IVI-WHO-Wellcome Trust Consultation On Non-typhoidal *Salmonella* Combination Vaccines 12 and 13 December 2024 Geneva, Switzerland

Meeting Report: Key Discussions, Deliberations, and Recommendations for Next Steps to Accelerate Development of invasive non-typhoidal *Salmonella* (iNTS)-Containing Vaccines for Vaccine Developers

Executive Summary

Fifty-eight (58) participants from 36 institutions from public, private, and academic sectors attended a consultation meeting convened by WHO and IVI as part of the iNTS Full Value of Vaccine Assessment (FVVA) project on 12-13 December 2024 in Geneva, Switzerland. The FVVA project is funded by the Wellcome Trust. The meeting gathered experts input on critical considerations for iNTS combination vaccines and outlined progress made towards the gaps identified during an iNTS meeting in Kigali, Dec 2023 to further inform the FVVA. The meeting also focused on advancing the development of questions for regulators regarding the clinical development pathway for iNTS and iNTS-typhoid conjugate vaccines, in preparation for a regulatory meeting scheduled for on 4-5 February 2025 in Nairobi, Kenya.

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 young 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%.

WHO has finalized the Preferred Product Characteristics (PPC) guidance and R&D Roadmap document to aid and inform decisions regarding characteristics of an iNTS vaccine that will support uptake in vulnerable populations living in LMICs.

Shift Health presented a demand and financial forecast model for a hypothetical trivalent iNTS-typhoid vaccine in sSA. The business case evaluates the vaccine's market potential and return on investment for developers, commercialization partners and global health stakeholders. With a $538 million USD risk-adjusted capital investment (and assuming a 10% industry-standard discount rate), the net present value (NPV) is negative, highlighting insufficient commercial viability without global health funding to de-risk investment. This model underscores the need for innovative financing to enable development and manufacturing of this vaccine to address a significant public health challenge.

IVI Policy and Economic Research department presented the overall progress of the FVVA for iNTS with a focus on the investment case work package consisting of four workstreams: systematic literature review, modeling economic burden of iNTS, stakeholders/policy survey, and cost-effectiveness analysis (CEA).

WHO presented an overview and outcomes from a workshop aimed to gather country-level perspectives and priorities to inform the development of iNTS vaccines, highlighting the potential challenges and strategies for their implementation in high-burden settings. WHO and IVI co-organized this workshop, held in Accra, Ghana in October 2024, involving delegations of between 2 and 5 immunization programme stakeholders from a total of 7 different African countries. Key takeaways from the workshop underscored critical data gaps regarding iNTS burden, which hinder vaccine prioritization. Participants favored combination vaccines for their logistical advantages, optimizing cold chain needs and minimizing injections. Ultimately, robust evidence on disease burden and vaccine characteristics is necessary for informed decision-making by policymakers.

The Fondazione Achille Sclavo presented the background and critical need for a sensitive and specific diagnostic available for use in low resource settings. The need for a reliable point-of-care Rapid Diagnostic Test (POC RDT) has been reported in the literature and in WHO expert meetings. Due to the non-specific nature of the disease and pre-existing morbidities, conditions, iNTS disease diagnosis is delayed, evolving into a disease rapidly fatal within 48 hours, with case fatality rates up to 28-35% in sSA. A consortium of research institutions with over a decade of experience in iNTS research and with integrated areas of expertise collaborated as part of the EU-funded Vacc-iNTS Project.

KEMRI provided a global overview of the gaps identified in iNTS vaccine development, addressing the challenges such as bivalent versus trivalent formulations, optimal administration schedules, the role of the Controlled Human Infection Model (CHIM) and the design of Phase 3 efficacy trials for regulatory approval. Dr. Kariuki underscored the challenges countries face as they transition out of Gavi support, highlighting the long-term need for improved WASH infrastructure. He emphasized a holistic approach, where vaccine development is just one component within a broader strategy that includes WASH and other critical interventions. Meanwhile, efforts to standardize assays are progressing, with the Gates Foundation funding collaborative work between GVGH and NIBSC. This initiative aims to leverage Phase 1 trial specimens to develop serovar-specific International Reference Standards (IRS)

CVD presented preliminary results of a Phase 1 in US adults and of a Phase 1/2a paving the way to pediatric trials for dose selection. Baseline antibody titers were evaluated, but there was some variability ("noise") in the data, especially in older infants, may reflect cross-reactive responses or prior lifetime exposure to other nontyphoidal *Salmonella* strains. Functional assays indicated killing activity (significant increases in antibody-dependent neutrophil phagocytosis (ADNP)) despite low fold rises in antibody levels. The results emphasize the need for additional functional and T-cell response analyses to address immunologic challenges in infants. 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 of the trivalent *Salmonella* conjugate vaccine (TSCV) aimed to determine optimal dosing, safety, and immunogenicity in sSA countries (Mali, Kenya, Mozambique). Immunogenicity was assessed using a mesoscale discovery (MSD) multiplex immunoassay, which demonstrated high seroconversion rates for the typhoid vaccine component across all ages. The study evaluated four investigational products doses (Full-strength TSCV (25 μg of each PS), half-strength TSCV (12.5 μg), Typbar-TCV, or placebo) across a stepwise age de-escalation trial, starting with 40 adult participants and expanding to 1,256 subjects, including infants (Step 1). In Step 2 toddlers 12-16 months of age (N=120) and older infants 8-11 months of age (N=120) were randomly allocated to receive one of the four investigational products. Older infants

(age 8 months and older) demonstrated seroconversion rates of >80% against *S*. Enteritidis and >30% against S. Typhimurium O-polysaccharide. In Step 3, the "low" seroconversion rates of ~10%with a single (first) dose of TSCV were observed in young infants (age 12-18 weeks) and to the *S*. Typhimurium O-antigen; the young infant response to the *S*. Enteritidis O-antigen was ~50% or higher. In the last Step 4, the dosing schedule includes a primary dose at 12–18 weeks and a single booster dose of TSCV at varying intervals (9, or 12, or 15-17 months). This raised concerns about a potential gap in protection between doses, as well as whether administering a second dose earlier might elicit a stronger amnestic response. It was suggested that a larger sample size might reveal a higher seroconversion rate. Breast milk interference, involving antibodies from maternal milk and systemic maternal antibodies, was also discussed.

IVI described the development process for the IVI trivalent iNTS/TCV vaccine. The bivalent invasive non-typhoidal *Salmonella* (iNTS) conjugate component was developed before incorporating the typhoid conjugate vaccine (TCV). Scalable O-specific polysaccharide (OSP) production, optimal carrier protein identification, and conjugation chemistry were key focus areas in early development, with CDAP conjugation achieving yields above 60%. Proof-of-concept studies demonstrated robust immunogenicity of *Salmonella* Enteritidis OSP (SE-OSP) and *Salmonella* Typhimurium OSP (ST-OSP) conjugates in animal models, with diphtheria toxoid (DT) selected as the carrier protein. The trivalent vaccine formulation showed strong immune responses in both mice and rats. Toxicology studies showed no adverse effects or formulation-related issues. For business consideration, the development with the TCV manufacturer was discontinued.

Oxford University presented the preliminary results of safety and immunogenicity of a bivalent GMMA-based iNTS vaccine, iNTS-GMMA, in healthy adults in the UK, Phase 1. 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 *S*. Typhimurium and *S*. Enteritidis antigens. Additionally, functional antibody activity, as measured by the serum bactericidal assay (SBA), increased following first vaccination. 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.

GVGH presented the design and preliminary results of an ongoing Phase 1/2a, observer-blind, randomized, controlled, two-stage, multi-country study evaluating the safety, reactogenicity, and immune response of the trivalent 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). The overall benefit-risk profile remains favorable, with no safety issues preventing further development. To assess potential interference between the TCV and the iNTS-GMMA vaccine 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 for a conference presentation. The next step will be a Phase 2a study that will involve evaluating non inferiority of the TCV when combined as an iNTS-TCV vaccine.

PATH highlighted the need for combination vaccines, because of the already crowded immunization schedule, challenges with adding vaccinations and program implementation in resource-limited settings, vaccine hesitancy and uptake concerns globally, and multiple priority pathogens with vaccine candidates in development. Regulators may be hesitant to accept lower

efficacy parameters without individual efficacy data for each component, which necessitates more flexibility in Phase 3 trial parameters. The complexity of combining different antigens and serotypes must be considered, as multiple components could lead to increased reactogenicity as well as decreased immunogenicity (and efficacy?) of specific components. Given the challenges of modifying vaccines post-licensure, decisions on combination vaccines and co-administration schedules must be made thoughtfully to ensure alignment with target age groups and disease burdens.

University of Otago key points included the significant burden of iNTS disease in children under 5 years, an age group for which deaths were estimated to be more than four times greater than those from paratyphoid and typhoid fevers combined. Malaria was noted to potentially contribute to up to 50% of iNTS disease, although data remains insufficient. The RTS, S malaria vaccine trials showed a non-significant reduction in iNTS bacteremia (30-40%) in the target age group. It was emphasized that for vaccine development and policy making, age-occurrence data including that available from large, long-term surveillance studies should be plotted by month of age for children under 5 years, since incidence data from incidence studies and the control arms of vaccine trials present data in broad age groups (e.g., two-year periods) with wide uncertainty bounds that seldom provide the needed granularity. The challenges of generating reliable incidence data from small case numbers were noted, which results in significant overlap and makes it difficult to draw definitive conclusions. The IVI EPIC study in underway in Kisantu, Democratic Republic of Congo (DRC), and will evaluate the impact of the R21 malaria vaccine on iNTS infection incidence.

The Bill and Melinda Gates Foundation addressed considerations and challenges of potential iNTS combination vaccines. The conclusions drawn emphasized a pivotal shift away from single-pathogen vaccines in pediatrics, with expectations that broad adoption will necessitate strategic combinations. A regimen of 2-3 immunizations per visit was deemed reasonable, acknowledging the significant number of disability-adjusted life years (DALYs) still to be prevented, and underscoring the need for an aggressive bundling approach. Post-licensure studies may be required to confirm that each vaccine component contributes positively to the combination. Additionally, regulators require clear guidance on acceptable margins and the potential impacts of wider coverage versus individual protection levels, along with a solid understanding of the public health benefits of such combinations, even if they offer lower per-component efficacy.

A Scorecard exercise was conducted to examine iNTS + TCV, iNTS + *Shigella*, iNTS + injectable rotavirus, followed by a roundtable discussion and Mentimeter virtual survey. Preferences leaned towards combinations of iNTS with *Shigella* and iNTS with TCV.

Health Canada, CVD, and EMA addressed further clinical development and regulatory pathway considerations for iNTS combination vaccines.

Regulators do not typically object to combinations unless issues arise, but in the case of PCV, a decrease in immunogenicity with increasing multivalency of serotypes could become problematic if overall immunogenicity falls below protective thresholds. There is uncertainty on how to justify a combination if it results in lower protection, and identifying clear benefits for such combinations is essential. The discussion highlighted that a trivalent iNTS vaccine might not be equivalent to a bivalent due to timing of administration / age considerations, particularly since TCV is not licensed for use below 6 months. Discussion was also engaged on the limitations and benefits of an NTS CHIM.

Participants stressed the importance of engaging regulators from endemic countries to clearly communicate the burden of iNTS to regulators and discuss issues related to iNTS single component or combination vaccine development. Regulators should be encouraged to consider several critical factors when evaluating combination vaccines, including efficacy of each component, selection of active comparator (i.e., should a licensed component be the active comparator, when applicable?), interference, and safety monitoring. Furthermore, it was noted that relatively few regulatory bodies in African countries currently operate at the ML3 level, although some African countries are planning to initiate vaccine manufacturing. Their involvement is essential for obtaining insights from those who will make critical regulatory decisions.

Background

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 young 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%.

WHO has finalized the Preferred Product Characteristics (PPC) guidance and Roadmap document to aid and inform decisions regarding characteristics of an iNTS vaccine that will support uptake in vulnerable populations living in LMICs. The PPC was endorsed by PDVAC in September 2024 and will be published in due course.

There are currently no licensed iNTS vaccines, but several iNTS vaccine candidates are under development, including candidates already in Phase 1 and 2 clinical trials. It is expected that safe and effective iNTS vaccines would reduce the burden of iNTS disease by preventing clinically important infections and deaths. However, understanding the pathway to marketing authorization has been a key challenge for vaccine developers and funders during early-stage development. Planning clinical development is further complicated by the growing urgency to develop combination vaccines to alleviate the increasingly crowded Expanded Programme on Immunization (EPI) schedules, particularly for children under 5 years of age.

This meeting provided a forum for developers to share interim data from ongoing trials, and for regulators, policymakers, and subject-matter experts to share advancements and new information in the field. These subject-matter experts provided input on critical considerations for iNTS combination vaccines and outlined progress made towards addressing the gaps identified during the Kigali consultation, Dec 4, 2023. Presentations and discussions focused on developing a framework for evaluating potential pathogens for an iNTS combination vaccine, examining epidemiological data and target populations, and evaluating selected combination vaccine options. This meeting also focused on developing the questions for regulators regarding the clinical development pathway for an iNTS vaccine (alone or as a combination vaccine), in preparation for a meeting scheduled for 4-5 February 2025. This meeting report, the output from the upcoming consultation with regulators, and the output from the ongoing full value vaccine analysis (FVVA) and related peer-reviewed publications will contribute significantly to shaping strategies for iNTS combination vaccine development.

Day 1, 12 December 2024

Opening

Dr. Jerome Kim (IVI) and Dr. Annelies Wilder-Smith (WHO) welcomed attendees and provided opening remarks, context and a review of the meeting objectives and expected output. Following participant round-table introductions, the meeting began with Session 1 with reports from the business case and economic evaluations and community and stakeholder analysis.

Session 1: Progress updates on the iNTS Full Value Vaccine Assessment (FVVA) – Jung Seok Lee (IVI), Chair

1.1. Trivalent iNTS-Typhoid Conjugate Vaccine business case update - Nicole Revie, Shift Health

Dr. Nicole Revie from Shift Health presented a demand and financial forecast model for a hypothetical trivalent iNTS-typhoid vaccine in sSA. The business case evaluates the vaccine's market potential and return on investment for developers, commercialization partners and global health stakeholders. The model includes projected vaccine demand, revenue and profit forecasts, and net present value (NPV) analysis, using established forecasting methodologies and assumptions based on characteristics current iNTS vaccine candidates, expert insights and WHO Draft PPCs. Ultimately, these forecasts aim to inform industry and global health funder decision-making relating to prioritization of a trivalent vaccine.

Shift Health evaluated single-dose or two-dose regimens with catch-up campaigns in Gavi-eligible countries and an optional booster dose. Based on typical R&D timelines, first licensure is projected for 2035 (2037 for Gavi-eligible countries), with all sSA countries adopting by 2045. Country-specific timing of introduction was determined by a scoring system incorporating disease burden, new vaccine adoption history and overall vaccine delivery infrastructure. By 2038, ~55% of countries are projected to introduce the vaccine, increasing to ~78% by 2040. By year 12 post-introduction, annual demand is projected to reach 47 million doses for a 1-dose regimen and 96 million doses for a 2-dose regimen, generating $118 million USD and $239 million USD in annual revenue, respectively. Gavi-eligible countries account for 65% of doses, with non-Gavi LMICs contributing an additional 25%, reflecting the higher disease burden across both groups. Inclusion of a booster dose[1] adds 30 million additional doses, increasing annual revenue by $76 million USD in year 12.

With a $538 million USD risk-adjusted capital investment (and assuming a 10% industry-standard discount rate), the NPV is negative, highlighting insufficient commercial viability without global health funding to de-risk investment. This model underscores the need for innovative financing to enable development and manufacturing of this vaccine to address a significant public health challenge.

[1] Note (as raised in the meeting) that a booster for the iNTS component is likely redundant due to the absence of iNTS disease cases in children older than 3 years, and currently there is no recommendation for a typhoid booster. If this situation changes, there will be multiple monovalent TCVs that could be used for boosting, rather than relying on a further dose of trivalent vaccine.

1.2 Economic evaluation of a hypothetical iNTS vaccine – Jung-Seok Lee, IVI

Dr. Lee presented the overall progress of the Full Value of Vaccine Assessment (FVVA) for iNTS disease with a focus on the investment case work package which has been led by the Policy and Economic Research (PER) department at the International Vaccine Institute (IVI). The investment case work package consists of four workstreams: systematic literature review, modeling economic burden of iNTS disease, stakeholders/policy survey, and cost-effectiveness analysis (CEA).

A systematic literature review was first carried out to identify existing evidence on the economic burden of iNTS disease and NTS. Together with field-based economic burden studies, the review resulted in a total of 52 data points which were fed into the second workstream: modeling the economic burden of iNTS disease. Given a lack of field-based iNTS disease economic burden studies, econometric models were developed to predict iNTS disease economic burden at the global-level (second workstream). Four econometric models with multiple country-level indicators were compared based on a series of model diagnostic tests. The iNTS disease economic burden per episode was estimated to be lower in Africa than the rest of the world as expected. However, when estimating the proportion of iNTS disease economic burden out of total health expenditure, the relative burden appeared to be much higher in sSA compared to the rest of the world.

The stakeholders/policy survey workstream consists of an online survey and offline workshop. The aim of the online survey was to inform stakeholders of the need, demand for, and feasibility of a vaccine against iNTS disease. There were eight countries in Africa participated in this survey: Burkina Faso, DR Congo, Ethiopia, Ghana, Kenya, Malawi, Mozambique, and Nigeria. Key findings included the awareness of iNTS disease symptoms, iNTS disease risk factors, preferred vaccine type (bivalent vs. trivalent), and vaccine priority among iNTS disease and 11 other existing vaccines. The offline workshop was elaborated in more detail in the next session.

The assumptions and preliminary outcomes on the cost-effectiveness (CEA) were also covered. Among the three regions (Middle East & North Africa, sSA, and South Asia) compared, the bivalent vaccine would likely be cost-effective in sub-Saharan Africa but not in the other two regions. However, with the trivalent vaccine, vaccination would become cost-effective in South Asia as well. It was also mentioned that the preliminary outcomes were based on the comparison with the "do-nothing" strategy. Further modeling work is ongoing by the IVI-Policy and Economic Research (PER) and Swiss-Tropical and Public Health Institute (TPH) teams.

Lastly, the outlines of the broader societal benefits analyses (LSHTM and NYU) were presented. The validated broader societal benefits vaccine analysis framework was finalized. The equity analysis (or extended CEA) is ongoing – estimating burden and vaccine impact by wealth quintile based on iNTS disease/typhoid risk factors and socioeconomic status. In addition, literature review is underway to examine AMR burden and antibiotic use for iNTS disease.

The discussion covered relatively high economic burden in China due to the baseline population size being large and the potential consideration for including other NTS strains (in addition to *S.* Typhimurium and *S.* Enteritidis) in the future. It was also noted that while including more criteria into the model would be possible, we should be cautious of setting up too many assumptions given that vaccines don't even exist.

1.3 Outcomes from a workshop on country-level perspectives and priorities to guide the development of Invasive Non-Typhoid *Salmonella* (iNTS) vaccines - Anna-Lea Kahn, World Health Organization

Dr Kahn presented an overview and outcomes from a workshop aimed to gather country-level perspectives and priorities to inform the development of iNTS vaccines, highlighting the potential challenges and strategies for their implementation in high-burden settings. WHO and IVI co-organized this workshop, held in Accra, Ghana, in October 2024, involving delegations of between 2 and 5 immunization programme stakeholders from a total of 7 different African countries. The workshop utilized the WHO vaccine innovation framework, structured into four phases: assessing the perceived iNTS burden, evaluating vaccine attributes, considering use cases and decision-making processes, and exploring feasibility scenarios. Participants identified 16 barriers to iNTS vaccination across various categories, with five significant barriers—including insufficient funding, inadequate logistics, and challenges in reaching remote populations—identified that would impact vaccine delivery. A major issue was the lack of data for burden of iNTS due to difficulties diagnosing invasive non-typhoidal *Salmonella* infections (resulting in misattribution of illness to other pathogens) and lack of routine data collection, highlighting the need for better integration of iNTS reporting into national surveillance systems.

Participants discussed the potential introduction of an iNTS vaccine into existing immunization schedules. The concept of a bivalent or trivalent iNTS vaccine, particularly in combination with TCV, was favored due to logistical advantages like reduced injection numbers and optimized cold chain management. Participants were concerned about a possible standalone iNTS vaccine, especially if it would require creating new immunization touch points, thereby increasing costs and program implementation burden. The workshop outlined the practical aspects of iNTS vaccine introduction, emphasizing the need for robust evidence regarding safety, immunogenicity, and efficacy. Proposed combination vaccines, such as iNTS with TCV or *Shigella*, were seen as viable solutions to logistical challenges. While iNTS + TCV was preferred, discussions highlighted challenges around age compatibility; the timing of TCV administration at 9 months contrasts with the 6-month[2] target for iNTS. This may necessitate strong advocacy to realign immunization schedules. Several criteria were suggested for co-administration decisions, including disease burden and cost-effectiveness, with malaria at 6 months and measles-rubella at 9 months receiving favorable consideration for co-administration. However, the potential for vaccine hesitancy due to multiple injections in a single visit was also acknowledged.

Key takeaways from the workshop underscored critical data gaps regarding iNTS burden, which hinder vaccine prioritization. Participants favored combination vaccines for their logistical advantages, optimizing cold chain needs and minimizing injections. Essential attributes for effective implementation include low injection numbers, alignment with immunization schedules, safety, and cost-effectiveness. Addressing challenges such as cold chain capacity, workforce training, and funding is crucial for successful vaccine delivery. Ultimately, robust evidence on disease burden and vaccine characteristics is necessary for informed decision-making by policymakers. Coordinated efforts are needed to bridge gaps and overcome barriers to the development and implementation of iNTS vaccines.

[2] TCVs are already licensed for use from 6 months of age.

1.4 The need for an iNTS point-of-care rapid diagnostic test - Gianluca Breghi, Fondazione Achille Sclavo

Dr. Breghi presented the background and critical need for a sensitive and specific diagnostic available for use in low resource settings. In recent years there has been substantial progress towards understanding iNTS epidemiology, characterization, burden and in developing and advancing iNTS vaccine candidates. Despite these advances in the field, there is still limited understanding of iNTS disease transmission dynamics and seroepidemiology, especially for clades restricted in distribution to sSA, which cause tens of thousands of deaths every year especially in children under 5.

iNTS disease is overshadowed and neglected in areas with the highest <5 mortality and is penalized by serious resource shortcomings, including lack of a rapid diagnosis, limited treatment options, and limited understanding of disease transmission and evolution. Moreover, the disease affects the most vulnerable: children under 5 in sSA, also affected by malaria, HIV, anaemia, malnutrition and high burden of HIV.

The need for a reliable point-of-care Rapid Diagnostic Test (POC RDT) has been reported in the literature and in WHO expert meetings. Due to the non-specific nature of the disease and pre-existing morbidities/conditions, iNTS disease diagnosis is delayed, evolving into a disease rapidly fatal within 48 hours, with case fatality rates up to 28-35% in sSA. Blood culture is the current means for diagnosing iNTS, however, blood culture has low sensitivity (50 to 60%), slow turnaround time, and access to blood cultures is generally limited in LMICs to larger referral hospitals. Misdiagnosis and mistreatment of iNTS Disease with inappropriate antibiotics has also caused a sharp increase in the number of iNTS disease cases due to MDR+XDR strains across sSA.

In addition to being needed for appropriate diagnosis and clinical management of iNTS in patients, a reliable diagnostic (preferably POC) is required for surveillance purposes to define burden of disease and eventually measure vaccine effectiveness and impact.

A consortium of research institutions with over a decade of experience in iNTS research and with integrated areas of expertise emerged out of the EU-funded Vacc-iNTS Project. These collaborators share the view that a POC RDT is needed and are committed to seek funding and pursue development of a diagnostic to fill this critical gap.

Session 2: Updates on iNTS Vaccine Clinical Studies – Sam Kariuki, Chair

2.1 Progress towards filling the gaps identified for iNTS vaccine development – Sam Kariuki, Kenya Medical Research Institute (KEMRI)

Dr. Sam Kariuki emphasized the need for safe, effective and affordable invasive nontyphoidal *Salmonella* (iNTS) vaccines, either as stand-alone vaccines or in regional combinations tailored to epidemiology. Trivalent vaccines (bivalent iNTS in combination with typhoid conjugate vaccine) for Africa and bivalent typhoid combined with Paratyphoid A vaccine for Asia were identified as priorities; while quadrivalent pan-*Salmonella* combinations were highlighted as less likely due to barriers of cost, complexity, and applicability of all components globally. Challenges to iNTS combination vaccine development include cost of goods, immunologic interference, regulatory hurdles, sparsity of burden data, age administration and (applicable to all new vaccines in development) the overcrowded EPI immunization schedule. Diagnostic limitations remain a key

barrier for iNTS clinical management, epidemiologic research, and product development. Recent data highlights waning typhoid immunity 3-4 years post-TCV vaccination in infancy, emphasizing the need for additional data and consideration for potential booster doses in preschool years and longer intervals between doses to enhance immunogenicity. Therefore, for both TCV and iNTS not only immunogenicity and efficacy but longevity of immune persistence and duration protection needs to be determined (noting that the epidemiology suggests that this is not an issue for iNTS vaccines). Dr. Kariuki discussed the role of CHIM as supportive but highlighted that Phase 3 efficacy trials in children aged 6 months to 5 years (little iNTS disease between 3 and 5 years of age) with sample size under 10,000 have been deemed feasible and will likely be required for regulatory approval. Perhaps, an efficacy trial in 6 months to 3 years in high disease burden setting could be targeted to start with and 3-5 years age group could be addressed post licensure. However, broader age range 6 months to 5 years strategy is preferred by manufacturers, regulators and policy makers. As per discussions with regulators in Kigali, one large efficacy trial should be sufficient Assay standardization, clear regulatory pathways, and market acceptability were identified as critical for vaccine development. Regulatory pathway to licensure is unclear, particularly in countries of introduction in sSA where disease is endemic, therefore there is a need for regulatory engagement for guidance to proposed iNTS clinical development path and to facilitate discussion with all the key players across countries, regions, vaccine manufacturers with WHO pre-qualification as the target. A consortium-led approach like TyVac was proposed to streamline multi-candidate trials, to address key research gaps, and to support manufacturers with burden data and demand forecasting. Integrating vaccination with WASH improvements was highlighted as essential for long-term disease control. Learning lessons from TCV introduction and Covid-19, there is a need to address vaccine hesitancy by engaging health care workers, EPI immunization program officers, ensuring awareness and education about iNTS disease. Being able to forecast the correct market demand is a very important aspect for countries that need the vaccine and manufacturers to minimize the supply and demand gaps. In conclusion, Dr. Kariuki highlighted the challenges for countries as they graduate from Gavi and the ultimate, long-term need for improvements in WASH, highlighting a holistic approach with vaccine development as one component of the whole.

Discussion highlighted the importance of understanding the dynamics of immune responses to vaccine dosing schedules. In early infancy, two doses are likely necessary based on experience with other conjugate vaccines, such as meningococcal and pneumococcal vaccines. Administering two doses close together in infancy can boost immunity effectively, while for vaccines given after one year of age, spacing doses a few years apart appears to enhance the immune response. Data from clinical studies with TCV in Nepal indicate that two doses of TCV given together yield comparable immunogenicity, but findings from Malawi suggest that longer intervals between TCV doses produce a stronger immune response. To address these gaps, attendees agreed that a consortium modeled on the TyVac initiative to facilitate clinical studies with multiple vaccine candidates would be important, as head-to-head trials by individual developers are unlikely. Efforts in assay standardization are underway, with the Gates Foundation funding collaborative work between GVGH and NIBSC to leverage Phase 1 trial specimens to develop a pan-*Salmonella* International Standard serum (ISS). This will be suitable for determining serum IgG levels to Vi of serovar Typhi and O-antigens of serovars Typhi, Paratypi A, Typhimurium and Enteritidis. This International Standard Serum will be submitted to the WHO Expert Committee on Biological Standardization for approval for global application.

2.2 Safety and immunogenicity of trivalent *Salmonella* conjugate vaccines (TSCV) in the Phase 1 trials of US Adults – Wilbur Chen, Center for Vaccine Development and Global Health, University of Maryland (CVD)

A Phase 1 study of the Center for Vaccine Development and Global Health, University of Maryland School of Medicine / Bharat Biotech (UMD/BBIL) Trivalent *Salmonella* Conjugate Vaccine (TSCV) conducted at the University of Maryland demonstrated promising safety and immunogenicity results in healthy U.S. adults (22 participants), despite interruptions caused by the COVID-19 pandemic. Preliminary data showed no significant safety concerns for either of the two doses tested. ELISA results indicated 100% seroconversion for all three primary antigens among 20 participants in the first study, with antibody responses persisting for over a year after a single dose. Memory B-cell responses and functional antibody activity, including neutrophil-dependent memory phagocytosis, were observed, along with evidence of gut mucosal immunity.

A Phase 1/2a study included 80 additional participants who received either the half-strength (12.5μg), target full strength (25μg), or dilutional half-strength dose (bridge to the Phase 1 first in human trial) doses, confirming robust antibody and antibody-secreting cell (ASC) responses through six months following single dose vaccination.

The discussion highlighted that no significant difference was observed between the half-strength (HS) and full-strength (FS) doses of the vaccine in healthy U.S. adults, but this is not the target population, so no dose decisions were made based on these results. Further studies in children in sSA are needed to guide dose selection. Baseline antibody titers were evaluated, but some variability ("noise") in the data may reflect cross-reactive responses or prior lifetime exposure to other nontyphoidal *Salmonella* strains. The adult study results provided sufficient positive safety and immunogenicity data to proceed with further clinical development.

2.3 Summary of initial steps of a Phase 2 age-descending trial to novel TSCV (WT and BBIL) in sub-Saharan Africa (Mali, Kenya, Mozambique) - Myron Levine, CVD

The Phase 2 age de-escalation study of the trivalent *Salmonella* conjugate vaccine (TSCV) aimed to determine optimal dosing, safety, and immunogenicity. The study evaluated four doses across a stepwise age de-escalation trial, starting with 40 adult participants and expanding to 1,256 subjects, including infants (Step 1). In Step 2 toddlers 12-16 months of age (N=120) and older infants 8-11 months of age (N=120) were randomly allocated to receive one of the four investigational products (Full-strength TSCV (25 μg of each PS), half-strength TSCV (12.5 μg), Typbar-TCV, or placebo.

After DSMB review of data from Step 2, the DSMB recommended proceeding to Step 3. Step 3 involved enrolling infants ~16-18 weeks of age (N=180) and infants 12-14 weeks of age (N=180). Step 1-3 in *Salmonella* Conjugates CVD 3000 CVD involves administration of a single dose.

In Step 4, priming doses were administered at 12–18 weeks, followed by a single dose booster administered at varying intervals (9, 12, or 15–17 months) in 465 subjects. Epidemiologic disease burden data collected over the years in the Mali site showed that case fatality ratios (CFRs) were notably high for *S.* Enteritidis (~30%) disease, with CFRs doubling those of *S*. Typhimurium across all age groups. While *S*. Enteritidis elicited strong immune responses in all age groups, responses to *S*. Typhimurium were weaker, particularly in young infants, which might suggest a suppressive role of maternal antibodies.

Immunogenicity was assessed using a mesoscale discovery (MSD) multiplex immunoassay, which demonstrated high seroconversion rates for the typhoid vaccine component across all

ages. Functional assays, conducted in collaboration with Galit Alter, indicated killing activity in children despite low fold rises in antibody levels. However, seroconversion rates for *S*. Typhimurium were minimal in young infants.

From the perspective of the safety data, the Data Safety Monitoring Board (DSMB) approved all three products to proceed to Step 4 as no coded product presented a safety signal indicating concern.

Maternal antibody interference and factors like breastfeeding, malnutrition, and endemic diseases like malaria were considered in the study. The results emphasize the need for additional functional and T-cell response analyses to address immunologic challenges in infants.

The discussion highlighted the value of functional assays in evaluating vaccine responses, particularly their ability to detect functional activity even with low antibody fold rises. A collaboration with Galit Alter has shown killing activity in children, though these findings remain unpublished. Maternal antibody interference was noted, with evidence suggesting that higher maternal antibody levels correlate with lower antibody concentrations in infants. Breast milk interference, involving antibodies from maternal milk and systemic maternal antibodies, was also discussed. This aligns with observations that baseline antibody levels decrease with decreasing infant age. While statistical analyses of antibody interference for *S*. Typhimurium have been conducted, equivalent data for *S.* Enteritidis are pending. Interestingly, the reverse was observed with flagellin antigens (FliCs).

In Step 4 of the trial, the dosing schedule includes a primary dose at 12–18 weeks and a booster at 9, 12, or 15 months. It is not known if the initial TSCV dose administered to infants 12-18 weeks of age immunologically primes them to mount a rapid anamnestic immune response should the infant encounter an iNTS pathogen. This raised theoretical concerns about a potential gap in protection between doses, as well as whether administering a second dose earlier might elicit a stronger amnestic response. Although the seroconversion rate of serum IgG anti *S*. Typhimurium COPS antibody in recipients of FS-TSCV and HS-TSCV was not discernable in infants 12-14 weeks and 16-18 weeks of age in the face of high titers of maternal antibody, in infants 8-11 months, 12-16 months, toddlers 16-23 months, and pre-school children 24-59 months of age the seroconversion rates were circa 50-100%. It was suggested that a larger sample size might reveal a higher seroconversion rate. It was recommended to evaluate T-cell responses to better understand the impact of breastfeeding and mixed feeding practices, as young infants are exclusively breastfed, while older children receive mixed feeding. The malaria burden in the trial areas and its potential interference were also considered. Children in the trial were tested for malaria using rapid diagnostic tests (RDTs) and treated for malaria even if asymptomatic before trial entry. The trial's timing aligns with reduced invasive non-typhoidal *Salmonella* (iNTS) burden and ongoing vaccine and control measures, which could influence results. The inclusion of functional assays or serum bactericidal assays (SBAs) to further evaluate vaccine responses was confirmed.

2.4 Development of iNTS conjugate and preclinical study for a novel trivalent iNTS/typhoid vaccine – So Jung An, IVI

Dr. An described the development process for the IVI trivalent iNTS/TCV vaccine. The bivalent invasive non-typhoidal *Salmonella* (iNTS) conjugate component was developed before incorporating the typhoid conjugate vaccine (TCV). Strains selected for the vaccine were clinical isolates from Ghana (*S*. Typhimurium) and Guinea Bissau (*S.* Enteritidis), obtained from 2011

blood culture samples. Scalable O-specific polysaccharide (OSP) production, optimal carrier protein identification, and conjugation chemistry were key focus areas in early development, with CDAP conjugation achieving yields of OSP conjugation above 60%. Proof-of-concept studies demonstrated robust immunogenicity of *S.* Enteritidis OSP (SE-OSP) and *S.* Typhimurium OSP (ST-OSP) conjugates in animal models (species and age), with diphtheria toxoid (DT) selected as the carrier protein. Process optimization improved OSP conjugate molecular weight and production yields, further enhancing immunogenicity. The trivalent formulation, including the iNTS conjugates and TCV, showed strong immune responses in both mice and rats. Following process optimization and successful proof-of-concept studies, the manufacturing process for the trivalent vaccine was transferred to SK bioscience, which produced materials for toxicology studies. These studies were completed without any adverse effects or test article-related issues.

Stress-testing of the vaccine has included accelerated temperature studies to evaluate stability, but transportation-related stress conditions have not yet been assessed. Critical data on free capsular polysaccharide (CPS), important for assessing vaccine stability, is not currently available. While initial tests were conducted at IVI, updates from SK bioscience are still pending.

2.5 Safety and Immunogenicity of a bivalent GMMA-based iNTS vaccine in healthy adults in the UK, Phase 1 - Leila Godfrey, Oxford Vaccine Group

SALVO was the Phase 1 first in human, double-blind, single-centre, randomised, placebo-controlled clinical trial conducted in healthy UK adults by the University of Oxford. Participants received placebo, low-dose, or high-dose groups of the iNTS-GMMA vaccine candidate at 0, 2, and 6 months. A total of 31 participants were enrolled, with 12, 4, and 15 in the placebo, low dose, and high dose, respectively. Participants in all groups had mild and transient systemic symptoms following each dose; most local adverse events were mild. Specifically, safety data from the high-dose group showed mild, transient systemic symptoms such as fever, malaise, and chills; one participant in the high-dose group had significant local swelling and neutropenia. Humoral response against S. Typhimurium and S. Enteritidis O-antigen peaked at day 28 following full dose, and antibodies persisted for one-year post-vaccination for both S. Typhimurium and S. Enteritidis antigens. Additionally, functional antibody activity, as measured by the SBA (serum bactericidal assay), increased following first vaccination. This study was performed as part of the Vacc-iNTS consortium.

The discussion highlighted several key areas for further investigation. One topic was the potential contribution of IgM to the bactericidal activity, which might explain the observed boost in response, with antibody functionality being considered critical and to be explored further. Reactogenicity data in this small sample size indicated more redness at the injection site in the low-dose group (4 participants) compared to the high-dose group, though this remains inconclusive due to small participant numbers. Additionally, while the data might suggest that a single dose could be sufficient, the small sample size in healthy adults in the UK (not the target population) limits definitive conclusions. The idea of exploring two-dose priming was raised, and it was emphasized that antibody functionality, rather than titre alone, should be further examined to understand the findings more fully.

2.6 GMMA-based vaccine against iNTS disease and interim safety data from a phase 1/2 a of trivalent iNTS-TCV vaccine in healthy European and African adults – Ashwani Kumar Arora, GSK Vaccine Institute for Global Health (GVGH)

The ongoing Phase 1/2a, observer-blind, randomized, controlled, two-stage, multi-country study is evaluating the safety, reactogenicity, and immune response of the trivalent vaccine against invasive nontyphoidal Salmonella (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). An age de-escalation study with the bivalent iNTS-GMMA is also ongoing in Ghana under the PEDVAC-iNTS consortium. Preliminary safety data from the iNTS-TCV Phase 1/2a in adults indicate no serious adverse events (AEs) related to vaccination and no AEs leading to withdrawal. Solicited events within 7 days post-vaccination were predominantly mild to moderate, with pain being the most common. Unsolicited AEs within 28 days also showed no safety concerns. Severe events considered related to vaccination were reported in 4 participants (3.0%) in the full-dose group, but all resolved. Safety laboratory test results were generally within reference ranges and non-clinically significant. The overall benefit-risk profile remains favorable, with no safety issues preventing further development.

The discussion covered several key points. These data confirm that GMMA and conjugate iNTS vaccines could have similar safety profiles. To assess potential interference between the Typhoid Conjugate Vaccine (TCV) and the iNTS-GMMA vaccine components in the iNTS-TCV combination vaccine, one group of participants was administered with the TCV and the iNTS-GMMA vaccines in separate arms in the Phase 1/2a. Immunogenicity data will be presented as soon as available for a conference presentation. The next step will be a Phase 2a that will involve evaluating non-inferiority of the TCV when combined in the iNTS-TCV vaccine.

Session 3: Potential iNTS Combination Vaccines – Bill Hausdorff (PATH), Chair

3.1 The importance of combination vaccines and considerations for global health – Bill Hausdorff, PATH

Dr. Hausdorff highlighted the need for combination vaccines, including the already crowded immunization schedule, challenges with adding vaccinations and program implementation in resource-limited settings, vaccine hesitancy and uptake concerns globally, as well as multiple priority pathogens with vaccine candidates in development (and others without vaccine candidates in the pipeline); discussed technical and scientific, policy, valuation, and regulatory barriers to combination vaccine development; Possible approaches were discussed, such as combination vaccines to address syndromic diseases with similar presentations (i.e., malaria and iNTS), combinations of vaccines to be given at the same or similar timepoints (i.e., Diphtheria, Pertussis, Tetanus, Hepatitis B and Hib pentavalent vaccine), combining new vaccine candidates with existing licensed vaccines, and combinations of new vaccine candidates. Regulators may be hesitant to accept lower efficacy parameters (such as wider confidence intervals) or even the absence of individual clinical efficacy data for each component, suggesting the need to introduce more flexibility in Phase 3 trial parameters. The complexity of combining different antigens and serotypes must be considered, as multiple components could lead to increased reactogenicity as well as decreased immunogenicity (and potentially efficacy) of specific components. Clear communication around the design and rationale for combination vaccines is critical, as once licensed, they may replace other vaccines, leading to challenges in

acceptability and increased reactogenicity. Dr. Hausdorff outlined the Combination Vaccine Policy Framework Project being conducted in partnership with WHO IVB and selected RITAG and NITAG members, and designed to develop an analytical framework that national, regional, and/or global vaccine advisory committees might use to prioritize future combination vaccine formulations. A key activity will entail devising and refining health and economic metrics appropriate for combination vaccines.

The discussion focused on the complexities of developing combination vaccines, which require careful prioritization across multiple levels. A clinical and epidemiologically attractive approach could target pathogens that each address a single clinical syndrome (e.g., acute otitis media), while alternative combinations may group vaccines targeting diverse diseases but which all may be administered at the same immunization visit. A successful combination must demonstrate clear value, as seen with pneumococcal conjugate vaccines (PCV), highly complex mono-pathogen combinations whose value comes from the aggregate protection against multiple individual serotypes. However, regulatory challenges persist, particularly in how to provide regulators with sufficient levels of confidence that each combination vaccine component has significant clinical efficacy. Combining vaccines may also involve efficacy trade-offs, raising questions about how to value these and manage associated risks. The RTS, S malaria vaccine's impact on antibiotic use may illustrate the potential value of combining vaccines targeting diseases that may present similarly, as combination of iNTS and malaria might result in additive or synergistic effects on antimicrobial use. The market history of acellular and whole-cell pertussis-containing pentavalent vaccines highlights the need for tailoring vaccine formulations to the specific needs of different populations; conversely, there is a growing interest in combinations comprising COVID-19, RSV, and influenza vaccines for the elderly in high-income countries. For LMICs, focus remains on vaccines for young children, such as iNTS and malaria. Given the challenges of modifying vaccines post-licensure, decisions on combination vaccines and co-administration schedules must be made thoughtfully to ensure alignment with target age groups and that sufficient protection is provided when it is most needed for each pathogen.

3.2 Epidemiological and target Population consideration for iNTS combination vaccines – John Crump, University of Otago

Dr. Crump's presentation covered several aspects of Nontyphoidal *Salmonella* (NTS), including invasive (iNTS) disease and diarrheal (dNTS) disease. Key points included the substantial burden of iNTS disease in children under 5 years, where deaths are estimated to be more than four times greater than those from paratyphoid and typhoid fevers combined. Malaria was noted to potentially contribute to up to 50% of the risk for iNTS disease, although data remain insufficient. The RTS,S malaria vaccine trials showed a non-significant reduction in iNTS bacteremia of approximately 15%. It was emphasized that for vaccine development and policy making, age-occurrence data including that available from large, long-term surveillance studies should be plotted by month of age for children under 5 years, since incidence data from incidence studies and the control arms of vaccine trials present data in broad age groups (e.g., two-year periods) with wide uncertainty bounds that seldom provide the needed granularity. The presentation also covered host risk factors, reservoirs, important data gaps on source and modes of transmission, and antimicrobial resistance, prevalence of NTS serogroups and serovars isolated from normally sterile sites, and the global burden of disease. Regarding modelled data on global burden of iNTS and typhoid fever by age, it was pointed out that the peak of iNTS disease incidence occurs earlier in life than the peak of typhoid fever incidence, but that both curves are further to the left than might be expected from available primary epidemiologic data.

The challenges of generating a clear picture of iNTS age-incidence from the small unadjusted case numbers often found in hybrid surveillance were noted, including the often overlapping uncertainty bounds and lack of detailed reporting by age make it difficult to draw definitive conclusions. However, age-specific occurrence data by month of age under 5 years including from large, long-term surveillance studies would offer another perspective on iNTS disease age-occurrence and should be collated and plotted. The subsequent discussion focused on the limited impact of malaria vaccines, such as RTS,S, on reducing invasive nontyphoidal *Salmonella* (iNTS) incidence, with estimates showing a non-significant effect of approximately 15%. The IVI EPIC study is underway in Kisantu, Democratic Republic of Congo, and will evaluate the impact of the R21 malaria vaccine on iNTS infection incidence, was also highlighted.

3.3 Considerations and challenges of potential iNTS combination vaccines - Chris Gill, Bill and Melinda Gates Foundation

Dr. Gill set the stage for discussions on specific candidate bacterial enteric combination vaccines. He outlined the most frequently proposed enteric combinations and highlighted that these generally share TCV as the central component, or "tugboat". The commonly proposed combinations include TCV with Paratyphoid A (PTA), TCV with 4-valent *Shigella*, TCV with 2-valent nontyphoidal *Salmonella* (NTS), TCV with Paratyphoid A (PTA), 4-valent *Shigella*, and 2-valent nontyphoidal *Salmonella* (NTS). While TCV was highlighted as a strong “tug” candidate for any of these, challenges such as PTA’s rarity, lack of correlates protection (or even immune markers, aside from for *S. sonnei and S. flexneri* 2a), and the risks of extrapolating efficacy from CHIM studies in healthy adults to infants remain prominent barriers to development of any of these combination vaccines. The complexity of a comprehensive enteric combination vaccine of TCV, PTA, 2v NTS, and 4v *Shigella* is a major barrier, however, it has potential appeal to ministries of health due to its ability to address combined burden of disease for these pathogens. Key questions arose about the sufficiency of efficacy evidence and whether these determinations hinge on the leading vaccine. The conclusions drawn emphasized a pivotal shift away from single-pathogen vaccines in pediatrics, with expectations that broad adoption will necessitate strategic combinations. A regimen of 2-3 immunizations per visit was deemed reasonable, acknowledging the significant number of disability-adjusted life years (DALYs) still to be prevented, and underscoring the need for an aggressive bundling approach. However, uncertainties around regulatory and marketing pathways remain, alongside the untapped potential of pneumococcal conjugate vaccines as platforms for incorporating non-pneumococcal antigens. The need to challenge the status quo and maintain flexibility in tackling these complex issues was a resonant theme throughout the presentation.

The feasibility of combining Pneumococcal Conjugate Vaccines (PCV) with iNTS is considered unlikely, mainly due to concerns over immunogenicity loss for PCV serotypes when adding components, which could undermine effectiveness. Major manufacturers are hesitant to take the risk, highlighting the need for mid-sized manufacturers from LMICs to pursue this avenue. While regulators are open to accepting a reduction in immunogenicity for a broader serotype coverage in PCVs, challenges arise when seeking to demonstrate efficacy for combined vaccines, particularly if individual efficacy data for components is lacking. Post-licensure studies may be required to confirm that each vaccine component contributes positively to the combination. Additionally, regulators require clear guidance on acceptable margins and the potential impacts of wider coverage versus individual protection levels, along with a solid understanding of the public health benefits of such combinations, even if they offer lower per-component efficacy.

3.4 Scorecard: iNTS + TCV – Melita Gordon, University of Liverpool

Dr. Gordon highlighted the increased likelihood of implementing a regional vaccine in response to the high burden of iNTS and typhoid disease among young children, including infants, in sub-Saharan Africa. She reviewed the very high likelihood of technical and regulatory success for this combination, since TCV is already being rolled out across Africa through catch up campaigns and routine immunization, and several candidate iNTS/TCV combinations are already in phase 1 and 2 clinical trials, with early results now becoming available (see earlier sessions, described above).

Uncertainties in relation to age distribution for both iNTS and typhoid complicates the understanding of their developmental roadmap, with considerations needed regarding vaccination timing particularly around the age of 6 months. Due to a lack of disaggregated data in many modelled estimates, assessing peak burden months in early life is challenging. She noted that the modelled data in the global burden of iNTS disease estimate from IHME appears to indicate a high level of neonatal disease but critically lacks resolution in the early months of life and may significantly over-estimate neonatal disease. Using examples of locations with granular age—incidence or case-count data that can be disaggregated by month, disease incidence rises markedly from around 6 months onwards in the DRC and Malawi, and at 9 months in Mozambique, and remains significant until the age of 4-5 years. Typhoid fever is common in pre-school children, and modeling indicates that early-life TCV vaccination could be beneficial. Improved resolution around age-incidence for iNTS and typhoid in the early months of life would be helpful.

It was noted that combined iNTS/TCV first given at the age of 6 months would meet the preferred vaccine characteristics for iNTS, and minimize concerns about immuno-bridging for TCV, since single dose TCV is already recommended by WHO from 6 months onwards. Emerging concerns about waning immunogenicity and efficacy in young children in Asia, vaccinated with a single dose of TCV, might indicate a need for a booster, perhaps in the second year of life or later. There may be a continued need for monovalent TCV for catch-up vaccination campaigns among older school-age children in countries wishing to introduce TCV, emphasizing the importance of cost-effectiveness analyses.

The discussion also addressed the concept of "force of infection," where higher bacterial exposure necessitates greater immunity. Natural immunity to nontyphoidal *Salmonella* is relatively strong, suggesting that early-life boosters (and priming through natural exposure to noninvasive cross-protective serovars) could enhance protection, while developing natural immunity to typhoid is less effective and requires multiple infections/exposures.

In summary, taken together, the scorecard indicated a very high likelihood of technical and regulatory success, but some caution was expressed in relation to the optimum age for first dose and booster scheduling.

3.5 Scorecard: iNTS + Shigella – Calman MacLennan, Independent Expert

Dr. MacLennan discussed the case for a combination iNTS/*Shigella* vaccine as a strong candidate for a syndromic combination vaccine against diarrheal/enteric disease. The burdens of disease due to NTS and *Shigella* are both significant according to IHME Global Burden of Disease (GBD) estimates. GBD 2019 estimates indicate that deaths due to *Shigella* and NTS (both diarrhea and invasive disease) surpass those for rotavirus, both for all ages (289,000 compared with 235,000) and among children under 5 years (183,000 compared with 152,000). While shigellosis has been

considered a particular problem during the second year of life, recent unpublished data from the WHO Global Pediatric Diarrhea Survey demonstrate an increasing proportion of shigellosis in children under 1 year of age such that half of cases in the first two years of life now occur in children under one year. Indeed, the WHO iNTS and *Shigella* vaccine Preferred Product Characteristics reports propose children from 6 months to 36 months as the target population for both diseases with primary immunization schedules of 1 or 2 doses. Regional approval in Africa for a *Shigella*/iNTS combination vaccine is supported by the high incidence of both iNTS disease and shigellosis in the continent, according to IHME 2021 GBD data. Moreover, if all-cause NTS disease (diarrhea and invasive disease) is considered, there is also a good case for such a combination vaccine in South Asia according to these data.

Probability of technical and regulatory success (PTRS) for an iNTS/*Shigella* combination vaccine is supported by platform compatibility with the most advanced standalone iNTS and *Shigella* candidates adopting the same parenterally administered technologies – glycoconjugates, the related 'multi-antigen presentation system' (MAPS) and outer membrane vesicles (also referred to as 'GMMA'). Formulation and preclinical animal studies at the Boston Children's Hospital indicate that such combinations are technically feasible, safe and immunogenic (unpublished findings, shared with permission of R Malley & Y Lu).

Moreover, several vaccine developers/manufacturers have both iNTS and *Shigella* vaccines in clinical development. Although, as for iNTS, there is as yet no licensed *Shigella* vaccine, there are strong vaccine pipelines for both diseases, with multiple candidates in Phase 2 studies. Phase 3 efficacy trials in high-endemic areas are the likely initial pathway to licensure for both iNTS and *Shigella* vaccines. However, PTRS is increase for *Shigella* vaccines by the demonstrated efficacy of a prototype *Shigella sonnei* vaccine in two phase 3 studies. Furthermore, these studies led to the identification of serum O-antigen IgG as a correlate of protection against shigellosis. A bivalent *Shigella* vaccine is currently in Phase 3 studies and could confirm this correlate of protection in the target population of LMIC infants and young children, facilitating the broader licensure of *Shigella* vaccines.

To finish, MacLennan pointed out the outcome of the Gavi Vaccine Investment Strategy (VIS) 2024 exercise where *Shigella* vaccines were shortlisted but not funded, with a strong recommendation from the Gavi steering committee that *Shigella* vaccines be incorporated into combination vaccines. Such a conclusion was also reached as part of the PATH-led Full Value of Vaccines Assessment for *Shigella* vaccines. Therefore, as for iNTS vaccines, there is strong political will for *Shigella* vaccines to be components of multi-pathogen combination vaccines.

Participants discussed the timing of protection, with the requirement for iNTS protection starting at 6 months, and concerns emerged about whether a single dose would provide adequate immunity. The need for careful assessment of clinical trial results was emphasized, with discussions on the implications if *Shigella* were to partner with a different entity in the future. Overall, the collaborative effort among developers in the field and the need for further research and clinical trials to solidify vaccine strategies were underscored.

3.6 Scorecard: iNTS + injectable rotavirus – Duncan Steele, Bill and Melinda Gates Foundation

Dr. Steele emphasized that, from his perspective, a combination vaccine for injectable rotavirus and iNTS will not be prioritized. Challenges with dose regimens and schedules were cited, as the rotavirus vaccination schedule occurs earlier than the currently recommended schedule for iNTS

in the Preferred Product Characteristics (PPC). Given the need for early and high protection against rotavirus, aligning vaccination ages presents an opportunity, especially with a potential rotavirus booster dose at 9 (months currently under evaluation). The discussion also highlighted the necessity of involving nutrition specialists to address the impact of foodborne illnesses on diarrhea mortality rates and the potential for higher Disability-Adjusted Life Years (DALY) if malnutrition is considered. Transitioning from oral to injectable rotavirus vaccines may complicate programmatic and cold chain logistics, although an injectable formulation with better efficacy could prove beneficial. Despite good uptake of oral rotavirus vaccines, rotavirus continues to be a leading cause of diarrhea-related hospitalizations and deaths, with vaccine efficacy varying significantly between high and low mortality settings. It was noted that while iNTS remains a regional concern, rotavirus vaccines could be universally applied. The need for three doses to ensure protection during the first year of life and up to age two was emphasized. Comments from participants reinforced the importance of ensuring protection against iNTS by 6 months and acknowledged the significant contributions of food safety to NTS diarrheal components. There was also recognition of the success of rotavirus vaccines in reducing global mortality rates, despite recent challenges, particularly in high-burden countries that have only recently introduced vaccination programs. Ongoing studies in Malawi were highlighted as crucial for informing policies and improving vaccine strategies, while concerns were raised about the implications for programs and for vaccine uptake and hesitance of reverting to injectable formulations instead of oral formulations.

3.7 Roundtable Discussions and Rankings: iNTS combination vaccines

Following the discussion on combination vaccines and the arguments and scorecards presented by Drs. Gordon, MacLennan, and Steele, a virtual survey was conducted using Mentimeter to solicit virtual feedback regarding proposed combinations. The meeting revealed substantial skepticism amongst participants regarding successful development of a standalone iNTS vaccine, with 88% of participants expressing doubts that a standalone iNTS vaccine would be successful (Figure 1). Preferences leaned towards combinations of iNTS with *Shigella* (21 votes) and iNTS with Typhoid Conjugate Vaccine (TCV) (14 votes). Participants suggested exploring additional combinations, such as pentavalent vaccines and various formulations with Hib, PCV, malaria, meningococcus, IPV, and potentially *Shigella*/TCV/iNTS. Advocacy emerged for a pan-salmonella plus *Shigella* combination (8-valent). Combination with PCV was again discussed, noting that incorporating iNTS with PCV would not necessarily increase the number of shots, given PCV's global implementation status. However, concerns were raised about the likelihood of major manufacturers entering this combination vaccine development space with vaccines that they have already licensed (potentially putting the existing licensed vaccines at risk) while smaller companies may have more interest. Regional vaccines could include diarrheal NTS as endpoints to support a global formulation, though the additional workload and efficacy demonstration pose challenges. The discussion highlighted the importance of starting with low-risk, high-certainty combinations and acknowledged the remaining timeline for developing a *Shigella* vaccine with an earliest *Shigella* vaccine licensure in 2029. There was consensus that adding more shots to vaccination schedules would be problematic, and data gaps on TCV's immunogenicity remain a significant concern. The communication challenges of introducing a regional pathogen like iNTS alongside universal vaccines like PCV were noted, emphasizing that large manufacturers are unlikely to support combinations due to potential drops in immunogenicity which have already been observed with increasing numbers of serotypes in the latest generation PCVs. Finally, integrating diarrheal NTS into endpoints with aspirations for a

global formulation raises questions about regulatory considerations, feasibility, and the prospects for combining multiple antigens to demonstrate efficacy.

Session 4: Clinical Development and Regulatory Pathway considerations for iNTS combination vaccines - Richard Isbrucker (Health Canada), Chair

PDVAC endorsed regulatory considerations for iNTS vaccines. These are now undergoing WHO publication and are provided below:

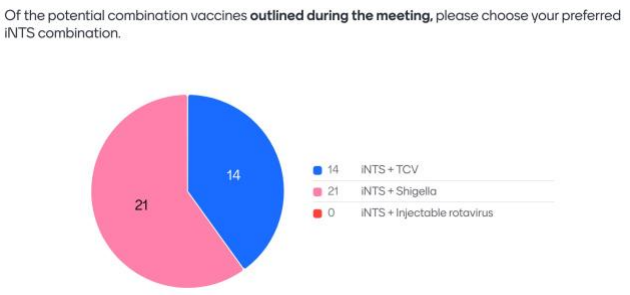
1. The WHO iNTS PPC indicates the target population is infants and young children 6 to 36 months of age though there is uncertainty particularly regarding the most appropriate lower age bound. One potential combination vaccine includes iNTS and TCV. TCV is not currently licensed in infants below 6 months. Could the regulatory community opine on whether non-inferiority for the TCV component (in the iNTS/TCV combination vaccine) should be determined in infants below 6 months given the vaccine is not licensed below 6 months?

2. If an iNTS vaccine was to be combined with a licensed product (for example TCV), could the regulatory community discuss whether the safety of the standalone iNTS vaccine would need to be established prior to demonstrating the safety, immunogenicity and efficacy of the combination iNTS + TCV vaccine in phase 1, phase 2a, and phase 3 studies, respectively.

3. A combination vaccine consisting of Typhi/Paratyphi/TCV/iNTS could potentially provide necessary coverage in Asia and Africa. However, each population would be immunized with a vaccine component not specific to their global region. Can the regulatory community provide insights into what benefit-risk analysis would be required to for market authorization/licensure?

4. What would be the key design considerations of a clinical study to determine vaccine efficacy for licensure?

4.1 Rationale for a Consortium Field Trial Approach to Assess Efficacy of iNTS Vaccine Candidates – Myron Levine, CVD



Dr. Levine highlighted historic WHO-sponsored and led trials comparing typhoid (parenteral and oral) and for SARS-CoV-2 vaccines and proposed a similar approach for evaluating several iNTS vaccines in the same field trial for optimal and efficient evaluation of candidates. Participants agreed on the necessity of developing serological assays to support the evaluation of iNTS candidates. However, the challenge of proposing a common protocol with standardized endpoints and assays was acknowledged, as each manufacturer sponsors their own products and has specific preferences. This makes it difficult to align evaluation of candidates under a single common platform protocol or to enforce common shared protocol elements. Despite the recognized support for a consortium approach that could create common protocols and a platform for collaboration and evaluation of candidates, participants noted that the complexities involved would make such an initiative very

challenging. Many attendees expressed optimism about the feasibility of forming a consortium but emphasized the significant hurdles that would need to be addressed.

4.2 Regulatory considerations for iNTS combination vaccines – Marco Cavaleri, European Medicines Association (EMA)

Dr. Cavaleri addressed the challenges associated with combining existing vaccines, particularly focusing on the major regulatory hurdles related to immune interference. Regulators do not typically object to combinations unless issues arise, but in the case of Pneumococcal Conjugate Vaccine (PCV), a decrease in immunogenicity for individual serotypes could become problematic if overall immunogenicity falls below protective thresholds. There is uncertainty on how to justify a combination if it results in lower protection, and identifying clear benefits for such combinations is essential. The European Medicines Agency (EMA) accepts waiving lot-to-lot consistency clinical trials if consistency in manufacturing is demonstrated, whereas the U.S. FDA mandates clinical testing for consistency. The discussion highlighted that for a trivalent iNTS vaccine age considerations impact the discussion, particularly since TCV is not licensed for use below 6 months.

The role of Controlled Human Infection Models (CHIM) was discussed for ensuring consistency of endpoints with field trials. The possibility of using TCV as a comparator vaccine for trivalent iNTS/TCV efficacy was raised. It was noted that microbiologic confirmation, requiring blood culture, is necessary for case definitions, and full protection against both components in a bivalent vaccine may not be required. A *Shigella* vaccine meeting held in Nairobi in March 2024 convened 35 global regulators to discuss primary clinical endpoints, case definitions, and antibiotic resistance matters. Some regulatory agencies do not accept CHIM data as primary efficacy data; however, immunogenicity and safety data from CHIM studies can still be used as supportive data, but in most cases do not serve in the critical pathway. Positive efficacy results from CHIM studies may increase confidence. But sometimes negative efficacy from CHIM does not rule out efficacy in real-world settings. Early engagement with regulatory bodies is recommended, and single trials testing multiple pathogens or valencies should not be dismissed. If one component of a combination is already licensed, only non-inferiority for that component needs to be demonstrated. To date, platform trials have not yet been successful for vaccine trials since all vaccine candidates must be available at the same time. Lastly, a robust safety database is essential for combination vaccines; early trials for safety of each component is helpful, but later stage testing of single components is likely not necessary.

The questions and discussion addressed key questions regarding regulatory hurdles and safety requirements for combining existing approved vaccines. The primary concern identified was immune interference; while there are limited additional regulatory challenges, the decrease in immunogenicity observed with increasing PCV serotypes raises uncertainties about the necessary protection thresholds and the justification for combination benefits. Regarding safety database requirements, regulators typically expect a larger sample size, often around 3,000 as a placeholder, although this could be reduced if no safety signals are detected or increased if concerns arise. Additionally, the cross-cutting discussion on value and sensitivity of lot-to-lot consistency clinical trials emphasize the importance of trying to resolve these technical aspects, particularly through potency studies.

4.3 Discussion Topics for Follow-on Regulatory Science Meeting

In the final discussion, participants examined the global precedent for combination vaccines that include antigens not typically found in specific regions, citing examples such as Pneumococcal Conjugate Vaccine (PCV) serotypes 1 and 5 and Meningococcal Conjugate Vaccine (MCV4) for Meningitis A utilized in high-income countries. Concerns over vaccination schedules (timing of first iNTS dose at or before 6 months of age) and questions about whether immunogenicity data would be required between priming dose at (or before) 6 months and at 9 months or 12-15 months were raised. The potential for an anamnestic response after a primary dose was acknowledged, but additional data are needed.

From a practical perspective, the current Target Product Profile (TPP) definition remains unclear and versatile. As highlighted above, the age of vaccination at 6 months or earlier as well as the reconciliation of the need for boosters and proper timing for a trivalent vaccine (peak times of *S*. Typhi and iNTS appear to be different) are still matter of debate. More data are however needed to trigger a policy recommendation (e.g., IVIR-AC, SAGE).

Participants stressed the importance of engaging regulators from endemic countries to clearly communicate the burden of iNTS to regulators and discuss issues related to iNTS single component or combination vaccine development. In preparation for the meeting in Nairobi on 4-5 February 2025, proposed points for consideration were submitted to the Product Development for Vaccines Advisory Committee (PDVAC). These were reviewed again here to solicit input on additional topics for discussion at the regulatory consultation on 4-5 February 2025, Nairobi. Data will be presented to and discussed with regulators to consider several critical factors when evaluating combination vaccines, including efficacy of each component, selection of active comparator (i.e., should a licensed component be the active comparator, when applicable?), interference, and safety monitoring. Furthermore, it was noted that relatively few regulatory bodies in African countries currently operate at the ML3 level, although some are planning to initiate vaccine manufacturing. Their involvement is essential for obtaining insights from those who will make critical regulatory decisions.

Closing

Dr. Wilder-Smith and Dr. Kim concluded the meeting with a heartfelt acknowledgment of the dedication and collaboration that made the event a success. Dr. Wilder-Smith emphasized the significant strides made since the Kigali meeting, particularly in refining the investment case, business case, and stakeholder analysis, underscoring the urgency of finalizing the FVVA within the next four months. The discussions on combination vaccines, scorecards, and voting outcomes highlighted the collective momentum toward transformative solutions for iNTS disease. She also commended the endorsement of the PPC for Combination Vaccines by the PDVAC, a milestone that underscores the potential of both bivalent and trivalent vaccines to address critical public health needs.

Dr. Kim echoed these sentiments, celebrating the substantial progress achieved and the collaborative spirit of the participants. He emphasized the importance of beginning work on the TPPs and committed to refining the FVVA modelling based on participant feedback. Looking ahead, he highlighted the pivotal role of the upcoming regulatory meeting in Nairobi as a critical step in shaping the future of iNTS vaccine development. With plans to refine regulatory questions and integrate perspectives on combination vaccines, the meeting in Nairobi represents an

opportunity to align global efforts toward actionable solutions. Dr. Kim concluded by inviting continued engagement and dialogue, reinforcing the shared commitment to overcoming barriers and delivering life-saving vaccines to those who need them most.