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Heterologous vaccine effects

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ABSTRACT

The heterologous or non-specific effects (NSEs) of vaccines, at times defined as "off-target effects" suggest that they can affect the immune response to organisms other than their pathogen-specific intended purpose. These NSEs have been the subject of clinical, immunological and epidemiological studies and are increasingly recognized as an important biological process by a growing group of immunologists and epidemiologists. Much remain to be learned about the extent and underlying mechanisms for these effects.

The conference "Off-target effects of vaccination" held in Annecy-France (June 8–10 2015) intended to take a holistic approach drawing from the fields of immunology, systems biology, epidemiology, bioinformatics, public health and regulatory science to address fundamental questions of immunological mechanisms, as well as translational questions about vaccines NSEs. NSE observations were examined using case-studies on live attenuated vaccines and non-live vaccines followed by discussion of studies of possible biological mechanisms. Some possible pathways forward in the study of vaccines NSE were identified and discussed by the expert group.

1. Introduction

Our understanding of the immunological landscape is changing dramatically. We now know that immune memory can be re-educated, that innate immunity can have "memory," that certain lymphocytes can exhibit innate-like responses, and that vaccines may have broader specificities that vary with age and sex. Classical immunology is being recast as we go from murine immunology to human immunology and as big data is stored, curated and investigated. Clinical, immunological and epidemiological studies appear to demonstrate that vaccines can affect the immune response to organisms other than their pathogen-specific intended purpose. For example, Bacille Calmette-Guerin (BCG), smallpox, measles, oral polio and yellow fever vaccines may reduce disease and/or mortality from infections other than tuberculosis, smallpox, measles, polio, yellow fever, respectively, and some vaccines have even shown promise when repurposed against certain cancers and/or autoimmune disorders. These heterologous or non-specific effects (NSEs) of vaccines, occasionally also termed "off-target effects", suggest that some vaccines can provide greater protection than their pathogen-specific intended purpose.

To examine vaccines NSEs, the *Fondation Mérieux* organized a conference from June 8–10 2015 entitled: "Off-target effects of vaccination" in Annecy, France ("*Les Pensières*" Conference Centre). The types of questions about NSEs considered at the workshop were:

- How does trained immunity (innate immune memory) influence the NSEs of vaccination?
- How does vaccine-induced NSE immunity vary with age and sex?
- What is the role of inter-pathogen cross-reactivity in the NSEs of vaccines?
- How do environmental antigens (such as the microbiome) influence cross-reactivity?
- Are there negative NSEs?

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In addition to addressing fundamental questions of immunological mechanisms, the workshop considered the following translational questions:

- How does understanding the NSEs/trained immunity change our view of host defence and immunological memory?
- Could NSEs lead to defining a new class of vaccines, or repurpose existing ones?
- What is the best way to determine whether the NSEs are real and important or whether their practical implications are minor? What data would be required (immunological, clinical, epidemiological, other) in order to assess the causal relationship?
- How could these "off label" observations be used to obtain new "on label" indications?

The intent of the workshop was to take a holistic approach to these questions, drawing from the fields of immunology, systems biology, epidemiology, bioinformatics, public health and regulatory science. The workshop was designed to not only advance the science of vaccinology, but also to consider the implications of verified NSEs: how could these previously underappreciated effects be utilized, and what would such an effort entail? The conference assessed the gaps in our knowledge and proposed an agenda for research.

This report provides a summary of the issues discussed, and the key findings and areas for future research and development.

2. Defining the scope of NSEs: Case studies

The hypothesis that vaccines have NSEs in addition to their actions against their targeted pathogens can be illustrated by studies that evaluated the impact on mortality of measles immunization in young children in West Africa [1,2] as well as the therapeutic effects of BCG on bladder cancer [3]. Nevertheless, major controversies remain. In 2012–2014, the Strategic Advisory Group of Experts (SAGE) on immunization requested that the World Health Organization (WHO) review the evidence concerning the possible NSEs of routine infant vaccines. SAGE asked the working group to determine whether the current evidence on NSEs of BCG, diphtheria–tetanus–whole-cell pertussis vaccine (DTP) and measles containing vaccines (MV) on all-cause mortality in children under 5 years of age is sufficient to lead to changes in policy recommendations or to warrant further scientific investigations and, if so, to define the path toward obtaining unequivocal evidence on these issues that would support future robust, evidence-based adjustments in immunization policies. Two separate systematic reviews of human immunologic and epidemiological studies were performed [4]. SAGE concluded that findings from the immunologic systematic review neither excluded nor confirmed beneficial or deleterious non-specific immunological effects of these vaccines on all-cause mortality. The heterogeneous data and the lack of high-quality evidence in children under 5 years of age did not allow for definitive conclusions. The systematic review of epidemiological studies suggested a possible reduction in all-cause mortality following the administration of BCG and MV, the effect of MV being stronger in females. The overall effect of DTP could not be determined due to methodological limitations of the available literature. Likewise, the results suggested that simultaneous administration of (DTP + BCG) versus BCG alone prior to DTP may be associated with lower mortality while co-administration of (DTP + MV) may increase the overall mortality rate (Table 1). The SAGE working group concluded that the overall evidence does not support a change to the existing policy for BCG, DTP and MV. Although there was a significant reduction in mortality following BCG and MV that was not explained by fewer deaths from tuberculosis or measles respectively, SAGE stressed the need for more high quality randomized controlled trials with embedded immunologic investigations that characterize underlying mechanisms of the NSEs of vaccines. Investigations should be performed across a number of countries using standardized protocols to address the well-recognized risks of bias, and to inform potential policy changes.

In this meeting report, we discuss NSE observations using live attenuated vaccines and non-live vaccines followed by possible biological mechanisms for these NSEs, proposed studies to help validate these observations, and some possible pathways forward in the study of NSEs of vaccines.

2.1. NSEs of live-attenuated vaccines

2.1.1. Measles containing vaccine

The NSEs of measles containing vaccines (MV) were first postulated following the analysis of studies comparing mortality in measles-vaccinated and non-vaccinated children in seven low-income countries [2]. Indeed, MV reduced all-cause child mortality after the age of measles vaccination and until 3 or 5 years of age by 30–86% in different studies, which is much greater than the proportion of deaths attributed to acute measles disease. One of the proposed mechanisms to explain this observation has been that MV reduces carriage of the bacteria that commonly cause pneumonia and sepsis in children in Africa. Other potential mechanisms that could contribute in countries with endemic measles infection may be the ability of MV to prevent the immunosuppressive effects of measles in depleting B and T lymphocytes [5]; however, this analysis used only population-based rather than individual data, and it does not explain the reduction in morbidity seen after MV in communities where measles has been eliminated [6] or the increased NSEs of MV (i.e. child survival rate) in the presence of maternal antibody [7]. In a cohort of children from rural Gambia, carriage of *H. influenzae* (OR = 0.36; 95% CI: 0.13, 0.99) and *S. pneumoniae* (OR = 0.25; 95% CI: 0.07, 0.90) was significantly reduced after vaccination against measles and yellow fever [8].

MV may also have beneficial effects in high-income countries. In a Danish population-based cohort study, hospital admission for any infection was significantly lower among recipients of measles, mumps, and rubella (MMR) vaccine compared to recipients of inactivated DTaP-IPV-Hib as the most recent vaccine [6]. These observations may be due to beneficial NSEs of MMR.

The beneficial effects of MV on mortality seem to be stronger in females. The female/male Mortality Rate Ratio (MRR) was 0.63 (95% CI: 0.47–0.84) in a meta-analysis of studies carried out in Guinea-Bissau; most studies had follow-up to 9 months of age after early MV or from 9 months to 3 years of age (P. Aaby, unpublished data). Earlier vaccination seems to further enhance the beneficial effect, possibly due to an increased NSE of MV in the presence of maternal antibodies. Indeed, in two randomized trials of two-dose MV vaccine schedules, the MRR at 5 years of age was 0.22 (95% CI: 0.07–0.64) in vaccinated children who still had maternal antibodies at time of measles vaccination compared with children of similar age who had no maternal antibodies when vaccinated [7]; this beneficial effect appears to be neutralized or reversed when an inactivated vaccine is given with or after MV. For example, immunization with inactivated DTP vaccine after MV was associated with higher MRR (MRR = 1.60; 95% CI: 1.14–2.24); most studies had follow-up to 9 months of age after early MV or from 9 months to 3 years of age. Analysis by sex showed that the aforementioned DTP/MV effect was statistically significant only in girls (MRR = 2.36, 95% CI: 1.4–3.9) [9]. Verification and mechanistic understanding of these observational studies are still required.

2.1.2. Oral polio vaccine

Inactivated polio vaccine (IPV) was first licensed in the USA in 1955 and trivalent live attenuated oral polio vaccine (OPV) was first licensed in the USA in 1963. OPV was chosen for widespread use to eradicate polio for several reasons: affordability, conferring gut immunity and ease of administration resulting in high routine coverage. As a consequence, polio infection has been reduced by 99% since 1988. The first OPV vaccination campaign in Guinea-Bissau in 1998 demonstrated that OPV vaccination was associated with lower MRR for children less than 5 years old irrespective of its impact on polio (MRR = 0.67; 95% CI: 0.5–0.9) [10]. In a subsequent randomized trial, OPV administered with BCG at birth was associated with 32% (0–57%) lower infant mortality than receiving only BCG [11]. Between 2002 and 2014, 15 national campaigns with OPV took place in this area. Mortality adjusted for age, season and calendar time was 19% (5–32%) lower after OPV-campaigns compared with mortality before vaccination campaigns. The benefit increased with additional doses of OPV (P. Aaby, submitted). Hence, one can speculate that the many polio eradication campaigns conducted in the last 15–20 years may have played a major role in reducing child mortality in low-income countries by non-antigen-specific effects.

2.1.3. Smallpox vaccine

Soon after the introduction of the live attenuated smallpox vaccine, it was suggested that it may reduce susceptibility to a number of other infectious diseases [12]. Due to its impact on reducing the overall mortality rate, smallpox vaccination was considered as one of the main reasons for population growth in Europe in early 19th century [13]. Vaccination campaigns eradicated smallpox, and the vaccine was stopped in 1980 but the effect on overall mortality was not examined. The Bandim Health Project is a health and demographic surveillance site in Guinea-Bissau that investigated the association between smallpox scar, survival, and HIV infection in both rural and urban Guinea-Bissau [14,15]. Compared with individuals without any vaccinia scar, those with a scar had MRR of 0.22 (95% CI 0.08–0.61) and 0.60 (0.41–0.87) in the two studies (age range: 25–90 years old). Presence of a vaccinia scar was associated with a significantly lower risk of HIV-1 infection in females (odds ratio OR: 0.49; 95% CI: 0.2–0.98) but had no observed effect in males (OR: 1.27; 95% CI: 0.5–3.6) (P. Aaby, unpublished data). Similarly, in a study conducted in Denmark, smallpox-vaccinated individuals had a reduced risk of hospitalization due to infectious diseases (hazard ratio HR: 0.84; 95% CI: 0.72–0.98) [16]. Such immune-enhancing effects of smallpox vaccine may also extend into the realm of oncology as the risk of developing melanoma in early childhood as well as survival in patients with malignant melanoma may also be significantly lower in individuals vaccinated against smallpox [17,18]. Not all features associated with the smallpox vaccine are positive, for instance, it is not a cure as its therapeutic use in HSV is not recommended by the Advisory Committee on Immunization Practices [19]. The vaccine was not effective in the treatment or prevention of recurrent herpes simplex infection, warts or any disease other than those caused by human Orthopoxviruses [20].

2.1.4. Bacille Calmette-Guerin

BCG is a live-attenuated vaccine that was first used for the prevention of tuberculosis (TB) in 1921. Several sub-strains of BCG have been developed since the original strain, named according to the site of origin or manufacturer. In low-income countries, BCG may reduce all-cause mortality in children before they are receiving other vaccines like DTP, primarily by reducing death from pneumonia and sepsis. BCG is recommended at birth in countries with a high incidence of TB but, in practice, administration is often delayed. Furthermore, BCG is not administered at birth to low-birth-weight (LBW) children in many countries. Randomized trials in LBW children [21,22] and community-based cohort studies [23,24] showed that the administration of BCG is associated with lower mortality in children and may contribute to better survival. A large randomized trial and several observational studies suggest that the effects of BCG vaccine vary between strains [25], but the mechanisms for such effects are still unknown. For example, a retrospective analysis of three birth cohorts indicated that the Japanese BCG vaccine, the Serbian BCG vaccine, and the Russian BCG vaccine were respectively 69%, 43%, and 22% effective against clinically diagnosed tuberculosis [26]. Further work is needed to better understand the differences between BCG vaccines when evaluating their NSEs.

Of note, BCG can act as a broad immune stimulant and generates anti-tumor activity [27–31]. Indeed, intravesical administration of BCG in bladder cancer demonstrated efficacy in reducing tumor recurrence in the majority of treated patients [3]. Almost 40 years later, BCG induction and maintenance therapy remains the predominant immunotherapy for the treatment of non-muscle invasive bladder cancer (NMIBC). The mechanisms of action of BCG for bladder cancer therapy is still under investigation as many immunological processes occur in parallel, but urothelial cells and both innate and adaptive immune system are thought to play crucial roles [32].

In summary, live-attenuated vaccines appear to reduce all-cause mortality in circumstances that cannot be explained by disease-specific effects, and this suggests that the NSEs may be useful in both low-income and high-income countries.

2.2. NSEs of non-live vaccines

2.2.1. Diphtheria–Tetanus–Pertussis (DTP) vaccine

Contrary to the beneficial NSEs reported for a number of live-attenuated vaccines, some studies report an excess in all-cause mortality among DTP-vaccinated children, especially among girls [9,24]. The systematic epidemiological review of NSEs conducted for WHO found an increase in mortality after DTP that was not statistically significant, with a hazard ratio (HR) of 1.38, 95% CI: 0.92–2.08 for DTP-vaccinated compared with DTP-unvaccinated children [4]. Though the majority of studies [7–10] suggested a deleterious effect of DTP, two studies reported a beneficial effect of DTP and the literature was therefore considered inconsistent [4]. However, re-analysis of the WHO/SAGE review with exclusion of studies with a poorly defined control group and an excessively high mortality rate in the control group due to survival or frailty bias found a twofold higher mortality (HR: 2.56; 95% CI: 1.74–3.76) in DTP-vaccinated compared to DTP-unvaccinated girls

Table 1
Systematic review of the sequence/order of DTP, BCG and MV vaccination and all-cause mortality in children: WHO Working-Group review [4].

Comparison (model)	Effect (95% CI)	I^2
BCG vs unvaccinated[a] (Random)	0.53 (0.40–0.72)	62%
DTP-after-BCG vs BCG[a,b] (Random)	1.38 (0.92–2.08)	71%
MV-after-DTP vs DTP[a] (Random)	0.54 (0.45–0.64)	49%
DTP-after-BCG[a] vs DTP-with-BCG (Fixed)	1.92 (1.25–2.93)	0%
DTP-after BCG[a] vs BCG-after-DTP (Fixed)	1.37 (0.84–2.25)	0%
MV-with-DTP vs MV-after-DTP[a] (Fixed)	2.30 (1.56–3.38)	0%
DTP-after-MV vs MV-after-DTP[a] (Random)	2.66 (1.04–6.81)	57%

www.who.int/immunization/sage/meetings/2014/April/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf.

[a] As in the WHO immunization schedule.

[b] Subsequent analysis excluding studies with a poorly-defined control group, severe frailty bias or severe survival bias gives an effect of 2.56 (95% CI 1.74–3.76) in girls.

(P. Aaby, unpublished data). Analysis by sex showed a HR of 1.50 (95% CI: 1.21–1.85) in DTP-vaccinated girls compared to DTP-vaccinated boys (P. Aaby, unpublished data).

While the specific effects of vaccines are additive and not affected by the sequence of vaccination, the NSEs might be determined by the order in which live and non-live vaccines are administrated [6]. For example, several studies in low-income countries including a randomized trial have found that receipt of DTP vaccine rather than MV as the most recent vaccine was associated with increased mortality in girls [9,33]. In addition, a study in Denmark noted that the incidence rate ratio for infection-related hospital admissions was significantly greater (1.62; 95% CI: 1.28–2.05) in children who received the third dose of the inactivated DTaP-IPV-Hib vaccine following live MMR vaccine [6]. A similar effect was found in the review of epidemiological studies in low-income countries by the SAGE working group, with an effect size of 2.66 (1.04–6.81) for DTP after MV compared to DTP before MV (Table 1). Recall that the overall effect of DTP could not be determined due to methodological limitations of the available literature by SAGE.

3. Basic biological mechanisms

Much remains to be learned regarding the mechanisms by which certain live attenuated vaccines could potentially reduce all-cause mortality. Immunological studies might elucidate the mechanisms by which NSEs occur, confirm their relative importance, and provide a guide to which randomized trials are likely to be most informative and confirmatory. Potential mechanisms for beneficial vaccine NSEs may include: (a) modulation (enhancement) of type 1 and type 17 T helper (Th1, Th17) cells, memory CD4 cells, cytokine responses (IL17, IL22, IL 1β, IL6, TNFα1, IFNγ), natural killer (NK) cell memory [34]; (b) enhancement of immune responses to unrelated antigens as has been observed regarding the impact of BCG in boosting geometric mean concentration (GMC) of antibodies against all serotypes of pneumococcus, *Haemophilus influenzae* type B and tetanus toxoid in a prospective non-randomized study in Australia [35]; (c) cross-reactivity between shared epitopes of seemingly unrelated pathogens; and (d) inducing "trained immunity" defined as long-term reprogramming of innate immunity that induces adaptive traits and provides protection against reinfection in a T/B-cell-independent but monocyte-dependent manner [36].

The concept of "trained immunity" has recently challenged the classic dichotomy of innate versus adaptive immunity by studies in plants, invertebrates and mammals that led to an increasing body of evidence for innate immune memory [36,37]. In human adults, immunization with BCG appears to trigger trained immunity [38]. Indeed, in addition to the classic specific immune response involving antigen-specific T cells and memory leading to protection against TB, BCG induces protective effects against unrelated pathogens (*S. aureus*, *C. albicans*, etc.), most likely through epigenetic reprogramming of monocytes, and non-specific production of cytokines such as TNF and IL-1β [38]. Of note, trained immunity has been observed in newborn mice wherein administration of TLR agonists enhanced cytokine responses to subsequent polymicrobial sepsis, associated with more robust phagocytic recruitment and enhanced survival [39]. Induction of trained immunity may also underlie similar phenomenon in human newborns in which bacteremia is associated with up-regulation of mononuclear cell pattern recognition receptors and histologic chorioamnionitis or early onset sepsis are associated with reduced risk of late onset sepsis [40]. Autophagy, i.e. proteolytic degradation of cytosolic components at the lysosome, is central to BCG induced trained immunity, by processing microbial PAMPs and presenting them to the intracellular NOD2 receptors [41].

Trained immunity may also be mediated via hematopoietic stem cells (HPSCs) and progenitors that detect and respond to cytokines, microbial agents, and as demonstrated for TLR2-induced innate immune memory [42]. Such an effect remains to be formally demonstrated for BCG or other vaccines. Following exposure to antimicrobial agents, epigenetic modifications may be propagated through myelopoiesis and could be maintained via programming of their precursor cells i.e. HSPCs, leading to macrophage memory and trained immunity. HSPCs express pattern recognition receptors (PRRs) including Toll-like receptors (TLRs). Detection of microbial components by HSPCs drive or promote production of "TLR-derived" cells (i.e. neutrophils, macrophages, dendritic cells and NK cells) that display myeloid cell characteristics such as phagocytosis, cytokine production and antigen presentation [43]. These results suggest that recognition of microbial components by HSPCs during infection may enhance the efficacy of host defence against infection. Exposure of HSPCs to TLR agonists alter the function of the macrophages they produce [42]. For example, bacterial lipopeptide (TLR2)-stimulated HSPCs generate a higher proportion of macrophages with reduced inflammatory response, but preserved phagocytic activity.

As discussed above, another mechanism to explain NSEs may include cross-reactive epitopes from antigens in the vaccine to other infectious diseases. This mechanism may have contributed, for example, to milder disease in the 2009 H1N1 influenza pandemic in older subjects. EpiMatrix analysis of conserved 9-mers suggested that there may be sufficient cross-reactivity in T-cell epitopes between the epidemic H1N1 strain and the seasonal influenza vaccine (2008–2009) that cross-react, potentially explaining reduced pathology in the elderly [44]. Accordingly, T-cell epitope clusters make excellent vaccine candidates with high immunogenicity [45]. Thus, NSEs may be due to shared cross-reactive epitopes between different pathogens.

As discussed earlier, in contrast to the beneficial effects noted after immunization with live vaccines, epidemiologic studies have raised the possibility of negative NSEs after inactivated/killed vaccines. Aluminum adjuvant is unlikely to explain these effects because some non-live vaccines that do not contain aluminum may have negative NSEs [46,47]. Much remains to be learned about the extent and mechanisms of such effects from a deeper biological perspective.

4. Resolving NSEs of vaccinations

As reported above, there is growing evidence that vaccines may affect mortality from causes other than the disease for which they were developed and vaccine NSEs are now recognized by a growing number of immunologists and epidemiologists [40,48]. However, the topic of NSEs of vaccines is contentious, with much of the evidence coming from one country (Guinea Bissau). Accordingly, greater insight is needed, as discussed next. Different trial types may be considered to build the needed scientific base and understanding of NSEs. Global molecular/big data approaches may characterize the influence of factors that may confound observed NSEs (e.g. frailty, demographics, vital signs, concomitant medications, medical history, etc.). In fact, confounding, i.e., failure to recognize or account for factor(s) other than the variable of interest that can affect outcomes, is near universal in non-randomized clinical studies. Typically these can lead to overestimated effects, and as such, the necessity for different variations of clinical trials was emphasized, as discussed below.

These include confirmatory, explanatory, estimatory or pragmatic/policy-oriented clinical trials. Confirmatory trials are in general designed to test a hypothesis, but can face an issue of prioritization: one should select the hypothesis with the greatest evidence from the literature or the one with "most thoughtful"

implications for public health and policy. Confirmatory clinical trials are often the most expensive. Explanatory trials can be performed to provide clarification of immunological mechanisms or outcomes (morbidity and mortality for example) which are closely linked because the knowledge of clinical outcomes may suggest immunological mechanisms and vice versa. Estimatory trials aim to measure the magnitude of an effect that would be important for risk–benefit and cost-benefit models. This type of trial has major implications for sample size as a function of the background, magnitude and duration of exposure, and requires multisite studies in ecologically different settings to reveal any heterogeneity. Pragmatic trials are intended to evaluate or compare different vaccine schedules that might be introduced into a given population. Intervention opportunities comprise new vaccines versus placebo or no vaccine, comparison of vaccines, or comparison of the timing or order of vaccination. No matter which trial is considered, it was emphasized that it is important for clinical trialists, statisticians and regulatory agencies to consult early, and question all assumptions – including event rates based on historical data.

In an era of global polio and measles eradication efforts, there is an opportunity to evaluate the impact of these two vaccines on childhood survival using data from vaccination campaigns undertaken since 2001. With these considerations in mind, the Bandim Health Project conducted several randomized trials with vaccines and vitamin A in Guinea-Bissau. The effect of measles vaccination campaigns (2006) on childhood mortality between 9 months and 5 years of age was assessed by following vaccinated children for 1 year after immunization. The adjusted mortality rate ratio was 0.80 (95% CI: 0.66–0.96) after the national campaign with a stronger effect in girls (MRR = 0.74, 95% CI: 0.56–0.97) and in children who had received both routine and campaign MV (MRR = 0.59, 95% CI: 0.36–0.99) [49]. A similar pattern of lower mortality (19% reduction) was observed following OPV vaccination campaigns in Guinea-Bissau (2002–2014). The benefit increased with additional doses of OPV (P. Aaby, submitted).

Turning now to animal studies, the concept of animal challenge models was discussed as a way to help build the evidence base for NSEs before engaging in human clinical trials. One important argument for protective NSE of previous infections or vaccinations comes from abundant literature demonstrating that previous infections can induce immunological memory in organisms lacking adaptive immunity, such as plants or invertebrate animals [36]. There is growing data to support NSEs in animal studies as well. For example, immunization of mice with BCG protects against secondary infections with *Candida albicans* or *Schistosoma mansoni*, at least partially mediated via T-cell-independent mechanisms [50,51]. Moreover injecting mice with an attenuated PCA-2 strain of *C. albicans* induced protection toward both a virulent CA-6 strain and the bacterium *Staphylococcus aureus* [52]. Importantly, this protection was also induced in athymic mice, demonstrating a T-cell-independent mechanism [53]. Following animal challenge studies, it could also be envisaged that human challenge studies may be of value to demonstrate NSE of vaccines in a relevant population. In this case, vaccinate the subjects and challenge with a different pathogen.

"OMIC" technologies, including proteomics, genomics, transcriptomics, etc. are tools that allow characterization of global molecular responses to perturbations, including infection or vaccines [54]. OMIC approaches may uncover molecular pathways that correlate with specific and NSE (off-target) protection. Such correlates of protection are useful biomarkers that may inform development of vaccines inducing beneficial NSE/heterologous effects. In addition, such biomarkers/pathways may potentially also provide insights into mechanisms of protection. Despite the fact that the majority of global vaccine use is in children [55], very little is known about age-specific global molecular changes in response to vaccines. OMICs studies of new-born and infant vaccines may define age-specific signatures that if correlated with trained effects and conventional correlate of protection (e.g. antibody response) will generate mechanistic hypotheses that can be tested *in vitro* and in animal models to inform future vaccine development. Using OMICs technologies, including transcriptomics and proteomics, a single-blinded, prospective study is ongoing in infants admitted to neonatal intensive care units in Guinea-Bissau. Blood is collected in pre- and post-BCG immunization to assess whether molecular signatures correlate with trained and adaptive responses.

5. The way forward

A growing body of research in the field of vaccinology suggests that certain vaccines may have heterologous or non-specific effects. As an example, major reductions in all-cause mortality have been consistently noted in observational studies of MV, OPV and BCG, and in randomized trials their NSEs on reducing mortality have been 26% for MV, 32% for OPV and 41% for BCG [22,11,33]. Similar effects have also been noted for hospital admission patterns in a high-income setting [6]. However, much of the evidence has related to all-cause mortality in a small number of low-income countries in West Africa, and changes in distal outcomes such as death and admission to hospital are multifactorial and susceptible to confounding in observational studies. SAGE concluded that further observational studies with an inherent risk of substantial bias would be unlikely to provide conclusive evidence about putative NSEs on mortality. However, it should be noted that in the field of NSEs, repeating observational studies has revealed consistent patterns eventually confirmed in randomized trials. If observational studies are contemplated, their design and analysis should mimic the design of a randomized controlled trial. To address well-recognized risks of bias, efforts should be made to develop standardized definitions of NSEs and their potential immunologic mechanisms (heterologous effects, trained immunity, cross-reactivity, etc.) as well as standardized protocols for both RCTs and observational studies of mortality effects.

By what process might vaccines be approved for use in inducing beneficial NSEs? The indications must be clearly defined and tested in adequately controlled and powered, ideally randomized, controlled trials for new regulatory indications. For a successful regulatory path, it may be reasonable to consider several different types of evidence. Cluster randomization, active controls with different designs such as comparing timing or vaccine sequencing (e.g. early versus delayed BCG, DTP3 before versus after MV, and BCG before versus after DTP1) and comparison of vaccines (e.g. acellular pertussis versus whole cell pertussis) are valid and potentially feasible approaches. The use of biology, including basic science, biomarkers, *in vitro* assay systems [56], network models and disease models, as regulatory science tools could also be helpful to identify both candidate mechanisms and correlates of benefit or harm. Any prospective trial is a critical opportunity for biomarker development; carefully designed immunological studies should be an important part of future randomized trials. Careful trial design and consensus about the immunological endpoints (what, when) are required to address the immunological questions. Systems biology approaches may be particularly informative in providing a profile of host immune response. A combination of system biology and clinical data may also help identify and study candidate NSEs. As an example, the European Research Infrastructure for biological data is an organization that pools the substantial volume of data being generated by publicly funded research, and provides facilities for testing hypotheses in very large databases. The use of nanopublication (i.e. the smallest unit of publishable information) could also allow the analysis of large, heterogeneous and decentralized data and the detection of new associations that

would otherwise be beyond the capacity of human reasoning [Nanopub.org].

Post-marketing surveillance is also critical. In particular, the effects of any change to the Expanded Program on Immunization (especially the introduction of a non-live vaccine or withdrawal of a live vaccine) should be carefully monitored. One of the major research questions relates to differential effects by age and by sex, and future studies should therefore be designed and powered to examine these key variables. Assessment of the effects due to adjuvants is also important. For example, the HPV vaccines Gardasil and Cervarix are similar non-live VLP-based vaccines that induce Th2 and Th1 skewed responses, respectively, because of the differences in their adjuvant. Theirs NSEs due to adjuvants could be evaluated, at least in preteens, in an RCT involving these two vaccines. Additional major research areas include characterizing the role of maternal antibodies (e.g. early priming, boosting effects) as well as vaccine interaction. Many candidate benefits/harms suggested during the conference could be well tested in adequately controlled and powered trials for new regulatory indications.

In conclusion, vaccine NSEs may lead to new uses for old vaccines, or new vaccines that combine induction of adaptive immune memory and trained immunity for new uses. However, this topic has not been high on the list of priorities of industrial research and development organizations. Current principles of evidence should govern the potential development and evaluation of NSEs of vaccines. Possible adverse NSEs should also be investigated. Indeed, high standards for evidence are critically important in NSEs of vaccines given the complexity of biology, big data, the high susceptibility to confounding factors, and the sensitivity of immunization across the population.

After a century, we still do not understand the mechanisms for the specific targeted effects of BCG and whole-cell pertussis vaccine, and we have no well-defined and accepted correlates of protection for these vaccines. However, a better understanding of the mechanisms for the NSEs would facilitate approval of new uses of these vaccines that exploit any off-target effects. Productive path forward may include (1) being specific with animal and/or human challenge models; (2) conducting age- and species-specific *in vivo*, *in vitro*, and *in silico* mechanistic studies [56]; (3) developing controlled human infection models to "demonstrate" effects and (4) once animal and human challenge models show effects, set-up multiple large studies in several countries from a wide range of geographic locations and burden of disease settings using common protocols and endpoints. Emphasis must be put on deciphering putative immunological mechanisms so that correlates of non-antigen specific protection can inform future studies. Building and testing the science chain from biology to biomarkers to clinical outcome may provide suitable data to be evaluated by regulatory agencies to consider approval for new vaccine indication.

Conflict of interest

MG and WLW are employee of Sanofi Pasteur. Other authors declare that they have no conflicts of interest to report.

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Annex

Speakers & Chairs

Peter Aaby: Denmark | Bandim Health Project, Statens Serum Institute
Yasmine Belkaid: USA | National Institute of Health NIAID
Christine S. Benn: Denmark | Statens Serum Institute
Anne De Groot: USA | EpiVax, Inc.
Michel De Wilde: USA | MDWConsultant, LLC
Paul Fine: UK | London School of Hygiene and Tropical Medicine
Ane Fisker: Denmark | Bandim Health Project, Statens Serum Institute
Jesse Goodman: USA | Georgetown University
Helen Goodridge: USA | Cedars-Sinai Medical Center
Michael Greenberg: France | Sanofi Pasteur
Mélanie Hamon: France | Institut Pasteur
Tobias Kollman: Canada | The University of British Columbia
Ofer Levy: USA | Boston Children's Hospital/Harvard Medical School
Jacques Louis: France | Fondation Mérieux
Nathalie Mielcarek: France | Institut Pasteur
Barend Mons: The Netherlands| Leiden University Medical Center
Mihai Netea: The Netherlands | Radboud University Medical Center
Terry Nolan: Australia | University of Melbourne
Stanley Plotkin: USA | University of Pennsylvania and Vaxconsult
Henrik Ravn: Denmark | Research Center for Vitamins and Vaccines, Statens Serum Institute
Frank Shann: Australia| Department of Paediatrics, University of Melbourne
Gary Steinberg: USA | University of Chicago
William Warren: USA | Sanofi Pasteur VaxDesign

Participants

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Ziauddin Ahmad: Bangladesh | Jalalabad Ragib Rabeya Medical College
S.Sohail Ahmed: Italia | GSK Vaccines
Christine Andreoni: France | Mérial
Brigitte Autran: France | CIMI Paris, UPMC/Inserm, U1135

Akhil Banerjea: India | National Inst Immunology
Mario Barro: USA | Biomedical Advance Research and Development Authority/Department of Health and Human Services
Natalia Bomchil: France| Merial
Nicolas Burdin: France | Sanofi Pasteur
Danilo Casimiro: USA | Merck
Lorena Charrier: Italia | University of Torino, Dept. Public Health and Paediatrics
Pascal Chaux: France | Sanofi Pasteur
Yahia Chebloune: France | PAVAL Lab. Université Joseph Fourier Grenoble
Karelle De Luca: France | Mérial
Arnaud Didierlaurent: Belgium | GlaxoSmithKline
Marc Girard: France | French Natl Academy of Medicine
Cindy Grasso: France | Fondation Mérieux
Nicola Groth: Italia | Novartis Vaccines & Diagnostic
Michel Joosten: The Netherlands | RIVM
Christiane Juhls: Germany | Mologen AG
Inca Kusters: France | Sanofi Pasteur
Cyrille Leperlier
France | Sanofi Pasteur
Christophe Longuet: France | Fondation Mérieux
Liz Miller: UK | Public Health England
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Valentina Picot: France | Fondation Mérieux
Farzin Roohvand: Iran | Pasteur Institute of Iran
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