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Immunogenicity and safety of the candidate RTS,S/AS01 vaccine in young Nigerian children: A randomized, double-blind, lot-to-lot consistency trial

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ABSTRACT

Background: For regulatory approval, consistency in manufacturing of vaccine lots is expected to be demonstrated in confirmatory immunogenicity studies using two-sided equivalence trials. This randomized, double-blind study (NCT01323972) assessed consistency of three RTS,S/AS01 malaria vaccine batches formulated from commercial-scale purified antigen bulk lots in terms of anti-CS-responses induced.

Methods: Healthy children aged 5–17 months were randomized (1:1:1:1) to receive RTS,S/AS01 at 0-1-2 months from one of three commercial-scale purified antigen bulk lots (1600 litres-fermentation scale; commercial-scale lots), or a comparator vaccine batch made from pilot-scale purified antigen bulk lot (20 litres-fermentation scale; pilot-scale lot). The co-primary objectives were to first demonstrate consistency of antibody responses against circumsporozoite (CS) protein at one month post-dose 3 for the three commercial-scale lots and second demonstrate non-inferiority of anti-CS antibody responses at one month post-dose 3 for the commercial-scale lots compared to the pilot-scale lot. Safety and reactogenicity were evaluated as secondary endpoints.

Results: One month post-dose-3, anti-CS antibody geometric mean titres (GMT) for the 3 commercial scale lots were 319.6 EU/ml (95% confidence interval (CI): 268.9–379.8), 241.4 EU/ml (207.6–280.7), and 302.3 EU/ml (259.4–352.3). Consistency for the RTS,S/AS01 commercial-scale lots was demonstrated as the two-sided 95% CI of the anti-CS antibody GMT ratio between each pair of lots was within the range of 0.5–2.0. GMT of the pooled commercial-scale lots (285.8 EU/ml (260.7–313.3)) was non-inferior to the pilot-scale lot (271.7 EU/ml (228.5–323.1)). Each RTS,S/AS01 lot had an acceptable tolerability profile, with infrequent reports of grade 3 solicited symptoms. No safety signals were identified and no serious adverse events were considered related to vaccination.

Conclusions: RTS,S/AS01 lots formulated from commercial-scale purified antigen bulk batches induced a consistent anti-CS antibody response, and the anti-CS GMT of pooled commercial-scale lots was non-inferior to that of a lot formulated from a pilot-scale antigen bulk batch.

1. Introduction

The RTS,S/AS01 candidate malaria vaccine targets the *Plasmodium falciparum* circumsporozoite (CS) protein, therefore acting at the pre-erythrocytic stage of the parasite life cycle [1]. This is a partially efficacious vaccine, which has shown protection against both clinical and severe malaria in young children and infants in a large phase 3 trial in Africa [2,3], and has an acceptable safety profile when co-administered with vaccines included in the routine Expanded Programme on Immunization [2–4].

Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; CS, circumsporozoite; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; HBs, hepatitis B surface antigen; Ig, immunoglobulin; MPL, monophosphoryl lipid A; QS21, *Quillaja saponaria* Molina, fraction 21; SAE, serious adverse event; SAS, Statistical Analysis System.

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For regulatory approval of a new vaccine, it is necessary to demonstrate the quality of the manufacturing process, including consistency in the manufacturing of vaccine lots [5–7]. The assessment is expected to be performed in confirmatory immunogenicity studies using two-sided equivalence trials [8,9]. This study evaluated the consistency and safety of three different RTS,S/AS01 vaccine lots formulated from commercial-scale purified antigen bulk lots. The co-primary objectives were to demonstrate lot-to-lot consistency in terms of anti-CS antibody responses and, if reached, subsequently to demonstrate non-inferiority of the commercial-scale lots to a RTS,S/AS01 vaccine lot derived from pilot-scale purified antigen bulk material.

2. Methods

2.1. Study design and ethics

This was a phase III, randomized, double-blind study (ClinicalTrials.gov, NCT01323972) conducted at two sites between May 2011 and May 2012: University of Nigeria Teaching Hospital in Enugu, which is located in south-east Nigeria, and Jos University Teaching Hospital in Jos, which is in north-central Nigeria.

The production scale of the RTS,S purified bulk antigen was increased from 20 litres-fermentation (pilot-plant scale, produced in January 2010; hereafter referred to as pilot-scale lot) to 1600 litres-fermentation (commercial-scale scale in commercial facilities, produced in October/November 2010; hereafter referred to as commercial-scale lots). The same starting material was used at both manufacturing scales, and the components of the final vaccine, including the adjuvant system, remained identical. Eligible children were randomized (1:1:1:1) to receive one of three different commercial-scale lots (lot 1, 2 or 3) or the pilot-scale lot (comparator) of RTS,S/AS01 vaccine according to a 0, 1 and 2 month schedule.

A randomization list was generated by the study sponsor via an internet-based system, and treatment allocation at each site was performed using MATEX, a program developed for Statistical Analysis System (SAS®; Cary, NC, USA).

The study protocol was approved by the ethics review committee of each study site and by the National Agency for Food and Drug Administration and Control in Nigeria and Western Institutional Review Board in the USA. Overall, this study was conducted in accordance with Good Clinical Practice guidelines and all applicable regulatory requirements, including the Declaration of Helsinki. The trial was conducted in partnership with the PATH Malaria Vaccine Initiative. An Independent Data Monitoring Committee oversaw the study's progress and safety of the children, assisted by a local safety monitor (an experienced physician) at each site.

2.2. Study population

Healthy children aged 5–17 months at the time of first vaccination were eligible for enrolment. As phase II evaluation of RTS,S/AS01 indicated that previous hepatitis B immunization may influence RTS,S-induced antibody responses in children [10], to be eligible for participation, all participants must have received three doses of hepatitis B vaccine before the study start. Exclusion criteria included a history of an immunodeficient or neurological condition, acute disease or fever (axillary temperature $\geq$37.5 °C) at the time of enrolment, and an acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality. Chronic administration of immune-modifying drugs was not permitted. Unapproved use of a drug or vaccine within 30 days before the first study vaccine dose and administration of a licensed vaccine within 7 days of the first dose were also exclusion criteria. Written informed consent was obtained from the children's parents or guardians. Illiterate parents indicated consent with a thumbprint and a signature was obtained from an independent literate witness.

2.3. Study vaccine

Each vaccine dose contained lyophilized RTS,S (25 μg) reconstituted with 500 μl of $AS01_E$ (referred to elsewhere in this paper as AS01), a liposome-based Adjuvant System containing monophosphoryl lipid A (MPL) and *Quillaja saponaria* Molina, fraction 21 (QS21, Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, Massachusetts, USA). The vaccines were administered intramuscularly to the deltoid muscle of the left arm and vaccine recipients were observed for at least 60 min following each vaccination with appropriate medical treatment available in case of anaphylactic shock.

2.4. Study objectives

The co-primary objectives of the study were to first demonstrate consistency of anti-CS antibody responses at one month post-dose 3 for three commercial-scale RTS,S/AS01 lots. If the first primary objective was met, then the second primary objective was to demonstrate non-inferiority of anti-CS antibody responses at one month post-dose 3 of the RTS,S/AS01 commercial-scale lots compared to the pilot-scale lot. The safety and reactogenicity of the vaccine lots were evaluated as secondary endpoints.

2.5. Immunogenicity assessment

Assessment of anti-CS and anti-hepatitis B surface antigen (anti-HBs) antibody titres were performed at the Centre for Vaccinology, Ghent University, Belgium, on serum samples taken before dose 1 and one month after dose 3. Antibodies against CS were measured by evaluating immunoglobulin (Ig) G responses to the CS-repeat region, using a validated enzyme-linked immunosorbent assay (ELISA) with R32LR as the capture antigen and a threshold for a positive titre of 0.5 EU/ml [11]. Anti-HBs antibodies were measured using an in-house sandwich ELISA. The cut-off for seroprotection was 10 mIU/ml [12].

2.6. Safety and reactogenicity assessments

Solicited local (injection site pain, redness and swelling) and general (drowsiness, irritability, loss of appetite and fever) adverse events (AEs) were recorded during the 7-day follow-up, and unsolicited AEs during the 30-day follow-up, after each vaccine dose. Serious AEs (SAEs) were reported throughout the study. Grade 3 (severe) solicited AEs were defined as follows: pain causing crying when limb is moved/spontaneously painful, swelling or redness >20 mm in diameter, drowsiness that prevented normal daily activity, irritability (crying that could not be comforted) that prevented normal activity, loss of appetite (not eating at all), fever with axillary temperature >39.0 °C, or any other AE that prevented normal daily activity. All solicited local reactions were considered causally related to vaccination; the relationship of other AEs was classified as possible or not causally related. Fever (temperature >37.5 °C) was evaluated for cause by study investigators.

2.7. Statistical analyses

Statistical analyses were performed using SAS version 9.2 on Windows and StatXact-8.1 procedure on SAS.

A sample size of 80 children per group was planned to have at least 70 evaluable children in each group (3 lots of commercial-scale and 1 pilot-scale lot). This sample size had >90% power to