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## **A plasmodium falciparum sporozoite's journey: through organs and across CD8+ T-cell challenges**

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# Chapter 7

## Single immunisation with genetically attenuated *Pf* $\Delta$ *mei2* (GA2) parasites by mosquito bite in controlled human malaria infection: a placebo-controlled randomised trial

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## Abstract

Malaria vaccines consisting of metabolically active *Plasmodium falciparum* (*Pf*) sporozoites can offer improved protection compared to currently deployed subunit vaccines. In a previous study, we demonstrated the superior protective efficacy of a three-dose regimen of late-arresting genetically attenuated parasites administered by mosquito bite (GA2-MB) as compared to early-arresting counterparts (GA1-MB) against a homologous controlled human malaria infection (CHMI). Encouraged by these results, we explored the potency of a single GA2-MB immunisation in a placebo-controlled randomized trial. Primary outcomes were safety and tolerability, time-to-parasitaemia and protective efficacy. Humoral and cellular immunological results were considered secondary outcomes. Here, we report safe administration of GA2-MB with no breakthrough malaria and sterile protection in 9/10 participants at six weeks after a single immunisation with 50 GA2-infected mosquitoes, compared with 0/5 mock-immunised participants, against a homologous CHMI. Immunisation increased circulating *Pf*-specific polyfunctional effector memory CD4<sup>+</sup> T-cells co-expressing TNF- $\alpha$  and IL-2. This unprecedented 90% protective efficacy after a single low dose immunisation holds great promise for the potency of GA2-MB immunisation. Future studies should demonstrate if GA2 is similarly efficacious in pre-exposed populations and if the favourable safety profile reported here holds up in larger groups. ClinicalTrials.gov identifier: NCT05468606

## Introduction

Each year more than 600,000 people die from malaria, mainly children under the age of five(1), making it the fifth leading cause of child mortality worldwide(2). Although the widespread deployment of both RTS,S and R21 subunit vaccines in regions with moderate-to-high transmission marks tremendous progress in reducing malaria-related morbidity and mortality(3, 4), their limited efficacy and need for boosters to sustain protection call for ongoing effort into development of improved vaccines with increased potency to achieve high level durable protection that can ultimately break transmission(5). Immunisation strategies based on the use of whole *Plasmodium falciparum* (*Pf*) sporozoites have the potential to provide this much sought after high level protection, particularly late liver stage arresting parasites that are attenuated genetically through the knock-out of genes that are crucial for the development of blood stage disease(6-9).

Previously, we demonstrated that genetically attenuated *Pf* sporozoites can be safely administered to humans by injection and mosquito bite(10, 11). Through targeted gene deletion, we created two different parasite lines based on the *Pf*NF54 strain: early-arresting *Pf* $\Delta$ *b9*/ $\Delta$ *slarp* (GA1)(12) and late-arresting *Pf* $\Delta$ *mei2* (GA2)(8). We demonstrated with three immunisations through mosquito bites that parasites arresting development late in the liver at day six post-infection are much more potent in inducing protection as compared to early-arresting counterparts (89% vs. 13% protection in a controlled human malaria infection [CHMI])(11). The high level protection was accompanied by potent circulating cellular memory responses, potentially against late-liver stage antigens(11).

In previous studies involving immunisation with sporozoites under chemoprophylaxis, detailed parasite detection by *Pf*qPCR during immunisation regimens indicated that 10-90% of previously malaria-naïve participants become parasitaemic after the second immunisation(13-18), suggesting that single immunisation with parasites that reach the late liver stage can provide varying levels of immunity. We hypothesized that GA2 might also have that potential, and we decided to assess the efficacy of a single GA2-immunisation regimen against a homologous CHMI.

## Methods

### Study design and recruitment

A randomised, double-blind, placebo-controlled trial with a controlled human malaria infection (CHMI) was conducted from February to November 2023 at the Leiden University Medical Center (LUMC), in Leiden, the Netherlands. Fifteen malaria-naïve participants aged 15 to 30 were included after a health assessment including medical history, physical examination, a general laboratory evaluation including haematology and biochemistry assessment, a drugs test to exclude cocaine and amphetamine use, and an electrocardiography. Female participants were counselled to use adequate contraception throughout the study and were tested for pregnancy with a serum beta-HCG test on both the day before immunisation (I-1) and the day before CHMI (C-1). All participants provided written informed consent.

Ten participants were immunised with the bites of 45 to 55 GA2-infected mosquitoes, and five participants received a mock-immunisation with uninfected mosquitoes as a placebo. Six weeks after immunisation, all fifteen participants underwent a homologous CHMI with the bites of five wild-type *Plasmodium falciparum* (Pf) 3D7-infected mosquitoes. From day 6 to day 21 after CHMI (C+6 to C+21), participants were closely followed with daily ambulatory visits for the collection of adverse events (AEs), safety assessment, blood sampling, and highly sensitive quantitative polymerase chain reaction (qPCR) analysis for Pf on whole blood as previously described (lowest limit of detection was 50 parasites/mL)(22). Participants were treated with a three-day regimen of atovaquone/proguanil when they exhibited parasitaemia (Pf<sub>qPCR</sub>>100 parasites/mL) or at day 28 after CHMI (C+28). AEs were recorded by participants in a diary. AEs were graded in four categories (mild, moderate, severe and serious) that were prespecified per protocol. Both participants and investigators were blinded to intervention. Mosquito cages were prepared by technicians independent from the clinical investigators. Randomisation was carried out by an independent member of the study team. Safety, time-to-parasitaemia and protective efficacy were the primary outcomes. Secondary study outcomes were humoral and cellular immunology results. Data capture was done using an electronic case report form (Castor CDMS version 2023.1.x.x).

The trial protocol was approved by the Dutch Central Committee for Research involving Human Subjects (CCMO, file number NL82130.000.22) and registered at ClinicalTrials.gov (NCT05468606), and EudraCT (2022-002646-40).

### **Parasite culturing, mosquito rearing and exposures**

The characterisation of GA2 and its generation from *PfNF54*, its genetic backbone, has been described previously(8). The wild-type parasite used for the CHMI (*Pf3D7*) is a clone of the *PfNF54* parasite strain. Parasites were cultured in standard conditions using semi-automated shaker culture systems(23) and subsequently fed to female *Anopheles stephensi* mosquitoes by standard membrane feeding(24). Mosquitoes were reared and infected following standard procedures at the insectary of the LUMC following established methods(24). The production process of the parasites and mosquitoes underwent strict quality control before release by a qualified person. Fourteen days after feeding the parasites to the mosquitoes, a sample of twenty mosquitoes was taken from every mosquito batch (consisting of 200 to 500 mosquitoes) to assess sporozoite yield in the mosquito salivary glands. Only batches that had an average yield of at least 1.000 sporozoites per mosquito were used for exposure to participants. For the immunization, the average yield of the batches was 34.000 and 67.000 sporozoites per mosquito and for the challenge the yield of the batches ranged from 11.300 to 35.300 sporozoites per mosquito.

Exposure of mosquitoes to participants was done using small cages with mesh-covered openings that were applied for 15 minutes to the deltoid region (immunisation) or inner lower arm (CHMI). After exposure, mosquitoes were dissected to confirm feeding. Additionally, after CHMI exposures, salivary glands were dissected and microscopically assessed for the presence of sporozoites. At immunisation, exposures were repeated until the target dose of 45 to 55 mosquitoes was reached or up to a maximum of three times. For CHMI, the procedure was repeated until five infected mosquitoes had taken a blood meal or up to a maximum of four exposures.

### **Pf antigen binding antibody measurements in ELISA**

ELISAs were performed as described before(11). In brief, half area 96-well high binding plates were coated overnight at 4 °C with 1 µg/mL of antigen at 25 µl/well in 0.1 M sodium carbonate buffer (pH 9.6). Upon blocking with 5% skim milk in PBS for two hours, serially diluted plasma samples (starting dilution of 1:500 serially diluted 8 steps each by 1:2.5) were incubated for two hours. Bound antibodies were detected with 450 nm absorbance using goat-anti-human immunoglobulin G (IgG) conjugated with horseradish peroxidase and 3,3',5,5'-Tetramethylbenzidine (TMB) substrate development stopped with 10% sulfuric acid. Standard curve developed using polyclonal IgG with known concentration was used for normalization. Measures from

at least two independent experiments with a coefficient of variance below 30% were considered for analysis.

### **T cell response measurement using flow cytometry**

Cellular response using *Pf* infected red blood cells (RBC) stimulation was performed as described before(5). In brief, PBMCs stimulated with RBCs from a healthy blood donor either as uninfected (unRBC) or infected with *Pf* (*Pf*RBC) for 24 h during which 10 µg/mL Brefeldin A (Sigma) was added at 4 h post stimulation. Cells were stained with a panel of antibodies (Extended Data Table 2) to identify T cell subsets (CD56, γδ-Vδ2 TCR, CCR7, CD3, CD4, CD8, CD25 and CD11c), cytokine expression (IFN-γ, TNF-α, IL-10, IL-2, IL-4, IL-5, IL-13) and phenotype (CD45RA and CCR7). For fixation and intracellular staining Intracellular Fixation & Permeabilization Buffer Set (Invitrogen) was used. To stain dead cells Aqua Live/Dead dye (Invitrogen) was used. Cells were acquired on the 3-laser spectral analyser Aurora (configuration 16V-14B-8R) and analysed using FlowJo 10.8.2 as described in Extended Data Fig. 2 and 3. Frequency of *Pf*-specific cytokine positive cells in *Pf*RBC stimulated samples after subtraction of the same gate on the same sample stimulated with unRBC are reported for analysis. Frequencies of cytokine positive CD4<sup>+</sup> and γδ<sup>+</sup> Vδ2 cells were calculated as a percentage of CD3<sup>+</sup> cells by using the frequencies of total CD4<sup>+</sup> and γδ<sup>+</sup> Vδ2 cells, respectively.

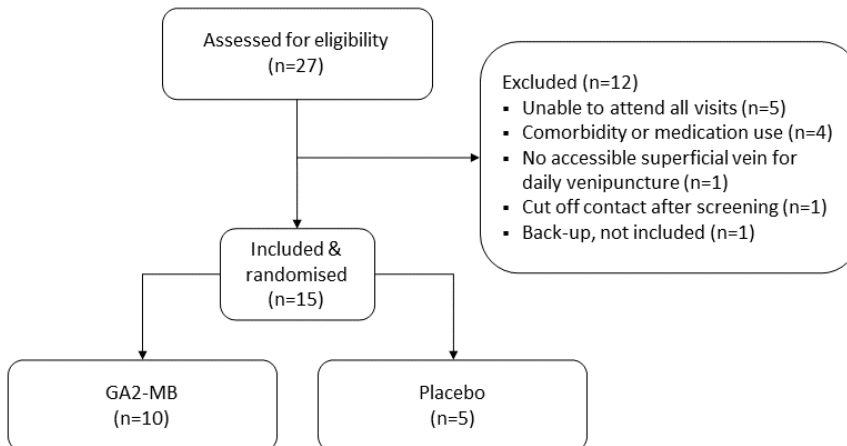
### **Statistical analysis**

Baseline characteristics of participants are reported as both means with standard deviations and medians with range for continuous variables and as frequencies with percentages for categorical variables. The incidence of AEs is reported as frequencies with percentages (risk). Time-to-parasitaemia is reported as a Kaplan-Meier graph and the difference between groups is evaluated using a log-rank test. Antibody concentrations are reported in µg per mL and frequency of responding cells as a percentage of the indicated population, both as arithmetic means with standard error of means. Antibody concentrations and cell populations are compared between groups with a two tailed Mann-Whitney test.

Figures were produced in GraphPad Prism (version 9.3.1) and RStudio (version 4.2.1).

## Results

We enrolled fifteen participants in a randomized double-blind placebo-controlled trial. Details on recruitment and participant characteristics can be found in Fig. 1 and Table 1. Participants were exposed to the bites of 50 ( $\pm 5$ ) GA2-infected or uninfected *Anopheles stephensi* mosquitoes (GA2-MB or placebo, respectively) on April 12<sup>th</sup> 2023 (Fig. 2A). For one participant in the placebo group, the target dose of 45-55 blood fed mosquitoes was not reached at immunisation (Fig. 2B). Six weeks later, all participants underwent CHMI through the bites of five mosquitoes infected with unattenuated homologous wild-type *Pf* parasites. After CHMI, blood feedings were confirmed in either five infected mosquitoes (6/10 GA2-MB participants and 4/5 in the placebo group) or four infected mosquitoes (4/10 GA2-MB participants and 1/5 in the placebo group) (Extended Data Fig. 1A).



**Fig. 1. CONSORT diagram on recruitment and inclusions.** Between 13 February 2023 and 17 March 2023, 27 persons were screened of which 15 were included, randomised, (mock-)immunised and infected with wild-type malaria parasites in a controlled human malaria infection.

**Table 1. Baseline characteristics of participants at screening.** BMI = body mass index, SD = standard deviation

	GA2-MB (n=10)	Placebo (n=5)	Total (n=15)
<b>Age</b>			
<b>Mean (SD)</b>	24 (3)	24 (5)	24 (4)
<b>Median (range)</b>	23 (21-30)	25 (19-31)	23 (19-31)
<b>Sex</b>			
<b>Male (%)</b>	4 (40.0)	3 (60.0)	7 (46.7)
<b>Female (%)</b>	6 (60.0)	2 (40.0)	8 (53.3)
<b>BMI</b>			
<b>Mean (SD)</b>	23.9 (3.3)	22.7 (2.1)	23.5 (2.9)
<b>Median (range)</b>	23.8 (19.4-28.6)	23.7 (20.3-25.0)	23.7 (19.4-28.6)

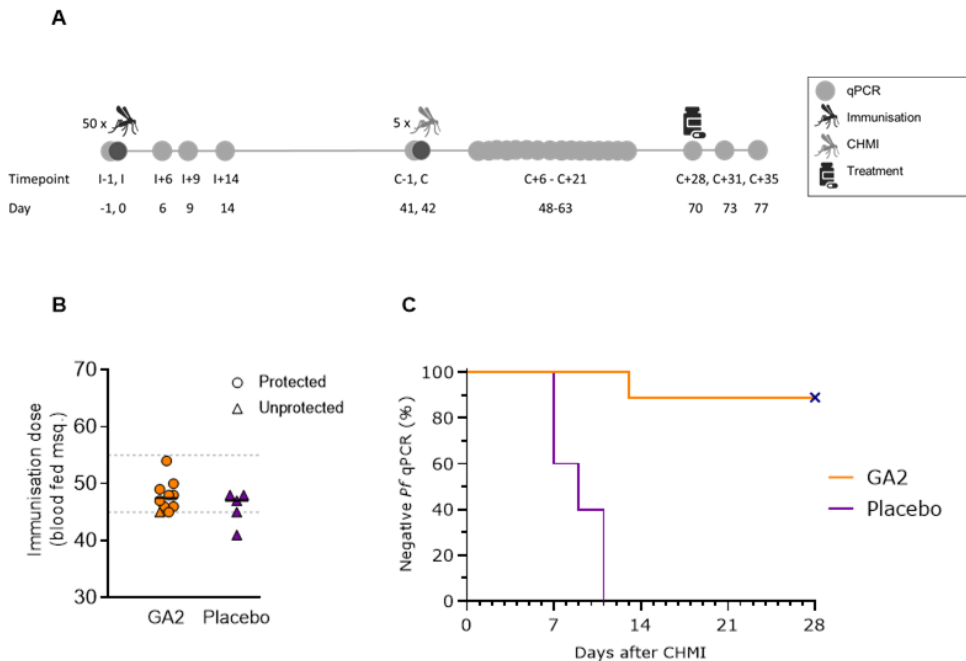
Study visits were held on the day before immunisation, on day 6, 9 and 14 post-immunisation, on the day before CHMI, daily from day 6 to 21 post-CHMI, and on day 28, 31 and 35 post-CHMI. During these visits, adverse events were collected, safety was assessed and a highly sensitive quantitative polymerase chain reaction (qPCR) analysis for *Pf* in whole blood was performed. The lowest limit of detection for the *Pf*qPCR was 50 parasites/mL blood. Escape treatment (three-day regimen of atovaquone/proguanil) was provided at a concentration of >100 parasites/mL or at day 28 after CHMI.

### Primary outcomes

The single immunisation with GA2-MB was safe and well tolerated with no study-related serious adverse events or breakthrough malaria. Neither were there any parasite concentrations  $\geq 50$  parasites/mL detected in any blood sample after immunisation. The mosquito bites led to severe itch in one participant and moderate swelling and mild blistering in another participant, for which topical corticosteroids were prescribed. All other adverse events after immunisation were mild to moderate. Further details on safety and tolerability can be found in Extended Data Table 1.

We found that 9/10 (90%) participants in the GA2-MB group were fully protected against *Pf* malaria and remained *Pf*qPCR-negative until day 28 post-CHMI (Fig. 2C). In contrast, all participants in the placebo group became parasitaemic (log-rank test:  $p < 0.0001$ ). While the median time-to-parasitaemia in the placebo group was 9 days (range: 7-11 days), detection of parasitaemia in the one unprotected GA2-MB participant was considerably delayed to day 13 (Extended Data Fig. 1B). When assessing

protection in solely the participants that received a dose of five infected blood fed mosquitoes at CHMI (not a pre-specified analysis in our protocol), we found a protective efficacy of 83% (5/6 GA2-MB participants protected vs. 0/4 placebo participants).

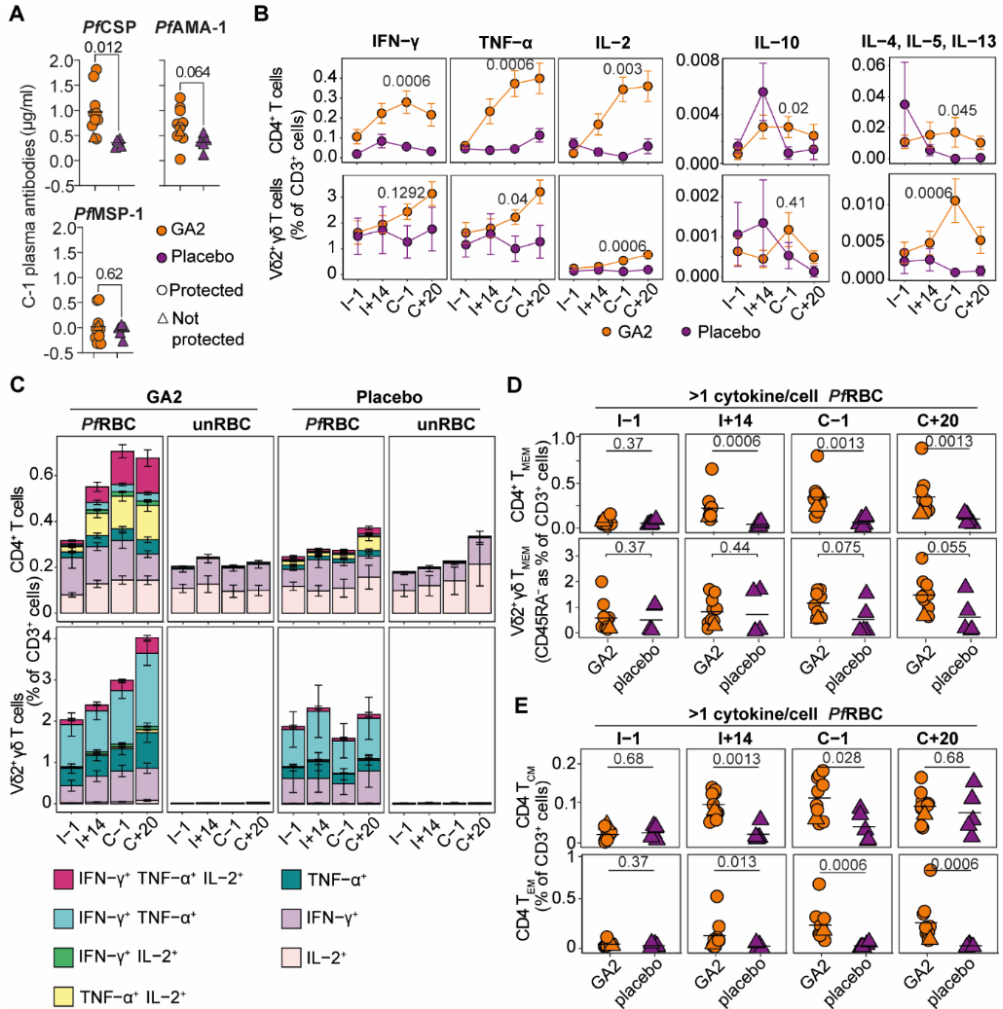


**Figure 2. Study design, immunisation dose and protection against controlled human malaria infection.** A. Schematic overview of study design. B. Number of blood fed mosquitoes per participant at immunisation. Orange represents GA2-MB, purple represents placebo. Circles represent protected participants and triangles unprotected. Black horizontal lines represent the median. Dotted grey line represents target dose (45 – 55). C. Kaplan-Meier curve of percentage of participants that had a negative *Pf*qPCR (<100 parasites/mL) in peripheral blood after CHMI. X = censored. Log-rank test =  $p < 0.0001$ .

### Secondary outcomes

We assessed antibody responses one day before CHMI (C-1) and detected significantly higher levels of antibodies targeting *Pf* circumsporozoite protein (*Pf*CSP) but not key late-liver and blood-stage antigens *Pf* apical membrane antigen-1 (*Pf*AAMA-1) and *Pf* merozoite surface protein-1 (*Pf*MSP-1) in GA2-MB participants compared to placebo (Fig. 3A). *Pf*-specific cellular immunity in GA2-MB participants assessed by stimulation of peripheral blood mononuclear cells (PBMCs) with *Pf*-infected red blood cells (*Pf*RBCs), surrogate for late liver stage antigens, and uninfected RBCs (unRBCs) showed a strong type-1 pro-inflammatory (IFN- $\gamma$ , TNF- $\alpha$  and IL-2), and a moderate type-2 anti-

inflammatory (IL-4, IL-5 and IL-13) and regulatory (IL-10) profile in CD4<sup>+</sup> and Vδ2<sup>+</sup> γδ T cells but not in CD8<sup>+</sup>, Vδ2<sup>-</sup> γδ and NK T cells (Fig. 3B, Extended Data Fig. 2-4). GA2-MB elicited higher frequencies of poly-functional CD4<sup>+</sup> and Vδ2<sup>+</sup> γδ T cells expressing more than one type-1 cytokine, in comparison to placebo (Fig. 3C). While CD4<sup>+</sup> T cells preferentially co-expressed TNF-α and IL-2 with or without IFN-γ, Vδ2<sup>+</sup> γδ T cells co-expressed high levels of IFN-γ and TNF-α with or without IL-2. We observed a relatively minor proportion of type-1 poly-functional CD4<sup>+</sup> and Vδ2<sup>+</sup> γδ T cells co-expressing type-2 cytokines (Extended Data Fig. 5). Poly-functional CD4<sup>+</sup> T cells, but not Vδ2<sup>+</sup> γδ T cells, were enriched among memory T cells (CD3<sup>+</sup>CD45RA<sup>-</sup>), indicating the capacity of single GA2-MB immunisation to form *Pf*-specific cellular memory (Fig. 3D, Extended Data Fig. 6A-F). These GA2-induced memory T cells preferentially acquired effector memory phenotype as early as 2 weeks post-immunisation and remained high during the post-CHMI time point, while central memory T cells were induced at much lower frequency (Fig. 3E, Extended Data Fig. 6G and H).



**Figure 3. Prominent polyfunctional CD4<sup>+</sup> memory T cell response in GA2-single-immunised participants.** A. Plasma antibody levels against the indicated *Plasmodium falciparum* (*Pf*) antigens on the day before CHMI (C-1). Values are log<sub>10</sub> transformed. B. Frequency of CD4<sup>+</sup> and Vδ2<sup>+</sup> γδ T cells expressing the indicated cytokines upon stimulation with *Pf* infected red blood cells (*Pf*RBC) corrected for uninfected RBC (unRBC) stimulation. C. Frequency of CD4<sup>+</sup> and Vδ2<sup>+</sup> γδ T cells expressing single or more than one of the indicated cytokines per cell upon *Pf*RBC and unRBC stimulation. D. Frequency of CD45RA<sup>-</sup> CD4<sup>+</sup> (top) and Vδ2<sup>+</sup> γδ (bottom) memory T cells (T<sub>MEM</sub>) among polyfunctional cells at the indicated time points upon *Pf*RBC stimulation. D. Frequency of central (T<sub>CM</sub>; top) and effector (T<sub>EM</sub>; bottom) memory cells among polyfunctional CD4<sup>+</sup> T cells.

## Discussion

In this study, we demonstrated the capacity of a single immunisation with 50 GA2-infected mosquitoes to protect 90% of malaria-naïve individuals against a homologous CHMI. So far, malaria vaccines have always been tested in regimens of two or more immunisations, but single immunisation has important potential advantages over multiple immunisations with regards to implementations in endemic settings as well as in travellers' indications. Although high level protective efficacy after immunisation with sporozoites has been observed before(11, 13-19), never has protective efficacy been demonstrated in a CHMI after one immunisation. Surprisingly, this high level of protective efficacy seems to be similar to three immunisations with GA2-infected mosquito bites(11), suggesting that the boosting effect of additional immunisations is limited. Further research into the dynamics of immune components in a larger cohort of participants is needed to understand changes on an individual basis and to evaluate how these changes relate to protection. Additionally, the longevity of the immune response and protective efficacy after both single and triple GA2-MB immunisation needs to be further evaluated and compared.

Blood stage break-through infections have been observed after immunising mice with *Plasmodium yoelli*  $\Delta mei2$ (20). When taking the current and our previous GA2-MB study(11) together, a total of 50 participants have now been exposed to GA2-MB: fifteen participants to 15 GA2-MB and thirty-five participants to 50 GA-MB (of which nine participants who underwent three exposures). None of these participants have developed breakthrough malaria. This is in line with results in mice with humanised livers that were exposed to *Pf* $\Delta mei2$  and did not develop break-through blood infections either(8). Future studies should demonstrate if this favourable safety profile of GA2 holds up in larger populations or if genetically attenuated parasites with more knockouts beside *mei2* are warranted to eliminate the risk of breakthrough infections after immunisations(21).

Due to the lack of knowledge on immunogenic late liver stage antigens and the technical limitations to generate large numbers of infected hepatocytes suitable for *in vitro* stimulation, *Pf*RBCs were used as a surrogate antigenic source in this study, similar to previously published clinical studies(10, 17, 19). Furthermore, preferential liver resident capacity of *Pf*-specific CD8<sup>+</sup> T cells may have thwarted our attempts to detect them in peripheral blood samples. In previous sporozoite immunisation studies, polyfunctional CD4<sup>+</sup> and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells expressing pro-inflammatory cytokines, particularly IFN- $\gamma$ , were associated with protection(11, 13, 17, 19). However, after single

GA2-MB immunisation we find the production of pro-inflammatory cytokines other than IFN- $\gamma$  (notably TNF- $\alpha$  and IL-2) by CD4<sup>+</sup> T cells to be more pronounced, in addition to an increase in effector rather than central memory phenotypes which differentiate early after immunisation and persist throughout the CHMI follow-up period.

A limitation of our study is the small sample size of healthy malaria-naïve participants that do not adequately represent the target population for malaria vaccines in endemic areas. Additionally, administration of GA2 through mosquito bites is not a feasible method for large scale immunisation campaigns. To translate the high-level protective efficacy of GA2-MB to an amenable way of vaccine administration through parenteral immunisation, future studies need to assess whether aseptically purified, vialled and cryopreserved sporozoites with the *mei2* deletion are as safe and as efficacious as GA2-MB in this study and similarly potent in malaria endemic areas.

Nonetheless, our finding that a single immunisation with GA2-MB can induce high-level protection against a homologous CHMI provides strong support for the further clinical development of potentially highly potent next generation single-immunisation malaria vaccines based on late-arresting genetically attenuated sporozoites.

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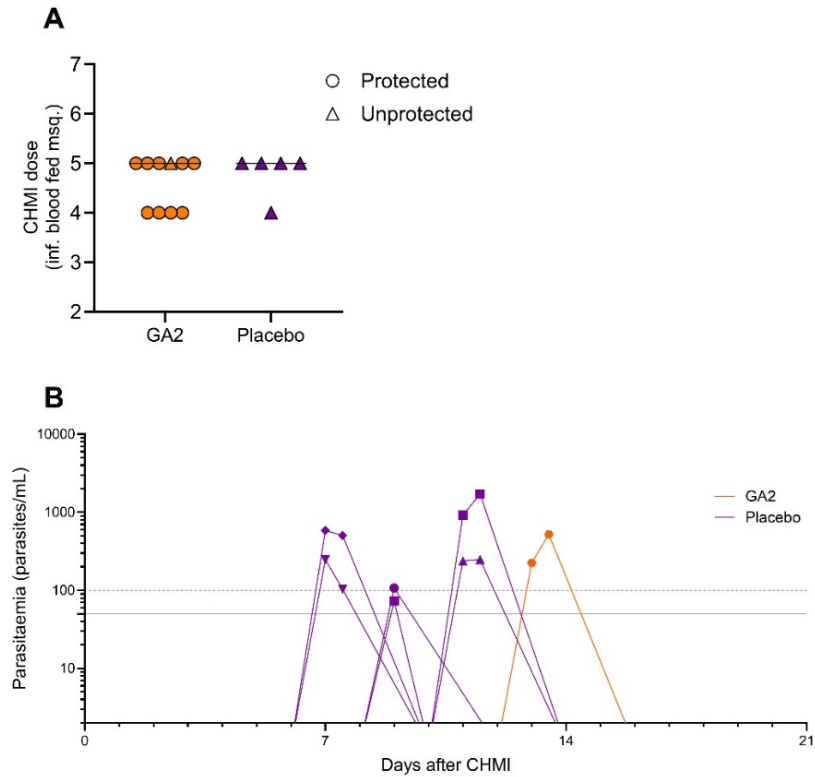
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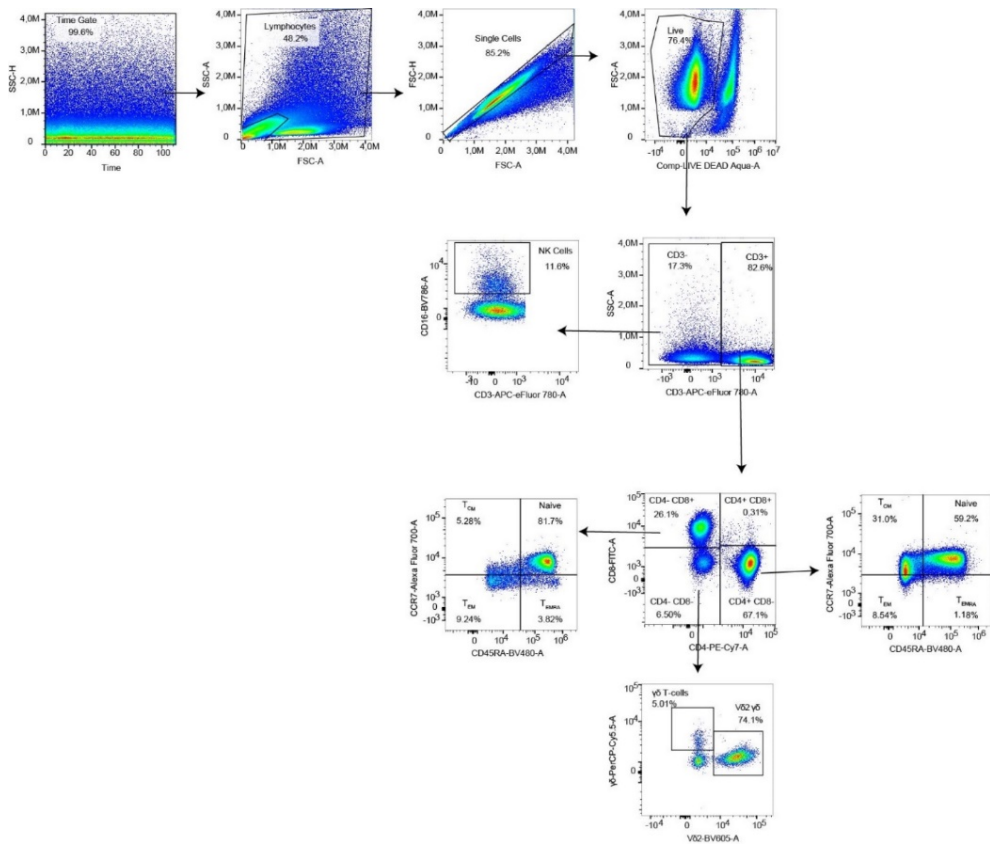
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## Supplementary

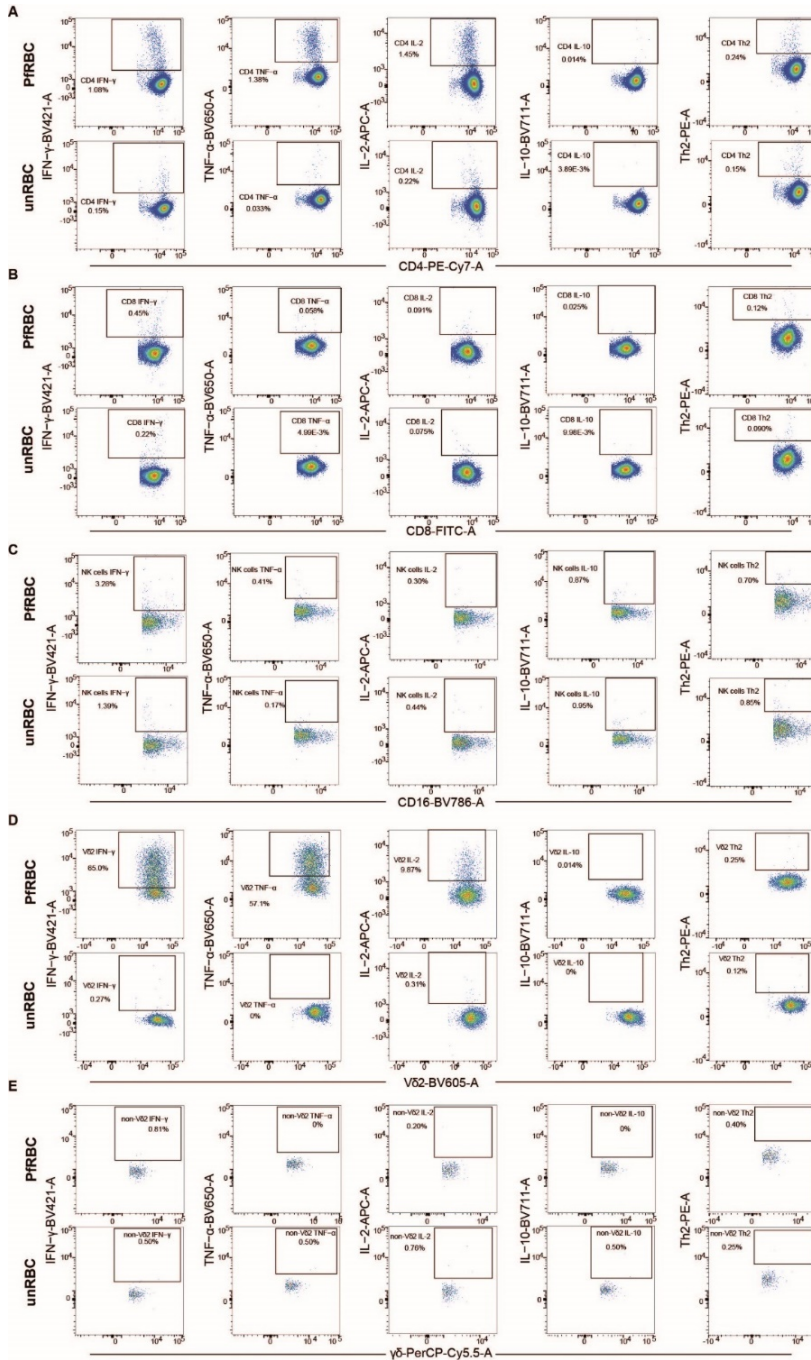


**Extended Data Fig. 1 | CHMI dose and time-to-parasitaemia.** A. Number of infected blood fed mosquitoes per participant at the controlled human malaria infection (CHMI). A black horizontal line represents the median. B. Development of parasitaemia per day after CHMI. Lines with different symbols represent different participants. The horizontal dotted grey line represents the cut-off for parasitaemia (100 parasites/mL blood) at which treatment is given. The horizontal continuous grey line represents the lowest limit of detection of the assay (50 parasites/mL blood).

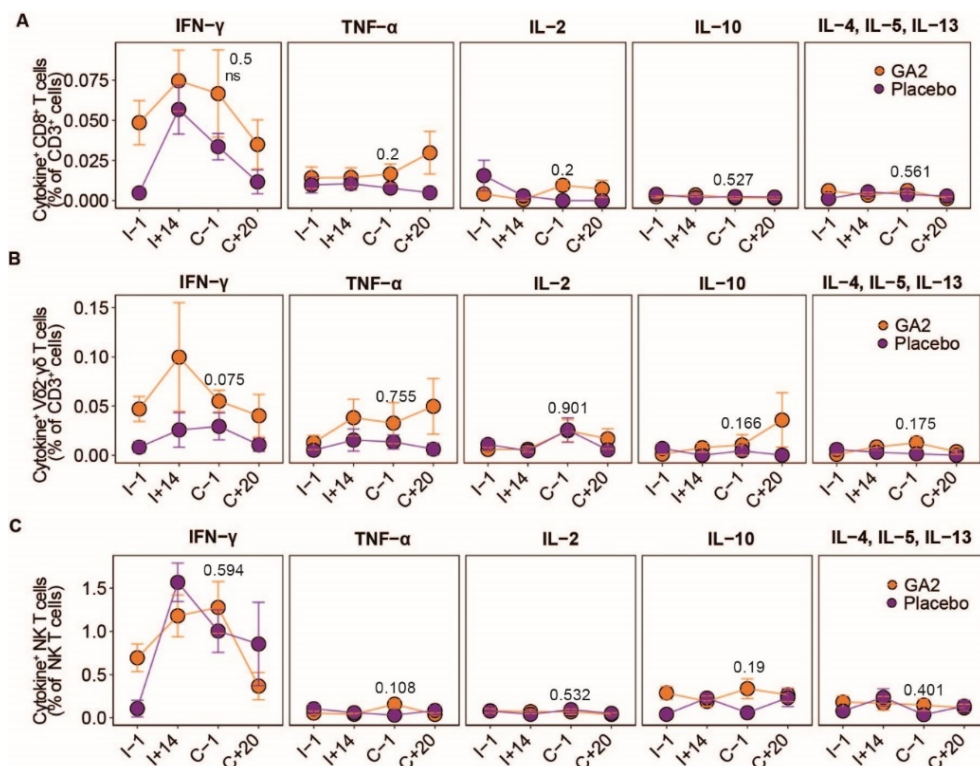
## Chapter 7



**Extended Data Fig. 2 | Representative gating strategy to define T cell subsets.** Live single cells were defined as NK cells (CD3–CD56+), CD4 or CD8 effector (CCR7–CD45RA–) and central (CCR7+CD45RA–) memory T cells and Vδ2+ or Vδ2– γδ T cells, based on the surface marker expression.

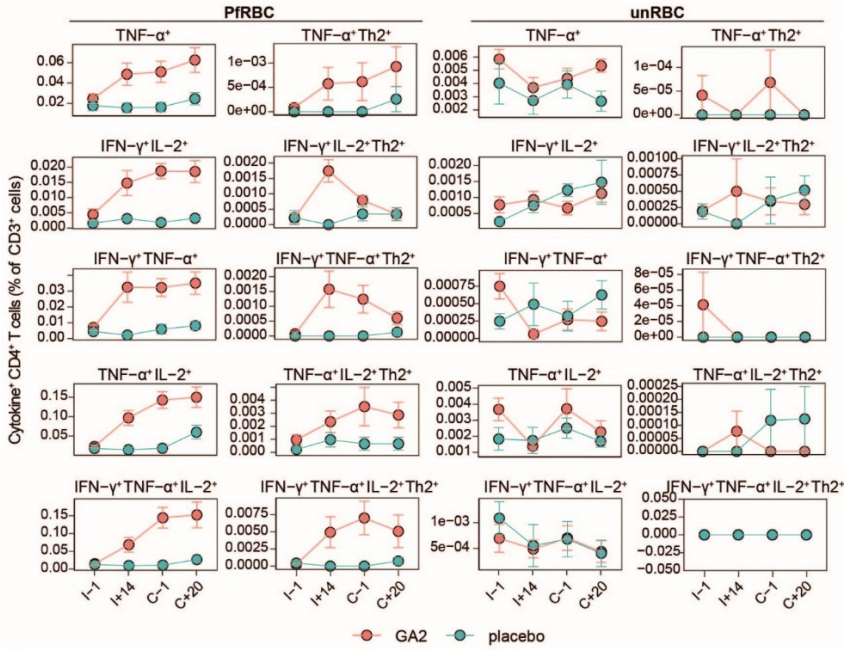


**Extended Data Fig. 3 | Representative cytokine expression gating strategy.** Gates defining the indicated cytokine expressing CD4+ (A), CD8+ (B), NK (C), V $\delta$ 2+  $\gamma$  $\delta$  (D) and V $\delta$ 2-  $\gamma$  $\delta$  (E) T cells upon stimulation with Pf infected (Pf RBC; top) and uninfected RBC (unRBC).

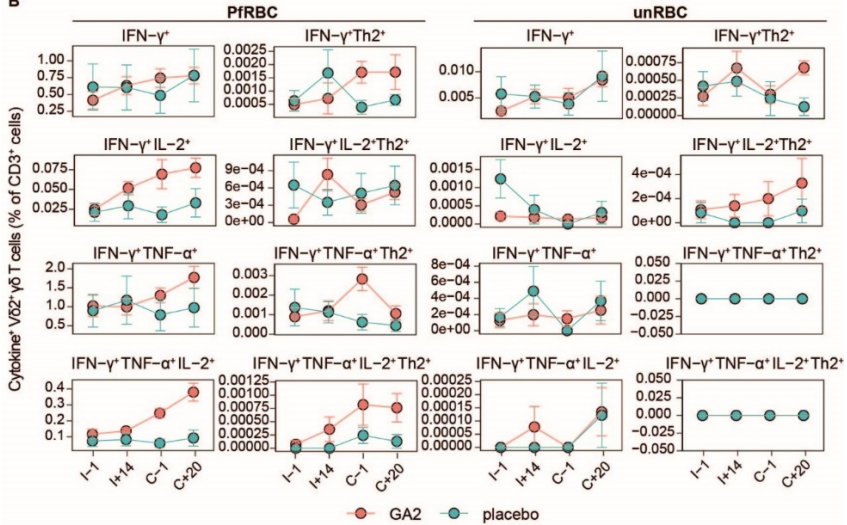


**Extended Data Fig. 4 | Cytokine expression in T cell subsets.** CD8<sup>+</sup> (A), V $\delta$ 2- $\gamma\delta$  (B) and, NK (C) T cells expressing the indicated cytokines upon stimulation with Pf infected RBC (Pf RBC) reference corrected for uninfected RBC (unRBC) stimulation. Data corresponding to GA2-MB and placebo groups are indicated in orange and purple, respectively (A–C). Filled circles and error bars indicate arithmetic mean and standard error mean, respectively. Two tailed Mann-Whitney test (at C-1).

A

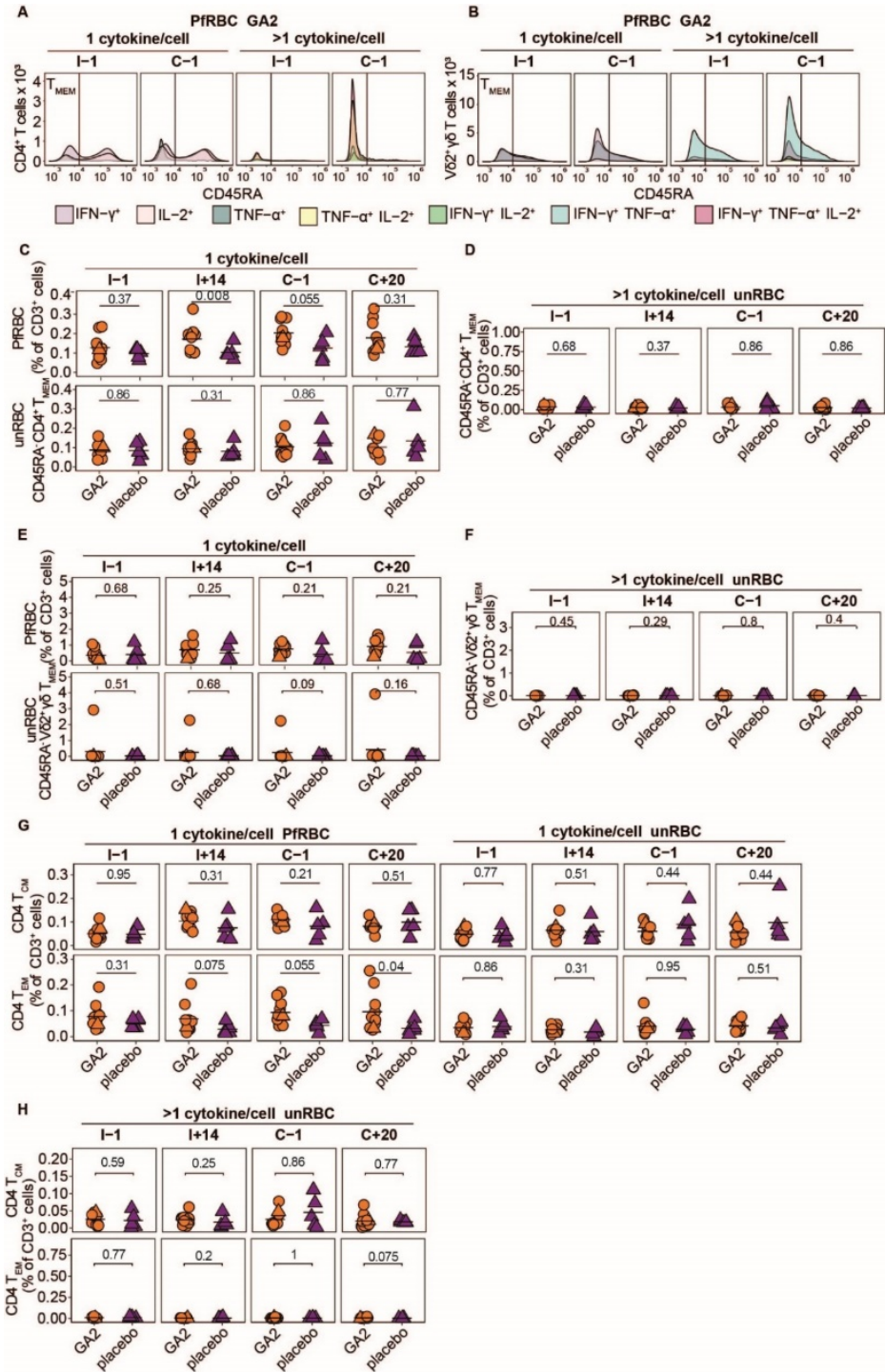


B



**Extended Data Fig. 5 | Mono and polyfunctional T cells moderately co-expressing Th2 cytokines.** Frequency of CD4<sup>+</sup> (A) and Vδ2<sup>+</sup>γδ (B) T cells expressing the indicated cytokines in combination with or without type-2 cytokines (Th2: IL-4, IL-5 and IL-13) among GA2-MB and mock-immunised participants upon Pf RBC (left) and unRBC (right) stimulation. Filled circles and error bars indicate arithmetic mean and standard error of mean of both intervention groups, respectively.

Chapter 7



**Extended Data Fig. 6 | Memory status of T cell subsets.** A,B. CD45RA expression in CD4+ (A) and Vδ2+ γδ (B) T cells among cytokine expressing cells at baseline (I-1) and C-1 upon Pf RBC stimulation. C-F. Frequency of CD45RA- mono- (C,E) and polyfunctional (D,F) CD4+ (C,D) and Vδ2+ γδ (E,F) memory T cells (TMEM ) upon Pf RBC and unRBC stimulation. G,H. Frequency of central (TCM ; top) and effector (TEM ; bottom) memory cells among monofunctional and polyfunctional in CD4+ (G) and Vδ2+ γδ (H) T cells. Each data point represents individual participant and data corresponding to GA2-MB and placebo groups are indicated in orange and purple, respectively (C-H). Horizontal lines indicate arithmetic mean (C-H). Two tailed Mann-Whitney test.

**Extended Data Table 1 | Risk of study related adverse events**

n (%)	GA2 (n=10)			Placebo (n=5)		
	Mild	Moderate	Severe	Mild	Moderate	Severe
<b>Mosquito bites – immunization (41 – 55 blood fed mosquitoes)</b>						
<b>Bullous skin reaction</b>	1 (10.0)	-	-	-	-	-
<b>Swelling</b>	-	1 (10.0)	-	-	-	-
<b>Pain</b>	1 (10.0)	1 (10.0)	-	-	-	-
<b>Itch</b>	5 (50.0)	-	1 (10.0)	3 (60.0)	-	-
<b>Insect bite reaction</b>	8 (80.0)	1 (10.0)	-	5 (100)	-	-
<b>Immunisation</b>						
<b>Increased liver transaminase</b>	1 (10.0)	-	-	-	-	-
<b>Chills</b>	-	1 (10.0)	-	-	-	-
<b>Myalgia</b>	-	1 (10.0)	-	-	-	-
<b>Malaise</b>	-	1 (10.0)	-	-	-	-
<b>Diarrhea</b>	-	1 (10.0)	-	1 (20.0)	-	-
<b>Fever</b>	2 (20.0)	-	-	-	-	-
<b>Headache</b>	1 (10.0)	2 (20.0)	-	1 (20.0)	-	-
<b>Mosquito bites – CHMI (4-5 infected blood fed mosquitoes)</b>						

<b>Itch</b>	5 (50.0)	1 (10.0)	-	3 (60.0)	1 (20.0)	-
<b>Insect bite reaction</b>	6 (60.0)	1 (10.0)	-	5 (100.0)	-	-
<b>CHMI</b>						
<b>Thrombocytopenia</b>	-	-	-	1 (20.0)	-	-
<b>Nausea or vomiting</b>	1 (10.0)	-	-	-	-	-
<b>Malaise</b>	1 (10.0)	-	-	1 (20.0)	-	-
<b>Fever</b>	1 (10.0)	-	-	-	-	1 (20.0)
<b>Myalgia</b>	-	1 (10.0)	-	-	-	-
<b>Abdominal pain</b>	-	1 (10.0)	-	-	-	-
<b>Chills</b>	-	-	1 (10.0)	-	-	-
<b>Headache</b>	3 (30.0)	1 (10.0)	-	1 (20.0)	2 (40.0)	-
<b>Venipuncture</b>						
<b>Pain</b>	1 (10.0)	-	-	-	-	-
<b>Hematoma</b>	4 (40.0)	-	-	1 (20.0)	1 (20.0)	-
<b>Anti-malarial treatment</b>						
<b>Dizziness</b>	-	-	-	-	1 (20.0)	-
<b>Abdominal pain</b>	-	-	-	-	1 (20.0)	-
<b>Flatulence</b>	1 (10.0)	-	-	-	-	-
<b>Diarrhea</b>	1 (10.0)	-	-	-	-	-
<b>Headache</b>	1 (10.0)	-	-	1 (20.0)	-	-
<b>Nausea or vomiting</b>	-	3 (30.0)	-	-	-	-

**Extended Data Table 2 | Staining antibody panel used in multi-parameter spectro-flow cytometry**

<b>Marker</b>	<b>Fluorophore</b>	<b>Dilution</b>	<b>Manufacturer</b>	<b>Cat. Number</b>	<b>Clone</b>
TCR $\gamma\delta$	PerCP-Cy5.5	1:50	Biolegend	331224	B1
CD56	BV786	1:200	BD	744222	R19-760
TCR V $\delta$ 2	BV605	1:200	BD	743751	B6
CCR7	AF700	1:100	Biolegend	353244	G043H7
CD3	APC-ef780	1:800	Invitrogen	47-0038-42	UCHT1
CD4	PE-Cy7	1:800	BD	557852	SK3
CD25	PE-fire 640	1:200	Biolegend	356148	M-A251
CD11c	AF532	1:100	Invitrogen	58011641	ITGAX
CD45RA	BV480	1:100	BD	746799	5H9
IFN $\gamma$	BV421	1:500	Biolegend	502532	4S.B3
TNF $\alpha$	BV650	1:500	Biolegend	502938	MAB11
IL-10	BV711	1:125	BD	564050	JES-9D7
IL-2	APC	1:100	Biolegend	500310	MQ1-17H12
IL-4	PE	1:20	BD	340451	3.010.211
IL-5	PE	1:250	Biolegend	504304	TRFK5
IL-13	PE	1:100	Biolegend	501903	JES10-SA2
FOXP3	PE-CF594	1:50	BD	562421	259D/C7
CD8	FITC	1:400	BD	555366	RPA-T8