Blood-stage malaria vaccine candidate RH5.1/Matrix-M in healthy Tanzanian adults and children; an open-label, non-randomised, first-in-human, single-centre, phase 1b trial

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Summary

Background A blood-stage *Plasmodium falciparum* malaria vaccine would provide a second line of defence to complement partially effective or waning immunity conferred by the approved pre-erythrocytic vaccines. RH5.1 is a soluble protein vaccine candidate for blood-stage *P falciparum*, formulated with Matrix-M adjuvant to assess safety and immunogenicity in a malaria-endemic adult and paediatric population for the first time.

Methods We did a non-randomised, phase 1b, single-centre, dose-escalation, age de-escalation, first-in-human trial of RH5.1/Matrix-M in Bagamoyo, Tanzania. We recruited healthy adults (aged 18–45 years) and children (aged 5–17 months) to receive the RH5.1/Matrix-M vaccine candidate in the following three-dose regimens: 10 µg RH5.1 at 0, 1, and 2 months (Adults 10M), and the higher dose of 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months (delayed-fractional third dose regimen; Adults DFx). Children received either 10 µg RH5.1 at 0, 1, and 2 months (Children 10M) or 10 µg RH5.1 at 0, 1, and 6 months (delayed third dose regimen; Children 10D), and were recruited in parallel, followed by children who received the dose-escalation regimen (Children DFx) and children with higher malaria pre-exposure who also received the dose-escalation regimen (High Children DFx). All RH5.1 doses were formulated with 50 µg Matrix-M adjuvant. Primary outcomes for vaccine safety were solicited and unsolicited adverse events after each vaccination, along with any serious adverse events during the study period. The secondary outcome measures for immunogenicity were the concentration and avidity of anti-RH5.1 serum IgG antibodies and their percentage growth inhibition activity (GIA) in vitro, as well as cellular immunogenicity to RH5.1. All participants receiving at least one dose of vaccine were included in the primary analyses. This trial is registered at ClinicalTrials.gov, NCT04318002, and is now complete.

Findings Between Jan 25, 2021, and April 15, 2021, we recruited 12 adults (six [50%] in the Adults 10M group and six [50%] in the Adults DFx group) and 48 children (12 each in the Children 10M, Children 10D, Children DFx, and High Children DFx groups). 57 (95%) of 60 participants completed the vaccination series and 55 (92%) completed 22 months of follow-up following the third vaccination. Vaccinations were well-tolerated across both age groups. There were five serious adverse events involving four child participants during the trial, none of which were deemed related to vaccination. RH5-specific T cell and serum IgG antibody responses were induced by vaccination and purified total IgG showed in vitro GIA against *P falciparum*. We found similar functional quality (ie, GIA per µg RH5-specific IgG) across all age groups and dosing regimens at 14 days after the final vaccination; the concentration of RH5.1-specific polyclonal IgG required to give 50% GIA was 14·3 µg/mL (95% CI 13·4–15·2). 11 children were vaccinated with the delayed third dose regimen and showed the highest median anti-RH5 serum IgG concentration 14 days following the third vaccination (723 µg/mL [IQR 511–1000]), resulting in all 11 who received the full series showing greater than 60% GIA following dilution of total IgG to 2·5 mg/mL (median 88% [IQR 81–94]).

Interpretation The RH5.1/Matrix-M vaccine candidate shows an acceptable safety and reactogenicity profile in both adults and 5–17-month-old children residing in a malaria-endemic area, with all children in the delayed third dose regimen reaching a level of GIA previously associated with protective outcome against blood-stage *P falciparum* challenge in non-human primates. These data support onward efficacy assessment of this vaccine candidate against clinical malaria in young African children.

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Introduction

The global burden of malaria declined between 2000 and 2015; however, progress has since stalled and even reversed.[1] In 2022, there were 249 million cases of malaria leading to 608 000 deaths, mostly due to *Plasmodium falciparum* in children younger than 5 years in sub-Saharan Africa.[1] Encouragingly, two vaccines targeting liver-invasive pre-erythrocytic parasites (sporozoite stage), RTS,S/AS01 (Mosquirix; GSK, London, UK) and R21/Matrix-M (Serum Institute of India, Pune, India) have been shown to reduce clinical malaria in young African children[2,3] and are now recommended by WHO. However, pre-erythrocytic vaccines necessitate sterile immunity to prevent the subsequent disease-causing blood-stage infection. Addition of a blood-stage vaccine component, if combined in a multi-stage vaccination approach, would provide a second line of defence to complement partially effective or waning pre-erythrocytic immunity.[4]

P falciparum reticulocyte-binding protein homologue 5 (RH5) is the most advanced blood-stage vaccine candidate antigen. RH5 is highly conserved and forms part of a pentameric complex essential for erythrocyte invasion.[5–8] RH5-based vaccine protection in *Aotus* monkeys correlates with a threshold level of functional anti-RH5 serum IgG antibody, measured by the in vitro neutralisation or growth inhibition activity (GIA) assay. Passive transfer of anti-RH5 monoclonal antibody (engineered not to engage complement or Fc receptor-dependent effector mechanisms) in *Aotus* validated GIA as a mechanistic immune correlate.[9] We previously reported that a two-dose, viral-vectored, RH5 formulation was well tolerated and induced cross-strain functional antibodies in malaria-naive UK adults.[10] We subsequently reported that an insect cell-expressed, full-length, RH5 soluble protein vaccine candidate, called RH5.1,[11] formulated in GlaxoSmithKline's $AS01_B$ adjuvant, induced superior levels of functional antibody after three doses in UK adults, compared with two doses of the viral-vectored RH5.[12] This activity led to a significantly reduced in vivo growth rate of *P falciparum* following

Research in context

Evidence before this study

A highly effective and durable vaccine against the human malaria parasite *Plasmodium falciparum* is needed. A multi-stage vaccine, such as combining a blood-stage-targeted vaccine with the approved anti-sporozoite vaccines (RTS,S/AS01 or R21/Matrix-M), is widely regarded as the most promising strategy to achieve this goal; however, development of an effective blood-stage vaccine has proved exceptionally challenging. We searched PubMed for research articles using the terms "malaria vaccine" AND "blood stage" AND ("phase 1-2b" OR "phase ii"). No date or language filters were applied. We identified six blood-stage vaccine candidates that have reached field efficacy trials: SPf66, Combination B, FMP1/AS02, AMA1-C1/Alhydrogel, FMP2.1/$AS02_A$, and GMZ2/Alum. These trials reported either no or minimal efficacy or evidence of strain-specific efficacy linked to target antigen polymorphism. Identification of the highly conserved and essential RH5 target antigen on the *P falciparum* blood-stage merozoite has since reinvigorated this field, with RH5 vaccination and *P falciparum* challenge studies in non-human primates showing in vivo efficacy that correlated with anti-RH5 antibody growth inhibition activity (GIA) measured in vitro. We subsequently reported that RH5 vaccination could reduce the in vivo blood-stage growth rate of *P falciparum* in a phase 1/2a, controlled, human malaria infection trial in healthy UK adults.

Added value of this study

This age de-escalation phase 1b trial reports the first data in a population in a malaria-endemic region for the adjuvanted protein subunit vaccine candidate RH5.1/Matrix-M. We showed that in healthy Tanzanian adults and 5–17-month-old children the vaccine candidate is well tolerated with a favourable safety profile across different dosing and vaccination regimens. Notably, 5–17-month-old children in this trial vaccinated using a 0, 1, 6-month (delayed third dose) regimen, with a 10 µg dose of RH5.1 protein with 50 µg Matrix-M adjuvant, showed the highest anti-RH5 serum IgG responses following third immunisation. This level of antibody resulted in the highest reported levels of GIA in vaccinated humans yet, with all children in this group exceeding the level of GIA associated with protective outcome in non-human primates.

Implications of all the available evidence

These data suggest the importance of a delayed third dose of RH5.1/Matrix-M in 5–17-month-old children to maximise induction of functional anti-malarial antibodies in this target age group for a blood-stage malaria vaccine. As a result of these data, RH5.1/Matrix-M has since progressed to a phase 2b trial in 5–17-month-old children in Burkina Faso to assess the efficacy of this vaccine candidate against clinical *P falciparum* malaria for the first time. These data also highlight the potential for important gains by optimising vaccine dose and regimen in early-phase clinical studies.

blood-stage controlled human malaria infection; however, the level of GIA remained below the *Aotus*-defined threshold of protection.[12] Anti-RH5 serum IgG responses were also more durable in UK adults when the third dose of RH5.1/AS01$_B$ was delivered at 6 months and at one-fifth of the initial dose (delayed fractional regimen), compared with responses seen after three full (identical) doses given 1 month apart.[12]

The RH5 viral-vectored vaccine candidate induced ten-times higher antibody responses in Tanzanian infants aged 6–11 months compared with in UK adults.[13] Here, we therefore assessed safety and immunogenicity of RH5.1 protein, now formulated with Matrix-M adjuvant from Novavax (due to GlaxoSmithKline withdrawing access to AS01$_B$ and to align with R21), in a population in a malaria-endemic region in Tanzania.

Methods

Study design and participants

We conducted a non-randomised, open-label, age de-escalation, dose-escalation, phase 1b trial of the RH5.1/Matrix-M blood-stage malaria vaccine candidate at the Ifakara Health Institute Clinical Trial Facility in Bagamoyo, Tanzania. Malaria incidence in Bagamoyo District was estimated to range from 539 cases per 1000-person years in higher-transmission areas to 76 cases per 1000-person years in lower-transmission areas in 2020.[14] Participants were recruited from Bagamoyo town, a lower-transmission area. To assess the potential effect of previous malaria exposure on RH5.1/Matrix-M immunogenicity, a fourth group of children with higher malaria pre-exposure was recruited. Before study start, a serological assay testing IgG activity against *P falciparum* schizont lysate was established using sera from children living in urban and rural wards within Bagamoyo District to define relative low versus high thresholds of malaria pre-exposure relevant to this study population. These thresholds were used to assign children to groups on the basis of malaria pre-exposure in this study.

Healthy, non-pregnant, non-breastfeeding adults aged 18–45 years and healthy children aged 5–17 months residing in Bagamoyo District, with a negative malaria blood film at screening, were eligible for inclusion in the study. Full inclusion and exclusion criteria are given in the appendix (pp 6–8). Participants (or their guardians) were asked to self-report their sex as male or female. Effective contraception was required for adult female participants (appendix p 6) as there is currently no information about the effect of the vaccine on a foetus. Each participant (or guardian) signed or thumb-printed an informed consent form at the in-person screening visit and consent was verified verbally before each vaccination. Enrolment was into six groups (two adult and four child groups) according to age, timing of enrolment, and malaria pre-exposure status (children only).

The trial was conducted according to the principles of the Declaration of Helsinki 2013 and Good Clinical Practice. It was approved by the Oxford Tropical Research Ethics Committee in the UK, and the following authorities in Tanzania: the Ifakara Health Institute Institutional Review Board, the National Institute for Medical Research, the National Health Research Ethics Sub-Committee, and the Tanzania Medicines and Medical Devices Authority. This trial is registered with ClinicalTrials.gov, NCT04318002, and is now complete.

Procedures

The RH5.1 soluble protein was produced to Good Manufacturing Practice by the Clinical Biomanufacturing Facility in Oxford, UK, as reported previously,[11] and was mixed with 50 µg Matrix-M, a potent, saponin-based adjuvant manufactured by Novavax AB (Uppsala, Sweden), immediately before administration via the intramuscular route.

Recruitment began with six adults who received 10 µg RH5.1 at 0, 1, and 2 months (monthly regimen; Adults 10M), followed by dose-escalation in six adults who received 50 µg RH5.1 at 0 and 1 months and 10 µg RH5.1 at 6 months (delayed-fractional third dose regimen; Adults DFx). Four groups of 12 children aged 5–17 months were subsequently enrolled. Those in groups Children 10M (monthly regimen) and Children 10D (10 µg RH5.1 at 0, 1, and 6 months; delayed third dose regimen) were recruited in parallel first, followed by the dose-escalation group, who received the delayed-fractional regimen (Children DFx). Finally, we recruited children in the higher malaria pre-exposure cohort, who also received the delayed-fractional regimen (High Children DFx). An independent data safety monitoring board (DSMB) reviewed the study progress according to a safety review schedule, including before age de-escalation (from adults to children) or each dose-escalation step (from 10 µg to 50 µg RH5.1).

Following each vaccination, each participant was visited at home on days 1 (children only), 3, 4, 5, and 6. On days 1 (adults only), 2, 7, 14, and 28 following vaccination, participants were seen at the clinical research facility. Additionally, participants attended the facility for follow-up on days 84, 112, 309, and 674 post-third vaccination. Blood samples for safety (full blood count, alanine aminotransferase, and creatinine) were carried out at screening and at all clinic visits except those occurring on days 1–2 post-vaccination and on day 28 post-second vaccination for the delayed third dose regimen groups. Blood tests for immunology were taken at all visits except those occurring 2 days after each vaccination and 7 days after the first vaccination. Solicited adverse events were collected at visits up to 7 days post-vaccination and unsolicited adverse events were collected at visits up to 28 days post-vaccination. Serious adverse events were reported for the duration of the trial. We assessed the probable cause and grade of adverse events (appendix pp 12–13, 28–31). Unsolicited adverse events

See Online for appendix

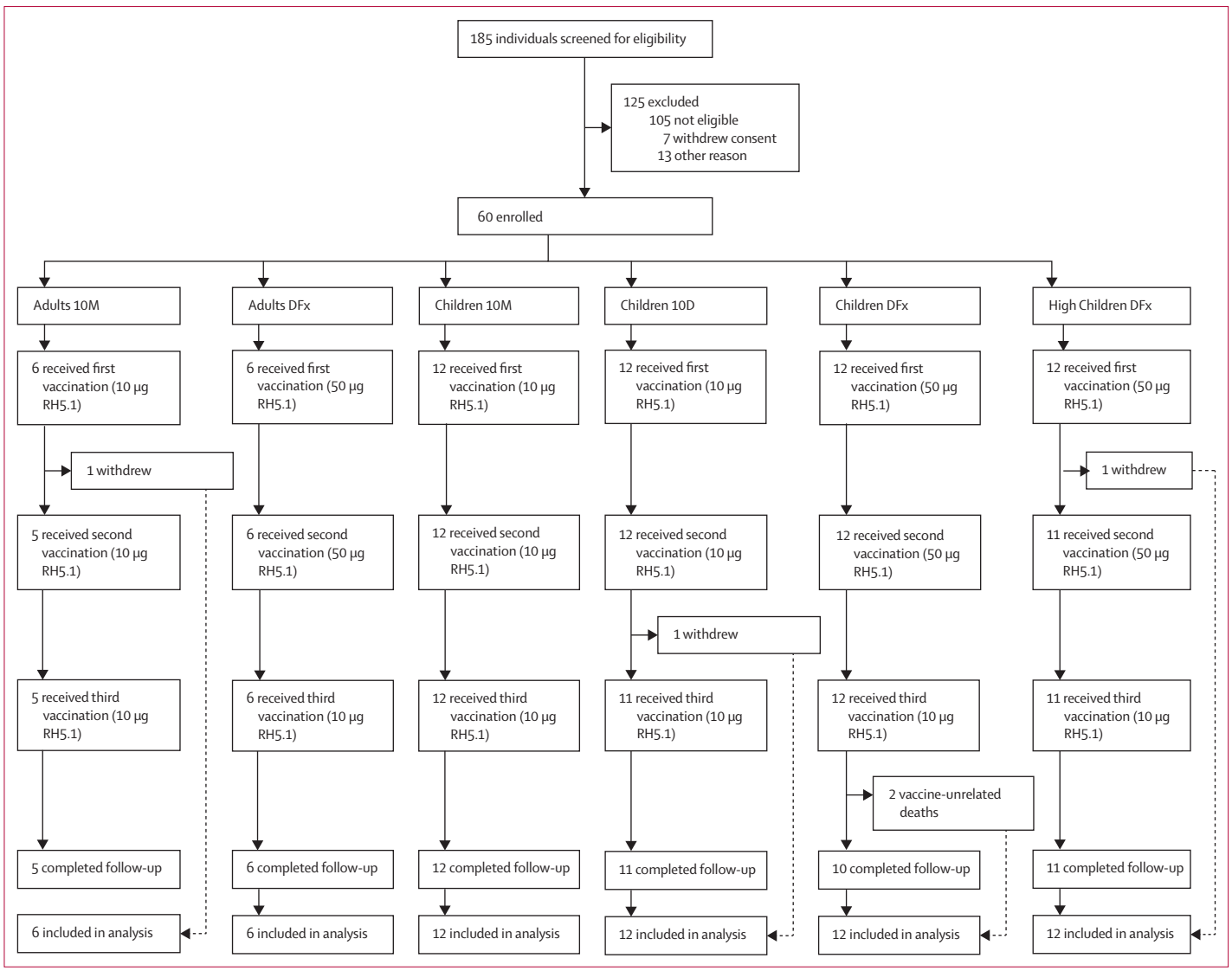

Figure 1: **Trial profile**
All indicated doses of RH5.1 were administered with 50 µg Matrix-M adjuvant. The main reason for participant withdrawal was consent withdrawal, which was not due to adverse experience. Two participants withdrew consent after the first study vaccination, one in the adults 10M and one in high children DFx groups, and one participant in the children 10D group withdrew after the second vaccination (due to the participant moving out of the study area). All participants who received at least a single dose of RH5.1/Matrix-M were analysed as part of the safety and immunogenicity cohorts. Adults 10M=adults who received 10 µg RH5.1 at 0, 1, and 2 months. Adults DFx=adults who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. Children 10M=children who received 10 µg RH5.1 at 0, 1, and 2 months. Children 10D=children who received 10 µg RH5.1 at 0, 1, and 6 months. Children DFx=children who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. High Children DFx=children with higher malaria pre-exposure who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months.

were classified according to the Medical Dictionary for Regulatory Activities (version 26.0).

Outcomes

The primary objective for this study was assessment of the safety and tolerability of RH5.1/Matrix-M in adults and children in a malaria-endemic country. Primary outcome measures were solicited adverse events for 7 days after each vaccination, unsolicited adverse events for 28 days after each vaccination, and serious adverse events during the study period. The primary outcome analysis was done in the safety analysis population, which included participants who received at least the first dose of vaccine.

The secondary objectives were to assess the magnitude, quality, and longevity of humoral and cellular immune responses to RH5.1/Matrix-M in adults and children in a malaria-endemic country (appendix pp 14–15, 17–18). The secondary outcome measures for immunogenicity were the concentration and avidity (overall strength of binding) of anti-RH5.1 serum IgG antibodies by ELISA and their percentage GIA in vitro using purified IgG, as well as cellular immunogenicity to RH5.1 as measured by ex vivo ELISpot assay[12] or flow cytometry (to be reported elsewhere).

Exploratory objectives were analysis of the frequency of RH5-specific plasma cells in the bone marrow of adults vaccinated with RH5.1/Matrix-M (reported elsewhere[15]) and to assess the effect of relatively higher malaria pre-exposure on vaccine-induced immune responses.

Statistical analysis

We aimed to assess the safety and immunogenicity of RH5.1/Matrix-M. Group size was chosen to allow safety assessment via descriptive analysis of adverse events after vaccination, rather than testing for statistical differences between groups. We analysed immunological data using GraphPad Prism (version 10.1 for Windows; GraphPad Software, Boston, MA USA). We applied non-parametric tests because of small group sizes. Analyses of ELISA and GIA data between groups were post hoc and used the Mann–Whitney test for the comparison of two groups, or Kruskal–Wallis with Dunn's multiple comparison test for three or more groups. All tests used were two-tailed. We used non-linear four-parameter regression to analyse ELISA versus GIA (constrained to >0 % and <100% GIA). A value of $p<0·05$ was considered significant. All participants who received at least one dose of RH5.1/Matrix-M were included in the analyses.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We recruited participants between Jan 25, and April 15, 2021. We screened 26 adults and enrolled 12 (46%) and screened 159 children, of whom 156 (98%) were tested for anti-parasite lysate IgG responses, with 36 (23%) recruited from those defined as having relatively low previous malaria exposure and 12 (8%) recruited from those defined as relatively high previous malaria exposure (figure 1; appendix p 42). Two participants in the high children DFx group were recruited with anti-parasite lysate IgG slightly below the serological threshold; following a review these participants continued to receive the remaining vaccinations and study visits as scheduled. There were three withdrawals during the vaccination period. Two participants withdrew after the first study vaccination and one withdrew after the second vaccination; no withdrawals were related to adverse experiences with study procedures. All other participants received the study vaccinations as scheduled. The final study vaccinations were administered on Sept 11, 2021. Two child participants died during the post-vaccination follow-up period for reasons unrelated to the study or the study vaccinations (figure 1). All other participants completed follow-up until 22 months post-final vaccination, with the final study visit taking place on July 27, 2023.

The median age of all adult participants was 23·5 years (range 20–30 years) and the median age of all child participants was 12 months (range 5–17 months), with similar ranges across the groups (table 1). Similar numbers of male and female participants were enrolled across the child groups; however, although there were no sex-based exclusion criteria, no adult female participants were screened or recruited.

	Adults		Children			
	10M (n=6)	DFx (n=6)	10M (n=12)	10D (n=12)	DFx (n=12)	High DFx (n=12)
Sex						
Female	0	0	8 (75%)	4 (25%)	4 (25%)	8 (75%)
Male	6 (100%)	6 (100%)	4 (25%)	8 (75%)	8 (75%)	4 (25%)
Age	25·5 years (23–29)	21 years (20–30)	12 months (6–15)	14·5 months (5–17)	8·5 months (5–15)	11·5 months (5–15)
Literacy						
Literate	6 (100%)	6 (100%)	NA	NA	NA	NA
Not literate	0	0	NA	NA	NA	NA
Education level						
Primary	3 (50%)	1 (17%)	NA	NA	NA	NA
Secondary	3 (50%)	5 (83%)	NA	NA	NA	NA
Tertiary	0	0	NA	NA	NA	NA

Data are n (%) or median (range). 10D=10 µg RH5.1 at 0, 1, and 6 months. 10M=10 µg RH5.1 at 0, 1, and 2 months. DFx=50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. High DFx=higher malaria and 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. NA=not applicable.

Table 1: **Baseline characteristics**

There were no safety concerns during the course of the trial. Most local solicited adverse events in both adults and children were graded as mild to moderate in severity (table 2; appendix pp 36–37). Severe local solicited adverse events were reported in one adult and three children. The most common local solicited adverse event across all groups was swelling, reported after 32 (18%) of 175 vaccine doses and in 24 (40%) of 60 participants, mainly occurring after the second and third doses (table 2). Severe swelling and induration occurred in one adult participant in the Adults 10M group after the final vaccine administration, which resolved spontaneously within 7 days. All other local adverse events in adults resolved by 72 h post-vaccine administration. In the groups children DFx and high children DFx, three children developed severe and one developed moderate induration after receiving their final delayed dose. Most local solicited adverse events in children resolved within 72 h post-vaccination, and all resolved by day 8 (appendix pp 36–37).

Most systemic solicited adverse events were mild in severity and none were severe (table 2; appendix pp 36–37); the most frequent were mild subjective fever (reported after 31 [18%] of 175 vaccinations) and mild objective fever (reported after 12 [7%] vaccinations). One case of moderately severe subjective fever and one of objective fever were reported in the higher-dose child groups after receiving the second vaccination. Fever occurred only in children, with subjective fever reported

	Adults						Children											
	10M			DFx			10M			10D			DFx			High DFx		
	Vaccination 1 (n-6)	Vaccination 2 (n=5)	Vaccination 3 (n=5)	Vaccination 1 (n=6)	Vaccination 2 (n=6)	Vaccination 3 (n=6)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=11)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=11)	Vaccination 3 (n=11)
Pain at the injection site																		
Mild	0	1 (20%)	0	3 (50%)	1 (17%)	5 (83%)	0	0	0	0	1 (8%)	0	0	0	0	0	1 (9%)	1 (9%)
Moderate	1 (17%)	1 (20%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limitation of arm movement																		
Mild	0	0	0	0	0	1 (17%)	0	0	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swelling at the injection site																		
Mild	0	4 (80%)	0	0	0	4 (67%)	0	0	0	1 (8%)	0	0	0	0	3 (25%)	0	1 (9%)	6 (55%)
Moderate	0	0	0	0	0	0	0	0	2 (17%)	0	2 (17%)	0	0	2 (17%)	0	0	6 (55%)	0
Severe	0	0	1 (20%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pruritus																		
Mild	0	0	0	0	1 (17%)	1 (17%)	0	0	1 (8%)	0	0	0	0	0	0	0	1 (9%)	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Induration																		
Mild	0	4 (80%)	0	0	0	0	0	0	2 (17%)	1 (8%)	1 (8%)	0	0	2 (17%)	2 (17%)	0	0	2 (18%)
Moderate	0	0	0	0	0	0	0	0	1 (8%)	0	0	0	0	0	1 (8%)	0	0	0
Severe	0	0	1 (20%)	0	0	0	0	0	0	0	0	0	0	0	2 (17%)	0	0	1 (9%)
Erythema																		
Mild	0	3 (60%)	0	0	0	2 (33%)	0	0	1 (8%)	0	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue/Malaise																		
Mild	1 (17%)	1 (20%)	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Severe	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Chills																		
Mild	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Severe	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(Table 2 continues on next page)

	Adults						Children											
	10M			DFx			10M			10D			DFx			High DFx		
	Vaccination 1 (n-6)	Vaccination 2 (n=5)	Vaccination 3 (n=5)	Vaccination 1 (n=6)	Vaccination 2 (n=6)	Vaccination 3 (n=6)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=11)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=11)	Vaccination 3 (n=11)
(Continued from previous page)																		
Myalgia																		
Mild	0	0	0	1 (17%)	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Severe	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Arthralgia																		
Mild	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Severe	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Subjective fever																		
Mild	0	0	0	0	0	0	0	1 (8%)	1 (8%)	1 (8%)	3 (25%)	4 (36%)	0	2 (17%)	1 (8%)	0	8 (72%)	10 (91%)
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (8%)	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Objective fever																		
Mild	0	0	0	0	0	0	0	1 (8%)	1 (8%)	0	1 (8%)	1 (9%)	0	3 (25%)	1 (8%)	0	4 (36%)	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (9%)	1 (9%)
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Allergic reaction																		
Mild	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nausea																		
Mild	0	0	0	0	1 (17%)	0	0	0	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache																		
Mild	1 (17%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Moderate	1 (17%)	1 (20%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reduced activities																		
Mild	NA	NA	NA	NA	NA	NA	0	0	0	0	1 (8%)	0	0	1 (8%)	0	0	0	1 (9%)
Moderate	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0
Severe	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0

(Table 2 continues on next page)

	Adults				Children									
	10M		DFx		10M		10D		DFx		High DFx			
	Vaccination 1 (n=6)	Vaccination 2 (n=5)	Vaccination 3 (n=5)	Vaccination 1 (n=6)	Vaccination 2 (n=6)	Vaccination 3 (n=6)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=12)	Vaccination 3 (n=12)	Vaccination 1 (n=12)	Vaccination 2 (n=11)	Vaccination 3 (n=11)
(Continued from previous page)															
Reduced oral intake															
Mild	NA	NA	NA	NA	NA	NA	2 (17%)	1 (8%)	0	0	0	1 (8%)	0	0	1 (9%)
Moderate	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Severe	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Vomiting															
Mild	NA	NA	NA	NA	NA	NA	1 (8%)	1 (8%)	0	0	0	1 (8%)	1 (8%)	0	1 (9%)
Moderate	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Severe	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Diarrhoea															
Mild	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	1 (8%)	1 (9%)	2 (18%)
Moderate	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Severe	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0

Data are n (%) of participants reporting local and systemic solicited adverse events in the 7 days following first, second, and third vaccinations. The maximum severity reported by each participant for each adverse events after each study vaccination is shown. NA=not applicable.

Table 2: **Safety summary**

in 22 (46%) of 48 and objective fever in 13 (27%; table 2; appendix pp 36–37). Vomiting, diarrhoea, reduced oral intake, and reduced activities were also experienced by children only and were all of mild severity (appendix p 36). Most systemic solicited adverse events resolved within 48 h of vaccination in adults and within 72 h in children; all resolved by day 8 (data not shown). Few unsolicited adverse events were deemed at least possibly related to study interventions, all of which were mild or moderate in severity (appendix p 38); all resolved by the end of the study period.

There were five serious adverse events involving four children during the trial, none of which were deemed related to vaccination (appendix pp 22–24, 39). Two of the five events had a fatal outcome: one child diagnosed with stage IV neuroblastoma after enrolling in the trial and one child with acute respiratory failure secondary to herbal intoxication. The independent trial DSMB was informed of both cases and determined there was no relationship to study vaccinations. There were no adverse events of special interest or suspected unexpected serious adverse reactions.

The most frequent laboratory abnormality identified in the 28-day post-vaccination period in adults was lymphopenia, occurring in one (17%) of six adults in the adults 10M group and two (33%) of six in the adults DFx group (appendix pp 24, 40–41). All were mild in graded severity and resolved by the end of the study period, apart from one participant in the adult 10M group who had mild lymphopenia who withdrew following the day-14 visit (post-first vaccination). The most frequent laboratory abnormality identified in the 28-day post-vaccination period in children was anaemia, occurring in seven (58%) of 12 participants in the children 10M, children 10D, and children DFx groups, and in five (42%) of 12 in the high children DFx group. The maximum severity of anaemia was moderate and, in all cases, had resolved by the final study visit (maximum duration 154 days; appendix pp 24, 40–41). There were three severe (grade 3) laboratory adverse events in children in the 28 days following vaccination, based on the relevant toxicity grading scales relevant to healthy HIV-negative participants with modification to suit local normal values (appendix p 30). Two of these events were episodes of severe neutropenia, probably related to concurrent bacterial infections, both of which completely resolved with treatment of the probable underlying trigger. One participant in the high children DFx group developed severe eosinophilia at 7 days after the third vaccination, which persisted at moderate grade until 28 days post-vaccination and had resolved by 84 days. There were no symptoms suggestive of allergy and stool microscopy did not show any evidence of helminth infection. This child was well throughout this time period with no concerns apart from an isolated episode of fever on day 22.

All participants tested negative for malaria by microscopy at screening and were subsequently tested by

microscopy at the second and third vaccination visits and monthly thereafter. Two participants in the high children DFx group (with higher previous malaria exposure) tested positive during study visits in the follow-up period (at 4 and 12 months following third vaccination); these participants were clinically well and received anti-malarial treatment in the community. Additionally, one participant in the children DFx group tested positive for malaria outside of the study during chemotherapy for neuroblastoma (appendix p 22).

The magnitude of the serum anti-RH5.1 IgG antibody response was measured over time by ELISA. At the time of first vaccination almost all participants showed background level responses (figure 2A), consistent with immuno-epidemiological data that suggest RH5 is not a dominant target of naturally acquired malaria immunity.[8,10] 14 days following two monthly vaccinations of either 10 µg or 50 µg RH5.1 with Matrix-M adjuvant, responses were similar across the two doses within the adult or child cohorts (figure 2A–C). However, the magnitude of the antibody response showed a clear age dependence, with significantly higher responses (around 4–5 times) observed in the 5–17-month-old children (figure 2D). After two doses of 10 µg RH5.1, adults showed a median response of 38 µg/mL anti-RH5.1 IgG (IQR 18–65) and, after two doses 50 µg RH5.1, adults showed a median response of 52 µg/mL anti-RH5.1 IgG (14–99), compared with children with median responses of 230 µg/mL anti-RH5.1 IgG (130–352; groups children 10M and children 10D combined) and 200 µg/mL anti-RH5.1 IgG (100–409; group DFx; figure 2D).

Responses then declined over time and were significantly lower before the third vaccination in all participants receiving the delayed or delayed-fractional boost at month 6, compared with those receiving a final boost at month 2 (figure 2A, C). Subsequently, 14 days following the third dose (day 70 for monthly cohorts and day 196 for delayed and delayed-fractional cohorts), significantly higher responses (around 2–3 times) were maintained in the 5–17-month-old children than in adults, regardless of vaccination with either the monthly or delayed-fractional regimen (figure 2E). Responses were moderately but significantly higher at 14 days after the third dose in the adults DFx (median response 138 µg/mL [IQR 110–211]) than in the adults 10M group (median response 81 µg/mL [51–99]; figure 2A). By contrast, responses at 14 days after the third dose in the 5–17-month-old children receiving the same regimens were similar, with median responses of 293 µg/mL (191–456) in the children 10M group and 290 µg/mL (209–338) in the children DFx group (figure 2C). We also found no difference in the serum anti-RH5 IgG response at any timepoint between the two child cohorts who received the same DFx regimen but who differed in baseline malaria pre-exposure status (figure 2B). However, participants in the children 10D group showed significantly higher responses at 14 days following the

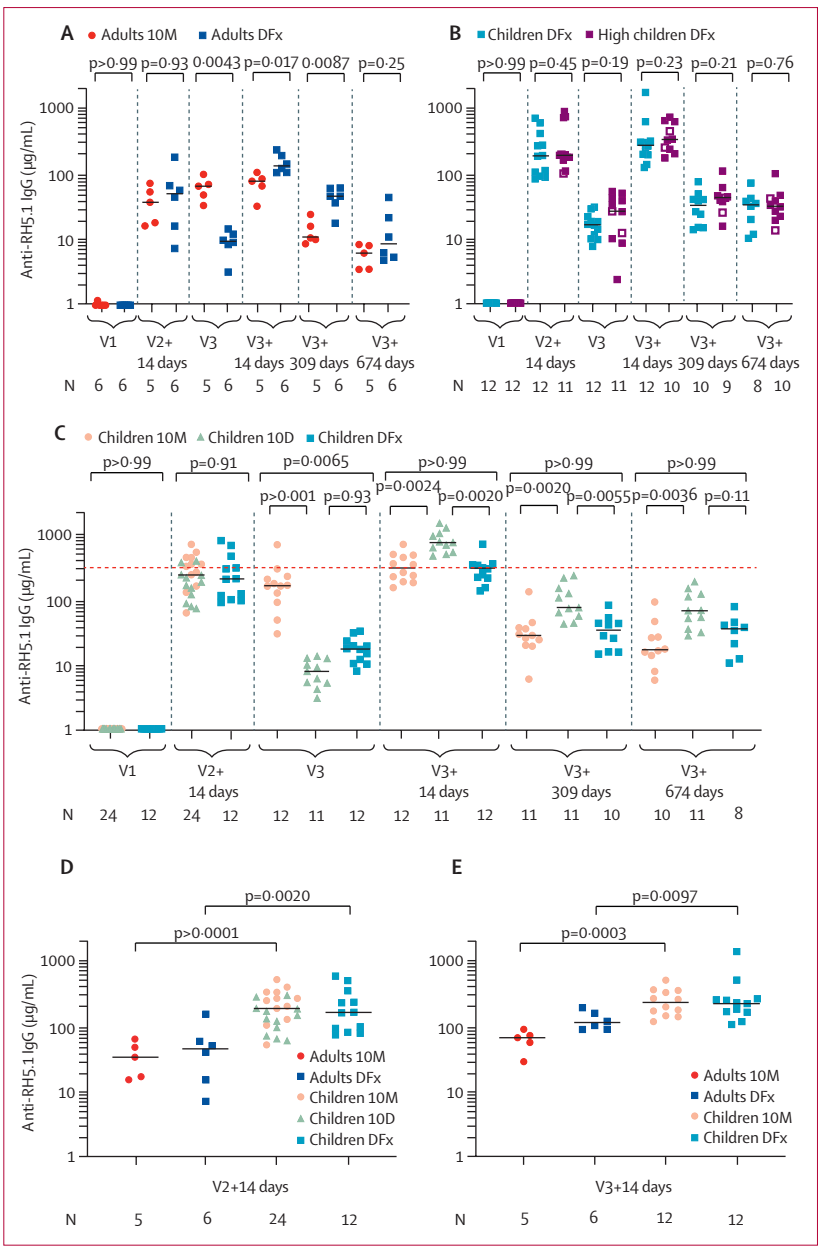

Figure 2: **Serum antibody response to vaccination**
Median and individual anti-RH5.1 serum total IgG responses as measured by ELISA are shown before V1; at V2 plus 14 days; day of V3; and at V3 plus 14, 309, and 674 days for Adults 10M and Adults DFx (A) and Children 10M, Children 10D, and Children DFx (C). Children 10M and Children 10D data were pooled at V2 plus 14 due to identical immunisation and dosing regimens at this timepoint. The red dotted line indicates the threshold associated with protection against *Plasmodium falciparum* blood-stage challenge in RH5-vaccinated *Aotus* monkeys (around 300 µg/mL).[16] (B) ELISA data for the Children DFx and High Children DFx groups. Open symbols indicate two participants who did not meet the high pre-exposure threshold. Group kinetic responses are shown in the appendix (p 43). Data from adult and child groups at V2 plus 14 days (D) and at V3 plus 14 days (E) replotted from panels A and B to show age comparison. Post-hoc analyses to compare between two regimens in panels A–C or age groups in panels D and E at specific timepoints used the Mann–Whitney test, except for V3, V3 plus 14 days, V3 plus 309 days, and V3 plus 674 days in panel B, which used Kruskal–Wallis with Dunn's multiple comparison test. Adults 10M=adults who received 10 µg RH5.1 at 0, 1, and 2 months. Adults DFx=adults who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. Children 10M=children who received 10 µg RH5.1 at 0, 1, and 2 months. Children 10D=children who received 10 µg RH5.1 at 0, 1, and 6 months. Children DFx=children who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. High children DFx=children with higher malaria pre-exposure who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. V1=first vaccination. V2=second vaccination. V3=third vaccination.

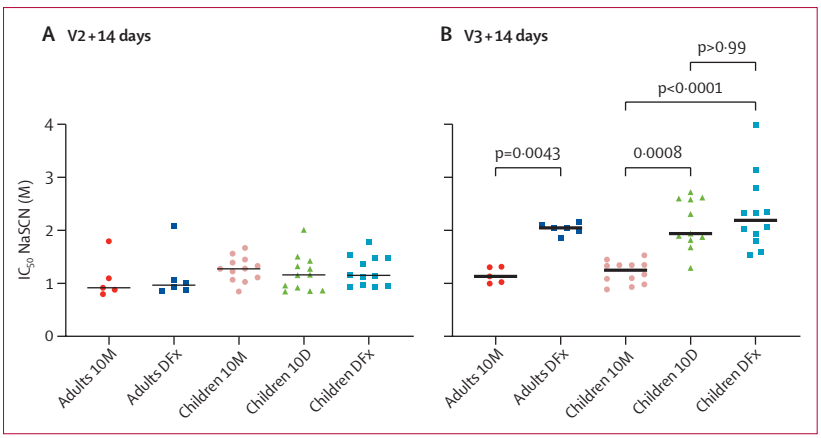

Figure 3: **Avidity of serum antibody responses**
Avidity of serum anti-RH5.1 total IgG responses was assessed by sodium thiocyanate (NaSCN) displacement ELISA at V2 plus 14 days (day 42; A) and V3 plus 14 days (day 70 for monthly regimens and day 196 for delayed third dose regimens; B). Post-hoc analyses in panel B to compare between the two adult groups used Mann–Whitney test, and the three child groups used Kruskal–Wallis with Dunn's multiple comparison test. Adults 10M=adults who received 10 µg RH5.1 at 0, 1, and 2 months. Adults DFx=adults who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. Children 10M=children who received 10 µg RH5.1 at 0, 1, and 2 months. Children 10D=children who received 10 µg RH5.1 at 0, 1, and 6 months. Children DFx=children who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. IC_{50}=the molar concentration of NaSCN required to reduce the starting optical density in the ELISA by 50%.

third dose, reaching a median of 723 µg/mL (IQR 511–1000) and all above the level associated with protection against *P falciparum* blood-stage challenge in *Aotus* monkeys[16] (figure 2C).

Anti-RH5 serum IgG antibody responses subsequently declined over time (appendix p 43), but a similar pattern of immunogenicity to the peak response was maintained at 309 days after the third dose, although we found no significant differences at 674 days after the third dose between the monthly and delayed-fractional regimens in adults or children (figure 2A, C). The highest responses were maintained at 674 days after the third dose in the children 10D group, with a median of 65 µg/mL (IQR 34–117); these responses were significantly higher than those in the children 10M group, with median of 16 µg/mL (12–30; figure 2C).

We also assessed the avidity of the anti-RH5 serum IgG response and the induction of RH5-specific interferon (IFN)-γ T cell responses. Administration of a delayed or delayed-fractional, but not monthly, third dose led to significantly more avid anti-RH5 IgG responses in adults and children (figure 3A, B). By contrast, the anti-RH5 IFN-γ T cell response was similar across all groups after the third vaccination, regardless of age or regimen (appendix p 44).

Serum samples were next tested for in vitro functional anti-parasitic activity in a blinded analysis at the GIA Reference Centre (National Institute of Allergy and Infectious Diseases, National Institutes of Health). Purified total IgG was normalised to a starting concentration of 10 mg/mL and tested against *P falciparum* (3D7 clone) parasites using the standardised single-cycle GIA assay. All baseline samples (pre-vaccination) showed negligible (<20%) GIA, except for two adults with 21% and 58% GIA (figure 4A); however, large increases in overall GIA were observed following RH5.1/Matrix-M vaccination, with all samples from children tested using 10 mg/mL total IgG showing greater than 80% GIA at 14 days after the third dose (figure 4B). All total IgG samples with greater than 40% GIA at 10 mg/mL were subsequently titrated using a 2-fold dilution series in the assay (appendix p 45). Using 2·5 mg/mL total purified IgG, the results were similar to the previous serology with children showing higher levels of GIA than those of adults (figure 4C), and with no differences seen between the two child delayed-fractional groups who differed in baseline malaria pre-exposure status (figure 4D). Moreover, participants in the children 10D group showed a median GIA level of 88% (IQR 81–94), significantly outperforming the children 10M (71% [59–82]) and children DFx (68% [51–84]) groups (figure 4C).

GIA showed a sigmoidal relationship to the concentration of RH5.1-specific IgG present in the total IgG used in the assay and as measured by ELISA (figure 4E). The concentration of RH5.1-specific polyclonal IgG required to give 50% GIA (EC_{50}) was similar across all the groups (regardless of age, vaccine regimen, and malaria pre-exposure status at baseline). Analysis across all groups combined showed a GIA EC_{50} of 14·3 µg/mL (95% CI 13·4–15·2).

Discussion

We showed that the RH5.1/Matrix-M blood-stage malaria vaccine candidate has a favourable safety and reactogenicity profile in healthy Tanzanian adults and 5–17-month-old children. Independent review concluded that none of the five serious adverse events, involving four children and two with fatal outcomes, were related to vaccination. These occurred against a similar backdrop child mortality rate (aged 1 to <5 years) of 26·5 per 1000 population and infant mortality rate (aged 0 to <1 years) of 53·0 per 1000 population in Bagamoyo District, as last recorded in 2012.[17] Data now reported for 109 adults, 66 children, and 18 infants vaccinated with RH5-based vaccines[10,12,13] all show similar safety and tolerability profiles, and Matrix-M adjuvant is now licenced in vaccines for malaria and COVID-19.[3,18] Ongoing phase 1/2 trials continue to monitor the safety of RH5.1/Matrix-M vaccination (ISRCTN95289709 and NCT05790889).

RH5.1/Matrix-M induced peripheral IFNγ T cell responses that were similar to those reported in UK adults immunised with the same regimen of RH5.1/AS01$_B$;[12] we also showed that RH5-specific circulating T follicular helper (Tfh) cell responses correlated with RH5-specific memory B cells and serum antibody.[19] Ongoing work is investigating B cell and Tfh cell response phenotypes in RH5.1/Matrix-M vaccine recipients.

Priming of Tanzanian adults with RH5.1/Matrix-M led to similar antibody responses after the second

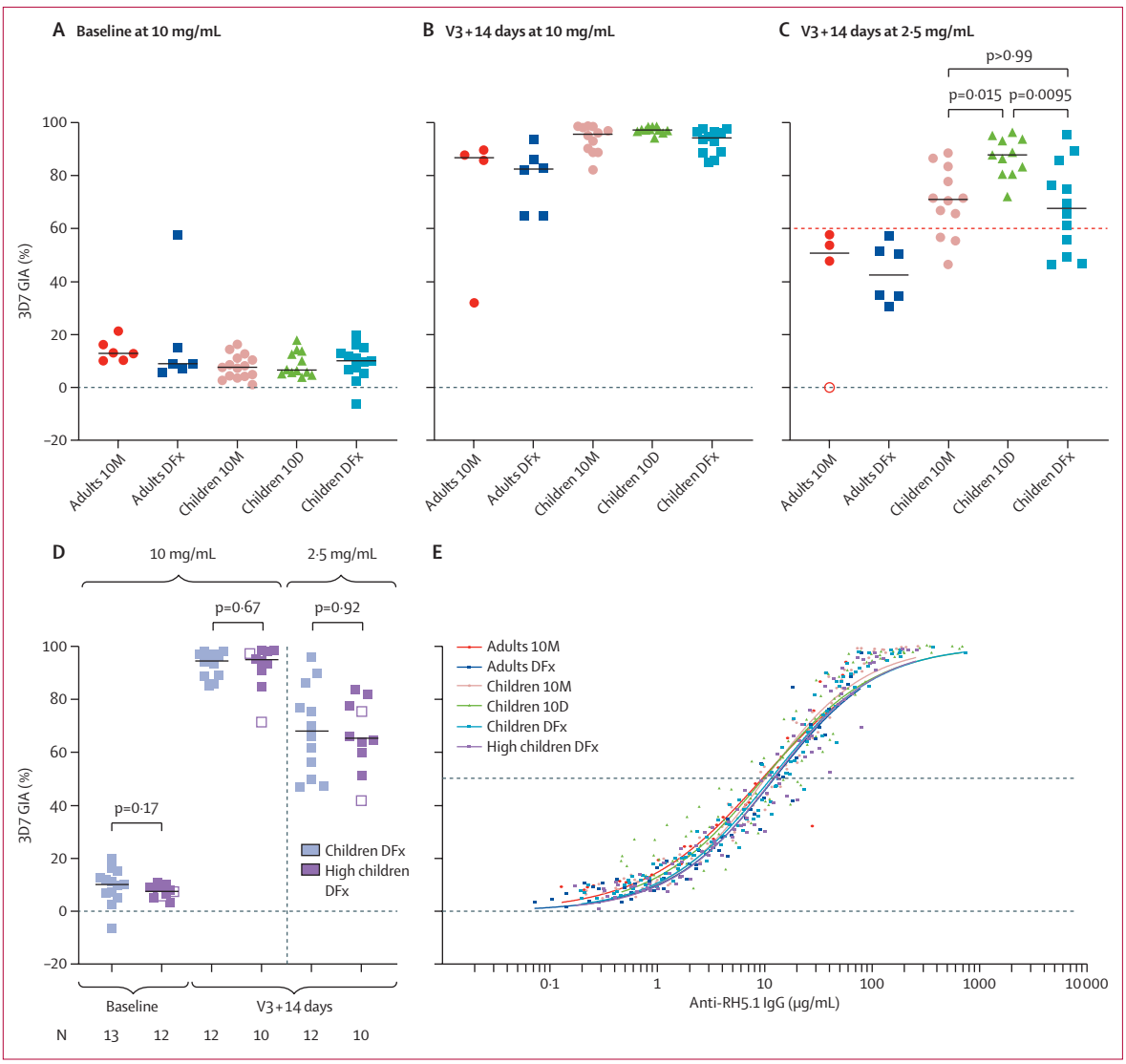

Figure 4: **Functional GIA induced by RH5 vaccination**
In vitro GIA of total IgG purified from serum was assessed against 3D7 clone *Plasmodium falciparum* parasites. Median and individual values are shown for each group at baseline (day 0) at 10 mg/mL total IgG (A); V3 plus 14 days at 10 mg/mL total IgG (B); and V3 plus 14 days at 2·5 mg/mL total IgG (C). The open circle datapoint in panel C was arbitrarily set at 0% GIA for the sample not titrated in the assay. The red dashed line indicates the threshold of 60% GIA associated with protective outcome in RH5-vaccinated and *P falciparum*-challenged *Aotus* monkeys.[16] Post-hoc analysis to compare between child dosing regimens used Kruskal–Wallis with Dunn's multiple comparison test. (D) GIA of samples at baseline and V3 plus 14 days tested at 10 mg/mL and 2·5 mg/mL total IgG in the children DFx and high children DFx groups. Open symbols indicate two participants who did not meet the high pre-exposure threshold. Post-hoc analyses to compare between the two groups used Mann–Whitney test. (E) Samples from V3 plus 14 day were titrated in the GIA assay using a 2-fold dilution series starting at 10 mg/mL. The relationship between GIA data from the dilution series and the concentration of anti-RH5.1-specific IgG used in the assay as measured by ELISA in the total IgG is shown. Non-linear four-parameter regression curves (constrained to >0% and <100 % GIA) are shown for each group. We calculated the concentration of anti-RH5.1 polyclonal IgG that gives 50% GIA (dashed line represents 50% GIA) using data points pooled across all groups (r^2=0·95; n=487). 3D7=*Plasmodium falciparum* clone 3D7. Adults DFx=adults who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. Children 10M=children who received 10 µg RH5.1 at 0, 1, and 2 months. Children 10D=children who received 10 µg RH5.1 at 0, 1, and 6 months. Children DFx=children who received 50 µg RH5.1 at 0 and 1 month and 10 µg RH5.1 at 6 months. GIA=growth inhibition activity.

vaccination, regardless of RH5.1 dose. However, a delayed-fractional (rather than monthly) third dose induced significantly more avid and higher concentrations of anti-RH5 serum IgG, with responses better maintained for around 1 year. These responses were consistent with our observations in UK adults vaccinated with RH5.1/AS01$_B$ using the same regimens,[12] where we also previously showed that the delayed-fractional (as opposed to monthly) dosing regimen in adults induces an improved circulating RH5-specific memory B cell response and a higher frequency of RH5-specific (and putatively long-lived) plasma cells in the bone marrow that correlated with the serum anti-RH5 IgG response.[15,20] However, these observations were only

partly replicated in children. Here, we observed higher serum antibody responses in children than in adults (a phenomenon seen with other malaria subunit vaccines, including R21/Matrix-M,[13,21–24] which bodes well for future assessment of multi-stage combination malaria vaccine strategies in children) but, unlike in adults, the monthly and delayed-fractional regimens performed similarly. By contrast, the highest anti-RH5 serum IgG responses were induced by a delayed, but not fractionated, third dose of RH5.1 protein at 6 months. The underlying reasons for this remain unknown, although circulating levels of vaccine-specific antibody might affect vaccine responsiveness[25] and this group (primed twice with 10 µg RH5.1 with Matrix-M) showed lower anti-RH5 serum IgG responses at the time of third vaccination (month 6) than in those in the delayed-fractional regimen group (primed twice with a higher 50 µg RH5.1 dose). Consistent with our observations, trials of RTS,S/AS01 showed improved outcome measures with a delayed-fractional third dose (as opposed to monthly) regimen in healthy US adults,[26] but no improvement in field efficacy when tested in 5–17-month-old children.[27] Notably, these trials fractionated the dose of adjuvant and antigen, unlike our trials in which a fractionated dose of antigen was delivered in a full dose of adjuvant.[12] Our data thus suggest a delayed, as opposed to delayed-fractional, third dose might be more optimal in children, which warrants further investigation.

We previously reported an association between the in vitro GIA assay and *in vivo* protection outcome following RH5 vaccination and *P falciparum* blood-stage challenge in *Aotus* monkeys, with protected animals showing greater than 60% GIA at 2·5 mg/mL purified IgG.[9,16] A similar association was observed following controlled human malaria infection in healthy UK adults vaccinated with RH5.1/AS01$_B$, although this threshold level of GIA was not reached in that study,[12] and GIA thus remains to be formally associated with protection against clinical malaria in vaccinated African children. Nevertheless, all four groups of vaccinated children in our study showed median GIA levels of greater than 60% at 2·5 mg/mL total IgG, with all 11 participants in the Children 10D group above this threshold at day 14 following the third vaccination. This level of GIA is the highest level of functional GIA observed in human vaccinees to date (appendix pp 46–47). This threshold was also reached in nine (75%) of 12 children vaccinated with the 0, 1, and 2 month regimen, suggesting RH5 vaccination could be aligned with the current monthly delivery schedule for the primary series of RTS,S/AS01 and/or R21/Matrix-M.

The functional quality readout of the RH5.1/Matrix-M vaccine candidate (ie, GIA per µg anti-RH5 IgG) was consistent with our previously reported data following vaccination of healthy volunteers in the UK or Tanzania with viral-vectored RH5 or RH5.1/AS01$_B$ (appendix p 45).[10,12,13] However, the improved anti-RH5 IgG avidity seen with delayed boosting here (and previously in UK adults vaccinated with RH5.1/AS01$_B$[12,20]) did not affect the functional quality of these IgG concentrations in the GIA assay. This finding has now been explained by analysis of anti-RH5 human mAbs that showed that delayed boosting leads to improvement in the anti-RH5 antibody dissociation, but not association rates, and only association rates strongly correlate with GIA potency.[28]

Work on historical blood-stage vaccine candidates targeting the AMA1 antigen suggested that naturally occurring anti-malarial antibody responses could interfere with vaccine-induced GIA,[29] whereas other work with anti-RH5 IgG has suggested such antibodies might interact additively or synergistically in terms of GIA.[30] Our data in children who had experienced relatively higher levels of malaria exposure before study recruitment indicated no apparent effect of these anti-malarial responses on RH5.1/Matrix-M immunogenicity or functional GIA, albeit group sizes were small in this trial. Whether much higher levels of naturally acquired anti-malarial antibodies might enhance or interfere with RH5 vaccine outcomes now remains to be investigated in larger field trials at sites of higher malaria endemicity.

Our study had limitations. First, the GIA assays only used 3D7 clone *P falciparum* parasites; future work should assess antibody function against various laboratory-adapted parasite lines and field isolates to assess for any variation in functional antibody potency. Second, we recruited small numbers of participants for this first-in-human trial and, in the adult groups, only men volunteered to participate. Finally, we did not include a control group; however, previous data on rabies vaccines recipients from the same study site,[13] low rates of malaria transmission, minimal immunogenicity of RH5 in the context of natural malaria infection, and the strong consistent relationship between anti-RH5 IgG and GIA all strongly indicate the immune responses observed were vaccine induced. Consequently, given RH5.1/Matrix-M is highly immunogenic, achieving the greatest magnitude of in vitro functional GIA in children, this vaccine candidate has now progressed to a randomised controlled phase 2b field trial in 5–17-month-old children in Burkina Faso (NCT05790889) to assess the safety and efficacy against clinical malaria following monthly or delayed third dose vaccination.

Contributors

SES, SJD, AIO, and AMM conceived the trial. AIO was the trial principal investigator, AMM was the chief investigator, and SJD was the senior laboratory investigator. SES, WFK, JS, IMM, FM, AD, CKB, NB, OH, CGM, SR, SM, NSL, BS, HM, MM, DMD, LM, GN, BM, OJ, TGM, IAS, RPM, JJK, JRB, MMH, CAL, CMN, KM, SJD, AIO, and AMM contributed to the implementation of the study and data collection. SES, WFK, JS, TA, KM, SJD, AIO, and AMM analysed the data. SA, CC, AML, REC, FLN, RR, and J-SC performed project management. LDWK, DP, and CC assisted with vaccine provision. SES, JS, KM, SJD, AIO, and AMM completed statistical analysis, interpreted data, contributed to writing the manuscript, and have accessed and verified the data.

Declaration of interests

LDWK and SJD are named inventors on patent applications relating to RH5 malaria vaccines. AMM has an immediate family member who is an inventor on patent applications relating to RH5 malaria vaccines. CC is an employee of Novavax, developer of Matrix-M adjuvant. All other authors declare no competing interests.

Data sharing

Data associated with this study are present in the paper or appendix and are available upon reasonable request that should be directed to the corresponding author. Proposals will be reviewed and approved by the Sponsor, Chief Investigator, and collaborators. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. Any shared data will be de-identified.

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