

SUPPLEMENTARY MATERIAL

Inclusion Criteria

- Healthy adults aged 18 to 50 years;
- Able and willing (in the Investigator's opinion) to comply with all study requirements;
- Willing to allow the Investigators to discuss the volunteer's medical history with their General Practitioner;
- Women only: Must practice continuous effective contraception for the duration of the study;
- Men only: Must use barrier contraception from day of any vaccination with CPG 7909, for 3 months;
- Agreement to refrain from blood donation during the course of the study and for 6 months after the end of their involvement in the study;
- Written informed consent.

Exclusion Criteria

- History of clinical *P. falciparum* malaria;
- Travel to a malaria endemic region during the study period or within the preceding six months with a significant risk of malaria exposure;
- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period;
- Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data;
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate;

- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed);
- Pregnancy, lactation or intention to become pregnant during the study;
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine e.g. egg products, Kathon;
- History of clinically significant contact dermatitis;
- History of a known allergy to nickel (volunteers may be enrolled in Group 5 if they have an allergy to nickel);
- Any history of anaphylaxis post vaccination;
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma *in situ*);
- History of serious psychiatric condition that may affect participation in the study;
- Any other serious chronic illness requiring hospital specialist supervision;
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week;
- Suspected or known injecting drug abuse in the 5 years preceding enrolment;
- Seropositive for hepatitis B surface antigen (HBsAg);
- Seropositive for hepatitis C virus (antibodies to HCV);
- History or evidence of pre-existing autoimmune or antibody mediated disease or laboratory evidence of possible autoimmune disease, defined as anti-dsDNA ≥ 25 IU/mL or a positive antinuclear antibody (ANA) result at screening;
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination;

- Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data;

Re-Vaccination Exclusion Criteria

The following AEs associated with vaccine immunization constituted absolute contraindications to further administration of vaccine:

- Anaphylactic reaction following administration of vaccine;
- Pregnancy.

The following adverse events constituted contraindications to administration of vaccine at that point in time:

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever.) All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of $<37.5^{\circ}\text{C}$ (99.5°F);
- Temperature of $\geq 37.5^{\circ}\text{C}$ (99.5°F) at the time of vaccination.

SUPPLEMENTARY METHODS

Vaccinations

34 volunteers were vaccinated with 5×10^{10} viral particles (vp) ChAd63 AMA1 (undiluted and administered in 300 μ L). Volunteers then received subsequent vaccinations according to group allocation (**Fig. 1**). 1.25×10^8 plaque forming units (pfu) MVA AMA1 was administered undiluted in 50 μ L. AMA1-C1 (80 μ g)/Alhydrogel (800 μ g) was either administered alone (total volume 0.5 mL) or mixed immediately prior to administration with 564 μ g CPG 7909 formulated in saline (total volume of 0.55 mL).

Clinical Follow-up and Safety Assessment

All volunteers were observed in clinic for 30 min following each vaccination. Volunteers were given a digital thermometer, injection site reaction measurement tool and symptom diary card to record their daily temperature, injection site reactions and solicited systemic AEs for 7 days following each vaccination. Volunteers attended clinic for review according to the schedules outlined in **Table S1**. A time window ranging between 12 hours and 28 days was allowed for vaccination and post vaccination follow-up visits.

Local and systemic reactogenicity was evaluated at subsequent clinic visits and graded for severity, outcome and association to vaccination as per the criteria outlined in **Tables S2-4**. Blood was sampled at all visits post vaccination. 'Safety bloods' including full blood count with differential, platelet count, electrolytes, urea, creatinine, bilirubin, alanine aminotransferase, alkaline phosphatase and albumin were measured at varying time points depending on group (**Table S1**).

SUPPLEMENTARY TABLES**Table S1: Schedule of clinical reviews according to group.**

Group	Vaccine 1	F/U visits	Vaccine 2	F/U visits	Vaccine 3	F/U visits
1 AMP+	d0*	d1, 14*, 28*	d56*	d57, 60, 63*, 84*	d112*	d113, 116, 119*, 140*, 168*, 196*, 280 [#]
2 AP+	d0	d1, 14*, 28*	d56*	d57, 60, 63*, 84*, 112*, 140*, 224 [#]	-	-
3 AP-	d0	d1, 14*, 28*	d56*	d57, 60, 63*, 84*, 112*, 140*, 224 [#]	-	-
4 A_P+	d0	d1, 14*, 28*, 56*	d112*	d113, 116, 119*, 140*, 168*, 196*, 280 [#]	-	-
5 A_M	d0	d1, 14*, 28*, 56*	d112	d113, 116, 119*, 140*, 168*, 196*, 280 [#]	-	-

* 'Safety bloods' (full blood count with differential, platelet count, electrolytes, urea, creatinine, bilirubin, alanine aminotransferase, alkaline phosphatase, albumin) performed.

[#] The last review could take place either in person in clinic or over the telephone, according to volunteer preference. Blood sampling occurred at every review except the last visit if this took place via telephone. F/U = follow-up.

Table S2. Assessment of relationship of AE to study intervention.

0	No Relationship	No temporal relationship to study product <i>and</i> Alternate aetiology (clinical state, environmental or other interventions); <i>and</i> Does not follow known pattern of response to study product.
1	Unlikely	Unlikely temporal relationship to study product <i>and</i> Alternate aetiology likely (clinical state, environmental or other interventions) <i>and</i> Does not follow known typical or plausible pattern of response to study product.
2	Possible	Reasonable temporal relationship to study product; <i>or</i> Event not readily produced by clinical state, environmental or other interventions; <i>or</i> Similar pattern of response to that seen with other vaccines.
3	Probable	Reasonable temporal relationship to study product; <i>and</i> Event not readily produced by clinical state, environment, or other interventions <i>or</i> Known pattern of response seen with other vaccines.
4	Definite	Reasonable temporal relationship to study product; <i>and</i> Event not readily produced by clinical state, environment, or other interventions; <i>and</i> Known pattern of response seen with other vaccines.

Table S3. Severity grading criteria for quantifiable AEs.

*erythema ≤ 3 mm is an expected consequence of skin puncture and is not therefore considered an AE.

Adverse Event	Grade	Intensity
Erythema at injection site*	1	>3 - ≤ 50 mm
	2	>50 - ≤ 100 mm
	3	>100 mm
Swelling at injection site	1	>0 - ≤ 20 mm
	2	>20 - ≤ 50 mm
	3	>50 mm
Fever (oral)	1	37.6°C - 38.0°C
	2	>38.0°C – 39.0°C
	3	>39.0°C

Table S4. Severity grading criteria of AEs.

Scale	Description	Definition
0		Absence of the indicated symptom
1	Mild	Awareness of a symptom but the symptom is easily tolerated
2	Moderate	Discomfort enough to cause interference with usual activity
3	Severe	Incapacitating; unable to perform usual activities; requires absenteeism or bed rest