlled human infection models (CHIMs) can be helpful to study pathogenesis and for the development of vaccines. As challenge agents are used to infect healthy volunteers, ethical considerations include that the challenge studies need to be safe and results should be meaningful. The meeting provided a state-of-the-art overview on a wide range of CHIMs, including viral, bacterial and parasitic challenge agents. Recommendations included globally aligned guidance documents for CHIM studies; further definition of a CHIM, based on the challenge agent used; standardization of methodology and study endpoints; capacity building in Low- and Middle-Income Countries, in performance as well as regulation of CHIM studies; guidance on compensation for participation in CHIM studies; and preparation of CHIM studies, with strong engagement with stakeholders.

As a follow-up from earlier meetings and workshops on human challenge trials (HCT¹) organized by the International Alliance for Biological Standardization (IABS, <https://www.iabs.org>) in Strasbourg, France, in 2014 [1], Rockville, MD, USA, in 2017 [2], and Langen, Germany, in 2019 [ref to be inserted once available], this workshop was organized around challenges identified previously: Good Manufacturing Practice (GMP) production of the challenge agent; CHIM ethics; the performance of HCT in children; environmental safety in CHIM; recruitment, engagement, advertising and incentive; pre-existing immunity; and clinical, immunological and microbiological endpoints.

The first session, on the role of challenge models, chaired by Andrew Pollard (University of Oxford, United Kingdom) was intended to set the scene.

In the first presentation, Myron M. Levine (University of Maryland School of Medicine, USA) discussed the role of challenge studies. Challenge studies can be performed to establish pathogenicity, elucidate pathogenesis, identify host risk factors, estimate the infective inoculum, assess infection-derived immunity, characterize the immune response, measure vaccine efficacy, and identify correlates of protection. The cholera CHIM has been used to study all these aspects, eventually leading to licensure of live *Vibrio cholerae* O1 vaccine strain CVD 103-HgR by the US Food and Drugs Administration (FDA) and the European Medicines Agency (EMA). Initial insight into the pathogenesis and host factors led to development of a pioneer toxin-deleted El Tor strain, JBK70. This strain elicited high titers of serum vibriocidal antibodies without stimulating cholera antitoxin, and conferred 89% protection against challenge, documenting the importance of anti-bacterial antibodies in the absence of antitoxin antibodies [3,4]. Attenuated strain CVD 103-HgR (a classical Inaba strain deleted of the gene encoding cholera toxin A subunit [*ctxA*] and harboring insertion into the chromosome of a gene encoding resistance to Hg⁺ as an indelible

marker) was tested in multiple challenge studies. These included challenges with virulent *V. cholerae* O1 of Inaba and Ogawa serotypes and El Tor and classical biotypes at early (8–10 days) and late (3–6 months) time points after a single oral dose. The significant high-level efficacy documented in these CHIM studies, in conjunction with results of large trials demonstrating safety and immunogenicity, resulted in FDA and EMA licensure. The correlation of efficacy of CVD 103-HgR with serum vibriocidal antibody seroconversion following ingestion of a single dose was another important observation derived from the cholera challenge trials.

In the second presentation, Joshua Osowicki (Murdoch Children's Research Institute, Melbourne, Australia) presented the role of a CHIM in accelerating vaccine development against *Streptococcus pyogenes*, also known as group A *Streptococcus* (GAS), the cause of scarlet fever. GAS causes a spectrum of disease, from mild and superficial to fulminant invasive infections, and post-infection immune complications. GAS also contributes to chronic non-communicable syndromes. More than 500,000 deaths are directly attributable to GAS every year. From the early 19th century there were attempts to induce experimental human scarlet fever, hoping to induce mild infections that might have an immunizing effect.

In the 1970s, studies were performed comparing a monovalent M-protein vaccine to placebo, for protection against experimental pharyngitis caused by a homologous strain swabbed directly on to the pharynx, with impressive efficacy. This unfortunately coincided with heightened concerns of vaccine-induced autoimmune complications, focused by a 1969 report of 1 probable and 2 definite cases of acute rheumatic fever in a group of 21 child siblings of rheumatic fever clinic patients, who received between 18 and 33 weekly doses of a partially purified M protein vaccine. This cast a pall on GAS vaccine development and led to an FDA ruling which had the practical effect of a ban,

¹ AE - adverse event; AUC - area under the curve; CE - Community engagement; CHIMs - Controlled human infection models; EMA - European Medicines Agency; FDA - US Food and Drugs Administration; GAS - group A *Streptococcus*; GBS - Group B *Streptococcus*; GMP - Good Manufacturing Practice; HCT - human challenge trials; HIC - High-Income Country; IABS - International Alliance for Biological Standardization; LMICs - Low- and Middle-Income Countries; MGIA - Mycobacterial Growth Inhibition Assay; PBMC - peripheral blood mononuclear cells; qPCR - quantitative polymerase chain reaction; WHO - World Health Organization.

halting vaccine development for more than 20 years.

A study protocol for a dose-ranging inpatient study in healthy adults was developed, with the primary objective to establish a new GAS pharyngitis CHIM, with an attack rate of at least 60% [5]. An *emm75* (M75) GAS strain was selected as the challenge agent [6]. This initial study has been completed with encouraging signs that it will be a safe and reliable platform for vaccine evaluation and pathogenesis research (publication forthcoming). As for other pathogens, a human challenge model for GAS is a means to an end, a tool with intrinsic limitations, the greatest of which is generalizability across strains, subjects, settings, syndromes, and severity. Recent years have seen new momentum building for GAS vaccine development, including the Australian Strep A Vaccine Initiative (ASAVI, www.asavi.org.au), with its goal of taking at least one vaccine forwards to a phase 2b field trial in 5 years, and with CHIM studies making an important contribution to the matrix for choosing between candidate vaccines.

In the third presentation, Andrew Pollard discussed early studies with vaccines against *S. Typhi*. A meta-analysis performed on clinical trials using the Ty21a vaccine showed 56% efficacy against typhoid fever after three years (risk ratio 0.44, 95% confidence interval 0.25–0.76) [7] and studies of Vi polysaccharide vaccines also showed modest and relatively short-lived immunity. Neither of these vaccines is suitable for use in the youngest children. With the development of new typhoid Vi conjugate vaccines on the horizon, the World Health Organization (WHO) indicated in their development guidelines that “successful typhoid challenge studies conducted in healthy adults using an appropriate and validated model could provide considerable supporting evidence of the efficacy of a Vi conjugate vaccine” [8]. A typhoid CHIM was established in Oxford in 2011 and subsequently used to challenge 103 participants (31 in the control group, 35 in the Vi-polysaccharide group (Vi-PS), and 37 in the Vi-conjugate (Vi-TT) group). The criteria for typhoid diagnosis was met in 24 (77%) of 31 participants in the control group, 13 (35%) of 37 participants in the Vi-TT group, and 13 (35%) of 35 participants in the Vi-PS group to give vaccine efficacies of 54.6% (95% CI 26.8–71.8) for Vi-TT and 52.0% (23.2–70.0) for Vi-PS [9]. These results were followed by a phase 3, randomized, controlled field trial in an endemic region (Nepal), in children (between 9 months and 16 years of age) who received either Vi-TT or a capsular group A meningococcal conjugate vaccine (MenA) as a control. In an interim analysis, blood culture-confirmed typhoid fever occurred in 7 participants who received typhoid vaccine (79 cases per 100,000 person-years) and in 38 who received MenA vaccine (428 cases per 100,000 person-years) (vaccine efficacy, 81.6%; 95% confidence interval, 58.8 to 91.8; $P < 0.001$) [10].

Similarly, a CHIM for *S. Paratyphi A* was set up. In a dose-finding study, two groups of 20 participants underwent oral challenge with *S. Paratyphi A* following sodium bicarbonate pretreatment at 1 of 2 dose levels (group 1: $1-5 \times 10^3$ colony-forming units [CFU] and group 2: $0.5-1 \times 10^3$ CFU). The dose of $1-5 \times 10^3$ CFU resulted in an attack rate of 60%, was well tolerated and associated with an acceptable safety profile [11]. Preliminary results seem to indicate that re-challenge with *S. Paratyphi A* leads to a significantly lower number of participants developing disease, suggesting (partial) immune protection following infection. For novel paratyphoid vaccines, field trials will need to be very large and might not be feasible, such that challenge studies might be the most likely route to licensure.

In the fourth presentation, Andrew Catchpole (hVIVO, United Kingdom) presented the RSV CHIM used to evaluate the efficacy of an RSV candidate vaccine. RSV infection is an increasingly recognized illness in high-risk adults, particularly those aged over 60 years, with a disease burden similar to that of non-pandemic influenza. A recombinant adenovirus serotype 26 vector with a full-length RSV-F protein stabilized in the pre-F protein conformation has demonstrated immunogenicity in older adults in stable health, with no significant safety concerns to date and an acceptable tolerability profile. The CHIM was conducted to de-risk the clinical development program before

progressing with expensive field trials. Because of frequent previous exposure, significant levels of pre-existing baseline antibodies against RSV can be present, so participants were tested and those with no or high levels of antibodies were excluded. Participants received a single shot of vaccine, 28 days before challenge. Challenged participants were kept in quarantine for 12 days and followed on an out-patient basis for another 155 days. Endpoints of the study were: RSV viral load area under the curve (AUC) as determined by quantitative RT-PCR, as well as by quantitative culture; peak viral load; AUC of total clinical symptoms; and AUC of the weight of nasal mucus produced. Immunization significantly reduced nasal viral load detected by quantitative polymerase chain reaction (qPCR) after RSV challenge versus placebo ($p = 0.012$). Similarly, the mean viral load as determined by culture remained consistently lower in vaccinated volunteers compared to controls. Also, the mean disease severity remained consistently low in vaccinated volunteers compared to controls. Finally, the mucus mean weight remained consistently lower in vaccinated volunteers compared to controls, with controls producing significantly more mucus between days 6–10. In a post-hoc analysis, a trend for increasing vaccine efficacy with increased severity of the disease endpoint was observed, despite a lower incidence of higher severity endpoints. To further increase the utility of the RSV CHIM by using subjects more aligned with the vaccine target population, a separate pilot study has been performed in 60–74-year-old subjects, showing that challenge was safe and well tolerated, and that the model is ready for product efficacy testing.

In the final presentation of the first session, Chad Porter (Naval Medical Research Center, Maryland, USA) discussed the development, use and refinement of a *Shigella* CHIM. He emphasized the need for standardization of all aspects of *Shigella* CHIMs, including the conduct of studies [12], the clinical primary and secondary endpoints in the study [13], and the immunological assays used [14]. This includes the challenge inoculum preparation, a long process which is much less cumbersome if a lyophilate can be used that only requires dilution, as is the case for *S. sonnei* (53G). A robust CHIM can help de-risk vaccine development, validate correlates of protection, and support licensure, for travelers' vaccines; however, the role of CHIM in licensure for an LMIC paediatric population remains uncertain at this stage.

In the second session, on regulation of challenge agents, chaired by Pieter Neels (IABS), Nele Berthels (Federal Agency for Medicines and Health Products, FAMHP, Belgium) presented data on an in-depth analysis of the literature regarding the regulatory framework, quality requirements for challenge agents, and safety aspects of these agents. Based on the study, three proposals were formulated: 1) regarding the regulatory framework, it was proposed that all studies with challenge pathogens must be notified to, and approved by, the regulatory authority; 2) regarding the quality requirements for challenge agents, it was proposed that they should be manufactured according to GMP; and 3) regarding the safety requirements of the challenge agents, it was proposed that having a clear, unique and recognized legal framework for CHIM studies will contribute to better safety monitoring. These proposals were shared with stakeholders for consultation, who generally agreed with the requirement for regulatory approval of the clinical study/trial and GMP of challenge agent, although it remained unclear how a challenge agent could be manufactured according to GMP for small-scale use. Generally, GMP requirements for CHIM are determined on a case-to-case basis, with the level of GMP requirements growing with the phase of the study. Finally, stakeholders also saw the added value of standardization, transparency and legitimacy.

The position of the regulators is that CHIM are innovative, promising tools to accelerate vaccine development, while remaining cautious (with the need for strong ethical and scientific justification). A strong benefit/risk assessment is needed in which ensuring the safety of study participants must remain a primary concern. For licensure, it is understood that CHIM do not replace large phase III safety and/or efficacy trials. Finally, publication, standardization, data-sharing, safety reports and registration will facilitate future CHIM studies and their

potential.

In the second presentation, Pieter Neels discussed global variation in regulation, pointing out that in the EU, licensure of Vaxchora based on CHIM efficacy data is not yet possible. According to the 2004 definition, a challenge agent is a medicinal product, as it changes your immune status. In the European Union, while CHIMs are a national responsibility, in the case of multi-country trials, ethics review for CHIM by one country is accepted.

Besides the aspects already discussed, other regulatory questions concern the relevance of CHIM data for the prevention of disease and the extrapolation of CHIM to the real-life situation, which can be very different for different challenge agents, due to route of infection, infectious dose and spread in society.

In some countries, based on ‘primum non nocere’, it is not permitted to infect someone deliberately, which obstructs the use of CHIMs. Moreover, the history of a country can be important in its attitude towards CHIM. However, no document on the positions of countries is currently available.

As worldwide acceptance of CHIM is far away, the advice from a regulatory viewpoint is to look for general information for the target countries, seek scientific advice with the local regulators and ethics committees, and find partners with authority and experience to promote capacity building and exchange of experience.

In the third presentation, Paul Kaye (University of York, United Kingdom) discussed the manufacturing issues for leishmaniasis. A leishmaniasis CHIM could help in the understanding of early stages of pathogenesis, providing insights for vaccine development and as a tool for candidate vaccine progression/down-selection. The first issue is the choice of parasite source: many strains are available, some with genotypic information, but most have minimal clinical data, and an uncertain history, which may impact on infectivity and safety. To ensure good provenance, it was therefore decided to use a fresh isolate of *L. major* from Israel, which is the mildest form. Good clinical history was available, parasite culture was standardized, early freeze stocks were made, and parasites were genome sequenced and characterised using a variety of *in vivo* tests (including for drug sensitivity).

The next issue was the pathway to deliver the parasite in a CHIM model. Three routes are possible from a vial GMP product: 1) the natural method, through the sand fly (which cannot be reared to GMP); 2) Growth and selection of infective stages of the parasite *in vitro*, which can be done under Good Laboratory Practice, leading to a standard dose but with less predictive power of vaccine efficacy due to missing sand fly components transferred during the bite; 3) Direct inoculation, with or without washing, where issues of viability may be encountered. It was decided to focus on the natural route and challenge agent production was outsourced to a Contract Development and Manufacturing Organization. Currently, vials are ready for final analysis. GMP production and release by a Qualified Person is expected by May 2020. This will eventually lead to a bank of approximately 500 vials, which is sufficient for the challenge of 1500 volunteers.

In the final presentation of this session, Robert Sauerwein (Radboud University Medical Center, Nijmegen, the Netherlands) discussed the regulation of the malaria CHIM in the Netherlands. CHIMs fall under the Central Committee on Research Involving Human Subjects, which has several legal tasks, including to review specific fields of medical research; to act as Competent Authority for the review of research with medicinal products; and to harmonize implementation of European legislation for medicinal products. The Central Committee oversees 12 accredited Medical Ethical Reviewing Committees. The interaction is very constructive but may suffer at times from loss of historical knowledge due to changes in committee composition.

In order to perform malaria CHIM at Radboud UMC, there is a high level of certification: from JCI accreditation of the Radboud University, ISO certification 15189 for the department of Medical Microbiology, and ISO certification 9001 of the central animal facility. Where certification is not possible, the Radboud Research Clinic abides to Good

Clinical Practice, while the Malaria Unit uses Good Laboratory Practices, laid down in 139 Standard Operating Procedures, covering the whole process including general procedures, mosquito breeding, parasite culture, clinical procedures, environment, health and safety procedures, and molecular and immunological methods. A flowchart of activities covers all steps from start to infected mosquitoes, a process that takes 30 days.

To further facilitate the use of valuable research tools, CHIMs and challenge agents will greatly benefit from specific EU legislation, which is currently lacking. Such a framework initiative should get a high priority.

In the third session, on ethics, chaired by Robert Sauerwein, Michael Selgelid (Monash University, Australia) provided the philosophical perspective on human challenge studies in High-Income Countries (HICs) and Low- and Middle-Income Countries (LMICs). Infamous cases of HCT performed in Nazi Germany and in the Japanese Unit 731 during World War II were cited as paradigm examples of blatantly unethical research. However, in these cases, it was not intentional infection of research subjects that made the studies unethical; these were brutally harmful studies conducted on prisoners, without consent.

Insofar as HCT involve intentionally doing something that may be harmful (to a degree), they are not unique. Phase 1 toxicity trials that aim to determine maximal tolerable dose similarly aim to bring about (at least a minor) toxic reaction in some participants. It may furthermore be argued that harms associated with HCT are foreseen, but not intended. The intention is to bring about infections in order to learn about the natural history of the infectious agent, or to learn how to treat or prevent the disease. It is foreseen that intentional infection may bring about (minor) harm—but harm is not itself intended. In any case, intentional harm should not be considered ethically off-limits. Intentionally doing harmful things is commonly accepted when the harms in question are outweighed by benefits thereby enabled.

Not only are HCT morally permissible, there may be an ethical imperative to conduct HCT, especially if they provide the best way of developing drugs for neglected diseases in LMICs. While HCT are commonly characterised as research in which participants do not have potential to benefit directly, HCT participants in endemic settings can sometimes benefit directly by gaining immunity (resulting from controlled infection or a vaccine that turns out to be effective) against a disease to which they would have otherwise been at risk.

However, HCT are ethically sensitive and could potentially have Public Relations problems. Therefore, extra vigilance is necessary in closely following existing research ethics guidelines, developing special ethics guidelines and oversight committees, building trust through community engagement, and addressing unresolved issues.

In the second presentation, Susan Bull and Michael Parker (University of Oxford, United Kingdom) discussed ethical issues in controlled human infection studies in LMIC settings. There are strong ethical arguments to do HCTs, to high ethical standards, which can be complicated by having many stakeholders with different sets of values. HCTs should be aimed at helping to reduce suffering, with respect (involving communities in planning the research, sensitive to local values) and in fairness (creating an inclusive and transparent process, with equitable distribution of benefits and burdens of research). Special attention should be paid to the frontline workers, as they are the ones dealing with the participants, and, hence, face the practical issues and questions.

Despite the disproportionate global burden of infectious disease in LMIC, to date over 98% of HCT participants have been from HICs, resulting in an inequitable distribution of the benefits and burdens of such research. Findings from HIC participants may have limited relevance to LMIC populations, promoting moves towards conducting more HCTs in LMICs. When conducting HCTs in novel LMIC contexts, care is needed to determine appropriate responses to contextual considerations.

In the third presentation, Hugh Davies (University of Oxford, United Kingdom) provided insight in how HCT can be reviewed. It is generally

agreed that these studies do not raise new or different ethical issues for participants (although there are environmental issues). As with all research they must follow what we would deem the “Principles of Good Research”. Some of these principles are important in this research and rise particular considerations:

1. There should be a clear research question and justified purpose;
2. The research team should be equipped to complete the study;
3. The research should incorporate patient and participant views;
4. Benefits, harms and burdens of the study should be properly addressed;
5. The choice and recruitment of participants must be justified, safe and fair;
6. Participants should be offered a fair choice (Informed consent) and understand what they are agreeing to;
7. There should be fair payments for participation and compensation.

More detail can be found on the Reviewing Research webpage [15].

Reviewers should also recognise that “one size won't fit all” and adopt a proportionate approach as (i) the major risk will depend on the chosen infecting agent and (ii) these studies may be conducted in very different locations.

In the fourth presentation, Susan Bull presented the roadmap to the WHO guidance document on HCT. Reasons to develop such a document are an increasing interest and undertaking of HCTs in endemic settings, the lack of guidance on ethical issues raised by doing HCTs in LMICs, and calls from the community for this guidance. The document and six to eight case studies are intended to be completed by the summer of 2020.

The guidance will touch upon the acceptability of deliberate infection, implications for research design, risks and burdens, and moral commitments. The guidance will address justification, research contexts and populations, consent, reimbursement and compensation, engagement, fair sharing and collaboration, and governance.

In the fifth presentation, Marcia Hobbs (University of North Carolina, USA) described the advances and challenges of experimental human gonococcal infection.

Since the late 1980's controlled human infection studies have been conducted in the USA under appropriate ethical oversight and with written informed consent. The human model is necessary as chimpanzees are susceptible but can no longer be used, while other animal models may not replicate important human-specific features of infection. Given the sexually transmitted nature of the infection, some extra steps are taken: participation in the study does not become part of the medical record, experimental infection is not reported to health authorities as it is not considered sexually transmitted, and participants are required to abstain from sex until after a negative test of cure. Written informed consent is obtained twice: at screening followed by a test of understanding, and on the day of inoculation.

The urethral gonococcal CHIM is safe and reproduces natural infection. The model has been instrumental in testing bacterial requirements for urethral infection in men, and the model will facilitate efficient proof of principle testing for vaccines, while analysis of samples from infected and uninfected participants will define correlates of protection. Finally, given the low incidence (approximately 5/1,000), efficacy field trials of experimental vaccines may be difficult.

There is great interest in establishing a CHIM for pharyngeal gonococcal infection to better understand this increasingly common and difficult-to-treat presentation. However, ethical concerns about the ability to treat such experimental infections have prevented progress.

In the sixth presentation, Anna Durbin (Johns Hopkins Bloomberg School of Public Health, USA) described dengue as a case study in ethics. Dengue is the most important arbovirus in the world. There are four serotypes, which are all capable of causing the full spectrum of disease. The virus is endemic in tropical and sub-tropical regions of the world, and most areas are hyper-endemic, with multiple serotypes

circulating.

No approved antiviral agent for dengue exists, so treatment of dengue illness is only supportive. Furthermore, it is difficult to predict who will progress to severe disease. Investigators must have expertise in the clinical course and treatment of dengue. On the other hand, DENV challenge studies could be an important tool in the development of dengue vaccines and therapeutics and these societal benefits are important.

To date, challenge studies have all been performed in non-dengue endemic areas. Previous experience (rotavirus and live polio vaccine) has shown that results from large scale efficacy trials of vaccines have differed in different populations. Therefore, it might have been more informative if the dengue CHIMs had been conducted in dengue-endemic populations. Furthermore, only dengue-naïve or dengue-vaccinated individuals have been challenged.

If conducted in dengue-endemic populations, additional ethical considerations need to be addressed because of the potential antibody-dependent enhancement. In highly endemic areas, most individuals are expected to have had one or more previous dengue infections by the time they reach adulthood and so potential challenge study participants will have pre-existing immunity.

The value gained from performing challenge studies in endemic areas should balance the risks of the study to the individual (risk of illness) and community (risk of transmission). Steps to minimize those risks are: ensure experienced investigators and appropriate facilities are available to conduct the trial, ensure the clinical trial and endpoints of the trial are well designed and powered for statistical significance, and ensure the clinical trial team is experienced in the conduct of challenge studies and the treatment of challenge illness.

Early community engagement is necessary, and a robust informed consent process must be in place.

In conclusion, while many challenges remain to performing dengue CHIMs in endemic areas, there is also great interest. As an example, dengue CHIMs are being proposed in Viet Nam, extensive engagement has been undertaken with many different regulatory, governmental, and community stakeholders to discuss the possibilities.

In the final presentation, Meta Roestenberg (Leiden University Medical Center, the Netherlands) presented the *Schistosoma mansoni* CHIM, using single sex cercariae to avoid egg production that causes tissue damage. A rigorous process was established to produce male *S. mansoni* cercariae that complied with current regulatory standards for human use. Despite the complex, unconventional production process that includes a snail host, production was in accordance with the principles of GMP, with clearly defined release criteria, which were supervised by a Quality Control officer and a Qualified Person responsible for advanced cell therapy medicinal products [16].

In an initial dose-finding study, 3 participants were infected with 10 cercariae, after which the dose was escalated to 30 cercariae. However, this resulted in too many adverse events (AEs), including moderate and severe AE. Therefore, the dose was de-escalated to 20 cercariae and tested in 11 participants. This resulted in a limited number of AEs that were mostly mild to moderate. Results from the CHIM showed that eggs are not necessary to cause Katayama syndrome [17], a self-limited illness that occurs several weeks after infection with schistosome cercariae that has symptoms typical of an acute inflammatory response. It was also shown that the worms produce Circulating Anodic Antigen, but at much lower levels compared to endemic regions.

A questionnaire study was performed, including CHIM participants and non-participating controls (medical students). For participants, the main drivers of participation were the contributions to science and to developing countries, whereas financial compensation ranked third. In contrast, controls ranked financial compensation first, with 90% indicating it as important or very important. Both participants (100%) and controls (82%) found it acceptable for a physician to make volunteers deliberately ill for the benefit of a trial. In both groups some commented that this was acceptable as it was what they voluntarily

signed up for, as long as possible symptoms were explained to them before the trial. A minority of controls (18%) felt that deliberate infection was not acceptable because it breached the principle of ‘do no harm’ or provided burdens that did not outweigh the benefits.

Even among those participants who experienced more symptoms than they expected beforehand, the vast majority said they would participate again, and most would advise others to participate. The CHIM is ready for use for vaccine testing. A female-only cercariae model will be developed.

In the fourth session, on HCT in children, chaired by Claudia Emerson (McMaster University, Canada), Kate Emary (University of Oxford, United Kingdom) described a systematic literature review on human challenge models in paediatric populations. Many vaccines have their origin in challenge research and vaccine research is routinely performed in children, but this is not the case for CHIMs. To elucidate to what extent children have been involved in experimental infection research, a systematic review was set up to look at paediatric challenge studies. Although a limited literature base was expected, so far, 118,000 papers have been found for title and abstract screening. The search is complicated by the fact that some early reports do not identify participants as children, and there is no clear and unequivocal search string for challenge studies. Development of more appropriate nomenclature and clarity in terminology would help future studies.

In the second presentation, Claudia Emerson explained that research involving children must strike a balance between offering protection without blocking valuable and appropriate research that is responsive to their needs. As the benefits of research are not always predictable, the researcher must be satisfied that the research is not contrary to the child participant's interests. The foreseeable risks should be kept as low as possible: the potential benefits from the development of treatments and furthering of knowledge must outweigh any foreseeable risks. Research in which children are submitted to more than minimal risk with only slight, uncertain, or no benefit to themselves requires serious ethical consideration and strong justification [18]. In general, guidance for research involving children is consistent across jurisdictions. In addition to an imperative to minimize risks and obtain a favourable risk/benefit calculus, there are requirements to demonstrate scientific necessity to perform the research, that the research is informed by previous studies conducted on adults, that proxy informed consent is obtained, and whenever possible, assent from the child.

Current paediatric challenge studies involve only licensed and live attenuated vaccine candidates, with a grey area between paediatric vaccine studies and challenge *qua* challenge studies that has prompted a debate about whether the former ought to be clearly distinguished from the latter.

There are a host of unsettled ethics issues relating to paediatric research that are controversial and need extra attention in the context of challenge studies. These issues include quantifying and minimizing risks and burdens, and assessing their impact, consent/assent, and the possibility to withdraw this during the study; recruitment and retention; incentives and compensation, which may undermine the interest of the child; public perception, public trust and transparency; data sharing, including lack of data, and selective reporting; and research infrastructure, including the need for strong networks.

In the third presentation, Dominique Ploin (Hospices Civils de Lyon, France) pointed out that EMA and WHO are outspoken against the inclusion of children in HCTs, as it is an absolute requirement that accepting such risks and providing voluntary consent are based upon being truly informed. For this reason, it is not deemed acceptable to consider conducting human challenge trials in children (or in any other vulnerable population with diminished capacity to give informed consent) [19]. While this is clear for HICs with a low disease burden, in LMIC settings the context may change the equation. As an example, in case of malaria, children under 5 years are the most vulnerable group, with 272,000 deaths per year worldwide, and the vast majority of those occurring in the African region. Vaccines developed with the support of

CHIMs could have a significant impact on this disease. On the other hand, equity would require the same strategies in LMIC and HIC although the burden, i.e. the expectation of an individual benefit, makes the situation different and conflicting with the: what can't be done in HIC, can't be done in LMIC. The discussion pointed out that there is a wide age range within children, going from neonates and infants to pre-adolescents. It might be interesting to investigate how children, e.g. pre-, and adolescent, feel about challenge studies.

In the fourth presentation, Alex Mann (hVIVO, United Kingdom) presented on the efforts of HIC-Vac and Wellcome to develop manufacturing guidance for challenge agents. The guidelines are intended to provide a reference document to facilitate the production of formal guidance issued by global health authorities and or regulatory bodies and provide the research community with robust and specific guidance on the principles to follow when manufacturing infectious challenge agents until subsequent formal regulatory guidance is issued by regulatory authorities.

The document is intended to be applicable to all infectious challenge agents, to be applicable internationally and equally to low- and higher-income countries, to propose international standards for the manufacture of infectious challenge agents, and to describe the consensus view of both the research community and regulatory bodies. The process will be concluded by wide consultation: input from the community is welcomed and needed in order to ensure the guidance document has full applicability to the range of challenge agents and geographical regions. Towards this end, representatives are sought with experience in manufacturing infectious challenge agents, performing challenge studies, or research in LMICs, or from regulatory or advisory bodies. The final document is aimed to be published in the first quarter of 2021.

In the fifth session, on threats to the community and environmental safety, chaired by Adrian Wildfire (SGS Life Sciences, Belgium), Gagandeep Kang (Christian Medical College, Vellore, India) pointed out that CHIM protocols need to be aligned with local circumstances. Taking Vellore as an example: it is estimated that household toilet coverage is 78% but the proportion of safely managed excreta is only 11%, as about half of household toilets are discharged directly to drains. Furthermore, waste collected from toilets by trucks is generally emptied into drains or downstream rivers. Surveillance of *S. Typhi* found 14% positivity in routine sewage samples, compared to 40% in post-hospital sewage, with a correlation between positive blood cultures and positive sewage samples within a radius of 2 km. However, surveillance was by PCR and culture was unsuccessful, so it cannot be ruled out that the organisms detected were non-viable as only DNA could be found.

This leads to challenges in ensuring safety in enteric challenge studies performed locally, including engineering challenges such as facility modification (quality of the sewage system and method of treating raw sewage) and disinfection protocols (treatment of sewage to get rid of the pathogen and proof needed to show that the pathogen is no longer infectious).

But also laboratory challenges exist (evaluation of cleaning and disinfection, and identification and measurement of carriage), as well as study challenges (participant isolation, ethics review and monitoring, and follow up and screening of contacts). Finally, the use of antibiotics to treat participants could result in resistance in bystander flora, although, so far, no antimicrobial resistance has been observed.

These challenges can only be tackled through protocols and training, including strict inclusion and exclusion criteria; no contact with vulnerable persons; close monitoring, staffing availability, facility and critical care access; enteric precautions training of medical staff, participants, and families; and facility contamination testing, indicating that planning and preparation is vital.

In the second presentation, Adrian Wildfire discussed containment of respiratory viruses, presenting a case study of unexpected human parainfluenza virus (HPIV) infection during an influenza challenge

study. Usually in CHIM, there is little variance or noise in the system, which helps to keep the required number of participants low. If outliers are found, they may be indicative of something unexpected, e.g. an unplanned infection. While the primary endpoint (viral AUC) will not be usually affected by another infection, the secondary endpoints, based on symptoms, may well be impacted.

Despite rigorous exclusion measures prior to inoculation, including a 2-day incubation period in containment to pick up sub-clinical diseases and PCR screening for adventitious agents (BioFire™), in a period spanning 3 years and 6 challenge studies, two CHIM participants have been diagnosed as co-infected, both with HPIV-1. Subjects were diagnosed at discharge from the healthcare unit (at day 10) following routine screening.

There were three possibilities regarding the route and timing of the infection for Subject 1: 1) d-2 sample was already positive for HPIV but not detected; 2) the subject became infected between d-2 and d1; 3) the subject was in the incubation period on d-2 and started shedding on day 1 - with options 2 and 3 being most likely. Subject 2 was most likely to have become infected between d1 and d5 or d6.

Following diagnosis, the following actions were undertaken:

1. Data from Subject 1 were removed from the efficacy dataset, while both subjects were included in follow-up for the general and safety analyses. The study protocol allowed for replacement of Subject 1.
2. The Infection control protocol and procedures were audited.
3. Additional (day – 1) adventitious agent testing was included in the screening program.
4. The CHIM protocol was revised to reflect subject and replacement criteria.
5. Data analysis protocols were revised.

In conclusion, unplanned upper respiratory tract infections during a controlled human infection study may confound secondary endpoints regarding reductions in symptom incidence and severity. Therefore, screening subjects for adventitious agents is essential to maintain data integrity, especially during the cold and flu season.

In the final presentation, Robert Frenck (University of Cincinnati, Ohio, USA) discussed the use of norovirus as a challenge agent. Norovirus (NoV) has a low infectious dose of about 1000 copies. Given the high number of viral copies, 1 µg of stool is sufficient for transmission. Furthermore, alcohol-based sanitization is not effective to disinfect, bleach is needed. Immunity to norovirus is not long-lasting, for a maximum of three years, and there is no cross-protection against other types. Shedding may occur from days to weeks with asymptomatic individuals also shedding virus.

In NoV CHIM, several precautions are taken to reduce the risk for participants, as well as the environment. No visitors are allowed to the inpatient unit. Good hand-washing practices are emphasized to participants and staff. Staff use a gown and gloves when working with participants, as well as eye protection if the participant is vomiting. Surfaces are wiped with bleach and participants are not allowed in the study kitchen. Food handlers wore gloves, disposable dinnerware is used, no left-over food returns to the kitchen and study staff have a separate eating area.

To decrease transmission after the end of the inpatient period, good hygiene practices are reinforced. Participants are restricted from jobs in patient care, food handling or day-care. And if participants or household members become ill, they need to notify study staff. Having enrolled over 100 participants in the NoV CHIM, only one study staff member had an infection while working in the inpatient unit. However, the virus may have transferred from her sister who was diagnosed with NoV two to three days before the study staff member became ill. No secondary outpatient transmission has occurred.

In the sixth session, on recruitment, engagement, advertising and incentive, chaired by Helen McShane, Olivia Grimwade (Monash University, Australia) discussed the attitudes towards payment and

payment practices in CHIM research. Often CHIM papers do not explicitly report payment details and there has been no formal research into the payment practices across different models. General guidelines on research ethics do not offer specific advice as to how payment levels should be determined, which is reflective of the ongoing ethical debate surrounding appropriate payment for research participants. Historically, a focus on the potential issues that high payments may evoke has led to payments being set lower rather than higher, and it has meant that risk is not standardly considered in payment formulas. The CHIM Academy of Science guidelines [20] recognise that payment in CHIM is “particularly sensitive” but only refer to more general guidelines when advising investigators on how to navigate payment. The WHO HCT regulatory considerations [19] don't offer any advice on payment. As a result of this, levels of payment may vary considerably across models and participants may be at risk of not receiving fair payment for their contribution to medical research.

Moreover, there has been no formal research into the attitudes towards payment in CHIM amongst either the general public or experts in CHIM. Attitudes are critically important as they can be used to inform both policy and ethics. As CHIM research is growing, public approval and confidence in CHIM is paramount, so we need first to identify the public's attitudes and address any concerns they may have.

With the aim to propose an ethical framework for payment of CHIM participants, a survey to assess attitudes towards payment was given to two groups, a representative sample of over 250 members of the UK public recruited online and more than 35 CHIM experts. A second survey assessing current payment practices and principles was undertaken in the CHIM expert group. Based on data from over 20 CHIMs (located both in LMICs and HICs), a wide range of payment per hour (0–30 £/hour) or per study (0 – over 3000 £ per study) was observed.

In response to a hypothetical scenario involving a CHIM study with four different levels of risk over two different risk categories (i.e. the risk of experiencing an adverse event and the risk of death), both the public and investigators indicated that they believe hourly rates for participation should be considerably higher than the standard minimum wage. For the hypothetical CHIM involving the highest risk (1 in 1000 risk of death as a result of participation) the mean payment indicated by experts was £21.13 per hour and £35.59 per hour indicated by the general public. Furthermore, respondents increased the required payment for a CHIM as the risk level in the CHIM increased, indicating that they believe the risk involved in CHIM participation should be reflected in the payment level.

While the public strongly agrees with payment for risk, the CHIM experts seem to be divided about whether risk should be taken into account in payment.

Based on the results a wage and risk payment model was proposed. This model includes a base hourly wage similar to that of an unskilled laborer with extra payment for factors such as risk, pain and inconvenience. Compensation for any actual harm that eventuates as a result of a CHIM must also be available to participants. Although it is difficult to assign a monetary value to risk, in sectors such as health economics and road safety economics, specific methods have been employed to determine the value of statistical life. Similar tools could be useful to determine how much should be paid for the risk in CHIM studies. Finally, it was suggested that the same CHIM study undertaken in different locations could adopt a minimum required level of payment for any one CHIM which is kept constant, regardless of the study location, to allow equal pay for equal work and to minimize the risk of exploitation of participants by underpayment.

In the second presentation, Blanché Oguti (University of Oxford, United Kingdom), discussed factors influencing participation in CHIMs. She described the volunteers' experience based on a pooled analysis from six enteric fever studies, including both *S. Typhi* and *S. Paratyphi* CHIMs. Challenge studies raise some ethical concerns given the controversy of deliberately infecting healthy volunteers. The consent process and risk of financial inducement are key themes in the bioethical

debate. As the views of the volunteers have not been examined, this study aimed to examine participant motivations, attitudes and factors influencing their decision to participate in human challenge research, using an anonymous, self-administered survey at a single time point after challenge. The semi-structured survey consisted of 48-questions utilising the Likert scale. A descriptive analysis was performed on these pooled data. The median age of participants was 27 years, 57% were educated to Bachelor's level or higher and 33% were students [21].

Of the participants, 84.6% “agreed” or “strongly agreed” that being able to contribute to the progress of medicine was a motivating factor. Similarly, 82.6% “agreed” to the statement that “financial reimbursement was a motivation for joining this study”. Moreover, if there had been no financial reimbursement offered, 65.5% of participants stated that they would not have taken part [21].

Although awareness of enteric fever was low before the study, participants felt prepared, highlighting the importance of pre-study counselling. Less than 5.0% of the participants were “very concerned” about possible study-related risks such as hospitalisation or complications from enteric fever infection, whereas 27.0% reported that the deterrent was the perceived risk of transmitting the infection to others. Notably, 94% of the respondents were very satisfied with the level of care received during the study, 90% would participate again, and 99% felt that the financial reimbursement was fair or generous [21]. While the survey may have suffered from a positive response bias, it was performed anonymously. Nevertheless, in-depth interviews may provide more nuances.

The role of financial reimbursement in CHIM studies needs further discussion among researchers considering the differing contexts, including pathogens location and socioeconomic infrastructure.

In the third presentation, Primus Chi (KEMRI Wellcome Trust Research Programme [KWTRP], Kenya) discussed the role of community and public engagement for challenge studies in Kenya. Community engagement (CE) in health research has one main focus - that research is carried out in a respectful manner where social value is maximized [22]. CE can provide insights into how best to tailor consent to context and can be an important component of consent processes. Engaging communities can also have intrinsic and instrumental value; for example, as a means of showing respect and identifying appropriate ways of working respectfully. This could range from information sharing (such as use of media, as a form of public engagement) to consultation activities (such as working in-depth with community advisory groups).

At KWTRP, CE has evolved over the past 15 years, now covering a range of activities with different aims, but all of which have an overall goal of strengthening mutual understanding between researchers and the communities that participate in research.

Social science research can inform CE planning, e.g. understand roles and relationships between different stakeholders (power, trust, etc.), identify marginalized groups and bring to light historical/political perspectives underpinning community attitudes to research/science. Social science methods can be used to support, monitor and evaluate CE.

To prepare for the first malaria HCT in Kenya, beginning three years before the study started in 2013, a number of meetings and discussions were organized with a wide range of stakeholders, including the Ministry of Health, the Division of Vaccines and Immunization, the Pharmacy & Poisons Board (country regulatory authority), the Ethics Review Committee, the National Council for Science & Technology, and the Consortium for National Health Research in Kenya (a non-governmental organization) [23].

Social science research to support CE in malaria HCT has covered a range of areas, including information dissemination, the consent process, decision making for participation, voluntary participation and the right to withdraw, experiences during participation in the study, perception around deliberate infection, and implication of participation on participant lives.

As an example, while many participants confirmed understanding of

the information shared during the informed consent process, concerns arose later over blood volumes sampled during the study. This led to the development of visual aids used as part of routine information sharing.

Initially, compensation for time (\$20 per 24h) was paid as a lump sum at the end. For a study with a longer follow-up, compensation will be given on a weekly basis rather than as a lump sum at the end, which is expected to reduce the potential for undue inducement, as well as potential social burdens, making it possible to support the family while being away.

Future areas of focus are benefits and burdens (including exploring issues around long in-patient stay, compensation and inducement); recruitment and fairness (including perceptions of fairness of the recruitment process and strategies for ensuring fairness throughout the study); and autonomy and decision-making (decision to participate and stay, including understanding and perception of the right to withdraw).

In the final presentation, Roma Chilengi (Centre for Infectious Disease Research, Zambia) discussed the issues with CHIMs in LMICs, at a time when CHIMs are increasingly performed in LMICs. These issues encompass awareness and education, the regulatory environment, technical gaps and resource gaps. Worldwide, funders, sponsors and the international community are concerned about potential exploitation and the need for individual informed voluntarism against risks, while locally, the mere language, how to communicate about CHIMs, the community perceptions of CHIMs, and getting the levels of compensation right are critical issues.

Their first step was to use a vaccine containing live attenuated viruses, using the CHIM logic. The use of a challenge agent that is licensed as a vaccine and widely used, reassured the ethics committee. The objective of the study was to quantify virus shedding, and to evaluate the use of saliva to detect specific antibodies, as a minimally invasive procedure. In total, 22 infants were recruited under the routine immunization system, and blood samples (days 0, 28, 56), stool samples (days 0, 3, 5, 14, 28, 31, 33, 42) and saliva samples (days 0, 3, 28, 56) were obtained. A significant reduction in the viral particles in stool was observed following the second dose, giving an indication of mucosal immunity pointing to vaccine efficacy. Furthermore, there was a clear correlation between antibodies in serum and saliva, suggesting blood draws in very young infants could potentially be replaced by saliva-based testing. However, further studies, powered to confirm the use of saliva as an indicator, will need to be performed.

In the seventh session, on pre-existing immunity, chaired by Peter Openshaw (Imperial College London, UK), Shobana Balasingam (Wellcome Trust, United Kingdom) discussed the need to perform CHIMs in endemic regions. Regardless of where research takes place, vaccine development is too long and too costly. The use of CHIMs may lead to considerably reduced timelines and cost, see Fig. 1.

The use of a CHIM could help in down-selecting vaccine candidates, leading to earlier failures, and a higher success rate at the highly expensive Phase 3 level. Furthermore, CHIM could help to generate efficacy data more quickly, using a lower number of volunteers, and at a much lower cost than Phase 3 trials, although safety data would still need to be generated in larger trials.

However, while these CHIMs are generally developed in HIC, they should be performed in endemic areas for several reasons: the host-pathogen or host-vaccine interaction can be different in LMICs as compared to HIC; genetics, infectious disease history, co-infections, immune status and environmental factors, amongst others, can only be appropriately tested in the targeted settings; and the target population most affected by the disease gain the potential benefits of the study.

Nevertheless, several challenges for endemic settings remain, including legal barriers; regulatory issues, e.g. lack of robust globally aligned regulatory guidance for challenge studies; ethical issues, concerning tailored volunteer recruitment, robust informed consent and a reimbursement strategy for participation; community perception and engagement; and infrastructure.

To reap the benefits of CHIMs in endemic areas, funders convened

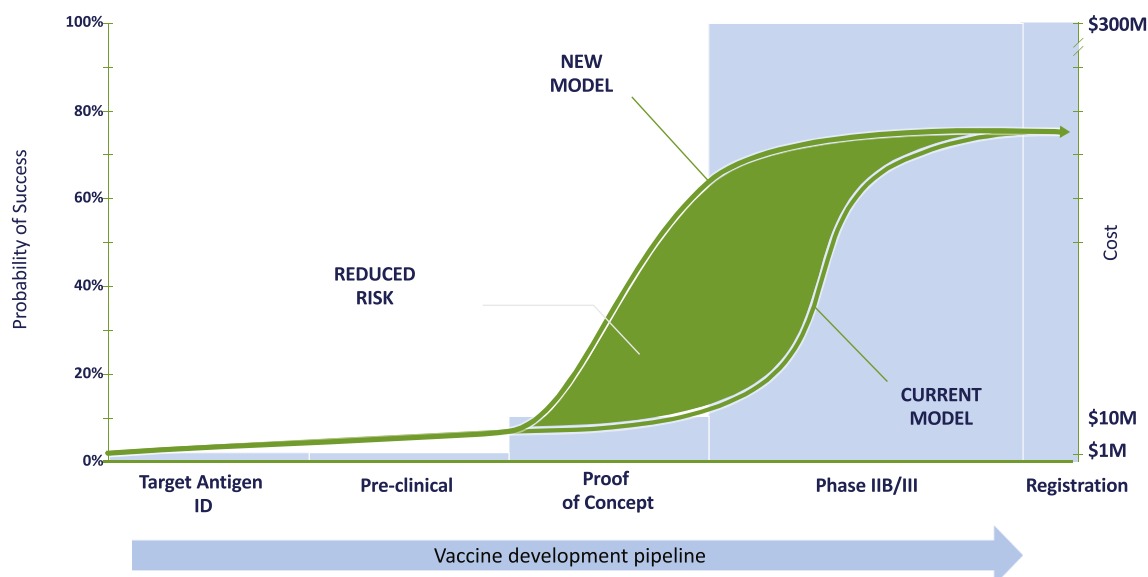


Fig. 1. Value of CHIMs in vaccine development.

in February 2018 and committed themselves to:

- Advance high-quality research that is scientifically and ethically justified
- Ensure the safety of participants and communities
- Engage meaningfully with participants, communities, and the public
- Support opportunities to maximize values of studies
- Share knowledge and data efficiently and responsibly

Wellcome hopes to achieve these commitments through supporting WHO in the development of a framework and guidance for studies in endemic areas to equip research ethics committees; equip National and Regional Regulatory Agencies to regulate and interpret human challenge studies; and, together with BGMF and HIC-Vac, develop a sharing platform for studies including protocols, Standard Operating Procedures, data and sample sharing, in order to encourage harmonization of studies.

In the second presentation, Philip Bejon (KEMRI Wellcome Trust Research Programme, Kenya) discussed a malaria CHIM study to examine naturally acquired immunity. Vaccine development starts off with demonstrating proof of concept in a preclinical setting, and developers often select a single antigen. However, genome sequencing has shown that there are 5000 antigens in the parasite. Nevertheless, most vaccines in the pipeline use antigens that were identified prior to the availability of the genome. Insight into naturally acquired immunity may help finding antigens that are strongly associated with protection.

For malaria in Africa, injection of cryopreserved sporozoites is much preferred over mosquito-bite challenge as it is difficult to import mosquitoes. Given the high level of purification and the reproducible infectious dose of 3200 *Plasmodium falciparum* sporozoites, this system works well. Using qPCR on daily blood samples, it was possible to look at the number of parasites in the blood. In North Kilifi, where most volunteers are also non-immune, parasites in the blood grow rapidly and some volunteers develop fever. Some individuals are parasite PCR positive but do not experience rapid parasite growth and need no treatment, and finally, some individuals remain PCR negative. These latter two categories are not found in challenge studies in non-endemic areas in HICs. In Kilifi South, where there is more malaria, more qPCR positive individuals not needing treatment are seen, as well as more qPCR negative volunteers. These phenotypes are more common upon prior exposure to malaria.

Dividing the volunteers according to place of residence shows a big

difference in time to reaching a threshold requiring treatment for volunteers from low transmission areas, compared to high transmission areas. Similarly, volunteers having anti-schizont antibodies, previously used as crude marker for exposure, are less likely to be diagnosed with parasites in the blood. In a field study, this difference would have gone unnoticed, because so many variables are unknown in the field study: the use of bed nets, the genotype of the infecting parasite, etc. The difference in the anti-schizont antibodies predicting CHIM outcomes, on the other hand, was highly significant, explaining 15% of the variability. This provides a model to look for other, protective antigens. If they are stronger than the anti-schizont antibodies they might be useful. These antigens could be found in high-throughput detection systems. Similar studies in subjects with naturally acquired immunity performed in Tanzania and Gabon have shown consistent results.

In the third presentation, Anna Durbin described the Zika virus as a pre-existing immunity case study. The Zika virus (ZIKV) is a member of the Flaviviridae family, genus *flavivirus*, with dengue virus, yellow fever virus, West Nile virus, and Japanese encephalitis virus as family members. Furthermore, Zika is primarily transmitted by *Aedes* mosquitoes, the same vector that transmits dengue and yellow fever. Finally, Zika is transmitted in all tropical and sub-tropical regions, and therefore overlaps with dengue endemic areas. Previous infection with one DENV serotype is a risk factor for more severe disease when infected with a second heterotypic antibody, a process known as antibody-dependent enhancement. Similarly, there is some evidence that previous JE infection may make subsequent dengue infection more symptomatic [24]. This leads to the question; can dengue antibodies promote Zika spread? *In vitro* data show that pooled serum from dengue convalescent individuals enhanced ZIKV infection *in vitro* [25], while, in contrast, monoclonal antibodies and convalescent serum from dengue patients also neutralized ZIKV [26]. When non-human primates previously infected with DENV or yellow fever virus ≥ 420 days previously were inoculated with ZIKV, DENV-immune serum enhanced ZIKV titers *in vitro*. However, no significant difference was found in ZIKV titer in non-human primates following ZIKV infection compared to DENV-naïve macaques [27]. A longitudinal paediatric cohort in Managua, Nicaragua, evaluating the effect of DENV-immune status on the clinical presentation of ZIKV patients during the ZIKV outbreak of 2015/2016, investigated more than 3,000 children and found that DENV-infection was significantly negatively associated with the risk of symptomatic ZIKV infection (incidence rate ratio 0.63; 95% confidence interval 0.48–0.81, $p < 0.005$) [28].

Can these conflicting data be better dissected using CHIM studies? The tetravalent dengue vaccine, when used in a challenge study, protected completely, whereas the trivalent did not protect completely against infection, nor against rash, indicating that the heterotypic antibodies induced by the trivalent vaccine did not protect; homotypic antibodies were needed. A ZIKV CHIM might help to study this, performing a DENV challenge followed by ZIKV and vice versa. This should make it possible to evaluate whether one infection modulates infectivity (either enhancement or protection) or modulates the immune response. Furthermore, samples obtained in CHIM can help to determine sensitivity and specificity of diagnostic tests, as cross-reactive antibodies can make diagnosis difficult.

Session 8 concerned clinical, immunological, and microbiological endpoints, and was chaired by Anna Durbin and Paul Kaye. Helen McShane discussed immunological endpoints in mycobacterial challenge studies. Mycobacterial challenge studies may provide a biological signal of efficacy with new vaccines, can be used to identify potential immune correlates of protection, and may act as a model of the immunobiology of disease. An *in vitro* assay, the Mycobacterial Growth Inhibition Assays (MGIA) using peripheral blood mononuclear cells (PBMC) measures a biologically relevant response that correlates with protection from *in vivo* human BCG intradermal challenge across two independent cohorts [29]. Control of mycobacterial growth in the MGIA is associated with a range of immune parameters measured post-BCG infection *in vivo* including the IFN- γ ELISpot response, frequency of PPD-specific IFN- γ or TNF- α producing CD4⁺ T cells, and frequency of specific sub-populations of polyfunctional CD4⁺ T cells. Distinct transcriptomic profiles are associated with good versus poor mycobacterial control in the MGIA, with good controllers showing enrichment for gene sets associated with antigen processing/presentation and the IL-23 pathway, and poor controllers showing enrichment for hypoxia-related pathways [29]. These data indicate that the adaptive immune response, particularly Th1 cells, strongly influences the outcome in BCG-vaccinated volunteers.

An aerosol-delivered challenge model is under development, better mimicking the natural route of infection. The aerosol infection model induces stronger CD4 and CD8 positive T-cell responses compared to the intradermal model, while the IgG and IgA responses in serum are higher after intradermal challenge. In contrast, while IgG is similar in BAL after aerosol and intradermal challenge, IgA response is significantly higher after aerosol challenge.

Further studies are needed to assess whether aerosol BCG is safe in historically BCG vaccinated individuals, and if a BCG vaccine effect can be detected in these previously vaccinated individuals.

In the second presentation, Peter Openshaw presented the RSV human challenge model.

While RSV is often seen as a paediatric disease, it is also a problem among older adults who are debilitated or suffering immune senescence. Inappropriate or dysregulated responses to RSV can be pathogenic, causing disease-enhancing inflammation that contributes to short- and long-term effects [30]. In recent studies, healthy volunteers aged between 18 and 55 years are challenged intranasally, with 10⁴ pfu RSV strain A Memphis 37. The volunteers are kept in seclusion from day 1 to day 10, with intensive sampling using ‘synthetic absorptive matrix’ (SAM) devices to absorb the mucosal lining fluid of the human respiratory tract. It is a non-invasive technique causing minimal discomfort; it yields reproducible results with the ability to frequently repeat sampling of the upper airway [31]. Just over half of the volunteers get infected, and, of these, two thirds develop symptoms, with no gender difference or relationship between age and infection or symptoms. In contrast to influenza, there is a lag period until day 3 before viral load or symptoms appear. Transcriptional analysis of pre-infection samples shows different patterns for those who will develop symptoms, mostly related to neutrophils. Although T cells only appear transiently in the blood, at a frequency too low to correlate with protection, an abundance of resident memory CD8⁺ T-cells before infection correlates

with reduced symptoms and viral load, implying that these cells can confer protection against severe respiratory viral disease when humoral immunity is overcome [32]. This CHIM is now being used to study healthy adults up to the age of 75, to establish safety and tolerability, and to determine differences in immune defense and responses to RSV infection.

In the third presentation, Kirsty Mehring-Le Doare (St. George's, University of London and MRC/UVRI @LSHTM, Uganda) described the need for a Group B Streptococcal (GBS) CHIM. GBS is commensal in the gut but in pregnant women, GBS may lead to foetal and placental inflammation and can cause stillbirth, or preterm birth. Infection during delivery may lead to pneumonia, bacteraemia or meningitis in the neonate. As higher functional antibodies were shown to be associated with non-colonized pregnant women [33], and higher concentrations of maternally-derived antibody-mediated complement deposition are associated with a decreased risk of GBS colonisation in infants up to day 60–90 of life [34], a vaccine given to the mother at or around week 16 could prevent infection.

A challenge model evaluating the immunizing effect of experimental GBS carriage in non-pregnant women could be the first step towards reducing GBS-associated preterm birth, stillbirth and early onset disease. Challenge would need to be done in women, due to the effect of the menstrual cycle on GBS colonisation.

In the fourth presentation, Rebecca Cox (University of Bergen, Norway) discussed the impact of pre-existing immunity on study endpoints in influenza CHIMs. Young children are particularly hard hit by influenza, as are the elderly and pregnant women. This is reflected in the changes in the immune system with age from being immature in young infants, immunosuppressed during pregnancy and exhibiting immunosenescence in the elderly, as well as under special circumstances such as in risk groups with comorbidities. In influenza CHIMs, pre-screening of volunteers is necessary, because previous exposure, as witnessed by antibody levels, may determine suitability for challenge. Haemagglutinin inhibition screening is a good indicator of susceptibility to infection and shedding, although with current H3N2 viruses the presence of neutralizing antibodies is superior. Similarly, high anti-HA stalk antibody titres reduce viral shedding, although they do not predict reduced clinical disease or disease severity. Furthermore, high anti neuraminidase antibody titers reduce viral shedding as well as the symptoms of disease [35]. Finally, nasal IgA protects against influenza challenge [36]. These findings indicate the importance of the humoral immune responses in providing a first line of protection. However, virus shedding is also inversely correlated to the number of CD8⁺ T cells [37], while CD4⁺ T cells correlate with disease protection [38], which indicate the importance of the cellular immune response. Hence, full characterization of pre-existing immunity in CHIM volunteers is necessary to improve the success of the challenge study.

In the final presentation, Robert Sauerwein discussed the parasite involved in and clinical endpoints of the malaria CHIM [39]. In fact, there are multiple malaria CHIM models defined by objectives and endpoints in relation to the parasite lifecycle. In addition, the parasite can have genetic or antigenic diversity, as well as differences in fitness or virulence, and infection can be with more than one strain simultaneously. In the human, host genetic diversity and immune history may also play a role, as does the presence of other underlying diseases and, possibly, the microbiome. Finally, the choice of endpoints influences the outcomes of the study, and the information to be collected while performing the study.

Parasitemia is seen in 100% of malaria naive volunteers, who become positive according to thick blood smear between days 7–12. However, qPCR gives a different picture, with 2 cycles of replication before thick smear diagnosis. Furthermore, there is a clear dose-dependent response. And finally, the infectivity of parasites is not the same: the NF54 strain produces fewer infected hepatocytes, compared to NF135 and NF166, resulting in longer prepatent periods. These data seem to suggest that the parasite determines the initial course of

infection rather than the malaria naïve host.

Using the qPCR, a lower threshold could be used for diagnosis and treatment, which would reduce the number of adverse events and maximize safety. However, this may go at the cost of the information gained by the experiments. In the malaria CHIM model for assessment of transmission, gametocyte carriage was induced by subcurative treatment with antimalarial drugs [40]. Participants were randomly allocated to four different treatment arms comprising low-dose piper-aquine or sulfadoxine-pyrimethamine, followed by a curative regimen upon recrudescence. Mature gametocytes were observed in all participants, appearing 8.5–12 days after the first detection of asexual parasites [40]. Exploratory mosquito feeding assays showed successful sporadic mosquito infections. This model can be used to evaluate the effect of drugs and vaccines on gametocyte dynamics, and transmission-blocking interventions against malaria.

The range of CHIMs, with deviations from natural infection as defined by challenge conditions, necessitates harmonization of endpoints in order to facilitate comparison between results.

Summary of the meeting

The third Human Challenge Trial Meeting brought together a broad range of international stakeholders, including academia, regulators, funders and industry, with a considerable delegation from Low- and Middle-Income Countries (LMIC).

Controlled human infection models (CHIMs) can be helpful to study pathogenesis and for the development of vaccines (or anti-infective drugs). CHIMs are an option to move away from animal models that are often imperfect and generally do not represent the human immune system. In CHIMs, challenge agents are used to infect healthy volunteers. Therefore, ethical considerations include that the challenge studies need to be safe and results should be meaningful, e.g. contribute to better cure, or at least better understanding of the disease.

While everyone agreed on ethics for CHIMs in the adult population, no consensus was reached on the performance of CHIMs in children. If alternatives are available, CHIMs on children should not be done. However, some participants felt that situations were conceivable under which CHIMs on children would be deemed acceptable, for example through the use of attenuated vaccine strains.

The meeting provided a state-of-the-art overview on a wide range of CHIMs, including viral, bacterial and parasitic challenge agents.

Recommendations

- There is a great and widely felt need for globally aligned guidance documents for CHIM studies, preferably endorsed by regional and global organizations such as AVAREF and WHO. The development of these EU guidance documents should be a priority.
- There was a strongly presented view that challenge studies should not be classed as different from other clinical development studies, indeed the risks are more likely to be foreseeable than in the case of phase I studies of novel drugs. Thus, it seems appropriate to use existing ethical and regulatory guidance with emphasis adapted for challenge agents.
- Further discussion is needed to specify the definition of a CHIM, based on the challenge agent used: a wild-type organism; a genetically modified organism (e.g. dengue); or an attenuated live vaccine (e.g. rotavirus oral vaccine)
- Standardization of methodology and study endpoints will make it easier to compare study outcomes and perhaps perform meta-analyses.
- CHIM studies performed in naïve participants from High-Income Countries may be less relevant as a model for non-naïve/pre-immune participants in endemic LMIC. But, for many diseases non-immune adults may be present in the LMIC communities (e.g. in malaria free cities) who may be more representative of the target

age group. However, CHIM for immune/semi-immune individuals will have to be performed in LMIC adults. Hence, there is a need for capacity building in these countries, in performance as well as regulation of the studies.

- As compensation for participation in CHIM studies may be perceived as undue inducement (reducing appropriate judgement of risks), compensation needs to be considered carefully. It should be related to local wages (i.e. accepted values for the minimum wage). There was a robust discussion of the need to consider appropriateness of additional compensation for actual harm.
- To be able to perform CHIM studies requires preparation well in advance. Strong engagement with stakeholders is needed, and trust takes time to build.

Disclaimer

The content of this paper and the presentations and discussions during the meeting do not reflect the views of the regulatory agencies that participated in the meeting but express the personal opinion of the participants.

Declaration of competing interest

The authors have no competing interests to declare.

Acknowledgements

The authors are grateful for the support of Wellcome, HIC-Vac, the Bill & Melinda Gates Foundation, SGS and GSK to organize the meeting; Abigail Charlett (International Alliance for Biological Standardization) for logistic management, Suzanne Penfold (P95) for critical review of the manuscript, and Ana Goios (P95) for editorial support.

References

- [1] Sheets RL, Fritzell B, Aguado de Ros MT. Human challenge trials in vaccine development: Strasbourg, September 29 - October 1, 2014. *Biologicals* 2016;44:37–50.
- [2] Baay MFD, Richie TL, Neels P. Human challenge trials in vaccine development, Rockville, MD, USA, September 28–30, 2017. *Biologicals* 2019;61:85–94.
- [3] Levine MM, Kaper JB, Herrington D, Ketley J, Losonsky G, Tacket CO, et al. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD 103 and CVD 103-HgR. *Lancet* 1988;2:467–70.
- [4] Levine MM, Kaper JB, Herrington D, Losonsky G, Morris JG, Clements ML, et al. Volunteer studies of deletion mutants of *Vibrio cholerae* O1 prepared by recombinant techniques. *Infect Immun* 1988;56:161–7.
- [5] Osowski J, Azzopardi KI, Baker C, Waddington CS, Pandey M, Schuster T, et al. Controlled human infection for vaccination against *Streptococcus pyogenes* (CHIVAS): establishing a group A *Streptococcus* pharyngitis human infection study. *Vaccine* 2019;37:3485–94.
- [6] Osowski J, Azzopardi KI, McIntyre L, Rivera-Hernandez T, Ong CY, Baker C, et al. A controlled human infection model of group A *Streptococcus* pharyngitis: which strain and why? *mSphere* 2019;4.
- [7] Milligan R, Paul M, Richardson M, Neuberger A. Vaccines for preventing typhoid fever. *Cochrane Database Syst Rev* 2018;5. Cd001261.
- [8] WHO Expert Committee on Biological Standardization. Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. Available from.
- [9] Jin C, Gibani MM, Moore M, Juel HB, Jones E, Meiring J, et al. Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of *Salmonella* Typhi: a randomised controlled, phase 2b trial. *Lancet* 2017;390:2472–80.
- [10] Shakya M, Colin-Jones R, Theiss-Nyland K, Voysey M, Pant D, Smith N, et al. Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. *N Engl J Med* 2019;381:2209–18.
- [11] Dobinson HC, Gibani MM, Jones C, Thomaidis-Brears HB, Voysey M, Darton TC, et al. Evaluation of the clinical and microbiological response to *Salmonella* paratyphi A infection in the first paratyphoid human challenge model. *Clin Infect Dis* 2017;64:1066–73.
- [12] 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.
- [13] MacLennan CA, Riddle MS, Chen WH, Talaat KR, Jain V, Bourgeois AL, et al. Consensus report on *Shigella* controlled human infection model: clinical endpoints. *Clin Infect Dis* 2019;69. S591–s5.
- [14] Kaminski RW, Pasetti MF, Aguilar AO, Clarkson KA, Rijpkema S, Bourgeois AL, et al. Consensus report on *Shigella* controlled human infection model:

- immunological assays. Clin Infect Dis 2019;69:S596–601.
- [15] Reviewing Research. Human infection challenge studies Available from <http://www.reviewingresearch.com/human-challenge-studies/> Last accessed: April 10, 2020.
- [16] Janse JJ, Langenberg MCC, Kos-Van Oosterhoud J, Ozir-Fazalalikhani A, Brienen EAT, Winkel BMF, et al. Establishing the production of male schistosoma mansoni cercariae for a controlled human infection model. J Infect Dis 2018;218:1142–6.
- [17] Langenberg MCC, Hoogerwerf MA, Janse JJ, van Lieshout L, Corstjens P, Roestenberg M. Katayama syndrome without schistosoma mansoni eggs. Ann Intern Med 2019;170:732–3.
- [18] Medical Research Council UK. MRC EWthics Guide: medical research involving children Available from <https://mrc.ukri.org/documents/pdf/medical-research-involving-children/>.
- [19] 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>.
- [20] Academy of Medical Sciences. Controlled human infection model studies summary of a workshop held on 6 February Available from <https://acmedsci.ac.uk/file-download/55062331>; 2018.
- [21] Oguti B, Gibani M, Darlow C, Waddington C, Jin C, Plested E, et al. Factors influencing participation in controlled human infection models: a pooled analysis from six enteric fever studies. Wellcome Open Res 2019;4:153.
- [22] Consent and community engagement in diverse research contexts. J Empir Res Hum Res Ethics 2013;8:1–18.
- [23] Hodgson SH, Juma E, Salim A, Magiri C, Njenga D, Molyneux S, et al. Lessons learnt from the first controlled human malaria infection study conducted in Nairobi, Kenya. Malar J 2015;14:182.
- [24] Anderson KB, Gibbons RV, Thomas SJ, Rothman AL, Nisalak A, Berkelman RL, et al. Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. PLoS Neglected Trop Dis 2011;5: e1311.
- [25] Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. Nat Immunol 2016;17:1102–8.
- [26] Swanstrom JA, Plante JA, Plante KS, Young EF, McGowan E, Gallichotte EN, et al. Dengue virus envelope dimer epitope monoclonal antibodies isolated from dengue patients are protective against Zika virus. mBio 2016;7.
- [27] McCracken MK, Gromowski GD, Friberg HL, Lin X, Abbink P, De La Barrera R, et al. Impact of prior flavivirus immunity on Zika virus infection in rhesus macaques. PLoS Pathog 2017;13:e1006487.
- [28] Gordon A, Gresh L, Ojeda S, Katzelnick LC, Sanchez N, Mercado JC, et al. Prior dengue virus infection and risk of Zika: a pediatric cohort in Nicaragua. PLoS Med 2019;16:e1002726.
- [29] Tanner R, Satti I, Harris SA, O'Shea MK, Cizmeci D, O'Connor D, et al. Tools for assessing the protective efficacy of TB vaccines in humans: in vitro mycobacterial growth inhibition predicts outcome of in vivo mycobacterial infection. Front Immunol 2019;10:2983.
- [30] Openshaw PJM, Chiu C, Culley FJ, Johansson C. Protective and harmful immunity to RSV infection. Annu Rev Immunol 2017;35:501–32.
- [31] Thwaites RS, Jarvis HC, Singh N, Jha A, Pritchard A, Fan H, et al. Absorption of nasal and bronchial fluids: precision sampling of the human respiratory mucosa and laboratory processing of samples. J Vis Exp 2018;131:56413.
- [32] Jozwik A, Habibi MS, Paras A, Zhu J, Guvenel A, Dhariwal J, et al. RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. Nat Commun 2015;6: 10224.
- [33] Kwatra G, Adrian PV, Shiri T, Buchmann EJ, Cutland CL, Madhi SA. Natural acquired humoral immunity against serotype-specific group B Streptococcus recto-vaginal colonization acquisition in pregnant women. Clin Microbiol Infect 2015;21: 568.e13–21.
- [34] Le Doare K, Faal A, Jaiteh M, Sarfo F, Taylor S, Warburton F, et al. Association between functional antibody against Group B Streptococcus and maternal and infant colonization in a Gambian cohort. Vaccine 2017;35:2970–8.
- [35] Memoli MJ, Shaw PA, Han A, Czajkowski L, Reed S, Athota R, et al. Evaluation of antihemagglutinin and antineuraminidase antibodies as correlates of protection in an influenza A/H1N1 virus healthy human challenge model. mBio 2016;7: e00417–16.
- [36] Gould VMW, Francis JN, Anderson KJ, Georges B, Cope AV, Tregoning JS. Nasal IgA provides protection against human influenza challenge in volunteers with low serum influenza antibody titre. Front Microbiol 2017;8:900.
- [37] McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. N Engl J Med 1983;309:13–7.
- [38] Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med 2012;18:274–80.
- [39] Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol 2011;11:57–64.
- [40] Reuling I, Van de Schans L, Coffeng L, Lanke K, Meerstein-Kessel L. A randomized feasibility trial comparing four antimalarial drug regimens to induce Plasmodium falciparum gametocytemia in the controlled human malaria infection model. eLife 2018;7:e31549.

Andrew J. Pollard

Department of Paediatrics, University of Oxford, United Kingdom
E-mail address: andrew.pollard@paediatrics.ox.ac.uk.

Robert Sauerwein

Radboud University Medical Center, the Netherlands
E-mail address: robert.sauerwein@radboudumc.nl.

Marc Baay

P95 Epidemiology & Pharmacovigilance, Leuven, Belgium
E-mail address: marc.baay@p-95.com.

Pieter Neels*

International Alliance for Biological Standardization, Belgium
E-mail address: pieter.neels@vaccine-advice.be.

on behalf of HCT3 speakers and session chairs

Shobana Balasingam

Wellcome Trust, United Kingdom
E-mail address: S.Balasingam@wellcome.ac.uk.

Philip Bejon

KEMRI-Wellcome, Kilifi County Hospital, Kenya
E-mail address: pbejon@kemri-wellcome.org.

Nele Berthels

Federal Agency for Medicines and Health Products (FAMHP), Belgium
E-mail address: nele.berthels@fagg-afmps.be.

Susan Bull

University of Oxford, United Kingdom
E-mail address: susan.bull@ethox.ox.ac.uk.

Andrew Catchpole

hVIVO, United Kingdom
E-mail address: a.catchpole@hvivo.com.

Primus C. Chi

KEMRI-Wellcome Trust Research Programme, Kenya
E-mail address: pchi@kemri-wellcome.org.

Roma Chilengi

Centre for Infectious Disease Research in Zambia, Roma, Zambia
E-mail address: chilengi@cidrz.org.

Rebecca Cox

University of Bergen, Norway
E-mail address: rebecca.cox@uib.no.

Hugh Davies

University of Oxford, United Kingdom
E-mail address: hugtdavies@gmail.com.

Anna Durbin

Johns Hopkins Bloomberg School of Public Health, USA
E-mail address: adurbin1@jhu.edu.

Kate Emary

University of Oxford, United Kingdom
E-mail address: kate.emary@paediatrics.ox.ac.uk.

Claudia Emerson

McMaster University, Canada
E-mail address: emerson@mcmaster.ca.

Robert Frenck

University of Cincinnati, USA
E-mail address: robert.frenck@cchmc.org.

Olivia Grimwade

Monash University, Australia

* Corresponding author.

E-mail address: orgri1@student.monash.edu.

Marcia Hobbs

University of North Carolina, USA

E-mail address: mmhobbs@med.unc.edu.

Gagandeep Kang

Christian Medical College, Vellore, India

E-mail address: gkang@cmcvellore.ac.in.

Paul Kaye

University of York, United Kingdom

E-mail address: paul.kaye@york.ac.uk.

Kirsty Le Doare

St. George's University of London, United Kingdom and [@lshtm](mailto:mrc/uvri),
Uganda

E-mail address: kiledoar@sgul.ac.uk.

Myron M. Levine

University of Maryland School of Medicine, USA

E-mail address: mlevine@som.umaryland.edu.

Helen McShane

University of Oxford, United Kingdom

E-mail address: helen.mcshane@ndm.ox.ac.uk.

Blanche Oguti

University of Oxford, United Kingdom

E-mail address: blanche.oguti@paediatrics.ox.ac.uk.

Peter Openshaw

Imperial College, United Kingdom

E-mail address: p.openshaw@imperial.ac.uk.

Joshua Osowicki

Murdoch Children's Research Institute (MCR), Australia

E-mail address: joshua.osowicki@rch.org.au.

Michael Parker

University of Oxford, United Kingdom

E-mail address: michael.parker@ethox.ox.ac.uk.

Dominique Ploin

Hospices Civils de Lyon, France

E-mail address: dominique.ploin@chu-lyon.fr.

Chad Porter

Naval Medical Research Center, USA

E-mail address: chad.k.porter2.civ@mail.mil.

Meta Roestenberg

Leiden University Medical Center, the Netherlands

E-mail address: m.roestenberg@lumc.nl.

Michael J. Selgelid

Monash University, Australia

E-mail address: michael.selgelid@monash.edu.

Adrian Wildfire

SGS Life Sciences, Belgium

E-mail address: adrian.wildfire@sgs.com.