Early Phase Clinical Trials with Human Immunodeficiency Virus-1 and Malaria Vectored Vaccines in The Gambia: Frontline Challenges in Study Design and Implementation

Muhammed O. Afolabi,* Jane U. Adetifa, Egeruan B. Imoukhuede, Nicola K. Viebig, Beate Kampmann, and Kalifa Bojang
*Vaccinology Theme, Medical Research Council Unit, The Gambia; The Jenner Institute, University of Oxford, United Kingdom;
European Vaccine Initiative, Germany; Disease Control and Elimination Theme,
Medical Research Council Unit, The Gambia*

Abstract. Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and malaria are among the most important infectious diseases in developing countries. Existing control strategies are unlikely to curtail these diseases in the absence of efficacious vaccines. Testing of HIV and malaria vaccine candidates start with early phase trials that are increasingly being conducted in developing countries where the burden of the diseases is high. Unique challenges, which affect planning and implementation of vaccine trials according to internationally accepted standards have thus been identified. In this review, we highlight specific challenges encountered during two early phase trials of novel HIV-1 and malaria vectored vaccine candidates conducted in The Gambia and how some of these issues were pragmatically addressed. We hope our experience will be useful for key study personnel involved in day-to-day running of similar clinical trials. It may also guide future design and implementation of vaccine trials in resource-constrained settings.

INTRODUCTION

Plasmodium falciparum malaria and human immunodeficiency virus (HIV) are the two most prevalent infectious diseases in sub-Saharan Africa. According to recent estimates, sub-Saharan Africa, which represents just over 10% of the world's population, accounts for 90% of malaria deaths¹ and two-thirds of all people living with HIV.² The social and economic impacts of these diseases are enormous and severe.³

As with other infectious diseases, safe and effective vaccines are needed to complement or enhance the success of existing strategies to control HIV/acquired immune deficiency syndrome (AIDS) and malaria, especially in developing countries.^{4,5} The development and final licensure of any vaccine requires clinical evaluation at various stages, with some of the necessary steps involving developing countries in ever increasing numbers. Challenges in selecting suitable vaccine candidates, clinical trial end-points, and changing epidemiological pattern of HIV and malaria in certain regions have been identified in the late phase clinical trials conducted in Africa.^{6–9} Recent increases in the number of early phase clinical trials being conducted in developing countries has brought a set of additional factors that pose considerable challenges to planning and implementation of clinical trials in these settings.^{10–13} Although some of these factors have been documented in general terms,^{14–17} we highlight here specific challenges encountered while hosting two phase I vaccine trials in our center. We discuss how we pragmatically addressed some of these issues with a view to providing guidance that may shape the design and implementation of future vaccine trials in similar settings.

Box 1: Profile of Medical Research Council unit, The Gambia

The Medical Research Council Unit (MRC), The Gambia is a UK government-funded research institution established over six decades ago to conduct biomedical and translational research. The research portfolio of MRC spans basic scientific research, clinical studies, large epidemiological studies and vaccine trials addressing malaria, hepatitis B, measles, TB, HIV, pneumonia amongst others. Since its inception, several intervention trials have been conducted in the prevailing context of high morbidity and poverty.

MRC has field sites located within rural and urban communities. One of these is Sukuta clinical trial site located within a government health centre, about 25 km south of Banjul, capital of The Gambia. Prior to conduct of the HIV vaccine trial, the site was upgraded to a 10-room facility which included 4 consultation rooms, 1 pharmacy, 1 vaccination room, 1 bleeding room, 1 resuscitation room, 1 administrative office and an anthropometry area. There were also a back-up generator, toilet and wireless internet facilities. At the time of conduct of the HIV and malaria vaccine trials, the site had 2 clinicians, 1 field supervisor, 5 nurses, 7 field assistants and 1 project driver. The clinical and field staff were trained in Good Clinical Practice (GCP), research ethics, HIV voluntary counselling and basic paediatric life support.

* Address correspondence to Muhammed O. Afolabi, Medical Research Council Unit, Atlantic Road, Fajara, P.O. Box 273, Banjul, The Gambia. E-mail: mafolabi@mrc.gm

STUDY PROFILES OF THE TWO CLINICAL TRIALS ARE SUMMARIZED BELOW:

Box 2: Infant HIV-1 vaccine trial

Goal: To develop an effective HIV vaccine to prevent mother-to-child transmission of HIV during breast-feeding.

Vaccine platform: Modified Vaccinia Ankara (MVA) delivering HIV-1 clade A immunogen HIVA was used to induce HIV-1-specific T-cell immunity early after birth

Trial design: Infants were recruited from birth. Study mothers underwent HIV antibody testing. The infants received EPI vaccines by study team and were screened for eligibility at age of 19 weeks.

Trial implementation: Eligible infants were randomised at age of 20 weeks at ratio of 1:1 into vaccine and no-treatment control groups. All study infants underwent HIV antibody testing at age of 28 weeks. Safety and immunogenicity data were collected at 21, 28 and 36 weeks of age.

Box 3: Malaria vectored vaccine trials

Goal: To evaluate the immunogenicity of malaria vaccines using a new simian adenovirus vector to possibly overcome the previous limited immunogenicity results of liver-stage malaria candidate vaccines.

Vaccine platform: Prime-boost approach using a combination of chimpanzee adenovirus ChAd63 and Modified Vaccinia Ankara (MVA) as vectors to sequentially deliver a liver stage antigen, multiple epitope thrombospondin adhesion protein (ME-TRAP).

Trial design: Series of age de-escalated, dose-escalation clinical trials involving 18-50 year old adults, 2-6 year old children, 5-12 month and 10 week old infants evaluated these vaccine candidates.

Trial implementation: Eligible participants were vaccinated with prime vaccine, ChAd63 ME-TRAP, at Day 0 and boost vaccine, MVA.ME-TRAP, at Day 56. Safety and immunogenicity data were collected at Days 14, 63, 90, 105 and 300.

LOGISTICAL ISSUES IN TRIAL IMPLEMENTATION

Recruitment of key study staff. Most early phase clinical trials require testing of the vaccines or investigational products in a small number of study participants ranging from 20 to 50 individuals. The small sample size is usually selected for assessment of safety, tolerability, and immunogenicity of the investigational vaccines⁵; this sample size balances the need to avoid exposing a large number of study participants to unknown risks with the need for data from an adequate sample. Although there is no doubt about the significance of safety and immunogenicity data at this vital stage of vaccine development, there is usually a tendency to think that these trials do not require a full complement of staff because the sample size is relatively small compared with efficacy trials. The general assumption is that the size of a clinical trial should dictate the number of staff. Our experience showed that the magnitude of workload in early phase trials is not proportional to sample size. This is because the same degree and quality of preparation, planning, and implementation strategies required for clinical trials with large sample size are also needed to successfully conduct early phase trials. We suggest that an appropriate number of clinical, field, and laboratory staff is used to execute early phase trials.

Furthermore, participation in early phase clinical trials represents excellent training opportunities for these cadres of staff. However, because of a low yield of scientific publications from these trials, it is important to identify additional research questions within the overall trial, which the team could address in collaboration with the main trial's Principal Investigator. This could maintain satisfaction within the team and prepare the staff for higher responsibilities in future trials.

Host community engagement. Unlike other African communities,^{18,19} a Community Advisory Board (CAB) does not exist in The Gambia, but the roles of participants' advocates are informally and efficiently played by the village heads called "Alkalos." Before commencement of the clinical trials, the host communities were sensitized by first introducing the studies to the village heads. This was accompanied by presentation of traditional kola nuts, which signified the symbolic introduction of the studies and to solicit support from the host community for smooth conduct of the studies. The study rationale and its justifications were subsequently explained to the "Alkalo" and his chiefs. Concerns like the issues of blood collections and required laboratory assays were explained to the community leaders in lay language. Subsequently, the "Alkalo" passed the information about the studies to potential participants through household heads and religious leaders. Apart from these initial visits, community sensitization meetings in the form of annual Open Days were organized to provide feedback on findings of previous studies and the introduction of the new trials. This provided ample opportunities for researchers and members of host communities to engage in mutual partnership that could contribute to success in the vaccine trials.

Recruitment of study participants. The excellent working relationship between the Medical Research Council (MRC) and host communities makes it easier to recruit study participants. The participants are usually keen to join MRC studies because of perceived medical benefits.^{13,20} However, this phenomenon changed during the HIV vaccine trial and it was difficult to recruit study mothers because of the belief that

the vaccine might cause HIV infection in the infants. This was further complicated by the likelihood of a “false HIV antibody positivity” elicited by the vaccine. As the majority of intending mothers were illiterate, it was practically challenging to explain this technical adverse event to them. We communicated this to the study mothers by using an illustration of a “real car” and a “toy car.” Just as the two cars look alike, a real car’s mobility is powered by an engine, whereas a toy car, which does not have an automobile engine has limited mobility; so also is the laboratory-synthesized Modified Vaccinia Ankara expressing HIVA immunogen (MVA.HIVA) vaccine, which could elicit the HIV antibody that is not truly positive. Although no study infants had false positive antibody results, communicating this technical information was a significant challenge.

Similar challenges were faced in translating the informed consent documents to local languages as the languages are only spoken and do not have standardized writing formats. After several attempts at forward and back translations of informed consent documents, very few literate participants could read and understand it. This persuaded our local ethics committee to recommend the use of trained field assistants in interpreting the contents of the informed consent documents to the potential participants.¹³ To ensure an objective assessment of understanding of participants before enrollment into the study, a study quiz covering the domains of “study purpose,” “study procedures,” “randomization,” “placebo,” “adverse events,” “confidentiality,” “compensation,” and “rights of withdrawal” was developed and administered to the participants. Satisfactory performance assessed by success in giving seven correct answers out of eight questions was used as an indicator of “good understanding” of the study. Participants who failed the first attempt received further education on the areas of deficiency and were allowed one more attempt at the quiz. This effort increased the participation rate and 100% retention of participants was recorded throughout the study period. This study quiz has been further developed into a digitized format. Finally, a multimedia informed consent tool has been developed in Gambian local languages to deliver study information to low literacy participants.

Cultural practices. Understanding and responding appropriately to the cultural beliefs and practices of the host communities play significant roles in the successful conduct of clinical trials.^{16,21,22} In many communities in The Gambia, newborns and the mothers should not be seen outside their homes until 8 days after birth. This caused delay in taking BCG vaccination for the neonates and may affect clinical trials that require crucial clinic visits within the first week of life.²³ Similarly, vaccination and follow-up visits during the malaria vaccine trial in adult men fell within the month of fasting in which all adult Muslims are expected to abstain from eating and drinking from dawn to dusk. All participants in the trial were Muslims and of fasting age. We communicated the requirements of the clinical trial to vaccinate the study participants with prime and boost vaccines and collect 30 mL of blood during each study visit during the informed consent process. We also informed them that these procedures might take place during the fasting period. All participants agreed with the arrangement but later refused to be bled or vaccinated during the fasting period. The clinic visits were subsequently rescheduled to evenings to allow vaccination and bleeding of study participants after the fast

had ended for the day. This development underscores the need for researchers to have back-up plans for executing every stage of clinical trials.

CHALLENGES AND EXPERIENCE FROM HIV VACCINE TRIAL

Study design. The clinical trial was based on the hypothesis that protective T cell-mediated responses could be elicited by vaccines in early life, and BCG as a vaccine vector might be an appropriate prime. Thus, all potential study infants were recruited at birth or within the first week of life to ensure the study team vaccinated them with BCG. As BCG vaccination was central to the success of the experimental vaccine, the presence of a BCG scar was actively sought on the left deltoid area of the infants, which was also the site of vaccination with the experimental HIV vaccine.

Although BCG has shown great promise as an HIV vaccine vector, some antigens in MVA.HIVA, such as HIV *env*, are poorly expressed in BCG and this may result in sub-optimal immune responses to the HIV antigen.²⁴ More important, infants have immature innate and adaptive immune responses that result in a diminished capacity to activate other immune cells which plays a major role in induction of the downstream adaptive immune responses.²⁵ In addition, infants’ T cells show diminished capacity to express Th-1 effector function caused by hyper-methylation of the proximal promoter of the interferon-gamma (IFN- γ) gene, which results in restricted IFN- γ responses to most stimulants.^{26,27}

In view of the aforementioned, we suggest that vaccine developers targeting infants in infectious disease-endemic countries should address the issues of immature infant immune system in early stages of the vaccine development. For example, the use of an improved strategy on BCG vector such as codon optimization and combination with viral vector boost^{24,28} have proved more useful in HIV vaccine development. Similarly, induction of broadly neutralizing antibodies remains critical toward successful HIV-1 vaccine development.^{29,30}

HIV antibody testing in study mothers and infants. One of the major inclusion criteria for the study was HIV antibody testing of study mothers at 6 weeks post-partum. Of the 65 mothers approached, 61 (93.8%) agreed to undergo voluntary counseling and HIV antibody testing by enzyme-linked immunosorbent assay (ELISA). The mothers attended immunization clinics with their infants until 19 weeks when the infants were enrolled in the study. Although the prevalence of HIV-1 was low in The Gambia,³¹ there are a number of traditional practices that could increase risk of HIV transmission during infancy. These include breast-feeding of infants by female relations whose HIV status are unknown and use of unsterilized instruments for male and female circumcisions.³² We recommend HIV antibody retesting of the study mothers within 6 weeks after the first test, according to international guidelines,³³ to avoid enrolling infants whose mothers were in a serological “window” period.

The possibility of a false “positive” HIV antibody test was documented as an adverse event related to vaccination with MVA.HIVA caused by cross-reactivity with the “*gag*” protein of the HIVA immunogen.³⁴ To detect this, all study infants had ELISA tests at 28 weeks of age and showed negative antibody results. Maternal antibodies are known to persist

beyond 28 weeks, hence the World Health Organization (WHO) recommendation of HIV antibody testing only in children 18 months of age and older. To conform to this guideline, we advise similar vaccine trials to use WHO recommended technology like the PCR test for this age group.³⁵

Absence of local hematology and biochemical references. Hematological and biochemical references are essential indices used in establishing safety parameters, especially in early phase intervention trials. The accuracy and appropriateness of these laboratory parameters are key to understanding the results from such trials. At the time of conduct of this trial, there were no local reference values for hematology and biochemical parameters for the pediatric age group. Previous studies conducted within the institution made use of values obtained from a pediatric population from an East African country. In view of differences in environmental, genetic, and nutritional factors that may influence these laboratory indices,^{36,37} we developed “in-house” laboratory references from previous infant studies, which compared favorably with similar references from neighboring West African countries. This challenge stimulated a comprehensive study, which is currently underway to determine the laboratory reference values for all age groups in different geographic regions of The Gambia.

Administration of supplementary EPI vaccines. In line with international recommendations to facilitate eradication of childhood vaccine preventable diseases, the Gambian Health Ministry organizes periodic house-to-house campaigns to immunize infants < 5 years of age, irrespective of whether they have received the Expanded Program on Immunization (EPI) vaccines before. Among the study infants, these additional immunizations could potentially act as confounding variables because of variations in age at vaccination and batches of vaccines administered. More important, these variations may distort findings about interference of the EPI and study vaccines. All Gambian children presenting for EPI vaccinations are issued an infant welfare card, which the mothers keep for the infants’ immunization record. We designed and placed an informative sticker on the health card indicating that the child was enrolled in an important vaccine trial, and should only receive EPI vaccines from the study team at the trial center. This greatly minimized the number of study infants who received the supplementary immunizations. The stickers were removed when the child exited the study, i.e., end of the study period, not eligible for randomization or dropped out. The study team ensured all infants received all recommended EPI vaccines according to Gambian immunization schedule. To further minimize potential disruptions in clinical trial schedules, we suggest that the clinical trial team should work with the local EPI team to plan and identify favorable dates for the supplementary immunizations.

Use of analgesics for post-vaccination fever. Fever is a common adverse event after infant vaccinations. Studies have shown the efficacy of acetaminophen prophylaxis after whole cell pertussis vaccination and also in treating febrile episodes after routine immunizations.^{38,39} Because of a study report that prophylactic or therapeutic use of acetaminophen may reduce immunogenicity to vaccines,⁴⁰ we encouraged mothers not to give the drug except when infants became irritable after vaccination. Study infants were observed for immediate local and systemic adverse events at the trial clinic for 1 hour after administration of the study vaccine. This was followed

by post-vaccination home visits by trained field staff that collected further adverse events on purpose-designed diary cards. Although most mothers reported fever in vaccinated and non-vaccinated infants within 6–24 hours after vaccination, the temperature records taken by trained field staff did not support the episodes of reported fever. Furthermore, the infants did not develop any clinical symptoms and signs necessitating indiscriminate use of analgesics. This observation lends credence to rational use of acetaminophen after infant vaccinations.

Infants’ weight faltering during follow-up. Weight faltering, a common growth pattern in developing countries, is characterized by undernutrition when complementary feedings are introduced to growing infants.⁴¹ Almost equal numbers of study infants in the vaccine and control groups had weight faltering after study intervention. Although these were documented as adverse events, they were considered not related to the study vaccine. The mothers were educated to use locally available food items as complementary diets. However, there was no appreciable improvement in weight of the study infants until locally produced food supplements were procured by the study team and distributed to the mothers of affected infants. Sustainability of such intervention was a major financial challenge as it was not factored into the clinical trial budgets. We suggest that clinical trials conducted on children in low-income countries should make appropriate budgetary provisions for malnutrition-related events that commonly occur in this age group.

CHALLENGES AND EXPERIENCE FROM MALARIA VECTORED VACCINE TRIALS

Malaria antigen testing. A report of attenuated immunogenicity in malaria vaccine volunteers from The Gambia and Kenya⁴² was considered to be caused by previous exposure to malaria. This was expected as the volunteers lived in malaria-endemic countries with high transmission rates. After this 2007 report, malaria transmission declined drastically in The Gambia resulting in low malaria parasitemia.^{43,44} Despite this development and to further ensure that immunogenicity to study vaccines is not distorted by level of parasitemia, we included a negative malaria antigen test as one of the eligibility criteria. The First Response histidine-rich protein-2 rapid diagnostic test was used to screen potential participants for presence of malaria antigens. The diagnostic accuracy of this test-kit was comparable with malaria microscopy.^{45,46} Of 140 children and infants screened over a period of 2 years, only 4 (2.9%) tested positive for malaria antigens. These children were treated with a full course of antimalarial drugs and excluded from participating in the trial. We considered enrolling them in the trial after treatment but decided against this because of evidence that malaria antigens could persist after treatment.⁴⁶ Nevertheless, none of the children/infants who had negative antigen tests developed clinical malaria throughout the study period, proving the value of testing before enrollment.

Narrow recruitment age. After showing satisfactory safety and immunogenicity profiles in adults and children trials; further studies were conducted in 5–12-month-old infants, closely followed by 10-week-old infants. To select optimal dosage of ChAd63 ME-TRAP for this age group, the study

was further divided into two stages with a low dose ChAd63 group paired with a no-treatment control group, after which infants in high dose ChAd63 group were recruited and screened for eligibility. Because the infants had to receive the prime vaccine at exactly 10 weeks of age, recruitment, screening, and randomization of eligible infants had to take place about 2 weeks before this period. This implied that 8-week-old infants presenting at the trial center for routine EPI vaccines were the target population for recruitment. Unfortunately, most mothers did not bring their infants to the clinic until they were about 10–12 weeks of age making pretrial procedures logistically challenging. We therefore recommend that enrollment age of participants in similar clinical trials should be more flexible to accommodate predominant health-seeking behavior and practices of potential participants, unless there is a scientific rationale to be very restrictive.

Required volume of blood. The adult trial had a total of eight study visits and the volume of blood required from each volunteer at each visit was 30 mL, whereas during the pediatric trials 5 mL of blood was collected at each visit. Some participants had concerns and pre-conceived ideas that MRC sold the blood collected from study participants abroad and used the proceeds to procure vehicles. We made use of purpose-designed visual aids to illustrate the relatively small volume of blood collected from each participant when compared with the total body volume of individual participants. The participants were invited to our laboratories where we demonstrated how the collected blood samples were processed. This practice allayed fear of uncertainties about volume of blood collected and improved participants' retention and adherence to study protocols.

Use of comparator vaccine as control. In the pediatric trial, human diploid cell rabies vaccine was used as a comparator with the malaria vaccines. The reconstituted anti-rabies vaccine was a pink liquid, whereas the study vaccines were colorless. The comparator and study vaccines were prepared separately using identical 27G 1 mL syringes that were covered with an opaque label to maintain blinding of study mothers and field staff who were involved in assessment of primary safety outcomes. In the control group, two doses of anti-rabies vaccines were designed to be given to the randomized participants. This raised an important ethical issue because of the concern that two doses of anti-rabies vaccines may confer limited protection against rabies. However, it was established that the two doses could confer optimal protection provided they are given 28 days apart, which suited the study design. Furthermore, because of a relatively high rabies risk in The Gambia and the prohibitive cost of general prophylactic vaccination, it was decided that participants randomized to receive malaria vaccines should also receive anti-rabies vaccine at the end of the study. Although this decision led to an increase in research cost, it was ethically acceptable as a form of compensation to participants involved in early phase trials that in most instances do not confer any immediate benefit to the participants. We suggest the inclusion of similar health benefits in future vaccine trials conducted in developing countries.

Dry ice supply. Study vaccines were required to be transported at -20°C or below to the trial site, which is located about 20 km away from the storage facilities. Unfortunately, dry ice is in limited supply in most developing countries. For instance, only one company produces dry ice in commercial

quantity in The Gambia and this supply is often unreliable. Attempts to produce dry ice within our host institution were not sustainable. On many occasions, we had to import dry ice from Europe. We suggest development of a sustainable "in-house" dry ice production in research institutions in developing countries. We also recommend development of cold-chain free vaccines for resource-constrained settings.

CONCLUSIONS

We have discussed some technical, logistical, managerial, ethical, and cross-cutting issues encountered in the conduct of early phase trials in our center. The lessons learned have strengthened our capacity to avoid common pitfalls and prepare us to adequately address challenging issues that are capable of frustrating smooth planning and execution of future vaccine trials. This experience may be useful in addressing specific challenges faced by investigators in other resource-limited settings. It may also improve the understanding of funders, sponsors, and other collaborators about these issues, so that a subsequent working relationship can enhance the realization of the development of safe and effective vaccines for the diseases.

Received October 24, 2013. Accepted for publication January 6, 2014.

Published online March 10, 2014.

Acknowledgments: We acknowledge with thanks the constructive reviews of the manuscript by Barbara Janssens of German Cancer Research Center, Germany; Maria Mavris of European Organization for Rare Diseases, France; and Adrian Hill of The Jenner Institute, University of Oxford, UK. We also appreciate the tremendous support of the following MRC staff: Ebrima Touray and the entire staff at Sukuta field site, Peter Dukes of Research Training and Development Department, Toyin Togun of the Vaccinology Theme, Jenny Mueller and Vivat Thomas-Njie of the Clinical Trials Support Office, and Dembo Kanteh of the Research Support Office.

Financial support: We thank European and Developing Countries Clinical Trials Partnership for funding the two trials: (EDCTP grant nos. IP.2008.31100.001 and CT.2006.33111.002).

Disclaimer: The authors declare no conflict of interest.

Authors' addresses: Muhammed O. Afolabi, Jane U. Adetifa, and Beate Kampmann, Vaccinology Theme, Medical Research Council Unit, Fajara, The Gambia, E-mails: mafolabi@mrc.gm, jadetifa@mrc.gm, and bkampmann@mrc.gm. Egeruan B. Imoukhuede, The Jenner Institute, University of Oxford, Oxford, UK, E-mail: egeruan.imoukhuede@ndm.ox.ac.uk. Nicola K. Viebig, European Vaccine Initiative, Heidelberg, Germany, E-mail: nicola.viebig@euvaccine.eu. Kalifa Bojang, Disease Control and Elimination Theme, Medical Research Council Unit, Fajara, The Gambia, E-mail: kbojang@mrc.gm.

REFERENCES

1. Hay S, Okiro E, Gething P, Patil A, Tatem A, Guerra C, Snow RW, 2010. Estimating the global clinical burden of *Plasmodium falciparum* Malaria in 2007. *PLoS Med* 7: e1000290.
2. WHO, 2012. Global Health Observatory-HIV/AIDS. Available at <http://www.who.int/gho/hiv/en/>. Accessed July 22, 2013.
3. Kilama WL, Wanga CL, 2007. Towards an African-driven malaria vaccine development program: history and activities of the African Malaria Network Trust (AMANET). *Am J Trop Med Hyg* 77: 282–288.
4. Mugenyi PN, 2002. HIV vaccines: the Uganda experience. *Vaccine* 20: 1905–1908.
5. Chilengi R, 2009. Clinical development of malaria vaccines: should earlier trials be done in malaria endemic countries? *Hum Vaccin* 5: 627–636.

6. Mwangoka G, Ogotu B, Msambichaka B, Mzee T, Salim N, Kafuruki S, Mpina M, Shekalaghe S, Tanner M, Abdulla S, 2013. Experience and challenges from clinical trials with malaria vaccines in Africa. *Malar J* 12: 86.
7. Girad M, Reed Z, Friede M, Kieny M, 2007. A review of human vaccine research and development: malaria. *Vaccine* 25: 1567–1580.
8. Moorthy V, Reed Z, Smith P, 2007. Measurement of malaria vaccine efficacy in phase III trials: report of a WHO consultation. *Vaccine* 25: 5115–5123.
9. Sullivan M, 2009. Moving candidate vaccines into development from research: lessons from HIV. *Immunol Cell Biol* 87: 366–370.
10. Ibang HB, Brookes RH, Hill PC, Owiafe PK, Fletcher HA, Lienhardt C, Hill AV, Adegbola RA, McShane H, 2006. Early clinical trials with a new tuberculosis vaccine, MVA85A, in tuberculosis-endemic countries: issues in study design. *Lancet Infect Dis* 6: 522–528.
11. Idoko OT, Kochhar S, Agbenyega TE, Ogotu B, Ota MO, 2013. Impact, challenges, and future projections of vaccine trials in Africa. *Am J Trop Med Hyg* 88: 414–419.
12. Jaffar S, Amuron B, Birungi J, Namara G, Nabiryo C, Coutinho A, Grosskurth H, 2008. Integrating research into routine service delivery in an antiretroviral treatment programme: lessons learnt from a cluster randomized trial comparing strategies of HIV care in Jinja, Uganda. *Trop Med Int Health* 13: 795–800.
13. Saidu Y, Odutola A, Jafali J, Ogundare O, Worwui A, Sey G, Thomas V, Stanley-Batchilly E, Afolabi M, Idoko OT, Owolabi O, Ota MO, 2013. Contextualizing the informed consent process in vaccine trials in developing countries. *J Clin Res Bioeth* 4: 141.
14. Bekker LG, Jaspan H, McIntyre J, Wood R, Gray G, 2005. Adolescents and HIV vaccine trials: what are the clinical trial site issues? *J Int Assoc Physicians AIDS Care (Chic)* 4: 93–97.
15. Fawzi WW, Msamanga GI, Spiegelman D, Urassa EJ, Hunter DJ, 1999. Rationale and design of the Tanzania Vitamin and HIV Infection Trial. *Control Clin Trials* 20: 75–90.
16. Lang TA, White NJ, Tran HT, Farrar JJ, Day NP, Fitzpatrick R, Angus BJ, Denis E, Merson L, Cheah PY, Chilengi R, Kimutai R, Marsh K, 2010. Clinical research in resource-limited settings: enhancing research capacity and working together to make trials less complicated. *PLoS Negl Trop Dis* 4: e619.
17. Grady C, Levine RJ, 2007. Commentary 13.2: shared responsibilities for treatment in the South African phase I HIV vaccine preventative trials. In Lavery JV, Grady C, Wahl ER, Emanuel EJ (Eds.), *Ethical Issues in International Biomedical Research: A Casebook*. New York: Oxford University Press, Inc., pp. 225–232.
18. Nyika A, Chilengi R, Ishengoma D, Mtenga S, Thera MA, Sissoko MS, Lusingu J, Tiono AB, Doumbo O, Sirima SB, Lemnge M, Kilama WL, 2010. Engaging diverse communities participating in clinical trials: case examples from across Africa. *Malar J* 9: 86.
19. Sista ND, Abdool Karim Q, Hinson K, Donnell D, Eshleman SH, Vermund SH, 2011. Experience in international clinical research: the HIV Prevention Trials Network. *Clin Investig (Lond)* 1: 1609–1618.
20. Leach M, Fairhead J, 2011. *Being 'with Medical Research Council': infant care and the social meanings of cohort membership in Gambia's plural therapeutic landscapes*. In: Evidence, ethos and experiment: the anthropology and history of medical research in Africa. Berghahn Series. New York: Berghahn Books, New York: Oxford, pp. 77–98.
21. Essack Z, Koen J, Barsdorf N, Slack C, Quayle M, Milford C, Lindegger G, Ranchod C, Mukuka R, 2010. Stakeholder perspectives on ethical challenges in HIV vaccine trials in South Africa. *Dev World Bioeth* 10: 11–21.
22. Moodley K, 2002. HIV Vaccine Trial participation in South Africa: an ethical assessment. *J Med Philos* 27: 197–215.
23. Ota MO, Idoko OT, Ogundare EO, Afolabi MO, 2013. Human immune responses to vaccines in the first year of life: biological, socio-economic and ethical issues ; a viewpoint. *Vaccine* 31: 2483–2488.
24. Chapman R, Chege G, Shephard E, Stutz H, Williamson AL, 2010. Recombinant *Mycobacterium bovis* BCG as an HIV vaccine vector. *Curr HIV Res* 8: 282–298.
25. Sepulveda RL, Arredondo S, Rodriguez E, Gonzalez B, Leiva LE, Sorensen RU, 1997. Effect of human newborn BCG immunization on monocyte viability and function at 3 months of age. *Int J Tuberc Lung Dis* 1: 122–127.
26. White GP, Watt PM, Holt BJ, Holt PG, 2002. Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD45RO-T cells. *J Immunol* 168: 2820–2827.
27. Kampmann B, Tena-Coki G, Anderson S, 2006. Blood tests for diagnosis of tuberculosis. *Lancet* 368: 282, author reply 282–283.
28. Matsuo K, Yasutomi Y, 2011. *Mycobacterium bovis* Bacille Calmette-Guerin as a vaccine vector for global infectious disease control. *Tuberc Res Treat* 2011: 574591.
29. Kumar P, 2013. Challenges on induction of broadly neutralizing antibodies for optimization of HIV vaccines development and vectored immunoprophylaxis. *J Immune Based Ther Vaccines* 2: 9–13.
30. Kwong PD, Mascola JR, Nabel GJ, 2011. Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. *Cold Spring Harb Perspect Med* 1: a007278. doi:10.1101/cshperspect.a00727.
31. Schim van der Loeff MF, Sarge-Njie R, Ceesay S, Awasana AA, Jaye P, Sam O, Jaiteh KO, Cubitt D, Milligan P, Whittle HC, 2003. Regional differences in HIV trends in The Gambia: results from sentinel surveillance among pregnant women. *AIDS* 17: 1841–1846.
32. Otti PO, Jallow C, 2003. *A Socio-Cultural Study of HIV/AIDS in The Gambia*. UNDP/UN System and Government of The Gambia. Available at <http://ergodd.zoo.ox.ac.uk/download/reports/Socio-culturalStudyERGOREvisedFinal.pdf>. Accessed October 18, 2013.
33. WHO, 2010. Delivering HIV test results and messages for re-testing and counseling in adults. Available at http://www.who.int/hiv/pub/vct/hiv_re_testing/en/index.html. Accessed August 4, 2013.
34. Goonetilleke N, Moore S, Dally L, Winstone N, Cebere I, Mahmoud A, Pinheiro S, Gillespie G, Brown D, Loach V, Roberts J, Guimaraes-Walker A, Hayes P, Loughran K, Smith C, De Bont J, Verlinde C, Vooijs D, Schmidt C, Boaz M, Gilmour J, Fast P, Dorrell L, Hanke T, McMichael AJ, 2006. Induction of multifunctional human immunodeficiency virus type 1 (HIV-1)-specific T cells capable of proliferation in healthy subjects by using a prime-boost regimen of DNA- and modified vaccinia virus Ankara-vectored vaccines expressing HIV-1 Gag coupled to CD8+ T-cell epitopes. *J Virol* 80: 4717–4728.
35. WHO, 2010. *WHO Recommendations on the Diagnosis of HIV Infection in Infants and Children*. WHO Library Cataloguing-in-Publication Data. Available at http://whqlibdoc.who.int/publications/2010/9789241599085_eng.pdf. Accessed December 7, 2013.
36. Quinto L, Aponte JJ, Sacarlal J, Espasa M, Aide P, Mandomando I, Guinovart C, Macete E, Navia MM, Thompson R, Menendez C, Alonso PL, 2006. Hematological and biochemical indices in young African children: in search of reference intervals. *Trop Med Int Health* 11: 1741–1748.
37. Kumwenda NI, Khonje T, Mipando L, Nkananena K, Katundu P, Lubega I, Elbireer A, Bolton S, Bagenda D, Mubiru M, Fowler MG, Taha TE, 2012. Distribution of hematological and chemical pathology values among infants in Malawi and Uganda. *Paediatr Int Child Health* 32: 213–227.
38. Dhingra B, Mishra D, 2011. Immediate versus as-needed acetaminophen for post-immunization pyrexia. *Ann Trop Paediatr* 31: 339–344.
39. Jackson LA, Peterson D, Dunn J, Hambidge SJ, Dunstan M, Starkovich P, Yu O, Benoit J, Dominguez-Islas CP, Carste B, Benson P, Nelson JC, 2011. A randomized placebo-controlled trial of acetaminophen for prevention of post-vaccination fever in infants. *PLoS ONE* 6: e20102.

40. Rose MA, Juergens C, Schmoele-Thoma B, Gruber WC, Baker S, Zielen S, 2013. An open-label randomized clinical trial of prophylactic paracetamol coadministered with 7-valent pneumococcal conjugate vaccine and hexavalent diphtheria toxoid, tetanus toxoid, 3-component acellular pertussis, hepatitis B, inactivated polio virus, and *Haemophilus influenzae* type b vaccine. *BMC Pediatr* 13: 98.
41. Shields B, Wacogne I, Wright CM, 2012. Weight faltering and failure to thrive in infancy and early childhood. *BMJ* 345: e5931.
42. Bejon PM, Kai O, 2007. The induction and persistence of T cell IFN-gamma responses after vaccination or natural exposure is suppressed by *Plasmodium falciparum*. *J Immunol* 179: 4193–4201.
43. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJC, Sesay SSS, Abubakar I, Dunyo S, Sey O, Palmer A, Fofana M, Corrah T, Bojang KA, Whittle HC, Greenwood BM, Conway DJ, 2008. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet* 372: 1545–1554.
44. Ceesay SJ, Casals-Pascual C, Nwakanma DC, Walther M, Gomez-Escobar N, Fulford AJ, Takem EN, Nogaro S, Bojang KA, Corrah T, Jaye MC, Taal MA, Sonko AA, Conway DJ, 2010. Continued decline of malaria in The Gambia with implications for elimination. *PLoS ONE* 5: e12242.
45. Ghouth AS, Nasseb FM, Al-Kaldy KH, 2012. The accuracy of the first response histidine-rich protein2 rapid diagnostic test compared with malaria microscopy for guiding field treatment in an outbreak of falciparum malaria. *Tropenmed Parasitol* 2: 35–37.
46. Abba K, Deeks JJ, Olliaro P, Naing CM, Jackson SM, Takwoingi Y, Donegan S, Garner P, 2011. Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane Database Syst Rev* CD008122.