HIC-Vac Annual Meeting

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Abbreviations

CAA - circulating anodic antigen
CFU - colony-forming units
ChAd63 - Replication-deficient chimpanzee adenovirus serotype 63
DENV - Dengue virus
EHPC - Experimental Human Pneumococcal Carriage model
EMA - European Medicines Agency
Fc – the crystallisable fragment of the immunoglobin
FDA - US-Food and Drug Administration
FFU - foci-forming units
GAS - Group A Streptococcus
GBS - Group B streptococcus
GMP - Good Manufacturing Practice
HAE - human airway epithelial cells
HIC - Human Infection Challenge
ICU - intensive care unit
IMP - Investigational Medical Product
iNTS - invasive disease with non-typhoidal *Salmonella* serovars
ISARIC4C - International Severe Acute Respiratory and emerging Infection Consortium: Coronavirus Clinical Characterisation Consortium
LAIV - live attenuated influenza vaccine
LFA - lateral flow assay
LMIC - Low- and Middle-Income Countries
MAb - monoclonal antibody
MHRA - Medicines and Healthcare products Regulatory Agency
MOSAIC - Mechanisms of Severe Acute Influenza Consortium
MVA - modified vaccinia virus Ankara
NGS - Next-Generation Sequencing
PERISCOPE - PERtussis Correlates of Protection Europe
(q)PCR – (quantitative) polymerase chain reaction
RHD - Rheumatic heart disease
RING - Response Induced by a Genotoxin
RPA - replication protein A
SBA - serum bactericidal activity
SIMON - Sequential Iterative Modeling "OverNight"
ssDNA - single-stranded DNA
TCID - Tissue Culture Infectious Dose
TN - toxin-deletion mutant
WT - wild-type

Introduction

HIC-Vac is an international network of researchers who are involved or interested in Human Infection Challenge (HIC) studies to accelerate the development of vaccines against pathogens, with the aim of reducing the burden of some of the world's most crippling diseases by supporting human infection studies. This is achieved by fostering an engaged and interactive community of international researchers, to promote open sharing of knowledge and expertise, generate new ideas, support and share best practice, and form new cross-disciplinary collaborations.

The network provides catalyst funding to develop early-stage research ideas and extend analysis of existing samples, grants to enable researchers to advance their career development through training opportunities, and public involvement and engagement awards. Advances made through HIC-Vac support are highlighted during the annual meetings of the network.

Research and influence on policy making during Covid-19

Peter Openshaw, Professor of Experimental Medicine at Imperial College and Director of HIC-Vac, pondered the role of scientists in providing scientific advice and policy making during the SARS-CoV-2 pandemic, by discussing questions such as: How did the UK prepare, and how ready were we? What did we get right, and what did we get wrong? What did we learn that we didn't know? How can we use the media? How did science advisors get on with policymakers? Have we brought the public with us? These questions are not only relevant to the UK, but to the whole world.

From the National Risk Register of Civil Emergencies from 2010, it was already clear that pandemic human disease was high on the agenda, judged to have a high likelihood and a strong impact. Globally, such a risk assessment should go hand in hand with the development of emergency plans and a system for scientific advice. In the UK, this is done by the Expert Advisory Network, but similar bodies do not seem to exist in many European countries.

A set of guidelines have been developed on the use of scientific advice in policy making, building on mutual respect, while allowing scientific advisers to publish and present their work and communicate with the media and the public (as long as it is clear in what capacity they are communicating). On the other hand, the government has the democratic right to take decisions that go against scientific advice.

When communicating with the public, this includes the members of parliament, a mere 16% of whom have a scientific background. While this percentage is much higher in the UK cabinet, only one person, Alok Sharma, has a background in science, technology, engineering, or

mathematics. It should therefore not be forgotten that the media are often a vehicle for science communication for politicians as well.
The UK developed a pandemic plan around 2008, mostly focussed on an influenza outbreak. The plan described how to prepare, how to contain, and how to treat the hypothetical outbreak.
During the 2009/2010 H1N1 pandemic, the Mechanisms of Severe Acute Influenza Consortium (MOSAIC) was set up. This consortium provided a blueprint for the International Severe Acute Respiratory and emerging Infection Consortium: Coronavirus Clinical Characterisation Consortium (ISARIC4C). This made it possible to quickly respond to the SARS-CoV-2 pandemic, with a tiered approach, with straightforward data collection initially, followed by stepwise increasingly complex biological sampling.
The major issue with science communication during the Covid-19 pandemic is the polarisation in the debate, further facilitated by social media. The best way for experts to contribute to the discussion is to remain objective, to understand and explain the bigger picture, to retain credibility, and to be consistent and brief.

Streptococcal challenge models

Controlled Human Infection for Vaccines Against *Streptococcus pyogenes* (CHIVAS)

Josh Osowicki, Paediatric Infectious Diseases Physician and postdoctoral researcher at the Murdoch Children's Research Institute, Melbourne, Australia, elaborated on setting up challenge models, focusing on strain selection, characterisation, manufacture, study protocols and sampling schedules, with the ultimate goal of evaluating preventive and therapeutic interventions and understanding host-pathogen interactions. In these models, we challenge human volunteers with pathogens that naturally infect humans, and then, naturally, we observe infections in humans. To quote Sydney Brenner, Nobel prize winner for pioneering the use of a tiny roundworm as a model for human diseases: "We don't have to search for a model organism anymore. Because we are the model organisms." In considering a human infectious challenge, the two key questions are: 1) Is it safe enough; and 2) Is it useful enough?
The long history of *Streptococcus pyogenes* – Group A Streptococcus (GAS) – experimental human infection studies includes work in the 1920s that proved GAS was the cause of scarlet fever. From this work flowed a skin test correlating with protection, passive immunisation products, and a vaccine including a mixture of GAS extracellular toxins with contemporaneous reports of protective efficacy against scarlet fever, invasive GAS infections, and post-infectious complications.
While there are no longer epidemics of scarlet fever and invasive GAS disease, and GAS remains susceptible to penicillin, the pathogen continues to cause a profound global burden of disease across its diverse clinical spectrum. In comparison to other leading causes of infection-attributable mortality and the Neglected Tropical Diseases, research and

development for GAS has been objectively underfunded relative to its impact in terms of death and disability [1][2].
The development of a GAS HIC model started with strain characterisation and manufacturing, including the production of single-dose vials [3, 4]. The primary pharyngitis ("strep throat") endpoint for the HIC matches the initial target of current vaccine development activities and is typically a mild self-limited disease in the young healthy adult participants recruited to this HIC. Locally invasive and disseminated infection following pharyngitis is very rare, and post-infectious complications, already extremely rare in healthy young adults in high income countries, are not seen with early antibiotic therapy as used in the HIC model. Expanding on the previously reported results of the initial dose-finding trial (Lancet Microbe, 2021), experimental human pharyngitis was characterised by an early and robust Th1 inflammatory response with elevation of serum IFN-γ, IL-6, IL-1Ra, IP-10 and IL-18; elevation of salivary IL-18, IL-6, IL-1β, and IL-1Ra; expansion of peripheral blood innate dendritic cell and monocyte populations; migration of B cells and CD4+ T cell subsets (Th1, Th17, Treg, TFH); and activation of unconventional MAIT and γδTCR + Vδ2+ T cell subsets.
Antibody responses across a panel of vaccine antigens were relatively modest, although a pattern of serum IgG responses was observed in subjects with pharyngitis, whereas a salivary IgA response was seen in subjects without pharyngitis, highlighting the importance of the understudied initial host-pathogen encounter.
On multiple levels, a GAS HIC model cannot reproduce the diversity of natural Strep A disease, but it can still have a strategic and scientific impact to accelerate vaccine development, most obviously in randomised double-blind placebo-controlled vaccine challenge trials evaluating vaccine efficacy, with the original M75 challenge strain and potentially additional strains to add to the generalisability of HIC findings. The HIC model will be used to highlight potential correlates of protection and test the relevance of new immunoassays. To inform development of new long-acting penicillin formulations, a HIC trial will shortly begin to establish the penicillin steady state level required to prevent GAS pharyngitis.

Group B *Streptococcus* human infection model (the TIMING study)

Kirsty Le Doare, Professor of Vaccinology and Immunology at St. George's, University of London, pointed out that Group B streptococcus (GBS) accounts for around 57,000 stillbirths and 90,000 infant deaths, more than the total number of deaths from mother-to-child transmission of HIV, or the combined neonatal deaths from tetanus and pertussis. Nevertheless, no vaccine against GBS is available, and it remains a neglected disease during the neonatal period. A vaccine may have significant benefits in terms of addressing neonatal morbidity and mortality and would also be better suited for low- and middle-income countries (LMICs), where screening for GBS and treatment with intrapartum antibiotics is often not available. Finally, the replacement of

intrapartum antibiotics by a vaccine would reduce the risk of antimicrobial resistance.
Several vaccines are now in phase 2 trials but there are problems in showing efficacy, and large study populations are needed. This can be circumvented by finding correlates of protection, or by showing efficacy in a GBS HIC model.
As a first step, we need to develop assays with which to measure antibodies in the vagina. The TIMING study developed assays to measure the concentration of total and serotype-specific IgG in vaginal secretions at baseline and at two-weekly intervals in GBS colonised and non-colonized women, as well as the IgG in serum, and the IgG at the vaginal and IgA at the nasal mucosa. The results should allow us to determine the likelihood of colonisation for a given amount of total and serotype-specific IgG/A in blood or mucosa. Finally, participants were asked about the acceptability of sampling methods (recto-vaginal and nasal swabs, and vaginal cups).
The GBS colonisation status was determined bi-weekly, and more than 50% of women changed their GBS status during the study. Serum antibody levels were consistent over time, regardless of the GBS status of the women, and the same was true for vaginal and nasal mucosal antibodies. Serum and vaginal IgG levels were not correlated to GBS status, but a correlation was found between vaginal IgG and serum IgG ($r = 0.61$) and nasal IgA and vaginal IgG ($r = 0.76$), which suggests that there is an interaction between the different mucosal and systemic immune systems. Although the serotypes detected over time changed, this did not impact the antibody levels, which remained constant.
The focus groups showed that the participants were aware that GBS is a neonatal disease, which generally does not impact the mother. Participants were divided in their feelings about maternal vaccination; some were positive, others were more concerned about the potential harm. Although there was agreement over the importance of vaccination trials, some would be hesitant to participate. Strikingly, when asked about human challenge trials, participants felt comparatively safe, as cure (antibiotics) was available. Finally, there were no problems with the sampling procedures or sexually transmitted infections screening involved, indicating that a GBS HIC model is feasible and acceptable.

Malawi Accelerated Research in Vaccines by Experimental and Laboratory Systems (MARVELS) *Streptococcus pneumoniae*

Ben Morton, Senior Clinical Lecturer at the Liverpool School of Tropical Medicine, shared that in Liverpool, over 20 HIC studies have been performed including more than 2000 volunteers, without any serious adverse events. Studies were focussed on dose ranging and reproducibility, vaccine testing, challenge and re-challenge (with both homologous and heterologous strains), co-infection with live attenuated influenza virus, and hands to nose transmission studies. Multiple strains

were used in these studies, including genetically modified organisms. Participants also included asthmatics and older adults.
The ideal vaccine will have directly protective effects on the vaccinated children, and indirect or herd effects on unvaccinated children and adults. While the indirect effect is seen in high-income countries (in vaccinated and unvaccinated age groups), despite clear direct effects of vaccination, indirect effects are not seen in a range of LMICs, including Malawi, where there is high residual carriage of *S. pneumoniae* types covered by the vaccine, 4 - 7 years after pneumococcal conjugate vaccine introduction [5]. The combination of the clinical need, the timely opportunity, and the right expertise (diagnostic, immunological and clinical expertise) make it feasible to set up the model in Malawi.
As HIC studies are new to Malawi, a framework was followed by which ethical, laboratory, scientific and governance issues may be addressed by investigators considering or planning HIC studies in LMIC [6].
Stakeholders perceived HIC studies to have potential population health benefits, but they also had concerns, particularly related to the safety of volunteers and negative community reactions. Stakeholder perceptions pointed to potential tensions, for example, balancing equity, safety, and relevance in inclusion criteria [7]. To quote Professor Joseph Mfutso-Bengo, a Malawian bioethicist: "HIC studies in Africa are ethically acceptable and scientifically feasible provided they are in accordance with more than minimum international human protection standard. A lower than equivalent human protection standard in HIC research would mean exploitation, therefore unethical and unacceptable". This pointed to the need for rigorous safety procedures, e.g., the 24/7 availability of a clinician, access to rescue medication, and the provision of accommodation after challenge. The accommodation is also a good opportunity to provide further information and training as health literacy is generally low.
The challenge agent used was the same agent used in Liverpool, for which the penicillin sensitivity and serotype were confirmed after transfer to Malawi.
Standard Operating Procedures were shared between Liverpool and Malawi, staff was trained, and a Contract Research Organisation did a pre-assessment visit to inspect the clinical and laboratory readiness. Finally, an audit was performed by a local governance team.
Initially, a dose-finding study was performed, including randomized allocation to sham inoculation. Twenty-four subjects completed the feasibility protocol with minimal side effects. Pneumococcal carriage was established in 0/6, 3/9 and 4/9 subjects in the saline, 20,000 colony-forming units (CFU)/naris and 80,000 CFU/naris groups, respectively. Experimentally induced type 6B pneumococcal carriage was associated with pro-inflammatory nasal mucosal responses prior to inoculation and altered mucosal recruitment of immune cells post bacterial challenge [8, 9]. As the 80,000 CFU challenge met the predefined target attack rate, there was no need to proceed to 160,000 CFU.

All testing was done locally, which helped in capacity building and gave the local scientists the opportunity to advance their careers.
The acceptability part of the study showed that the likelihood of participation in HIC studies rests on three essential conditions: motivation to participate, compensation and advocacy. The motivation and decision to participate was based on reasons including altruism, patriotism, monetary and material incentives, and while compensation was deemed appropriate, concerns about unanticipated research-related risks were raised. Participant advocate groups were recommended for increasing awareness and educating others in the broader community about HIC studies [10].
The first HIC study to test a pneumococcal vaccine in Malawi was built upon the protocol of a study performed in Liverpool [11]. The Malawi study will determine potential immunological mechanisms for the differential effects of PCV13 on nasal carriage between healthy Malawian and UK populations. A double-blinded randomised controlled trial will be conducted to vaccinate participants with either PCV13 or control (normal saline) (1:1). After a period of one month, participants will be inoculated with *S. pneumoniae* serotype 6B to experimentally induce nasal carriage [12]. Subsequently, participants will be invited for a second inoculation after one year to determine longer-term vaccine-induced immunological effects. The enrolment is on track, with currently 67 people screened. Covid-19 positive people are not enrolled, which affected the recruitment rate.
Future research questions in Malawi are: which vaccines can prevent pneumococcal carriage? Which vaccines are most effective in vulnerable populations, particularly those with exposure to air pollution, HIV or malnutrition? What are the critical mechanisms of respiratory tract defence needed for an effective mucosal vaccine suitable for LMIC use? Beyond pneumococcal challenge, plans exist to transfer other HIC models on infections that are relevant to LMICs, and especially the sub-Saharan setting. Amongst these could be the TB and Salmonella HIC model.

Activation of epithelial and innate immune cells following challenge with *Streptococcus pneumoniae*

Caroline Weight, a Senior Research Fellow in Cellular and Molecular Pathogenesis, Division of Infection and Immunity, University College London, explained that the transition from asymptomatic pneumococcal carriage to invasive disease is not well understood. Data from an experimental human pneumococcal carriage model (EHPC) show that *S. pneumoniae* colonisation is associated with epithelial surface adherence, micro-colony formation and invasion, without overt disease. An epithelial-innate immune response was detected by RNAseq around the time of bacterial clearance. A human epithelial cell model recapitulated *in vivo* findings and further revealed that interactions between different strains and the epithelium also shaped the host transcriptomic responses. This indicates that, rather than being confined to the epithelial surface and the overlying mucus layer, the pneumococcus undergoes micro-invasion of

the epithelium that enhances inflammatory and innate immune responses associated with clearance [13].
To investigate how micro-invasion events influence epithelial responses, double mutant strains (AS1 and AS2*)* in a 6B strain background were developed by the Brown lab. In a mouse model, the mutants retained the ability to colonise, but were strongly reduced in systemic virulence in comparison to the wild-type strain [14], suggesting that these mutants do not lead to active invasive disease. In epithelial cell culture models, whereas the AS1 and AS2 were less able to associate with the cells compared to wild type 6B, micro-invasion of both the mutant strains was enhanced compared to wild type. When these mutants were used in the EHPC model, micro-invasion of the mutants was also more readily detected compared to the wild type, in line with the *in vitro data*.
Using RNAseq from the epithelial cell culture model, upregulated differentially expressed genes after infection were largely the same between 6B and AS1, leading to an epithelial innate immune-like transcriptomic profile. In contrast, the profile for AS2 was unique and suggested a cellular metabolism profile linked with intracellular stress responses.
Using conditioned media from infected primary epithelial cell cultures, an upregulation of inflammatory markers from monocyte derived macrophages were detected. This indicates that there is a soluble factor secreted by epithelial cells that stimulates innate immunity.

Human challenge funding landscape

Funding landscape Wellcome Trust

Shobana Balasingam, Research Lead for the Human Investigations and Challenge Programme at the Wellcome Trust, gave an overview of the human infection study programme, which was set up in 2017. The goal of the Programme was to expand the use of human infection studies in endemic settings to understand the efficacy of vaccines on the target populations so that they could be tailored for use. With a view to increase success of the studies funded Wellcome took a holistic approach to strengthen the ethics, regulatory and policy pathways governing human infection studies. Another aim of the programme was to promote harmonisation of challenge protocols and to increase the potential for comparability of data regardless of where studies were conducted. To this end a Community of Practice was established on The Global Health Network site, together with HIC-Vac and the Bill & Melinda Gates Foundation. To date there are already more than 4400 members. So far, Wellcome have funded 7 human infection studies to be established for a range of diseases including *Vivax malaria*, *Pneumococcus*, *Shigella* to name a few in Asia, Africa and South America. Wellcome are also supporting activities in Vietnam to determine the feasibility of establishing human infection studies here as it was found that these studies are deemed to be illegal. The team are engaging with Government, key

stakeholders and the potential volunteer population to determine acceptability in addition to the legality of these studies.
With respect to SARS-CoV-2 challenge studies, Wellcome is supporting the Good Manufacturing Practice (GMP)-based manufacture of a SARS-CoV-2 challenge agent using Delta, which will be released in Q1, 2022. This will be available to the global field through Imperial College assuming a predetermined set of criteria are met.
Wellcome has also been active at a national level and a regional level (e.g. with SEARO and AFRO) to facilitate countries to strengthen and harmonise the regulatory framework for human infection studies through the development of guidance and tools. Additional activities have also included (1) the development of a considerations document for manufacturing of challenge agents outside of GMP guidelines which was supported by both Wellcome and HIC-Vac and produced by Hvivo and (2) a report into the utility of human infection studies as part of the emergency licensure pathway which Wellcome are supporting Boyds to produce.
Among the key achievements of Wellcome supported projects is the transfer of the *Pneumococcal* human infection study model to Malawi, and the start of the PCV-13 vaccine efficacy study. Similarly, in Thailand a malaria human infection study was established in 2021, with the first volunteers challenged and a bank of challenge inoculum established. Finally, through supporting meetings and workshops on human infection studies which were also attended by national regulatory agencies from Asia and Africa, human infection study guidelines have now become part of the clinical trial guidance in Kenya and Zambia.
In the future programme, Wellcome hopes to fund the establishment of more human infection studies. This is aimed at a broader roll out of HIC studies in LMIC's for escalating infectious diseases, focussed on preventive and therapeutic measures, but also to fund the development of new models, and to delve more into protective immunity. In addition, tackling barriers at the ethical, regulatory and acceptance level remains a priority, as does harmonisation of studies, to facilitate comparison of studies and combination of data.

Considerations on the principles of development and manufacturing qualities of challenge agents for use in human infection models

Emma Smith, HIC-Vac Network Manager and Communications Lead, stated that it has become clear that variability in regulation of human challenge agents has left uncertainty regarding the minimum standards that should be met during challenge agent manufacture, particularly agents made outside of GMP. To address this, a Considerations Document has been produced, after consultation with CMC and GMP manufacturing experts, representatives of regulatory agencies from the EU, US, Africa, and Brazil, representatives of ethics committees from Asia, HIC researchers from around the globe, and industry.

The Considerations Document is a living document, which covers challenge agent selection and characterisation, design of the manufacturing process, and specific manufacturing considerations. The aim of the document is to come to a harmonised, international approach, without being prohibitive to research. The document follows the essence of GMP to maintain safety of challenge studies yet leaving the flexibility to allow small-scale and non-GMP processes (especially for vector-requiring agents). The document is intended to provide a solid foundation to engage with regulatory authorities where appropriate.
The next phase will be to develop a smart practices document, based on the considerations document with condensed practical support.

Innovations to accelerate vaccine development and manufacture (INNO4VAC)

Meta Roestenberg, Professor in Vaccinology and Clinical Head of the Controlled Human Infection Centre at the Leiden University Medical Centre, provided an overview of the Innovative Medicines Initiative-sponsored public-private partnership is set up to accelerate and de-risk the development of new vaccines, and consists of four pillars: to create an open-access and cloud-based platform for *in silico* vaccine efficacy assessment and development; to develop new and improved controlled human infection models against influenza, RSV and *C. difficile*; to develop cell-based human *in vitro* 3D mucosal models that resemble the *in vivo* situation of an infection at the mucosa, to more reliably predict immune protection; and to develop a modular one-stop computational platform for *in silico* modelling of vaccine bio-manufacturing and stability testing. The partnership includes 24 public research institutes and universities, four vaccine manufacturers, nine small- or medium-sized enterprises, and four international non-profit organisations from 11 European countries.
Subtopic 2 on HIC models has four aims - first, to develop a new influenza challenge strain focused on the induction of clinical disease endpoints; second, to develop a novel contemporary RSV B strain that fills an important gap for HIC studies; third, to develop a novel model for *C. difficile*-associated diarrhoea that replicates natural disease; and finally, to establish an optimized framework to position HIC models in the vaccine development pipeline, with involvement of regulators.
The development of new HIC models follows a stage gating process, with a go/no-go decision to move from the model exploratory phase to the prototype development phase (e.g., the manufacturing of challenge agent of sufficient quality), and a go/no-go decision to move from the prototype development phase to the prototype consolidation phase.
Extensive interaction is expected between subtopic 2 and subtopic 3, which is hoped to result in state-of-the-art innovations in human *in vitro* 3D mucosal models and assays. In the validation phase of that subtopic, the samples collected in subtopic 2 would be extremely valuable.

Discussion

Is there a large difference between the EU and the UK in the role of GMP in challenge agent production? There is a clear difference between regulatory roles of the European Medicines Agency (EMA) and the Medicines and Healthcare products Regulatory Agency (MHRA), and also US-Food and Drug Administration (FDA). The FDA sees a challenge agent as an Investigational Medical Product (IMP), whereas MHRA sees it as an Auxillary Medical Product, and the EMA sees it as a non-IMP. In the end each manufacturing episode is a case in itself, which should be performed according to the principles of GMP. The manufacturing plan should adhere to GMP-like practice. There is a common pathway between the different regions.
In the Netherlands, for instance, any manufacturing done outside the GMP room is considered non-GMP but is nevertheless done according to GMP standards. The considerations document has been very helpful in providing guidelines for manufacturing.

Do LMICs look at this differently? Using Malawi as an example, the local regulatory body is not well resourced and does not have the expertise. Hence, they follow the advice from the MHRA.

Is training of LMIC regulators part of the future plans? This was pursued pre-Covid, and the intention is to pick this up again as soon as possible. Capacity building is a necessary step moving forward.

Are GMP needs different according to route of administration? This is also part of the considerations document. Clearly, the route of administration is extremely important. An agent that is delivered intravenously requires higher standards than an agent which is administered orally, intranasally or topically. Furthermore, some challenge agents cannot be manufactured under GMP. But the principle is to come to the safest agent possible. The inclusion of examples in the considerations document may help to make this clear.

HIC models are important tools, which should be available to academic institutions as well as industry. This may lead to conflicts. How do we make sure models and challenge agents remain available to the global research community? In case of the Covid-19 challenge agent, an access management group has been set up. Investigators who would like to use the agent need to provide a protocol, which will be reviewed, to see if the capacity and expertise is present to handle and use the challenge agent appropriately. Such a system can be put in place for other challenge agents as well, moving forward.
Nevertheless, there may be conflicts, as the capacity for HIC studies is limited. How to decide which are more important studies; academic studies or industry-sponsored studies? This needs to be discussed. A partnership model would be an option, in which vaccines or drugs can be

studied, but where samples are collected to maximise scientific value. The experience so far is that there is good interaction between academia and industry, with plenty of scientific output.

Dengue HIC model in Dengue vaccine development

Anna Durbin, Professor of International Health at the Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA, stated that Dengue virus (DENV) 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. The risk of severe disease is highest at the second infection with a different strain, resulting in antibody-mediated enhancement of infection [15]. Third and fourth infections produce less symptoms, suggesting the need for a tetravalent vaccine. This leads to the question whether multiple heterologic infections result in broad protective immunity, and whether a quadrivalent DENV vaccine is necessary for complete protection?

Despite inducing high levels of neutralizing antibodies, the Dengue vaccine Dengvaxia was shown to have a variable efficacy, dependent on the age at vaccination, the serostatus at vaccination, and the most prevalent serotype in the country. Long-term safety follow-up demonstrated an increased risk of hospitalization and severe dengue in vaccinated subjects compared to controls in year 3, with the highest risk in younger subjects (7.5-fold increase), whereas no increased risk was observed in subjects aged nine years and older [16].

DENV challenge studies could be an important tool to down select Dengue vaccines at an early stage and may provide a platform upon which to understand immune correlates of protection. A HIC model, which is an infection model rather than a disease model, must induce reproducible endpoints that occur with a sufficient frequency to keep the number of participants at a minimum. HIC models have been developed for DENV-2 Tonga/74 virus and DENV-3 Slemen/78 virus, with both showing 100% viremia at 3 $\log_{10}$ PFU/ml, and rash in 80% for DENV-2 and 100% for DENV-3, with mild or moderate adverse events.

In addition to Dengvaxia, two other Dengue vaccines are in phase 3, Tak-003 (Takeda) and TV003 (NIH/Butantan). The latter was selected for continued development after a HIC study to evaluate protective efficacy showed that TV003 protected against viremia, neutropenia, and rash [17]. However, because of the high efficacy the chance of finding a correlate of protection is limited.

Interestingly, participants with sterilizing immunity (antibody fold rise of less than 4) showed a higher frequency of memory B cells, compared to

participants with non-sterilizing immunity (with a four-fold increase or higher in antibody titers) [17].
An antibody repertoire analysis was done agnostically. Out of the 100 monoclonal antibodies (MAbs) chosen, 60 bound to DENV-2, of which 80% were DENV-2-specific and 20% were cross-reactive to other types. However, out of the 100 MAbs, only three were neutralizing antibodies. The key question is whether a sequential second infection induces a different immune response from the tetravalent immune response. To investigate this a study was set up to vaccinate with a trivalent vaccine and challenge with the fourth serotype after six months. On the day of challenge, seven DENV-2 naive trivalent vaccine recipients had DENV-2 neutralizing antibodies, which had to be heterotypic antibodies. As expected for a serotype that is not present in the vaccine, the peak geometric mean titer against DENV-2 was lower than for the three viruses in the vaccine. Four participants were viraemic by culture post-challenge, and 12 had virus detectable by PCR. Finally, three participants had a rash. This is strikingly different from the results after tetravalent vaccination, in which two extra DENV-2 proteins are present: prM and E. This indicates that a tetravalent vaccine is necessary to confer full protection.
Differences in onset and mean duration of viremia between trivalent and placebo may be due to T cell immune response, which is currently under investigation.
Data from the DENV-3 HIC are very similar; TV005 completely protected against viremia and rash, as well as neutropenia and retro-orbital pain. The viremia peaked at 4-6 days and quickly disappeared afterwards. Strikingly, when a higher dose of DENV-3 is given, a lower percentage of participants show viremia. The higher dose may lead to an earlier and stronger innate immune response, which in turn eradicates the infection sooner. The TV003 is now in phase 3 in Brazil, and results are expected by mid-2022.
A Zika virus HIC model is being developed and has been approved by the FDA. The model is necessary to perform vaccine efficacy studies, although phase 3 safety studies will need to be done in the field. A first Zika virus HIC study will start enrolment end of November 2021. The study will only enrol women, as inpatients.

Covid challenge

Development of a SARS-CoV-2 challenge virus

Adrian Wildfire, Director Scientific and Business Strategy at hVIVO, posed that although Beta coronaviruses are well known, SARS-CoV-2 is relatively unknown, so careful titration studies had to be performed. Triage was done on original samples, ensuring the sample was collected in the right matrix and that sufficient residual sample was available to do the necessary testing and avoid attenuation of the virus by passaging, retaining a 'hot' virus. Triage was focussed on the 20A clade, with the

D614G mutation, to be representative of the circulating strain at the time, to appropriately inform vaccine studies.
Vero cells are not all equally susceptible to the virus. While this will have a limited impact on production, it may have an impact on the viral titration, potentially leading to a one log miscalculation, with potential implications for challenge studies. This can be prevented by testing a battery of cell lines initially to find the most appropriate match. The well-characterised GMP Vero cells were used for infection for viral production. The virus was also tested in human airway epithelial cells (HAE). While delayed growth was seen in HAE, at 72 hours the levels were similar in Vero and HAE.
The final selected sample propagated well on the Vero cells and had no atypical growth characteristics or immunogenic changes. Furthermore, informed consent and the medical history were available, showing that the patient had no discouraging outcomes.
The sample was taken through three passages to obtain enough virus to prepare a master virus bank, which was aliquoted, frozen, and stored. The master bank vial was then diluted to produce different titre inoculum vials and stability testing was performed.
The adventitious agent and release testing plan was reviewed and approved by MHRA. Molecular-based testing was done for bacteria, fungi, mycobacterium and other viruses, including pathogen-specific PCR and Next-Generation Sequencing (NGS) for non-bias viral screening. NGS also served to analyse the virus and to demonstrate that no changes were introduced during the production process.

Safety, tolerability and viral kinetics following SARS-CoV-2 human challenge of healthy seronegative adult volunteers

Christopher Chiu, Professor of Infectious Diseases and Lead of the Imperial Network for Vaccine Research, shared that even early in the pandemic, there was a vision and an effort to define the feasibility and appropriateness of applying challenge studies to battle the pandemic [18, 19]. In the UK, the Vaccines Taskforce set up a consortium, led by Imperial College, together with the Royal Free London NHS Foundation Trust and hVIVO, with a wide range of other contributors.
Initially, when no vaccines or antivirals were available, the rationale of using a HIC model was straightforward. However, treatments became rapidly available, and at the time the challenge studies could start, vaccines were approved and being given. Therefore, the consortium had to rethink the rationale for using challenge studies. In the short term, there still was limited understanding of the viral kinetics and dynamics, which are key to the transmission and pandemic spread. This was especially true for asymptomatic and mild infections in the community, which are hard to capture and investigate systematically. In the medium- to long-term, there are still large numbers of vaccines in development, for which it will be difficult to demonstrate efficacy in clinical trials.

The major issue in the challenge model is the risk for the participants. Throughout the pandemic, the risks of ending up in the intensive care unit (ICU) or of death have been very low in otherwise healthy 18–30-year-olds [20, 21]. Mild symptoms such as loss of smell, persistent cough, fatigue, and headache generally resolve within 30 days, and even in outliers, resolve within 90 days, suggesting that the risks of long COVID are limited, and challenge studies are feasible.

Both surveys and focus groups indicated that a large majority of respondents agreed that human challenge studies should take place in the UK as a means to battle the pandemic.

To set up the studies, risk mitigation was carefully performed, including participant selection, the use of a well-characterised challenge agent, and starting with the lowest quantifiable dose (10 Tissue Culture Infectious Dose $(TCID)_{50}$ or 55 focus-forming units (FFU)) and following a careful dose escalation plan. Furthermore, the model had virological endpoints rather than clinical endpoints to reduce the risk of more severe symptoms, with pre-emptive treatment (Remdesivir) and early rescue treatment (Regeneron) if necessary. Participants were housed in a quarantine unit during the study, with strict discharge criteria to reduce the risk of environmental shedding, and were followed up carefully after the study, with specialist referral when needed.

The changing pandemic landscape resulted in three rounds of ethical review, with extensive revision of the protocol and informed consent process along the way. In February 2021, the study was finally approved. Before enrolment, participants were extensively screened using radiology, blood tests, smell, and cognitive tests, and after eligible participants provided informed consent, they were inoculated intranasally, aiming for an attack rate of between 50% and 70%, to balance the number of participants needed to power future efficacy trials with the possibility of obtaining insights into correlates of protection. The output was maximised with a large number of different samples taken, including nose and throat swabs, saliva, breath and environmental sampling, as well as blood samples for immunology testing. Due to the strict release criteria, participants were quarantined for 16 days on average, with an upper limit of 19 days.

Showing massive public support, 27,000 individuals registered their interest in participating; 6,000 were telephoned for pre-screening, 187 were seen in person, and from these, 36 were enrolled and inoculated. Eighteen developed a PCR-confirmed infection after inoculation with the lowest dose and, with 2 participants excluded due to the development of antibodies against SARS-CoV-2, this resulted in an infection rate of 53%. Therefore, it was decided to do no further dose-escalation.

In the non-infected group, despite the absence of neutralizing antibody responses, isolated virus detections were seen in the nose and throat of some individuals, suggesting abortive infection likely due to early inflammatory or immune responses.

In the infected individuals, it could be observed that the incubation period was on average 40 hours, which is much shorter than the five days that

are generally communicated. Very high viral loads were seen, with up to 10^{10} copies per ml, with a strong correlation between the viral loads in the throat and nose, especially when tested by qPCR. This suggests that even a single respiratory droplet could contain enough virus to infect a new individual.

In comparison to other viral challenge models, e.g., RSV or flu, the viral load curves were very consistent, especially in the early stages. Individual differences did exist, with delayed viral detection in the nose (despite the fact that this was the site of inoculation). On average, the peak viral load was delayed by 48 hours in the nose, compared to samples taken from the throat. However, the viral load was a full log higher in the nose than in the throat. The duration of viable virus varied widely between individuals, ranging from 2-9 days for the throat and 3-12 days for the nose. This does show, however, that the vast majority of shedders should be covered by the 10-day self-isolation rule.

Symptoms, mostly respiratory symptoms such as rhinitis and nasal stuffiness, as well as systemic symptoms, such as malaise and headaches, were seen in 16 out of the 18 individuals who got infected. These symptoms were mild to moderate, with only occasional grade 3 symptoms. Smell disturbance was seen in 80% of participants, peaking later than other symptoms, and generally resolved by day 28, although one participant had residual smell impairment at day 90.

While symptoms correlated with viral load in time, especially with viral load in the nose, there was no correlation between viral load and symptom magnitude, showing that individuals with high viral titres can remain entirely symptom-free. This underscores the need for strategies to deal with asymptomatic infection and transmission. Part of the strategy may be the use of lateral flow assays (LFA); these are known to be less sensitive than PCR, and their accuracy was therefore tested during the challenge infection. The general guideline is to use LFAs for self-testing twice a week and go into isolation if needed. In the challenge study, LFA was shown to strongly predict qPCR and cell culture positivity. The specificity of LFA was consistently 100% when looking at qPCR. The sensitivity was high in the acute phase of the infection, but the performance was less good in the early and late stages. Modelling this, taking LFAs every three days, you would capture almost 80% of the infections, and so self-isolation then would interrupt ~80% of viral transmission. Taking swabs once a week would still capture 50% of the virus before it is produced.

Finally, according to the data, there were no superspreading individuals; all individuals generated similarly high viral loads. This suggests that superspreading events are most likely to occur when environmental circumstances are right. The first paper detailing these results is now available as a pre-print at www.researchsquare.com/article/rs-1121993/v1.

Mucosal and systemic inflammatory and humoral immune responses in a human SARS-CoV-2 infection model

Ryan Thwaites, Lecturer in Respiratory Immunology within the National Heart and Lung Institute, Imperial College London, pointed out that data from the RSV challenge model showed that individuals who did not develop a cold had an early mucosal response to the virus, while in individuals who did develop symptoms, a robust response was seen, but only after initial repression of host mediators [22]. In the live-attenuated influenza virus challenge model, nearly all participants seroconverted, both in the blood and in the nasal mucosa, although the responses were not necessarily coordinated between the two compartments. However, these HIC studies show that the tools are available to profile mucosal and systemic inflammatory responses to inoculation and infection and to identify baseline factors associated with protection or susceptibility for the SARS-CoV-2 model.

Participants provided nasosorption samples twice daily and blood samples daily. Nasosorption samples from both nostrils were combined and used to detect cytokines and chemokines, while plasma was tested for antibodies against SARS-CoV-2 spike and nucleocapsid proteins, SARS-CoV-1 spike, and four endemic coronaviruses.

Data on mucosal interferon (IFN)-a showed a clear type 1 immune response in those infected, starting on day 3-4, with a one-log increase on average. Non-infected individuals showed fairly stable IFN-a levels. Very similar responses were seen for IFN-b and IL-29. The response lasts for approximately ten days. This leaves a timeframe for the virus to replicate before the adaptive immune system kicks in, leading to the early high viral loads, as described above.

Analysing plasma samples from hospitalised patients and outpatients with mild disease, the progressive elevation of levels of numerous inflammatory cytokines and chemokines (including IL-6, CXCL10, and GM-CSF) were associated with severity and accompanied by elevated markers of endothelial injury and thrombosis [23]. Principal component and network analyses demonstrated central roles for IL-6 and GM-CSF in COVID-19 pathogenesis. Comparing these profiles to archived samples from patients with fatal influenza, IL-6 was equally elevated in both conditions, whereas GM-CSF was prominent only in COVID-19. In the HIC participants, the GM-CSF and IL-6 levels did not match those in severe disease patients.

When looking at baseline antibody levels, there is no evidence of pre-existing immunity protecting non-infected participants.

When looking at IgA in the mucosa, the antibody levels started waning from day 20, while the antibody levels were still rising in the blood. Although seroconversion is seen in all infected participants, there is large variability in the response, the significance of which will be further investigated.

The differences between the immune responses to RSV and influenza on the one hand and SARS-CoV-2 on the other hand may be largely due to

SARS-CoV-2 being a new virus to which people have not been previously exposed.

Using a SARS CoV2 human challenge model to define protective immunity

Helen McShane, Wellcome Trust Investigator and Professor of Vaccinology at the University of Oxford, shared data on a study intended to look at individuals who had been infected with SARS-CoV-2 at least three months before enrolment into the challenge study to look at natural protective immunity. From 181 individuals showing interest in participating online, 21 were eligible, and 17 had been enrolled into a dose-escalation study to date, with the aim of trying to find the infective dose needed for a 50% attack rate. In the 10^1 $TCID_{50}$ group, in which all participants were unvaccinated, 25% were transiently PCR positive. In the 10^2 $TCID_{50}$ group, in which all participants had received at least one vaccination, one was transiently positive at a very early stage, but this was deemed inoculum contamination. So far, two volunteers have been inoculated with 10^3 $TCID_{50}$, and both individuals have been released from quarantine without signs of infection. None of the individuals have shown any adverse events.

In the single individual who showed a transient viral infection, as detected by focus forming assay, a mild leukopenia was observed, which resolved very quickly.

When looking at serum IgG, it was impossible to tease out differences in responses in the transiently infected individuals. However, IgG in the mucosa showed that the two individuals with transient infection had the lowest response against all antigens, although these data need to be carefully interpreted because of the low number of individuals in the study. IgA and IgM responses in group 1b, where no infections were observed, showed very limited variations between individuals.

This study has shown that exposing healthy, previously infected subjects to a controlled dose of SARS-CoV-2 is safe. It also shows that immunity induced by prior infection results in robust protective immunity against controlled human infection. This impacts the ability to determine immune correlates of protection unless a safe dose can be identified, which results in infection in at least some subjects. This can be done by carefully increasing the dose or by looking at the delta variant, which is more infectious. Similarly, comparison with non-infected, vaccinated individuals may provide insight into natural immunity due to infection.

Discussion

What would have been the effect of pre-emptive treatment on the study outcomes? And how was it decided to go from pre-emptive treatment to rescue treatment?

Because it was a new challenge agent, the trials started out ultra-cautious. In the trial with SARS-CoV-2-naïve participants, the first 10 patients who were confirmed as infected received Remdesivir as a

precaution. However, as it became clear that they did not suffer from any severe disease, pre-emptive Remdesivir was removed (and would instead be used as a rescue therapy if necessary) so as not to confound study results.
Although the numbers were low, there was a hint of effect of Remdesivir on viral load, with a delay in peak viral load in the nose in treated participants. To avoid this, rescue treatment is better if this option is safe. The trial with previously SARS-CoV-2-infected participants started four months later, and by then, Remdesivir was replaced by Regeneron as pre-emptive therapy. Because of a slight delay in the testing of swabs, it turned out that both participants who were positive and received Regeneron only received it after they had become negative again.

How was the process of getting ethical approval for the studies?
Both study protocols were seen by the same ethical committee, which was larger than usual. Both protocols went through three review rounds with open discussion. For the study with previously SARS-CoV-2-infected participants, two extensively discussed topics were the potentially discriminatory nature of the selection of study participants, and the justification for CT scans.
This same ethical committee will also review all future SARS-CoV-2 challenge studies.

How was it decided when to give rescue therapy?
Criteria for rescue treatment were persistent fever, altered observations such as hypertension, tachycardia, or a modest oxygen saturation drop; but these healthy participants never came close to these criteria.

What kind of swabs were obtained from the participants?
Separate mid-turbinate and throat swabs were obtained. In hindsight, it was very helpful to have these swabs separately, as they showed the time difference between the throat and the nose.

The study with previously SARS-CoV-2-infected participants clearly shows that it is not easy to re-infect those participants. Have you thought of stratifying the sero-positive participants?
If enough volunteers had been available, one could select those with lower antibody levels. Going forward, further dose-escalation to 10^4 $TCID_{50}$ will be discussed with the Data Safety Monitoring Board. Once the final dose has been decided, some stratification according to antibody levels should be possible when enrolling larger numbers. Moreover, waning antibody levels are expected in participants with increasing time since natural exposure/vaccination, unless booster doses are given.

Will the challenge studies also be used to look at new treatment options?
Discussions with the MHRA are ongoing. It would involve using the medication off-licence, as the participants are not the target population for treatment.

Enteric challenge models

Live-attenuated rotavirus challenge to assess next-generation vaccines in Africa

Nicholas Grassly, Professor of Infectious Disease & Vaccine Epidemiology in the Department for Infectious Disease Epidemiology, Imperial College London, explained that while the oral rotavirus vaccine has been introduced widely in Africa [24], this has not impacted the number of rotavirus-associated cases of acute gastroenteritis as much as hoped [25]. A systematic review confirmed the protective efficacy and effectiveness of rotavirus vaccination against rotavirus diarrhoeal outcomes among children under 5, globally. However, vaccine effectiveness was lowest in sub-Saharan Africa [26]. Many potential causes of poor oral vaccine effectiveness have been described [27], and effectiveness may also differ by setting and by product. Attempts to improve effectiveness have largely failed, except for the delay of the first dose by four weeks, which led to a significant increase in seroconversion [28]. There is a healthy pipeline of new generation rotavirus vaccines based on a large number of platforms [24]. These are parenteral vaccines, which may overcome the oral-intestine barrier to the oral vaccines. However, placebo-controlled trials are no longer ethical, and phase 3 trials with an active comparator need to be large, and hence expensive, slow, and difficult to perform. Therefore, a rotavirus HIC model may help to assess these new vaccines, especially when conducted in the correct target population and age group.

The advantages of using a live-attenuated oral rotavirus vaccine (RV1 or Rotarix) as a challenge agent are that it has an established safety profile and it is a widely available GMP product that can be used in children (the target population) with relatively uncomplicated ethics. However, it will only be possible to study infection, not disease, as an end point. Finally, the attenuated challenge is different from wild-type exposure. A pilot study funded by HIC-Vac in Zambia showed that shedding was significantly reduced after the second dose, which was seen as the challenge [29]. This opened the way to assess the parenteral, trivalent (P[4],P[6],P[8]) P2-VP8 subunit vaccine (VP8) [30]. A study with four arms has been funded by the MRC to see if the addition of parenteral vaccines to oral vaccines might improve effectiveness: RV1/RV1 (6 and 10 weeks, routine vaccination schedule) versus VP8/VP8/VP8 (6, 10 and 14 weeks, phase 3 trial schedule), RV1/RV1/VP8 (6, 10 and 14 weeks), and RV1+VP8/RV1+VP8/VP8 (6, 10 and 14 weeks). All schedules were followed by challenge with RV1 at week 18. Currently, no correlates of protection are available at the individual level. So far, 349 individuals have been screened, 327 have been randomised, 64 have been challenged, and 43 have completed all study procedures. Covid resulted in a two-month break, and a limitation to 20-30 new enrolments per week, delaying completion of the trial. The major challenge was the transfer of assays and reagents/equipment to Zambia to be able to

perform all tests locally. There were no ethical challenges or issues within the local community.

Investigating senescence responses to *Salmonella* Typhi

Daniel Humphreys, UKRI Future Leaders Research Fellow, School of Biosciences, University of Sheffield, explained that typhoid is initiated by ingestion of *Salmonella* Typhi, which infects the intestinal mucosa and releases virulence factors, including the typhoid toxin, that takes over the DNA repair mechanism to age cells and boost aggressiveness of infection. Typhoid is a waterborne disease driven by a lack of clean water and poor sanitation, is endemic in many low- and middle-income countries and causes many fatalities. *S.* Typhi has become very drug-resistant, resulting in treatment failures in the most vulnerable LMIC. Diagnostics are another major barrier, as are asymptomatic chronic carriers, who contaminate the food chain and transmit disease.

S. Typhi activates the host DNA damage response through the typhoid toxin, causing typhoid symptoms and chronic infections. A non-canonical DNA damage response, RING (Response Induced by a Genotoxin) is characterised by the accumulation of phosphorylated histone H2AX at the nuclear periphery [31]. RING is the result of persistent DNA damage mediated by toxin nuclease activity. The toxin overloads the replication protein A (RPA) pathway (a sensor of single-stranded DNA (ssDNA) and DNA replication stress) with ssDNA substrate, causing RPA exhaustion and senescence. Senescence, an innate defence requiring tight regulation, is transmitted to non-intoxicated bystander cells by an unidentified senescence-associated secreted factor that enhances *Salmonella* infections, i.e., a remodelling of the infection niche [31].

When studying the role of typhoid toxin in acute infection, no significant difference in the rate of infection was observed between participants challenged with wild-type (WT) or a toxin-deletion mutant (TN) *S*. Typhi. The clinical syndrome was indistinguishable between wild-type and TN groups too. These results suggest that the typhoid toxin is not required for infection nor the development of early typhoid fever symptoms within the context of a human challenge model [32]. However, the duration of bacteraemia was significantly longer in participants challenged with the TN strain.

A HIC study was set up to look at host senescence responses to typhoid toxin in samples from human participants challenged with WT and TN *S.* Typhi. While the upregulated proteins were similar in both arms, WT *S.* Typhi caused downregulation of more proteins than TN *S*. Typhi. When looking at participants individually, several proteins were differently regulated, such as ApoC3 and Lysozyme. Supporting lab experiments using the Caco2 cell line also showed that ApoC3 was induced by the WT toxin but not the TN toxin, suggesting DNA damage responses are activated. This can be reproduced by infection of cells with *S. javiana*, although with a 48-hour delay. The next step will be to take the findings

back to a HIC study to look at the host secretome during acute typhoid fever.

Typhoid vaccine-induced antibody glycosylation as a correlate of protection

Lisa Stockdale, Postdoctoral Immunologist at Jenner Institute, University of Oxford, pointed out that, unlike subclass selection, which irreversibly changes the constant region (in the crystallisable fragment, Fc) of antibodies, the addition of sugar residues in the CH2 domain of the Fc represents a mechanism by which the immune system can fine-tune antibody effector function. Post-translational modifications to the conserved *N*-linked glycan at asparagine position 297 of the Fc region of human IgG have been used in therapeutic monoclonal antibody development to improve the management of cancer or autoimmune diseases. Manipulation of antibody glycosylation can impact immunoglobulin stability, pharmacokinetics, efficacy, and immunogenicity. Notably, these alterations can affect Fc receptor binding, resulting in altered antibody effector functions.

Reactive, non-neutralising antibodies, enriched for afucosylated IgG1, triggered platelet reduction *in vivo*, which is a significant risk factor for thrombocytopenia. Thus, therapeutics and vaccines restricting the production of afucosylated IgG1 during infection may prevent antibody-induced disease enhancement.

Afucosylated antibodies are known to be formed in response to infection with enveloped viruses and were found to be associated with increased severity of symptoms to SARS-CoV-2 [33]. The mechanism by which changes in antibody glycosylation are affected is not known. It has been hypothesised that diet, age, and genetics could play a role in the range of glycoforms formed in response to antigenic stimuli but also the context in which the antigen is presented (type and infectious dose of pathogen, or formulation and route of administration of vaccination).

The *S.* Typhi HIC model was used in UK individuals to quantify Vi-specific glycans to investigate differences in glycosylation induced by vaccines Vi-PS and Vi-TT given prior to challenge. This made it possible to see whether any vaccine-induced glycans were associated with protection or disease severity.

Prevaccination samples from an adult cohort from the UK, vaccinated with either Vi-PS or Vi-TT, were compared with prevaccination samples from a cohort of children vaccinated with Vi-TT from Nepal. Fucosylation and galactosylation were high, with IgG1 galactosylation being significantly higher in the UK, IgG2/3 fucosylation higher in Nepal, and IgG2/3 galactosylation higher in the UK. However, these differences may be due to the age difference between the cohorts. After vaccination, IgG1 fucosylation, galactosylation and sialylation increased for Vi-specific IgG compared to total IgG, whereas bisection decreased. For IgG2/3 there was no difference in fucosylation, but galactosylation and sialylation increased for Vi-specific IgG compared to total IgG. The differences were

more pronounced for Vi-TT vaccinated individuals compared to Vi-PS vaccinated individuals. Comparing UK and Nepalese individuals vaccinated with Vi-TT showed similar changes in relative abundance, with higher galactosylation but lower sialylation in Nepalese versus UK individuals. Differences in vaccine-induced glycosylation did not correlate with protection, as there are no clear differences between those who developed typhoid symptoms and those who did not, regardless of the type of vaccine received.
In summary, Vi-specific glycan changes are distinct from total IgG glycosylation. Both the Vi-PS and Vi-TT vaccines induce increased fucosylation, galactosylation, and sialylation and decreased bisection, compared to total IgG. Where there is a difference between Vi-PS and Vi-TT, Vi-TT is associated with the larger magnitude change. However, vaccine-induced glycosylation does not predict challenge outcome.
IgA glycosylation has not been looked at extensively and might result in more differences.

Development of an iNTS human challenge model

Malick Gibani, Clinical Lecturer and Specialist Registrar in Infectious Diseases and Medical Microbiology at Imperial College, pointed out that invasive disease with non-typhoidal *Salmonella* serovars (iNTS) is common across Africa, with an estimated 535 000 cases and 77 500 deaths per year. The case-fatality rate is high, estimated at approximately 15-20%, which is partially due to the strong association with immunodeficient states.
The highest risk is in sub-Saharan Africa, and particularly in the age group 0-5 [34].
There is a growing interest in the development of multi-valent *Salmonella* vaccines, including Typhi, Paratyphi, and iNTS. The iNTS vaccines are in early-stage clinical testing e.g., a Trivalent COPS-FliC/Vi-TT conjugate vaccine and a Bivalent GMMA *S.* Typhimurium/*S*. enteritidis vaccine.
Large scale phase 3 trials are possible but challenging in these settings. Human challenge studies have a track record of accelerating some enteric vaccine candidates through licensure, including *Vibrio cholerae,* and *Salmonella* Typhi. Therefore, the development of an iNTS human challenge model could represent a novel approach to study host-pathogen interactions and accelerate vaccine development for iNTS. One particularly challenging issue is the decision regarding the measured endpoint, which must be both safe and relevant.
A consortium was set up to develop the iNTS HIC model, with five work packages including strain manufacture and characterisation, the clinical trial, and the definition of correlates of protection.
Two challenge strains will be used: ST19 (4/74 or 14028s), the archetypal "diarrheagenic" strain, and ST313 D23580, the archetypal "invasive" strain. 300 vials of each strain will be manufactured, of which 225 will be set aside for clinical trial purposes.

A careful dose-escalation study will be performed to determine the response of healthy individuals to challenge with *S*. Typhimurium ST19 and ST313. This study will be done with intensive screening of participants and in an inpatient setting.
The primary disease endpoint will be Salmonellosis, defined as a clinical illness with documented *S. typhimurium* infection, occurring within 14 days of challenge. This can be an infection with *S. typhimurium* based on a positive stool culture for *S. typhimurium* occurring >72 hours after challenge regardless of symptoms and/or blood culture positive for *S. typhimurium* at any time point. It can also be a clinical illness, e.g., bacteraemia, or diarrhoea, or two recorded fevers (≥38°C) without diarrhoea but with at least two associated gastrointestinal symptoms (vomiting, abdominal cramping, tenesmus). Finally, it can be diarrhoea, defined as mild (one loose/ liquid stool ≥300 g, or ≥2 loose/liquid stools ≥200 g in any 48-hour period, or ≥3 loose/liquid stools in a 24-hour period), moderate (4–5 diarrhoeal stools in 24 hours or 401–800 g within 24 hours) or severe (≥6 loose/liquid stools in 24 hours or >800 g of loose/liquid stools in 24 hours). Dysentery will be defined as ≥2 episodes of gross blood in a loose stool.

Respiratory challenge models

Controlled human infection with *Neisseria lactamica*

Diane Gbesemete, Clinical Research Fellow at the NIHR Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, Southampton, explained that *Neisseria lactamica* is a completely harmless commensal organism. What makes this bacterium especially interesting is its relationship with *N. meningitidis*. Both organisms colonise the nasopharynx, but the non-capsular, non-invasive *N. lactamica* appears to protect against *N. meningitidis*, which can invade and cause meningitis. The mechanism of this protection can be investigated in human challenge studies. Previous HIC studies have shown that safe, longstanding colonisation with *N. lactamica* can be produced in 33% - 85% of healthy adults, depending on factors such as smoking habits. The presence of *N. lactamica* reduces acquisition of *N. meningitidis* but also displaces pre-existing colonisation. Challenge induces a specific humoral immune response, with some cross-reactivity against *N. meningitidis* but no serum bactericidal activity (SBA) against *N. meningitidis* [35, 36].
The Neisseria adhesin A (NadA) gene from *N. meningitidis* was inserted in *N. lactamica*, with lacZ as a reporter gene. Studies in mice showed that this did not result in any pathogenicity signals but induced SBA against *N. meningitidis.* Because the resulting challenge agent is a genetically modified organism, extreme care was taken in the challenge trial design, with an in-patient observation period. Furthermore, to investigate the risk of environmental spreading, contact volunteers (bedroom sharers of challenge study participants) were also enrolled to assess onward transmission of *N. lactamica* [37]. The controlled infection model was

safe, with very few and mild solicited adverse events. The study showed a high colonisation rate but no transmission to contacts during the 90-day period. After the end-of-study treatment, all participants were negative for *N. lactamica* [38]. A significant increase in *N. lactamica*–specific immunity was shown in participants, regardless of inoculation with empty vector or NadA expressing cells. However, 50% of the NadA inoculated participants versus 0% of the control participants showed a two-fold or higher increase in NadA-specific IgG, as detected by ELISA. Moreover, a significant and sustained increase in NadA-specific IgG memory B-cells was shown using ELISpot in the study participants versus the control participants. Finally, an increase in SBA was found in both groups inoculated with *N. lactamica,* but the effect was larger in the NadA inoculated group [38].

This model is potentially a useful platform in the meningitis belt, where outbreaks of *N. meningitidis* occur. In order to facilitate this, the challenge agent was adapted from a frozen stock to a freeze-dried stock with defined amounts of bacteria that can be easily reconstituted, thus no longer requiring a cold chain and providing more accurate dosing. The new challenge agent was first tested in the UK to show that it was safe, that participants would be colonised and that an immune response would occur. In a dose-finding study, 10^5 CFU were found to give 100% colonisation. Few adverse events were observed, other than a burning/stinging feeling immediately after inoculation, which was resolved by replacing the medium for reconstitution from water to saline. Similar to the challenge with frozen stocks, a clear immune response was seen in volunteers who were colonised, with a 2.3-fold increase in IgG levels, whereas no change was observed in volunteers who were not colonised.

The next step was setting up a challenge study in Mali. Because of the differing host and environmental factors, a further dose-escalation study was done. Because of Covid, the Mali team had to run the study without on-site support. The colonisation rate was lower than in the UK, even using the highest dose of 10^7 CFU. There was also more spontaneous clearance, possibly due to the antibiotics given to participants during the study period for other reasons. Around 30% of colonised participants remained colonised at day 168 (end of study).

Ongoing and future work includes a study of the feasibility and acceptability of *N. lactamica* HIC studies in Mali, a study of the transmission within households, and the potential use of the challenge agent in an epidemic setting.

Changes in upper respiratory tract microbiomes following *Bordetella pertussis* challenge

David Cleary, Career Track Post-Doctoral Fellow in the Faculty of Medicine and the Southampton NIHR Biomedical Research Centre, shared that given the high incidence and mortality caused by *B. pertussis* worldwide, the PERtussis Correlates of Protection Europe (PERISCOPE) study was set up. The objectives were: to accelerate the development of improved vaccines

against *B. pertussis* or vaccination strategies that can be used to control *B. pertussis* in humans; to foster scientific innovation and rebuild the ecosystem and technical infrastructure needed in Europe to evaluate novel pertussis vaccine candidates; to improve understanding of the pathogenesis of *B. pertussis* infection and its potential impact on the recently observed changes in pertussis epidemiology. This has led to the development of a *B. pertussis* HIC model, existing samples from which have been used for a microbiome study, intending to investigate the following: does the existing microbiota determine resilience to colonisation? Does the upper respiratory tract microbiome alter in response to *B. pertussis* colonisation? And what impact does azithromycin have on the microbiome? Pre-, and post-inoculation samples were obtained by nasal wash and nasopharyngeal swabs (days 0, 4, 7, 9, 11, 14, 15, 16 + 28). When looking for *B. pertussis* in 16S rRNA sequencing data, *B. pertussis* is present in most participants, but high in only one participant. When looking at the microbiome and genus-level abundance, all individuals showed a distinct microbiome, however no clear impact of *B. pertussis* colonisation on the intra-individual microbiome diversity was observed.
Similarly, using beta diversity to look at community composition, there was no clustering, neither by patient nor by colonisation status. There is some clustering based on time points, with clustering of points after the use of azithromycin. Based on hierarchical clustering using Bray-Curtis Dissimilarity, eight groups with distinguishing dominant taxa could be defined (and one group without clear prominent organisms, the so-called bin-group). When looking at groups over time points, there were few shifts after colonisation, but changes were pronounced after azithromycin treatment.
Future studies should ensure bespoke sampling for microbiome analysis.

System-level analysis of the protective immunity to influenza in children

Adriana Tomic, Marie Curie Fellow in systems immunology, Oxford Vaccine Group, University of Oxford, noted that the influenza virus is highly successful because of its potential to infect multiple species, its high mutation rate (allowing escape from the immune system), and the potential for antigenic shift by which genetic materials are mixed, leading to new subtypes. These phenomena give the virus pandemic potential, as new strains that have not been seen by the human immune system before may evolve.
Data from the 2009 H1N1 pandemic demonstrated that influenza immunity lasts for a lifetime, because elderly people (those born in the 50's and before) were protected against H1N1 due to previous exposure. Therefore, in the 2009 pandemic, elderly people were not seen as a high-risk group, and vaccination was targeted at the young population.
Children play an important role in an influenza pandemic; they have close contact with other species, they infect 2.4 other children, as well as their

(grand)parents. By vaccinating 70% of the children, a pandemic can be avoided [39]. For example, based on surveillance data from England, vaccination with live attenuated influenza vaccine (LAIV) reduced hospitalization of children by 93% and influenza-like illness in adults by 59% [40]. Similarly, the mandatory vaccination of school children in Japan lowered the mortality in elderly people soon after introduction, while mortality went up again once the programme was stopped [41]. Inactivated influenza vaccines (given to older age groups) have a very good safety profile but low immunogenicity: antibodies wane over time, and no CD8+ T-cells are activated. This results in variable seasonal vaccine effectiveness, ranging between 20% to 60%. This was the reason behind the development of LAIV, which elicits both humoral and T-cell responses. However, because of this, it can only be given after the age of 2 (when the adaptive immune system is developed). Furthermore, no correlates of protection are known. Due to low LAIV vaccine efficacy in the 2015/2016 season, the CDC recommended not to use the vaccine. Simultaneously, in three independent studies, the efficacy of LAIV was moderate to high, based on which the UK and Finland recommended the use of LAIV for the 2016/2017 flu season.

A child will get its first influenza infection within the first three years of life, with many more exposures before the age of ten. During the remainder of life, the immune system will be boosted by vaccination or exposure to other influenza types.

As haemagglutination inhibition antibodies correlate with protection against influenza-like disease, these can be used to divide high and low responders. By comparing the immunological parameters of these two groups, an algorithm can be trained and a model built, which will predict if a person will or will not respond to vaccines.

A unified database, FluPRINT, was created with 740 individuals to enable large-scale studies exploring the cellular and molecular underpinnings of successful antibody responses to influenza vaccines. Over 3,000 parameters were considered, including serological responses to influenza strains, serum cytokines, cell phenotypes, and cytokine stimulations. This database facilitates the application of machine learning algorithms for data mining [42]. A novel approach, Sequential Iterative Modeling "OverNight" (SIMON), was developed, an automated machine learning system that compares results from many algorithms and is particularly suitable for datasets containing many missing values [43]. SIMON was applied to data from clinical studies of seasonal influenza vaccination. The results revealed previously unrecognized $CD4^+$ and $CD8^+$ T cell subsets strongly associated with a robust Ab response to influenza antigens [43].

To investigate how exposure to divergent influenza subtypes impacts influenza cellular immunity, a challenge study has been set up using LAIV as the challenge agent in children (4-6 years old). Using SIMON, integrative analysis will be performed to find the most relevant components.

The Covid pandemic has resulted in a natural experimental cohort, because a large number of children have not yet been exposed to

influenza due to lockdowns. Those samples would be extremely interesting for further research.

Parasite challenge models

The challenge of *Plasmodium vivax* CHMI

Jetsumon Sattabongkot Prachumsri, Head of the Mahidol Vivax Research Unit, Mahidol University, Bangkok, Thailand, explained that for controlled human malaria infection, three routes of infection are possible: mosquito bite, injection of sporozoites, or blood-stage injection (injection of blood from an infected person).

As *P. vivax* cannot be cultured *in vitro*, either infected mosquitoes need to be kept, or a blood bank of infected donors needs to be set up. However, to get blood from infected donors to create a blood bank, mosquito bites will have to be used as the initial route of inoculation. The preparation of the infected mosquitoes is delicate, as it involves many steps and needs to be aligned in time with the availability of volunteers to be bitten and infected. The transportation of mosquitoes between the Bangkok laboratory and the field stations as well as the transportation of patients' blood from different clinics to feed mosquitoes is challenging in normal times, but even more so during the covid 19 pandemic.

Volunteers for the study were healthy people between the ages of 20 and 55, weighing more than 50 kilograms, and with blood type O. Volunteers were screened for blood-borne or vector-borne diseases and previous malaria exposure. Of the 24 initial volunteers, only four remained eligible after intensive screening. The first two patients have been infected from different batches of mosquitoes. They both provided blood to set up the parasite banking. The sporozoite rate per mosquito differed substantially between volunteers, but the patient being bitten by the lower sporozoite rate mosquitoes had higher blood stage parasites per microliter at the time for donation. Moreover, both rings and trophs were detected, whereas the other volunteer only had rings in the blood.

For the banking of the infected blood, blood processing followed the GMP guidelines, with the use of a GMP grade cryoprotectant and extensive sterility testing. The next step will be to evaluate the blood inoculum as a challenge agent. Once that has been shown to work, the model can be used to perform a clinical trial to evaluate a *P. vivax* blood-stage vaccine.

Efficacy of *Plasmodium vivax* malaria blood-stage vaccine candidates

Mimi Hou, Clinical Research Fellow in Vaccine Development and Antibody Immunology, University of Oxford, shared that the landscape of malaria vaccines in development shows that the field is dominated by vaccines against *P. falciparum*, with only a few *P. vivax* vaccines in development [44]. Red blood cell invasion by the *P. vivax* merozoite depends on an interaction between the Duffy antigen and region II of the parasite's Duffy-binding protein. Replication-deficient chimpanzee adenovirus

serotype 63 (ChAd63) and modified vaccinia virus Ankara (MVA) viral vectored vaccines targeting PvDBP_RII have been developed. When delivered by the intramuscular route in a ChAd63-MVA heterologous prime-boost regimen using an 8-week interval, both vaccines were well tolerated and demonstrated a favourable safety profile in malaria-naive adults [45]. Similarly, in a phase 1 dose-finding study of a protein-based vaccine, a 50µg dose elicited antibodies against PvDBPII with the highest binding-inhibitory titres [46]. However, to avoid large-scale phase 3 efficacy trials, a *P. vivax* HIC model would be helpful. To this end, two healthy malaria-naive United Kingdom adults with universal donor blood group were infected by mosquito bite. Parasitaemia developed in both volunteers, and prior to treatment, each volunteer donated blood to produce a biobank of infected blood. Blood from one donor was used to inoculate six volunteers with different dilutions, all of whom developed pparasitaemia [47]. The dilution selected for final use was a 1:10 dilution. A pilot vaccine study was started in September 2019, vaccinating three volunteers with the ChAd63-MVA PvDBP vaccine before challenge. This was to be followed by a larger efficacy trial to look at both ChAd63-MVA PvDBP and PvDBPII/Matrix-M, but before completion of vaccination and challenge, the trial was halted because of Covid. When it was possible to restart, six volunteers were given a much delayed third PvDBPII/Matrix-M vaccine dose, followed by challenge 2-4 weeks later. Vaccination with PvDBPII/Matrix-M reduced parasite growth by 50%. This is reflected in anti-DFB IgG levels, which were higher after PvDBPII/Matrix-M compared to ChAd63-MVA PvDBP. The antibody levels correlated with the time to diagnosis ($r = 0.84$). This is the first demonstration of efficacy of any *P. vivax* vaccine. Future work will further explore the interval between vaccine doses.

Human infection challenge and the challenges of ethical approval

Rodrigo Correa-Oliveira, Professor of Immunology and Vice-President for Research and Biological Collections at Fundação Oswaldo Cruz, explained that in Brazil a hookworm vaccine is needed, as the current control tool (mass drug administration) is not a sustainable solution. People are treated and get reinfected. Children especially would benefit from a vaccine, as infection with hookworm can result in delayed physical and cognitive growth. Because of the challenges of hookworm vaccine phase 2/3 efficacy trials (sample size, cost and duration), a hookworm HIC model would accelerate vaccine development. The original hookworm HIC model was developed in Nottingham and transferred to George Washington University, and will now be transferred from GWU to Brazil. The first step in Brazil will be to establish a non-exposed donor cohort to be infected with *Necator americanus* from GWU. The hookworms isolated from these individuals will then be used in a dose-escalation study of volunteers previously exposed to hookworm. Once a safe dose with a reproducible attack rate has been defined, vaccine efficacy trials can be

performed, including the vaccines Na-GST-1 and NA-APR-1. These vaccines have been shown to be safe, but efficacy data are lacking. Obtaining ethical approval has been a major hurdle. The need for vaccines was questioned, as treatment is available. Moreover, challenge was seen as a deviation of the 'first, do no harm' principle.
As with any medical research study, informed consent was obtained after detailed information on research procedures, risks, and benefits was provided. The volunteers' willingness to participate and right to leave the study at any time was respected. Finally, through community engagement and participant support, conscious decision-making was encouraged. And this showed that in a population with low literacy, there was good understanding of the infection and the potential impact of a vaccine.
Human challenge research has to balance benefit for society with risk for the participants. Risks must be anticipated, communicated to participants, and plans must be available to avoid, minimize, or address those risks. The Ministry of Health supported the development of vaccines, including the use of HIC studies, to increase the quality of life of affected populations, and the Secretary of Health Research confirmed it is doing research of strategic interest for the Unified Health System. With this support, the Ethical Review Committee will hopefully be convinced to allow the HIC studies.
In the Brazilian system, it is not possible to remunerate volunteers, similar to phase 1 studies. Compensation of costs is possible, but remuneration is not allowed.

Establishing a single-sex *Schistosoma mansoni* controlled human infection model for Uganda

Moses Egesa, Scientist at the MRC/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit, shared that in Uganda, there is a high prevalence of *S. mansoni* infection, especially near freshwater bodies. Although there is a robust control programme, rapid reinfection occurs, and the development of drug resistance is a concern. No licensed vaccine is currently available. To accelerate vaccine development, an *S. mansoni* HIC model, based on the Leiden single sex cercariae HIC model [48] was being set up in Uganda. To establish the HIC model in Uganda, a roadmap was developed, of which most steps have been completed, including protocol approval. This also includes community engagement, which was done with a) a fishing community with high transmission and intense prior exposure and b) a Nkumba University community, with minimal prior exposure. Both communities had a clear knowledge of schistosomiasis and vaccines but had not heard of human challenge studies.
The local infrastructure and technical capacity were developed. A snail laboratory was set up for the production of the challenge agent. This laboratory has been inspected twice by the Institutional Biosafety Committee and will be inspected by the National Biosafety Committee. As

Biomphalaria glabrata (the non-human host in the parasite's life cycle) is not endemic in Uganda, a local snail was selected from Lake Victoria: *B. sudanica*. Finally, a research clinic was set up for patient care, with trained staff and laboratory support.
Working on the hypothesis that the dose of cercariae required to achieve infection is higher among healthy Ugandan adults than among *S. mansoni*-naïve Dutch volunteers, and higher in healthy Ugandan adults with intense prior exposure to *S. mansoni* than among those without, two groups of 33 volunteers with minimal or intense prior exposure will be challenged.
Primary endpoints are the frequency and magnitude of adverse events and the number of male cercariae needed for all the volunteers to show detectable *S. mansoni* circulating anodic antigen (CAA). Exploratory endpoints are the time to a positive serum CAA test and the humoral and cellular responses directed against *S. mansoni* antigens.
One challenge is the potential of natural infection of volunteers with *S. mansoni* during the study from contact with lake water. Volunteers and staff are sensitised to help avoid this, but it cannot be completely ruled out. Secondly, the intense prior exposure volunteers may need treatment before the start of the study to ensure that they do not have a current infection. Successful treatment will be checked, and challenge can be performed one week later. Finally, in intense prior exposure volunteers, the chance of natural immunity against *S. mansoni* is high. This will be checked in baseline samples.

Understanding immune responses in schistosomiasis with controlled human infection

Emma Houlder, Scientific Researcher at the Leiden University Medical Centre, the Netherlands, explained that in *S. mansoni* infection, the cercariae penetrate the skin and circulate in the bloodstream and the lungs between days 4 and 8 before moving to the liver to mature (days 9 to 35). The mature worms then pair and lay eggs in the mesentery from day 35 onwards. This approximate timing is based on baboon studies, as this data cannot be elucidated from humans.
Ex vivo skin infection has showed that cercariae induce a predominantly regulatory immune response (interleukin (IL)-10, IL-6 and macrophage inflammatory protein (MIP)-1α), whereas radiation-attenuated cercariae fail to achieve this [49]. In the egg-laying phase, the type-1 inflammatory response changes to a more regulatory/type-2 response, consistent with a chronic infection phenotype. However, little is known about the larval migration and maturation phase, which potentially affects the lungs. Morbidity is seen during these phases, including Katayama fever, which occurs 2-8 weeks post-infection in non-endemic individuals. Schistosomiasis has also been associated with other lung diseases, potentially because schistosomes migrate through the lung.
In sputum samples obtained 11-14 days after challenge, pro-inflammatory cytokines were increased, as well as the proportion of

dendritic cells. This was not observed in nasosorption samples nor in blood at these early stages. At a slightly later stage, maturing or adult worms seem to trigger an increase in serum pro-inflammatory cytokines, including interferon gamma induced protein (IP)-10 (also known as CXCL10) and MIP-1b, and an increase in antigen-specific T-cell cytokine secretion.
CyTOF performed on PBMCs obtained at weeks 0, 4, 8 post-infection identified lymphoid and myeloid cell clusters, with strong upregulation of CD45 and CD8a at week 4.
A case-control study was performed on sputum and blood samples from infected and non-infected individuals in Uganda to look at changes at the patent phase. In the infected individuals, an increase in dendritic cells in sputum was observed and an increase in serum IP-10. However, it remains unclear how the cellular response changes in infected individuals, which will be the focus of future work. For this, bronchoscopy might be an alternative for induced sputum, as the latter is generally not well appreciated by the participants, although bronchoscopy is difficult in the Covid situation.

Conclusion

Sir Andrew Pollard, Professor of Paediatric Infection and Immunity, Director of the Oxford Vaccine Group, University of Oxford, noted that one of the success stories of the use of challenge studies for the advancement of a vaccine is in the field of typhoid/paratyphoid. This started with the observation that in Nepal, in the summer period, 15% of the children visiting the outpatient clinic with fever had a *Salmonella* Typhi infection [50]. Moreover, antimicrobial resistance was increasingly observed with this organism, with global dissemination, e.g., the *S*. Typhi clade H58 [51]. This triggered the set-up of an *S*. Typhi HIC model, supported by WHO, which had produced guidance indicating that such a model could provide supporting evidence of the efficacy of a Vi conjugate vaccine. Despite ethics, regulation and manufacturing challenges, the HIC model was set up successfully and was used to show a vaccine efficacy of 55% for the Vi-TT vaccine [52]. This was sufficient proof to launch the Typhoid Vaccine Acceleration Consortium, which set up large phase 3 safety and efficacy trials in Nepal, Bangladesh, and Malawi, where more than 100,000 children were enrolled [53-55]. The efficacy in these countries was between 79-85%. This has now led to a number of countries in the highest-burden regions being interested in introducing the vaccine, ranging from the decision-making phase to introduction, e.g., in Pakistan, where, despite Covid, up to a million children were vaccinated per day in early February 2021.
This leads to the question of how challenge studies could have helped Covid vaccines develop even more quickly. At the early stages, it was widely claimed that vaccines would not be available for years to come, yet vaccines were authorized within one year. One year after the first

authorization, 7.6 billion doses have been administered, with more than 50% of the world population having received at least one dose. However, this figure is only 5% in low-income countries.
One important reason why vaccine development was so fast was that SARS-CoV-2 is a member of a well-known family of viruses, which may not be the case in the next pandemic. If the next pandemic virus is entirely unknown, challenge studies can be important to identify possible targets for the human immune response to select antigens for vaccines. At the same time, challenge studies are extremely difficult in the early stages, as so many things are unknown: can volunteers be safely challenged? With which dose? Are there treatment options? What could be the long-term effects (e.g., long Covid)? Furthermore, with SARS-CoV-2, mortality turned out to be relatively low (less than 1%), especially compared with previous outbreaks such as MERS (35%) and the plague (50%). A higher mortality may make challenge studies more difficult, but at the same time even more urgent to perform. To be appropriately prepared for the next pandemic, several things are needed: a government sleeping plan for funding the early steps of challenge trial development; early challenge strain development to GMP standards (even before the decision has been taken to embark on challenge studies so the agent is ready when the studies are approved); (sleeping) protocols for a range of pathogens held centrally for activation; and expertise in the field of challenge studies to take leadership.
HIC-Vac has played a clear role in driving innovation in the field of challenge studies, facilitating capacity building, and moving the field from a competitive to a collaborative field, by building a community. Although the future role of HIC-Vac needs to be contemplated, given the importance of HIC studies in improving health, some continuity is necessary.

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