IVI-WHO Consultation on Regulatory Considerations to Support Licensure of invasive Non-Typhoidal *Salmonella* (iNTS) Vaccines for use in children in Low- and Middle-Income Countries

4-5 February 2025
Trademark Hotel
Nairobi, Kenya

Meeting Report: Key Discussions, Deliberations, and Recommendations on Regulatory Considerations for the development of invasive non-typhoidal *Salmonella* (iNTS)-Containing Vaccines

Executive Summary

Dr. Jerome Kim and Dr. Jean-Louis Excler, IVI, welcomed attendees and provided opening remarks, context and a review of the meeting objectives and expected output. Non-typhoidal serovars of *Salmonella enterica* (NTS) were estimated to cause over 500 000 cases of invasive disease leading to more than 79 000 deaths in 2019. iNTS disease occurs mostly in infants and pre-school children in low- and middle-income countries (LMICs) in sub–Saharan Africa (sSA). A total of 45 participants attended the meeting including 23 participants from 14 sub-Saharan African countries.

John Crump, University of Otago, New Zealand, highlighted the substantial burden of invasive nontyphoidal *Salmonella* (iNTS) disease in children under five years of age, where annual deaths are estimated to exceed those of paratyphoid and typhoid fevers combined by more than four times. Malaria may account as a host risk factor for up to 50% of cases of iNTS disease. Vaccines targeting *Salmonella* Typhimurium and *Salmonella* Enteritidis belonging to serogroups O:4 (B) and O:9 (D), respectively, are projected to provide coverage for 94% of iNTS disease based on available NTS serotype data. Discussion also focused on the need for more granular analysis of surveillance data by age, particularly in infants less than 6 months of age to effectively define the epidemiology and burden of iNTS disease.

Annalies Wilder-Smith, World Health Organization, Geneva, Switzerland, summarized the key points and outcomes from the WHO and IVI consultations on *Salmonella* combination vaccines, held in Kigali (December 2023) and Geneva (December 2024), discussing he applicability and limitations of controlled human infection model (CHIM) studies for iNTS vaccines, standardization of immunological assays, and different combination strategies, highlighted preferences for iNTS-TCV and iNTS-*Shigella* combinations.

Robert Kaminski, World Health Organization, Geneva, Switzerland, reviewed the Preferred Product Characteristics (PPC) for iNTS vaccines, and the establishment of a R&D technology roadmap that identifies knowledge gaps and prioritizes appropriate research activities. The iNTS vaccine PPC and R&D Roadmap have been endorsed by the Product Development Vaccine Advisory Committee.

Maheshi Ramasamy, University of Oxford, United Kingdom, presented preliminary results of the Phase 1 safety and immunogenicity of a bivalent GMMA (Generalized Modules for Membrane Antigens)-based iNTS vaccine, iNTS-GMMA, in healthy adults (18-55) in the UK (SALVO). This study was performed as part of the Vacc-iNTS Consortium.

Ashwani Kumar Arora, GSK Vaccine Institute for Global Health (GVGH), Siena, Italy, presented the design and preliminary results of the ongoing Phase 1/2a of the trivalent GMMA vaccine against iNTS disease and Typhoid Fever, iNTS-TCV, in healthy European and African adults. The study is being conducted in Belgium and Malawi. The next step planned is a Phase 2a study evaluating non-inferiority of TCV when combined as an iNTS-TCV vaccine in the target age population in an endemic country.

Wilbur Chen and Myron (Mike) Levine, Center for Vaccine Development and Global Health, University of Maryland, Baltimore, USA (CVD, UMB), presented preliminary safety and immunogenicity results of Phase 1 and Phase 1/2a trials in healthy U.S. adults and of an ongoing Phase 2 age de-escalation trial of the Trivalent *Salmonella* (*S*. Typhi/*S*. Enteritidis/*S*. Typhimurium) Conjugate Vaccine (TSCV) in sub-Saharan Africa. The study ongoing in sub-Saharan Africa is evaluating two-dose regimens of half-strength TSCV, at 12–18 weeks of age and a booster at either 9 months, 12 months, or 15–17 months of age.

Marco Cavaleri, European Medicines Agency (EMA), Amsterdam, The Netherlands, presented key requirements for vaccine approval regarding efficacy studies and emphasized the necessity for assessing absolute protective efficacy through randomized controlled trials, where the incidence of the targeted infectious disease in vaccinated individuals is compared to a placebo group. For the primary analysis, case definitions should include clinical signs or symptoms indicative of the disease, paired with laboratory confirmation. The role of CHIM in early vaccine development was discussed. The duration of protection and the need for booster doses should be investigated after vaccine approval. For iNTS vaccine development, a pivotal efficacy study with clinical endpoints is needed for now.

iNTS Phase IIb and Phase III Efficacy Study Design Considerations

Myron Levine and Wilbur Chen, CVD, UMB, and participants discussed primary and secondary endpoint considerations for an iNTS Phase IIb and III efficacy study. Discussions particularly focused on the following points: discrimination of serogroup or serovar/serotype for the primary endpoint, case definition and case ascertainment strategy, criteria for inclusion and exclusion. A recommendation was made to consider subgroup analyses to see vaccine efficacy according to different risk-factor profiles, while an immunogenicity sub-study would be needed to allow inclusion. Potential secondary endpoints included: immunogenicity measures, bacterial shedding, severity of infection and diarrheal disease.

Melita Gordon, University of Liverpool, United Kingdom, presented a broad panel of potential exploratory endpoints for a Phase IIb/III trial, suggesting three main areas of potential interest, i) the importance of exploratory functional immunogenicity measures, particularly to understand how risk factors and susceptibility in sub-groups might affect vaccine efficacy, ii) the importance of the influence of vaccination on antimicrobial use and antimicrobial resistance, iii) the

importance of asymptomatic carriage in human transmission, and the possibility that vaccination might reduce disease transmission. Regulators highlighted the need to discuss exploratory endpoints and which will be most compelling for licensure.

iNTS Phase 2b and 3 Efficacy Study Design Considerations – Participants were divided into six groups to discuss primary endpoints, diagnostic considerations and enrolment criteria, and other various topics including lot-to-lot consistency testing, validated assays, lower bound of efficacy, non-inferiority margins, and safety considerations.

Roundtable Discussion with Regulators, Co-chaired by Marco Cavaleri (EMA) and Kwasi Nyarko, African Vaccine Regulatory Forum (AVAREF). Regulators and other participating stakeholders expressed appreciation for being engaged and involved in the discussion and in trial planning and agreed that early engagement with regulators and National Immunization Technical Advisory Groups (NITAG) is crucial for vaccine development. The role and activities of AVAREF were explained as well as its technical support to the Africa Medicines Agency (AMA), further echoed by **Sam Kariuki (KEMRI).**

1. Opening Remarks and Overview – Jerome Kim and Jean-Louis Excler, IVI, Seoul, Republic of Korea

Dr. Jerome Kim and Dr. Jean-Louis Excler welcomed attendees and provided opening remarks, context and a review of the meeting objectives and expected output. It was reminded that non-typhoidal serovars of *Salmonella enterica* (NTS) were estimated to cause over 500 000 cases of invasive disease leading to more than 79 000 deaths in 2019. While iNTS disease is reported globally, most infections occur in infants and pre-school children in low- and middle-income countries (LMICs) in sub–Saharan Africa (sSA), where host risk factors for iNTS disease such as recent or current malaria, acute malnutrition, HIV infection, and moderate to severe anaemia are common. Across all ages and settings there is a high case fatality ratio (CFR) of approximately 14.5%.

This meeting is a follow-up of two previous meetings in Kigali (December 2023) and Geneva (December 2024) that focused on regulatory requirements, clinical efficacy, and immunogenicity data needed for iNTS vaccine development and emphasized the absolute and critical need to engage regulators, developers and manufacturers very early in the vaccine development process.

A total of 45 participants attended the meeting (See list in **Appendix**) including 23 participants from 14 sub-Saharan African countries: Republic of the Congo, Burkina Faso, Mozambique, Democratic Republic of Congo, Mali, Ghana. Kenya, Malawi, Gambia, Nigeria, Uganda, South Africa, Tanzania, and Zimbabwe.

The agenda of meeting is provided in **Appendix**.

2. iNTS Epidemiology and Burden of Disease – John Crump, University of Otago, New Zealand

Dr. Crump's presentation highlighted the substantial burden of invasive nontyphoidal *Salmonella* (iNTS) disease in children under five years of age, where annual deaths are estimated to exceed those of paratyphoid and typhoid fevers combined by more than four times. Malaria may account as a host risk factor for up to 50% of the cases of for iNTS disease, although the data remains insufficient; and there is likely a large residuum of iNTS disease not attributable to currently recognized host factors. The presentation emphasized the importance of plotting age-occurrence data from long-term surveillance studies by month of age, as existing data from incidence studies and vaccine trial control arms typically present information in broad age groups with wide uncertainty, that may obscure critical insights needed for vaccine development and policy making. Additionally, the presentation addressed host risk factors, reservoirs, data gaps regarding sources and modes of transmission, antimicrobial resistance, and prevalence of NTS serogroups and serovars isolated from blood, cerebrospinal fuid, synovial flui, and other normally sterile sites worldwide. It was noted that modeled data indicate the peak incidence of iNTS disease occurs earlier in life compared to typhoid fever, with both incidence curves shifting leftward relative to what might be expected based on current primary epidemiologic data.

Vaccines targeting *Salmonella* Typhimurium and *Salmonella* Enteritidis belonging to serogroups O:4 (B) and O:9 (D), respectively, are projected to provide coverage for 94% of iNTS disease based on available NTS serotype data. Caveats to these data include inadequate description of serogrouping and serotyping methods in many reports.

Meeting participants discussed strategies for investment in Africa, debating whether the primary focus should be on preventing transmission through methods like Water, Sanitation, and Hygiene (WASH) or on vaccine development and implementation. Dr. Crump emphasized the importance of both approaches, with WASH as a long-term solution and vaccines potentially providing shorter-term intervention. He also noted that although reservoirs and modes of transmission for *Salmonella* Typhi are well understood, a better understanding of NTS transmission and non-vaccine preventive measures is needed. The impact of malaria vaccine introduction was further discussed, including ongoing secondary analyses of RTS,S/AS01 malaria vaccine trial data. Concerns were raised about current diagnostics and about antimicrobial resistance (AMR). Currently, the standard of care for diagnosis are blood culture and bone marrow culture, both lack sensitivity, and neither are universally available. Reported antimicrobial resistance (AMR) data estimate nearly 70% of isolates in Kenya are resistant to first-line treatments, indicating a need for new diagnostics and/or improved access to culture and susceptibility testing. AMR data presented show an apparent reduction in AMR after 2010; however, this likely stems from gaps or inconsistencies in data collection. Overall, the discussion underscored the necessity for improved diagnostics and access to current culture capabilities, improved reporting of iNTS cases (including antimicrobial susceptibility profiles/AMR and robust serogrouping and serotyping strains). Discussion also focused on the need for more granular analysis of surveillance data by age, particularly in infants less than 6 months of age to effectively define the epidemiology and burden of iNTS disease.

3. Summary of outcomes of the Kigali (December 2023) and Geneva (December 2024) meetings - Annalies Wilder-Smith, World Health Organization, Geneva, Switzerland

Dr. Wilder-Smith summarized the key points and outcomes from the WHO and IVI consultations on *Salmonella* combination vaccines, held in Kigali (December 2023) and Geneva (December 2024). These meetings focused on regulatory requirements, clinical efficacy, and immunogenicity data needed for invasive iNTS vaccine development. Key discussions revolved around the feasibility of using Controlled Human Infection Model (CHIM) studies, the standardization of immunological assays, and the potential benefits and challenges of combining iNTS vaccines with Typhoid Conjugate Vaccines (TCV). The meetings aimed to address scientific, regulatory, and policy challenges to accelerate vaccine development.

A major concern identified was the applicability of CHIM studies for iNTS vaccines, given that iNTS primarily affects children with HIV, malaria, and malnutrition, whereas CHIM studies typically recruit healthy adults. Despite potential limitations, CHIM data could help support clinical efficacy findings, but large-scale clinical trials in endemic regions remain necessary. Standardization of immunological assays emerged as a priority, with efforts underway to establish an international reference serum through collaboration with regulatory agencies like the Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom.

The meetings also evaluated the feasibility of an iNTS-TCV combination vaccine, given the already crowded Expanded Program on Immunization (EPI) schedule. While a combination approach may enhance vaccine uptake, potential mismatches in age recommendations and dosing schedules need further consideration. Regulatory success for such a combination appears promising, as TCV components already have an established approval pathway, whereas iNTS components still require efficacy data for licensure.

A scorecard exercise comparing different combination strategies, including iNTS + TCV, iNTS + *Shigella*, and iNTS + injectable rotavirus, highlighted preferences for iNTS-TCV and iNTS-*Shigella* combinations. Future steps involve continued regulatory engagement in endemic countries, further clinical evaluations, and addressing technical and policy challenges. The meetings underscored the importance of aligning vaccine development with disease burden, target populations, and implementation feasibility to ensure successful deployment.

Questions and discussion following Dr. Wilder-Smith's presentation focused on combination vaccines. Two potential approaches were discussed: developing two vaccines separately before combining them or evaluating them concurrently with co-primary endpoints. Participants discussed merits and concerns of TCV versus *Shigella* for a combination vaccine with iNTS. Concerns were raised that differing endpoints would be required for iNTS and Shigella and that a combination with *Shigella* would be higher risk given that *Shigella* vaccine candidates are still in development (no Phase 3 data available yet). The conversation also explored the philosophical and practical implications of combining vaccines, with debates on the merits of combining TCV with *Shigella* or iNTS with PCV or meningococcal vaccines, particularly in African contexts. It was noted that different regions may have varying market needs, requiring manufacturers to produce both conjugate and non-conjugate vaccine options. Overall, while the concept of a combined iNTS/TCV vaccine demonstrates potential, its success was noted to hinge on understanding specific regional market dynamics and the timing of introduction.

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4. Review of the WHO preferred product characteristics (PPC) for iNTS vaccines - Robert Kaminski, World Health Organization, Geneva, Switzerland

The World Health Organization (WHO) has developed Preferred Product Characteristics (PPC) for iNTS vaccines, which aims to guide vaccine development from early research to regulatory approval and implementation. This initiative is part of a broader strategy to accelerate the availability of vaccines addressing diseases prevalent in low- and middle-income countries (LMICs). The PPC outlines essential parameters, including vaccine indications, target populations, and safety and efficacy considerations, to inform investment and policy decisions.

To facilitate vaccine development, WHO has also established an R&D roadmap that identifies knowledge gaps and prioritizes research activities. This roadmap produced by WHO are developed in collaboration with global stakeholders and aim to highlight priority activities to accelerate the pathway to licensure, availability and access in low- and middle-income countries. The target audience is vaccine researchers, funders and product developers. The present roadmap outlines strategic goals for iNTS vaccine development by addressing evidence gaps, accelerating vaccine development, and maximizing health impact. These efforts ensure

that vaccine candidates undergo rigorous scientific evaluation and align with global public health needs.

The timeline for the iNTS vaccine PPC development spans multiple years, with key consultations and revisions taking place from 2021 to 2024. The process involves collaboration between WHO, researchers, and policymakers to refine the vaccine attributes and address implementation challenges. The goal is to produce safe, effective, and accessible vaccines that reduce the burden of iNTS infections, particularly in vulnerable populations such as children in LMICs. iNTS vaccine PPC and R&D Roadmap have been endorsed by the PDVAC and will be soon posted on the WHO website.

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5. (Virtual) Safety and Immunogenicity of bivalent GMMA-based vaccines against iNTS Phase 1 in the UK – Maheshi Ramasamy, University of Oxford, United Kingdom

Dr. Ramasamy from the University of Oxford presented preliminary results of the Phase 1 safety and immunogenicity of a bivalent GMMA (Generalized Modules for Membrane Antigens)-based iNTS vaccine, iNTS-GMMA, in healthy adults (18-55) in the UK (SALVO). All participants received 3 doses of vaccine at 0, 2, 6 months; participants were followed for 1 year.

The vaccine was safe and generally well tolerated. Humoral response against *S.* Typhimurium and *S.* Enteritidis O-antigens peaked at day 28 following full dose, and antibodies persisted for one-year post-vaccination for both antigens. Additionally, functional antibody activity, as measured by the serum bactericidal assay (SBA), increased following first vaccination for both antigens. It was emphasized that antibody functionality, rather than titre alone, should be further examined to understand the findings more fully. This study was performed as part of the Vacc-iNTS Consortium.

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6. GVGH iNTS-TCV vaccine clinical development plans and available data – Ashwani Kumar Arora, GSK Vaccine Institute for Global Health (GVGH), Siena, Italy

Dr. Ashwani Kumar Arora from GVGH presented the design and preliminary results of the ongoing Phase 1/2a, observer-blind, randomized, controlled, two-stage, multi-country study evaluating the safety, reactogenicity, and immune response of the trivalent GMMA vaccine against iNTS disease and Typhoid Fever, iNTS-TCV, in healthy European and African adults. The study is being conducted in Belgium (Stage 1) and Malawi (Stage 2). Low dose (in Belgium only) and Full dose are being tested with a 0,2,6-month vaccination schedule. Overall, the anticipated benefit-risk profile continues to be favorable with no safety concerns precluding further development. To assess potential interference between the TCV and the iNTS-GMMA components in the iNTS-TCV combination vaccine, one group of participants was administered with the TCV and the iNTS-GMMA vaccines in separate arms. Immunogenicity data will be presented as soon as available. The next step planned is a Phase 2a study evaluating non-inferiority of TCV when combined as an iNTS-TCV vaccine in the target age population in an endemic country.

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7. CVD/Bharat Biotech International Ltd (BBIL, Hyderabad, India) TSCV current clinical development plans and available data – Wilbur Chen and Myron (Mike) Levine, Center for Vaccine Development and Global Health, University of Maryland, Baltimore, USA (CVD, UMB)

Wilbur Chen and Myron Levine from CVD presented preliminary safety and immunogenicity results of Phase 1 and Phase 1/2a trials in healthy U.S. adults and of an ongoing Phase 2 age de-escalation trial of the Trivalent *Salmonella* (*S*. Typhi/*S*. Enteritidis/*S*. Typhimurium) Conjugate Vaccine (TSCV) in sub-Saharan Africa. The TSCV includes Typbar TCV Vi conjugate (Typhi Vi polysaccharide linked to tetanus toxoid), *S*. Enteritidis conjugate (Enteritidis core and O polysaccharide [COPS] linked to Enteritidis FliC Phase 1 flagellin subunits), and *S*. Typhimurium COPS conjugated to Typhimurium FliC. The results of the studies in adult volunteers provided sufficient positive safety and immunogenicity data to proceed with further clinical development. The Phase 2 age de-escalation study, conducted at field sites in Mali, Kenya, and Mozambique is aiming to determine optimal dosing, safety, and immunogenicity of the TSCV. In the initial Steps of the study in Africa, by random allocation subjects received a single dose of either: Full-strength TSCV formulation (25 mcg of each of the three conjugates); half-strength formulation (12.5 mcg each of Enteritidis and Typhimurium conjugates and 25 mcg of Vi conjugate); Typbar TCV alone; or placebo. In descending age groups, starting with 40 adults, safety was documented stepwise in 40 children age 5-9 years, 40 children age 24-59 months, 80 toddlers age 16-23 months, 120 toddlers age 12-16 months, 120 infants age 8-11 months, and 360 young infants 12-18 weeks of age. Serum IgG antibody responses to the COPS antigens were considered along with safety data and manufacturing/supply considerations in selecting the half-strength TSCV formulation as the product to be advanced into a comparison of two-dose regimens.

In the initial age-descending single-dose part of the Phase 2 trial, serological results exhibited a hierarchy of responses, with anti-Vi being more robust than anti-Enteritidis COPS, and anti-Typhimurium COPS responses being least robust. There was also a pattern for all three anti-antipolysaccharide antibody responses to be lower in the youngest infants 12-14 weeks and 16-18 weeks of age. This was most evident for *S*. Typhimurium where these two youngest age groups did not manifest a $\geq$ 4-fold rise in GMT between baseline and day 29 post-vaccination. In contrast, infants immunized at 8-11 months of age exhibited a > 4-fold rise in GMT of Day 29 over Day 1. It was shown statistically that baseline IgG anti-Typhimurium antibody titers (acquired transplacetally) interfered with seroconversion in young infants. Thus, it is not known if those young infants who did not exhibit rises in anti-Typhimurium COPS following the priming dose of TSCV would nevertheless be protected in the face of encounter with virulent iNTS by mounting an accelerated anamnestic protective anti-Typhimurium antibody response. However, it should also be noted that some of the most protective polysaccharide-protein conjugate vaccines used on a global scale in the Expanded Programme on Immunization, such as *Haemophilus influenzae* type b capsular polysaccharide PRP (polyribosylribitol phosphate) linked to tetanus toxoid, do not exhibit a significant rise in anti-PRP antibodies following the first dose administered to young infants. At the time of the Nairobi meeting there were no immunologic data yet available to know whether the initial dose of TSCV primes the infant immune system to respond vigorously following receipt of a second dose administered several months subsequently. That information should become available in the second semester of 2025 when serological data from the two-dose

regimens of TSCV have been analyzed (vide infra). Moreover, there are as yet no B memory cell responses recorded in very young infant recipients of TSCV.

In the study ongoing in sub-Saharan Africa that is evaluating two-dose regimens of half-strength TSCV, 456 infants were randomly allocated to receive an initial injection of either half-strength TSCV (N=228) or of placebo (N=228) at 12–18 weeks of age. The ~228 recipients primed with half-strength TSCV were thereupon further randomly allocated to receive a single additional injection of Half-strength TSCV or of placebo administered at either 9 months, 12 months, or 15–17 months of age. The ~228 infants who received placebo as their initial injection were also further randomly allocated to receive a single subsequent injection with Typbar TCV at either 9 months, 12 months, or 15–17 months of age.

n a non-inferiority immunogenicity analysis and stakeholder evaluation of manufacturing and implementation considerations, the half strength dose formulation was selected for further clinical development. CVD is planning for a Phase 3 efficacy trial (target 70% efficacy with lower bound of 30%).

Questions and discussion centered on the challenges and considerations in developing vaccines for the youngest infants, particularly noting lower anti-COPS immune responses in that age group and the hypothesis that maternal antibodies and immunologic immaturity may play a role. Concerns were raised about the significant gap in anti-COPS between the initial dose administered at 12-18 weeks of age and the booster dose given at 9 months of age (or later), potentially impacting immune response and leaving infants unprotected during peak-risk age periods. On the other hand, significant rises in anti-FliC (the carrier protein for the COPS conjugate were observed in these youngest infants (12-18 weeks of age). In animal models, passively administered anti-FliC antibodies protect infant animals against fatal iNTS challenge.

A question posed to regulators was whether an anti-O antigen correlate of protection determined for conjugate vaccines would be acceptable for GMMA technology formulation vaccines. Definitions for determining non-inferiority were also discussed, and it was emphasized that investigators can define in early clinical development, but regulators will be more stringent and will evaluate non-inferiority definitions in late-stage development. Participants expressed concern over the low level of immune response in young children, proposing that this could be attributable to vaccine interactions, maternal antibody, nutrition. The possibility that natural exposure could potentially bolster (vaccine-induced and natural) immunity. Overall, the discussion underscored the need for careful consideration of immune response metrics and close communication to determine regulatory requirements and expectations throughout vaccine development.

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8. Regulatory considerations in iNTS combination vaccines – Marco Cavaleri, European Medicines Agency (EMA), Amsterdam, The Netherlands

Dr. Cavaleri presented key requirements for vaccine approval regarding efficacy studies as outlined in WHO TRS 1004, Annex 9. He emphasized the necessity for assessing absolute protective efficacy through randomized controlled trials, where the incidence of the targeted infectious disease in vaccinated individuals is compared to a placebo group. In some cases, the

control group may receive a licensed vaccine that addresses some, but not all, pathogen types, allowing this group to serve as an unvaccinated control for the specific types targeted by the candidate vaccine. For the primary analysis, case definitions should include clinical signs or symptoms indicative of the disease, paired with laboratory confirmation. If the candidate vaccine targets multiple subtypes of a pathogen, it is acceptable for primary endpoints to focus on disease cases linked to any of these subtypes. Additionally, if the analysis is limited to specific organism types, supplementary assessments should evaluate efficacy across all cases. However, it is generally impractical to design trials capable of assessing efficacy for each individual subtype.

The role of Controlled Human Infection Models (CHIM) in early vaccine development was highlighted, noting the importance of CHIM for proof-of-concept and assessing potential immune correlates of protection, despite limitations concerning dose relevance and target populations. Dr. Cavaleri provided examples of potential endpoints for Salmonellosis in CHIM: isolation of *Salmonella* Typhimurium from stool at determined times, evaluation of severe/moderate diarrhea with fever and other symptoms such as abdominal pain, nausea, vomiting, etc., *Salmonella* Typhimurium bloodstream infections, and others. He also highlighted the importance of CHIM in the EMA approval of the Vaxchora vaccine, a vaccine to prevent cholera disease in adults and children aged 2 years and older. The EMA approved this vaccine based on data from a CHIM efficacy study in healthy adult volunteers (divided into vaccinated and unvaccinated subgroups) who were challenged with live *Vibrio cholerae* at defined timepoints; the study demonstrated the vaccine's efficacy under the tested conditions. Dr. Cavaleri mentioned *Shigella* vaccine development and regulatory strategies as another example which would require different regulatory strategies than what was seen with cholera vaccine, particularly as a vaccine for children under 5 years of age in LMICs. CHIM data could aid in regulatory approval for adult populations, but alternative approaches are required for children. The WHO Regulatory Science Workshop on the *Shigella* vaccine was referenced as a key forum for discussing safety profiles and clinical endpoints to support policy-making decisions. For combination vaccines, distinct regulatory strategies may apply, especially if one of the components is a licensed product. For example, Typhoid and Paratyphoid combination vaccine could be evaluated using CHIM. Non-inferiority of the Typhoid component could be demonstrated, as there are already licensed vaccines for Typhoid. Protective efficacy of the Paratyphi A component could be evaluated in CHIM to help establish a correlate of protection that could be used to infer protection and could be applied in immunogenicity clinical trials. Safety should be evaluated in at least 3,000 participants.

In conclusion, the regulatory approach and development of iNTS vaccines must consider the specific combinations being proposed, intended indications, and the target population. Early clinical trials must justify the selection of doses within combinations. If an already approved component, such as TCV, is not licensed for the target age group, bridging immunogenicity data from older children should be provided. CHIMs can play a significant role in the early stages of development, particularly for identifying correlates of protection. The feasibility and study design for clinical efficacy trials in the target population, specifically children under 3 years of age in LMICs, should be discussed with regulators. The duration of protection and the need for booster doses should be investigated after vaccine approval.

The question was raised where licensure could be achieved based on correlates of protection/seroconversion followed by a real-world effectiveness study? Dr. Cavaleri responded that this can be explored and discussed, but regulators need a starting point (must have clearly established correlates of protection). For iNTS vaccine development, a pivotal efficacy study with clinical endpoints is needed for now.

9. iNTS Phase IIb and Phase III Efficacy Study Design Considerations

The next sessions of the meeting were designed to facilitate discussion and solicit input from regulators and stakeholders participating in the meeting to inform study design and the regulatory pathway for vaccine licensure in high-burden countries. The first session was conducted as full group discussion, facilitated by subject matter experts, to solicit input on primary, secondary, and exploratory endpoints. Following the large group discussion, participants divided into small groups of 5-8 people for in-depth discussions about clinical trial design and endpoints and the PDVAC questions.

9.1 Primary and Secondary Endpoints – Myron Levine and Wilbur Chen, CVD, UMB

Stakeholders discussed primary and secondary endpoint considerations for an iNTS Phase IIb and III Efficacy Study, with the discussion led and facilitated by Prof. Levine and Dr. Chen from CVD. Discussions particularly focused on the following points: discrimination of serogroup (B or D) or serovar/serotype for the primary endpoint, case definition and case ascertainment strategy, criteria for inclusion/exclusion of children presenting at healthcare facilities with acute disease, co-morbidities and risks factors.

The discussion highlighted that the primary microbiologic endpoint should focus on disease caused by serogroup B and D organisms, identified by culture (blood or from sterile site). Disease caused by B and D serogroups could be combined as an endpoint, and disease caused by *Salmonella enterica* serogroups other than B or D should not be considered as primary endpoint. Cross-protection is expected by experts, which is why some suggest to instead discriminate by serotype/serovar (*S.* Typhimurium and *S*. Enteritidis) for the primary endpoint; however, this cross-protection is hypothesized and there is not yet evidence supporting this. Cross-protection against other serogroups was suggested to be included as secondary or tertiary endpoints. Regulators emphasized that it is of utmost importance to measure what is specifically covered by the vaccine as well as additional protection provided that is not included in/covered by the vaccine. Therefore, serotyping/sequencing need to be done, but not as a primary endpoint.

Regarding case definition, experts suggested a stringent clinical definition (not merely fever) and suggested clinical syndromes such as meningitis, septic arthritis, and sepsis triggering blood culture (and possibly PCR). The group discussed the need to use international sepsis (or systemic inflammatory response syndrome (SIRS) age-specific case definition guidelines as the protocol-defined triggers for blood culture should differ by age category. A reminder to consider biological differences between children population's age group (notably infants vs toddlers) was made, notably when defining sepsis. It was suggested that international pediatric working groups have

specific recommendations for case definition and clinical management of sepsis which can be utilized to inform study design; laboratory parameters (WBC, inflammatory markers, biochemistry) should be used as well. Dr. Wilbur reminded that the vaccine will not be tested in neonates (first 28 days of life) and likely (based on the PPC and the ongoing discussion) will be tested in infants aged 6 months and above; however, the 6-month lower age range is still yet to be agreed upon as iNTS is also prevalent in younger children and developers have room to decide. One expert mentioned that the definition of sepsis is "too" reliant on blood culture results which might not be obtained before antibiotic administration in children, therefore very ill children might be blood culture negative and then would be missed as cases. Concern was also raised about making case ascertainment (criteria triggering blood culture collection) too stringent and proposed criteria for close follow-up and testing early so as not to miss cases. The importance of close participant follow-up and engagement to ensure (as much as possible) that participants seek evaluation at the study site/facility was discussed. The potential role for PCR and case adjudication was discussed. One participant queried whether only the first case of iNTS in a participant would be an endpoint (in case a child has multiple infections during trial participation).

The regulatory perspective that a systematic ascertainment strategy (for active case finding) beyond a mere case definition is especially important in the context of a clinical trial was raised. This needs to be clearly defined in the protocol to avoid missing cases (misdiagnosis or inadequate sampling/diagnostics) of children seeking care outside of the study team. A joint consultation involving clinicians from across Africa to discuss case ascertainment strategies was raised as an important step in designing the study. Ultimately, clinical case definition AND culture confirmation (from blood or from another sterile site (ie. CSF, joint) needs to be clearly defined and there appeared to be consensus that the trial should designed to detect efficacy in preventing invasive disease, not diarrhea.

There was extensive discussion surrounding risk factors for NTS disease and inclusion/exclusion criteria considerations to be sure at-risk infants and children are represented in the trial and that endpoints will be captured while also ensuring participant safety in the context of a pivotal preventive vaccine clinical in resource limited settings. The group discussed extensively whether infants and children known to be at increased risk of invasive salmonellosis (ie. malnutrition, history of recent malaria, sickle cell, and immune deficiency like HIV infection) should be eligible for and included in the study. The conversation was quite controversial at first balancing between excluding any child with co-morbidities and later evaluating the real-life effectiveness of the vaccine on one side and including a population that is representative of the population intended for use on the other side. Advocates for including children with co-morbidities clarified that they do not advocate recruitment/eligibility screening in acute care clinics or hospitals, rather they are referring to children living in the community who have co-morbidities. The discussion went forward towards a consensus on the need to define precise eligibility criteria to conduct a safe and ethical trial and to enrol a study population representative of the intended target population. Some examples discussed included infant exposed to HIV, infant living with HIV on ARVs with CD4 above a defined value/percentage, asymptomatic child with malaria RDT positive AND with history of treated malaria infection within the past 4 weeks or with a negative blood smear, infant with mild malnutrition with acceptable mid upper arm circumfence (MUAC) ranges defined in the

protocol. It was proposed that children who do not need immediate acute medical care should be considered for inclusion and that children who need acute care could be treated and rescreened. Concern about including children with immune deficiency was raised as response to vaccine could be impaired; the need to enroll only children with controlled disease was emphasized. Additionally, a recommendation was made to consider subgroup analyses to see vaccine efficacy according to different risk-factor profiles, perhaps within an immunogenicity sub-study. The option to identify cases post-mortem with minimally invasive autopsy at one trial site was also suggested; participants thought that this would be quite difficult in most sites but perhaps could be done by sites who had participated in the CHAMPS study (already have capacity and community engagement).

Endpoints discussed to include as potential secondary endpoints included: immunogenicity measures, specifically ELISA anti-O Ag IgG (working correlate of protection); stool culture (assess shedding and efficacy to prevent transmission); functional assays (SBA); severity of infection (deaths, hospitalizations, complications, duration of illness); diarrheal disease.

9.2 Exploratory Endpoints - Melita Gordon, University of Liverpool, United Kingdom

Prof. Gordon presented potential exploratory endpoints for a Phase IIb/III trial, emphasizing the importance of understanding how risk factors and susceptibility in sub-groups affect vaccine efficacy and influence antimicrobial use and antimicrobial resistance. She discussed exploratory functional immunological endpoints (humoral and cellular) that might be confirmatory correlates of protection, alongside a primary binding-assay measure of immunogenicity, and distinguished between mechanistic correlates, which are statistically correlated and causative of protection, and non-mechanistic correlates, which, while convenient to measure, only associate with protection but are not necessarily causative. She presented a systematic review identifying malaria and anemia as major risk factors in children. One benefit of exploratory immunological endpoints is to provide reassurance that functional vaccine responses are generated in these children who are most at-risk, highlighting the importance of considering the inclusion of children with risk factors such as recent malaria in efficacy trials There was considerable discussion about the possible benefits and logistics of this approach. Dr. Gordon highlighted key protection markers (IgG, CD8 T-cell, B-memory cell) against iNTS, noting that peak incidence occurs between 6 and 24 months, followed by a sharp decline in incidence.

Additionally, Prof. Gordon shared findings from the double-blind randomized phase 3 TyVAC clinical trials of TCV, on the impact on microbial use. This revealed a correlation between TCV vaccination and decreased prescription rates for antimicrobials among randomized children. Specific reductions in WHO Watch antimicrobials, including fluoroquinolones and ceftriaxone, were noted in Nepal, Bangladesh, and Malawi, highlighting the value of further exploratory evidence on vaccines as tools for combating antimicrobial resistance (AMR). She also presented a separate study demonstrating that increased fluoroquinolone prescription rate in Malawi led to the new emergence of fluoroquinolone resistance genes among *Salmonella* Typhi. Gathering exploratory information on antimicrobial usage and on antimicrobial resistance among NTS isolates in the context of a randomized efficacy trial could be similarly valuable.

She finally pointed out the significance of asymptomatic enteric nontyphoidal *Salmonella* (eNTS) as a precursor to invasive disease, and emphasized findings from a study in Malawi showing that invasive strains are associated with human household transmission rather than animal or environmental sources. Understanding the impact of vaccination on asymptomatic carriage and thus on disease transmission might be valuable exploratory information.

Regulators provided feedback that specific (and somewhat limited) objectives and endpoints are needed in Phase 3 trials, cautioning against over-expanding exploratory endpoints. There is a need to choose wisely which of these exploratory areas should be prioritized the the design of future phase 3 efficacy trials, and to receive feedback from regulators and other stakeholders which will be most compelling for licensure.

Overall, stakeholders agreed that the presentation and data were extremely helpful and provided a robust framework and comprehensive list (included below) for selecting exploratory endpoints for iNTS candidate vaccine efficacy trials.

Potential exploratory endpoints proposed for consideration:

Immune correlates of protection (COP) in differing settings / contexts putative mechanistic (m)COP / non-mechanistic (n)COP to be validated cell-free killing and cellular killing informed by current work	**Antimicrobial usage** number and days of prescriptions iNTS antibiotics and all antibiotics relationship to malaria RDT / malaria
Susceptibility subgroups **age-stratified** ****Malaria rapid diagnostic test (MRDT) positive children**** (smear negative) **MUAC** **Hemogobin/ haemocue** primary immunogenicity measure exploratory functional mCOP / nCOP vaccine efficacy	**AMR** Blood culture genomic pathogen surveillance AMR patterns LPS antigenicity serovar replacement Bystander AMT surveillance (phase 4 impact)?
****Stool surveillance**** eNTS and diarrhoeal episodes by culture and PCR frequency / duration serovar pattern of carried strains (replacement?) household transmission	**Severity** deaths - NTS (14.7%) and all-cause febrile presentations (secondary and tertiary) hospitalisations (NTS and all-cause) complications – sepsis 57% (definition), anemia 47% duration of illness (secondary and tertiary) diarrheal disease (with microbiological lx)

10. iNTS Phase 2b and 3 Efficacy Study Design Considerations – Small Group Discussion and Summary Presentations

Participants were divided into 6 groups, each with a facilitator to further discuss Phase 2b/3 trial endpoints/design and PDVAC questions. Each group identified a rapporteur to summarize and present the discussion to the larger group (See **Appendix** for group assignments and composition). The groups were asked to discuss the following:

Question 1: Trial Design and Endpoints

Comment on key design considerations for a clinical study to determine iNTS vaccine efficacy for licensure (endpoints discussed in Part 1):

a. Clinical and microbiological endpoints
b. Safety endpoints
c. Immunogenicity endpoints

Question 2: Evaluating safety and efficacy of iNTS combination vaccines

– Are there additional study design considerations if NTS antigens are combined with a licensed product (example TCV)?
– Or if iNTS is combined with another new vaccine (example shigella)?
– Would the safety and efficacy of the standalone iNTS vaccine need to be established first before demonstrating safety, immunogenicity and efficacy of the combination vaccine?
– Propose evaluating safety and efficacy of the combination vaccines. Please comment.

Question 3: Evaluating TCV non-inferiority in infants under 6 months of age (iNTS/TCV combination vaccines)

– WHO iNTS PPC indicates target population of 6-36 months of age, but there is uncertainty about the most appropriate lower age bound.
– How should non-inferiority for the TCV component (in the iNTS/TCV combination vaccine) be determined in infants below 6 months of age given the vaccine is not licensed below 6 months?
– Propose bridging immunogenicity from older children to infants below 6 months to determine non-inferiority of TCV in infants. Please comment.

Question 4: Typhi/Paratyphi/iNTS combination vaccine

– Potential to provide coverage in Asia & Africa. However, each population would be immunized with a vaccine component not specific to their region.
– What benefit-risk analysis would be required for market authorization/ licensure?

Question 5: Controlled human infection model (CHIM)

– Please comment on the role of data (safety and efficacy) from CHIM studies in iNTS vaccine development.
– Which endpoints from CHIM studies are clinically relevant for an NTS vaccine: colonization? fever? diarrhea?

Group 1: Issiaka Soulama from The Agence Nationale de Regulation Pharmaceutique (ANRP), Ouagadougou, Burkina Faso. The group focused on clinical trial design and provided in-depth feedback for Question 1. The group recommended a randomized controlled double -blind Phase 3 trial and queried whether a 2b is needed. They emphasized importance of selecting sites with documented iNTS incident cases (especially as iNTS incidence has been decreasing in some areas, likely because of improved access to ARVs for HIV treatment and malaria control (including malaria vaccine introduction).

Population: general and representative pediatric population, irrespective of concurrent medical conditions (HIV, malnutrition, sickle cell, etc) with clear definitions of risk cases and emphasized the need to define the standard of care and to bring benefit to participants and the community (ie. potential for RTS,S or R21).

Sample size calculation/efficacy: power the trial to detect 70% efficacy with a 30% lower bound of the confidence interval following the primary dose. They also noted the importance of analysing efficacy following the booster dose and assessing closely for a rebound of incidence between the prime and boost doses. **TCV efficacy** should be evaluated since the vaccine is a combination vaccine.

Case ascertainment should focus on severe cases (such as death, illness severe enough to bring child to health care, meningitis, septic arthritis, septicemia) triggering blood culture and possibly also PCR. **Case definition** should then be severe case with microbiologic confirmation.
Design: 2 groups, consider potential of co-administering with RTS,S: **1) RTS,S + rabies vs 2) RTS,S + iNTS/TCV** (proposed as an option for discussion). Regarding dose administration schedule, they noted the complexity with the EPI schedule and suggested considering EPI schedules in target countries; interval for booster dose (at 9, 12, or 15-17 months) will depend on Phase 2 results. Potential for interference with EPI vaccines needs to be evaluated (and avoided).
Lot-to-Lot consistency the group queried whether this should be included in the Phase 3 or evaluated separately (not required by EMA; required by US FDA and for WHO prequalification).
Safety endpoints should be standard safety assessment (AEFI, MAAE, SAE) and definition of Adverse Events of Special Interest (AESI).
Immunogenicity endpoints should be evaluated using international standard assays (NIBSC/WHO): ELISA and Serum Bactericidal Assay (SBA) (qualified assays sufficient for Phase 2b (validated assays preferred); validated assays required for Phase 3). They noted that the threshold for protection is still unknown.

Group 2: Emmanuel Masunga from Tanzania Medicines & Medical Devices Authority (TMDA), Dar es Salaam, Tanzania. The group recommended a less stringent clinical case definition of fever/illness plus microbiologically confirmed infection (culture) with an NTS serotype contained in the vaccine isolated from a normally sterile site such as blood. Secondary endpoints proposed included infection with other serotypes (not included in the vaccine), severity of illness (ie. sepsis), death, and moderate to severe diarrhea. Recommended safety endpoints include: local and systemic reactions, solicited adverse events for 7 days following vaccination, non-solicited AEs for 28 days from the last vaccination, serious adverse events, medically attended adverse events (MAAE); adverse events of special interest (AESI to be tailored based on safety from previous trials) should be collected for 6 months. The group recommended immunogenicity endpoints should include validated assays for measuring binding and functional antibodies, with duration of protection and durability of immune response as secondary endpoints and cellular immunity measures as exploratory endpoints. Group 2 recommended that combination vaccines, specifically iNTS combination vaccines, can be evaluated in the same clinical trials by measuring separate safety and efficacy endpoints for the vaccine components within one trial. The group specifically considered iNTS + Shigella and iNTS + TCV; they agreed that evaluating safety of the combinations (rather than evaluating safety of the components independently) can give a meaningful safety evaluation and comparison. Regarding iNTS + TCV combination, the group felt that a separate, dedicated immunogenicity bridging study should be done for TCV in infants below the age of 6 months BEFORE the Phase 3 trial evaluating an iNTS+TCV combination to assess non-inferiority of safety and immunogenicity of TCV in infants less than 6 months of age as compared to infants 6 months and above (for which TCV is licensed). The group concluded that a quadrivalent *Salmonella* vaccine (iNTS + TCV + Paratyphi) would be beneficial, if the safety and immunogenicity profiles were found to be acceptable. They commented that this could be acceptable regardless of the geographic differences in burden and epidemiology and drew parallels with other vaccines that are administered in EPI programs in Africa (including meningococcal vaccine and pneumococcal vaccine) in which the serotypes included are not all relevant for every country and region. They raised concern that the cost of goods (COGs) may be

a concern for manufacturers and for the GAVI market, but that this is not an ethical or acceptability issue. Lastly, they concurred that CHIMs are important for exploring correlates of protection and could potentially support approval, but that CHIM is not on the critical development pathway.

Group 3: Mary Kaniu from the Kenya Medical Research Institute Wellcome Trust Research Programme (KEMRI WT). Group 3 proposed that the clinical case definition for the primary endpoint for a Phase 2b/3 iNTS vaccine trial should include illness (fever, sepsis, meningitis, septic arthritis) with Serogroup B or D NTS isolated from blood culture or another sterile site. The group deemed that bone marrow aspirate is not feasible/practical in most LMIC health settings (even at experienced and well-equipped clinical trial sites in SSA). Secondary endpoints discussed included: immunogenicity (binding Ab, validated functional assays), stool surveillance for asymptomatic carriage, and severity of illness. PCR testing was discussed as a potential exploratory endpoint (perhaps in a subset of positive and negative blood culture samples). The group concurred that it is preferred to evaluate combination vaccines together in the same trial rather than developing the vaccines independently before evaluating the combination. They discussed risk to both products with this approach but concluded that the overall costs and the time to develop the combined product would be less if the vaccine components could be evaluated together as a combination. Regarding evaluating non-inferiority for the TCV component in an iNTS + TCV combination in infants below 6 months of age, Group 3 expressed that some NRAs may not accept bridging studies; they discussed the importance of AVAREF in this type of situation and queried whether AVAREF might be able to issue an opinion on this topic and regulatory bodies to reach consensus in order to accept/approve a bridging study. The group also highlighted that this might be more feasibly in countries with TCV programs already in place. The group concluded that there is no need to demonstrate efficacy for all four components of a Typhi/Paratyphi/iNTS combination vaccine in each geographic region; if clinical efficacy for Paratyphi is demonstrated in Asia, there is not need for clinical efficacy in Africa (where Paratyphi is not endemic). Trials would need to be conducted in both regions and safety and immunogenicity data need to be demonstrated from all trial sites/ geographic areas. They also highlighted the need to demonstrate risk management plans for co-morbidities and co-infections and a to conduct a comprehensive review of the literature in terms of co-morbidities and how geographic illnesses and co-morbidities prevalent in Africa would interact with components in the vaccines. Lastly, the group shared that many NRAs are not using CHIM and are therefore not familiar; however, they felt that there is a role for CHIM in generating safety data and that as CHIM becomes increasingly used for different pathogens and more data are generated, regulators may start to consider the data. Overall, the group suggested involving regulators at the study design stage for CHIM and working to help them understand that CHIM data may lead to identification of correlates of protection and other useful data for hypothesis generation.

Group 4: Ashwani Kumar Arora from GSK Vaccine Institute for Global Health (GVGH), Siena, Italy. The group agreed that the clinical and microbiologic endpoint should be the first episode of invasive disease with culture positive for vaccine serotypes, according to a consensus definition for severe illness (such as from Integrated Management of Neonatal and Childhood Illness

(IMNCI). They recommended secondary endpoints to include broader iNTS clinical case definitions and narrower, more stringent definitions. Safety endpoints suggested were any safety signal or AESI according to review of final Phase 2 data. They recommended that IgG and IgM and functional assays (such as SBA) should be measured as immunogenicity endpoints and that exploratory immunogenicity endpoints discussed in Part 1 of the discussion should be considered for inclusion in trial design. They commented on considerations for combination vaccines with a licensed and a non-licensed component (ie. iNTS + TCV) and with two new/non-licensed vaccine components (such as iNTS / *Shigella*). They raised concern with iNTS / *Shigella* combination that the two address opposite endpoints but BOTH need efficacy data (invasive disease for iNTS vs diarrhea for *Shigella*) and suggested that that combination would be better for later development following iNTS + TCV licensure. They proposed that safety, immunogenicity and efficacy of the combination vaccine can be evaluated at the same time (rather than evaluating the components independently first). For efficacy, iNTS efficacy can be bridged to iNTS + TCV efficacy and supported by non-licensed TCV component. The group proposed an approach for bridging TCV immunogenicity and evaluating TCV non-inferiority in infants (less than 6 months of age) by evaluating non-inferiority at 9 months plus one additional timepoint. They also proposed that regulators might consider that a potential decreased level of Vi immunogenicity in infants less than 6 months of age (if that were to be seen) is commensurate with the significantly decreased risk of typhoid in this age group. This brought some controversy and discussion on whether the commensurate risk could be incorporated and weighed in this manner by regulators. Generally, the regulators in the room disagreed with this as a viable argument/rationale for regulators. For the quadrivalent *Salmonella* vaccine, the group advised that the benefit of efficacy against relevant components Africa iNTS and Paratyphi in Asia would need to be weighed and that the safety profile and cost would need to be evaluated (and no significant increase in cost of goods) .

Group 5: Florence Wanyenza from the National Drug Authority (NDA), Kampala, Uganda. Group 5 recommended illness (such as fever, low blood pressure, etc) and positive blood culture as the primary clinical and microbiologic endpoints. They recommended comprehensive safety data, including adverse events of special interest (AESI). They recommended using validated immunoassays, stating that validated assays are ideal and would be necessary for immunobridging. The group focused on inclusion and exclusion criteria highlighting the importance of managing at risk populations carefully. They emphasized that drug interactions need to be monitored (eg. malaria treatment and vaccine interactions) and suggested and an adaptive study design in which healthy participants are enrolled first to establish a safety database prior to moving to at risk populations. This group also did highlight that children are not tested for asymptomatic malaria infection to confirm negative mRDT or microscopy/malaria blood film routinely prior to EPI immunization administration. For evaluating the safety of iNTS combination vaccines, Group 5 recommended conducting a Phase 2 study demonstrating safety of a new combination vaccine consisting of two unlicensed products prior to Phase 3 study. An alternate approach could be an adaptive study design in which safety of the combination is assessed first in a subgroup from a Phase 3 study and thoroughly reviewed by DSMB prior to proceeding/enrolling larger cohorts. This group also addressed evaluating non-inferiority for TCV (iNTS/TCV combination vaccine) in infants below 6 months of age and highlighted the evidence

that TCV may have waning immunity when administered to infants and young children (data under evaluation now). This was discussed in the context of a potential need for TCV booster dose in the future (but data is still lacking). Data from CVD supports TCV at earlier age (< 6 months) for immunogenicity but building on that data set is important. Regarding the potential quadrivalent *Salmonella* vaccine, this group recommended that combination vaccines should offer benefits to the population being immunized or specific combinations would not be acceptable. Discussion also included giving multiple doses of TCV if the licensed product is currently a one dose schedule; the group highlighted that acceptability of multiple doses may not be very favorable in this case. This may be overcome if TCV recommendations are changed to include a second dose (booster) and is aligned with combination vaccine dosing schedule. Finally, the discussion about CHIM highlighted that national regulatory agencies are largely separate for drugs/vaccines as compared to CHIM use for immune correlates (not vaccination/challenge studies). CHIMs for iNTS may not be applicable since target population for vaccine is infants whereas CHIM enroll adults. Concerns were expressed over disease severity of iNTS CHIM and whether CHIM fully mimics iNTS invasive disease.

Group 6: Gracian Harawa from the Public Health Institute of Malawi (PHIM), Lilongwe, Malawi. Group 6 emphasized the importance from a regulatory perspective that the case ascertainment and case definition be well-defined, pre-specified, and clear in the clinical protocol. They proposed: isolation of an O:4 or O:9 *Salmonella* isolate in a positive blood culture or culture from a normally sterile site in a child who presents with illness at least **x** days after vaccination (period to be decided). They recommended collecting solicited local and systemic reactions 7 days post vaccination (active surveillance to monitor symptoms); the group did not identify any special safety endpoints for consideration. Regarding immunogenicity endpoints, this group specified that it would be preferable to have a central reference laboratory responsible for the conducting immunogenicity assays for the Phase 3 study using a validated assay (following international standards). The microbiological endpoint assessment for the Phase 3 efficacy study could be based on site-specific microbiological blood culture and organism isolation and identification, which adheres to an internationally recognized standard (such as CLIA) and is quality assured. Other trial design considerations discussed and presented by this group included:

I. **Pre-specified interim analysis**: it is highly preferred to have interim analyses (including futility analysis) pre-specified.
II. **Prioritize development in high burden countries**: No, rather a mixture of high and low burden areas. High burden sites would improve power but epidemiology changes even within a country. Regulator's point of view was that it is preferable to include countries across African regions (e.g., West, East, and Central) and that this approach would gain more support than selecting only high-burden sites from a single region.
III. **Phase 1 First in Human (FIH) in adults in LMIC with vaccine ML3 NRA instead of in HIC?** There has been recent increasing NRA rigor and expertise that should allow for more Ph1 FIH studies to be performed in LMIC, rather than the traditional HIC then LMIC approach.

IV. **Placebo vs active comparator for Ph1 adult studies**? From the African regulator's point of view, an active comparator is preferred compared to a placebo. This is due to ethically ensuring that study participants may gain some benefit by participating in the study and acknowledging that study participants are more likely to have low levels of health literacy/knowledge.

V. **Considering one-to-one randomization vs. a cluster-randomized field efficacy study:** If there is indirect protection with iNTS vaccines, then 1:1 randomization may diminish the power of the study through a reduction of incident cases among both vaccinated and controls.

VI. **Considerations for combination vaccine trial design**: No need to demonstrate efficacy to each component of an iNTS combination vaccine. However, with a novel unlicensed non-iNTS component (e.g., *Shigella*), there would need to demonstrate efficacy for both iNTS and the novel component. Furthermore, the study would need to address case ascertainment for two different disease entities (e.g., *Shigella* disease/dysentery in older children and iNTS bacteremia in younger children).

VII. **Additional considerations for selecting combination for iNTS combination:** the group felt that combination with an already licensed product would be simpler than the design considerations needed for combination with a novel unlicensed vaccine, especially if disease (case ascertainment) and age incidence do not overlap.

This group considered whether multiple doses of TCV in a combination vaccine would need to be justified if the safety profile is acceptable. They concluded that this would likely be justifiable if there is supporting data, but that it could be difficult since the overall push is to reduce the number of routine visits for vaccines and to reduce vaccine doses. The group felt that the age incidence data for iNTS should be the major determinant for whether vaccinations below 6 months of age should be pursued. If needed (if TCV needs to be given below 6 months of age), bridging immunogenicity data from older children to infants should be performed with a validated assay from a "central" lab that ensures comparability and bridging of the immunogenicity.

Summary of key points raised and discussed following group presentations

Discussions on **primary endpoints** led to consensus regarding microbiologic case definition, but the clinical case definition needs further refinement as consensus regarding whether symptoms should be specified and narrow or broad was not reached. The group agreed that the primary microbiologic endpoint should be **serogroups B & D (O:4 and O:9)** rather than specific serovars in the vaccine, with serological cross-reactivity/cross-protection as another key measure. The **CVD TSCV vaccine** includes FlyC (flagellin), but serogroups B&D should remain the primary microbiologic endpoint.

- **Diagnostic considerations** for further evaluation of asymptomatic (afebrile, well) infants and children with positive malaria rapid diagnostic test (mRDT) during screening were debated. Several possible approaches were discussed, including treating all infants empirically, performing malaria microscopy for all infants/children with positive mRDT during screening, or using a varied approach based on history of recent diagnosis and treatment for malaria (within past 4 weeks). For enrolment criteria, **asymptomatic**

mRDT-positive cases with no treatment history in the past month likely require **microscopy testing/confirmation**, with treatment only if positive.

- **Lot-to-lot consistency testing** was deemed unnecessary in field efficacy studies. However, inferiority studies may be conducted separately as per NRA and WHO prequalification requirements
- **Regulatory perspectives** regarding efficacy were reviewed:
    - **US FDA** requires validated assays for Phases 2b and 3 and a **25-30% lower bound** for efficacy, while
    - **EMA** allows a lower bound of zero of the confidence interval depending on case frequency, focusing instead on the **point estimate**.
    - **Non-inferiority margins** were discussed, particularly for **serogroups B&D** and typhoid conjugate vaccine (TCV). The assessment of TCV in infants under **6 months of age** was debated, considering whether non-inferiority should be assessed **after a single dose or multiple doses**.
    - **Safety considerations** focused on **specific safety signals**, while **immunogenicity endpoints** included **IgG/IgM and functional assays (e.g., SBA)**, with many exploratory endpoints proposed. Including ***Salmonella* Paratyphi in a quadrivalent invasive *Salmonella* vaccine was not seen as a major concern for African settings** unless safety concerns arise.
    - **Controlled human infection model (CHIM) data** may be useful but colonization was seen as a high bar.
- **Vaccine schedule alignment** with malaria programs was suggested, possibly **starting at 5-6 months with boosters at 9, 12, or 15-17 months**, given that iNTS burden peaks at **9-12 months**. Further discussion on the **starting age (6 months?)** and burden in **low-malaria regions** was encouraged.

11. Roundtable Discussion with Regulators, Co-chaired by Marco Cavaleri (EMA) and Kwasi Nyarko, African Vaccine Regulatory Forum (AVAREF)

A robust discussion highlighted key points and take-aways from the meeting. Regulators and other participating stakeholders expressed appreciation for being engaged and involved in the discussion and in trial planning and agreed that early engagement with regulators and National Immunization Technical Advisory Groups (NITAG) is crucial for vaccine development. NITAG representatives expressed enthusiasm for involvement in trial design discussions and deliberations and debates on target age, number and timing of doses, and candidates for combination vaccines.

Kwasi Nyarko explained the role and activities of AVAREF, a regional body established in 2006 which is now providing technical support to the Africa Medicines Agency (AMA). AVAREF, which integrates ethical and regulatory considerations, aligns with the AMA and the African Medicines

Regulatory Harmonization (AMRH) initiative. AVAREF is intensifying efforts to harmonize processes and mentor NRAs, particularly in French-speaking countries. WHO oversees the AVAREF secretariat, offering scientific advice for clinical trials and submitting joint reports to individual countries.

AVAREF is positioned to play an important role for multi-country studies. Engaging every National Regulatory Authority individually is impractical and inefficient; AVAREF can assemble a committee and facilitate a multi-country joint review process. AVAREF can also coordinate and arrange scientific advice (like a pre-IND process). They provide training with an aim to build capacity of regulators. Dr. Nyarko assured participants that iNTS will be part of the portfolio and will have support from AVAREF for scientific input and joint review. EMA is also available to support but Dr. Cavaleri acknowledged that the actual use of an iNTS vaccine will be in Africa; EMA gives scientific opinion for products that will not be used in Europe. WHO provides scientific advice specific to prequalification.

Key feedback, Chaired by Sam Kariuki (KEMRI)

Dr. Kariuki emphasized the role and importance of AVAREF, which brings together ethical agencies and regulatory authorities from multiple countries together within the same committee for a joint review, as a key enabler for collaborative research. Several attendees expressed appreciation for being involved in the discussions and debate and for sharing in the planning process. There were multiple requests to share **presentations and key information points** from the meeting, with the option to **redact sensitive slides if necessary**. To enhance engagement in the future, one attendee (representing Wellcome Trust) recommended that **meeting materials, including key questions for discussion, be shared in advance of the meeting** so attendees can gather input from subject-matter experts in their respective agencies and countries prior to attending. The organizing team informed participants that a **summary or report of the meeting** will be shared to ensure broader dissemination of key discussions and outcomes.

Closing

Dr. Jerome Kim and Dr. Jean-Louis Excler thanked all speakers and regulator participants who contributed to the success of this meeting. Thanks went also to AVAREF for their critical role in coordinating efforts from National Regulatory Authorities in Africa and linking developers with regulators. iNTS vaccine development will benefit of this new dynamic that emerged from this meeting, emphasizing the need to keep this new momentum.

Special thanks went to the rapporteurs (Jessica Cowden, Camille Dauvergne, Tarun Saluja, Francis Reisdorfer, Robert Kaminski) and to Somyoung Cho and Jieun Kim, IVI Secretariat, for the excellence of the meeting organization.

Appendices

Agenda

Day 1: Tuesday, 4 February

Location: Conference room 3, 2nd Floor

Time	Activity	Speaker/Chair
08:30 – 09:00	Registration	-
09:00 – 09:20	Opening remarks & overview of meeting context and objectives	Jerome Kim (IVI), Jean-Louis Excler (IVI)
09:20 – 09:30	Participant introductions	-
09:30 – 10:00	Review of iNTS epidemiology and burden of disease	John Crump (Otago U)
10:00 – 10:20	Summary of the outcomes of the Kigali (Dec 2023) and Geneva (Dec 2024) meetings	Annelies Wilder-Smith (WHO)
10:20 – 10:40	Review of the WHO preferred product characteristics for iNTS vaccines	Robert Kaminski (WHO)
10:40 – 11:00	(Virtual) Safety and Immunogenicity of bivalent GMMA-based vaccines against iNTS Phase 1 in the UK	Maheshi Ramasamy (Oxford U)
11:00 – 11:25	Current clinical development plans and available data	Ashwani Arora (GVGH)
11:25 – 11:50	Current clinical development plans and available data	Mike Levine (CVD)
11:50 – 12:00	***Group photo***	-
12:00 – 13:00	***Lunch*** *Location: Atrium, 1st floor*	-
13:00 – 13:30	Regulatory considerations on iNTS combination vaccines	Marco Cavaleri (EMA)
13:30 – 14:15 14:15 – 15:15 15:15 – 15:45	iNTS Phase IIb and III Efficacy Study Design Considerations - Part I <u>Primary endpoints</u> • Clinical endpoints • Clinical case definitions and case ascertainment • Microbiologic case detection (culture, PCR) <u>Secondary endpoints</u> • Safety endpoints • Immunogenicity endpoints (ELISA, multiplex, functional assays) <u>Exploratory endpoints</u> • Immune correlates of protection • AMR and usage of antibiotics	Mike Levine & Wilbur Chen (CVD) Mike Levine & Wilbur Chen (CVD) Melita Gordon (Liverpool University)
15:45 – 16:00	***Coffee break***	-

16:00 – 17:40	iNTS Phase IIb and III Efficacy Study Design Considerations - Part II Breakout sessions Constitution of breakout groups and working modalities *The format will be breakout groups, with 7-8 people per group. Working modalities will be presented at the meeting.* *An opportunity for regulators and investigators to share insights on the presentations and to discuss PDVAC questions.*	Chair: Jessica Cowden (IVI) Group Facilitators: Jessica Cowden (IVI) Jean-Louis Excler (IVI) Tarun Saluja (IVI) Rob Kaminski (WHO) Sushant Sahastrabuddhe (IVI) John Crump (Otago U)
17:40 – 17:50	Day 1 adjourn	Jean-Louis Excler (IVI)
17:50	***Dinner*** *Location: Back Terrace, 2nd floor*	-

Day 2: Wednesday, 5 February

Location: Conference room 3, 2nd Floor

Time	Activity	Speaker/Chair
09:00 – 09:10	Introduction to Day 2	Jean-Louis Excler (IVI)
09:10 – 10:40	iNTS Phase IIb and III Efficacy Study Design Considerations - Part II (cont'd) Breakout sessions: Report back Group presentation & discussion *A representative from each breakout group to provide a brief 10-min summary of their discussion.*	Moderator: Tarun Saluja (IVI)
10:40 –11:30	Expectations and requirements from regulators for new vaccines for use in LMICs Roundtable discussion with regulators *The session will feature a roundtable discussion focusing on the presentations, breakout sessions, and additional topics (not limited to), e.g.,* • Study designs – core protocol elements, combination vaccines • Comparator vaccine • Surrogate immunological endpoints (for Typhoid) • Target groups	Co-Chairs: Marco Cavaleri (EMA), Kwasi Nyarko (AVAREF)
11:30 – 11:40	Meeting Summary	Sam Kariuki (KEMRI), Kwasi Nyarko (AVAREF)
11:40 – 12:00	Meeting wrap-up and adjournment • Identification of next steps • Adjourn	Jerome Kim (IVI), Jean-Louis Excler (IVI)
12:00 – 14:00	***Lunch & networking*** *Location: Atrium, 1st floor*	-

iNTS Phase 2b and 3 Efficacy Study Design Considerations - Group assignments and composition

Note:
The assigned group lead is requested to lead the group. A representative who will report back can be decided within the team.
The assigned note-taker will select a co-writer in the group and will be primarily responsible for ensuring the notes for reporting back.

		Group 1			Group 2			Group 3		
	Role	Name	Affiliation	Country	Name	Affiliation	Country	Name	Affiliation	Country
1	Group lead	Kwasi Nyarko	AVAREF Coordinator	Congo	Marco Cavaleri	EMA	Netherlands	Sam Kariuki	Kenya Medical Research Institute	Kenya
2	Facilitator	Jean-Louis Excler	IVI	Korea *(French speaking)*	Tarun Saluja	IVI	Korea	Jessica Cowden	IVI	Sweden
3	Note taker	Camille Dauvergne	IVI	Korea *(French speaking)*	Francis Reisdorfer	IVI	Brazil	Ondari Daniel Mogeni	IVI	Kenya
4		Issiaka Soulama	Agency The National Regulation Pharmaceutique (ANRP)	Burkina Faso	Jamal Mário Paulino	Autoridade Nacional Reguladora de Medicamento (ANARME), Mozambique	Mozambique	Malebelo Mabowe	South African Health Products Regulatory Authority (SAHPRA)	South Africa
5		Donatien Kabamb Kabey	Autorite Congolaise de la Reglementation Pharmaceutique (ACOREP)	DRC	Emmanuel Masunga	Tanzania Medicines and Medical Devices Authority (TMDA)	Tanzania	Ganesh Mishra	Bio E	India
6		Badri Patnaik	BBIL	India	Subhash Thuluva	Bio E	India	Brian Ngwira	Malawi Liverpool Wellcome Programme (MLW)	Malawi
7		Dominique Arama	Directorate of Pharmacy and Medicines	Mali	Mtwalo Duncan Jere	Pharmacy and Medicines Regulatory Authority (National Regulatory Authority of Malawi)	Malawi	Fatou Njie Sey	Medicines Control Agency, The Gambia	Gambia
8		Mike Levine	CVD/University of Maryland School of Medicine	USA	Gugu Mahlangu	Wellcome Trust	Zimbabwe	Mary Kaniu	KEMRI-Wellcome Trust Research Programme	Kenya

		Group 4			Group 5			Group 6		
	Role	Name	Affiliation	Country	Name	Affiliation	Country	Name	Affiliation	Country
1	Group lead	AnneliesWilder-Smith	WHO	Switzerland	Melita Gordon	University of Liverpool	UK	Beno Yakubu	National Agency for Food and Drug Administration and Control (NAFDAC)	Nigeria
2	Facilitator	Sushant Sahastrabuddhe	IVI	Korea	Robert Kaminski	WHO	USA	John Crump	University of Otago	New Zealand
3	Note taker	Johnathan Meriakol	Pharmacy and Poisons Board (PPB), Kenya	Kenya	Ernest Agyei Kwame	Ghana FDA	Ghana	Cecilia Mbae	Kenya Medical Research Institute	Kenya
4		Mariama Simah	Medicines Control Agency, The Gambia	Gambia	Florence Wanyenze	NDA, Uganda	Uganda	Nilsa Stela Francisco Basilio	Autoridade Nacional Reguladora de Medicamento (ANARME), MZ	Mozambique
5		Ashwani Kumar Arora	GSK	Italy	Rocio Canals Alvarez	GSK	Italy	Wilbur Chen	CVD/University of Mary	USA
6		Macpherson Mallewa	NITAG	Malawi	Jerome Kim	IVI	Korea	Gracian Harawa	Public Health Institute of Malawi	Malawi
7		Azeem H Walele	South African Health Products Regulatory Authority (SAHPRA)	South Africa						
8										

List of participants

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