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Supplementary Materials for

Infectivity of *Plasmodium falciparum* sporozoites determines emerging parasitemia in infected volunteers

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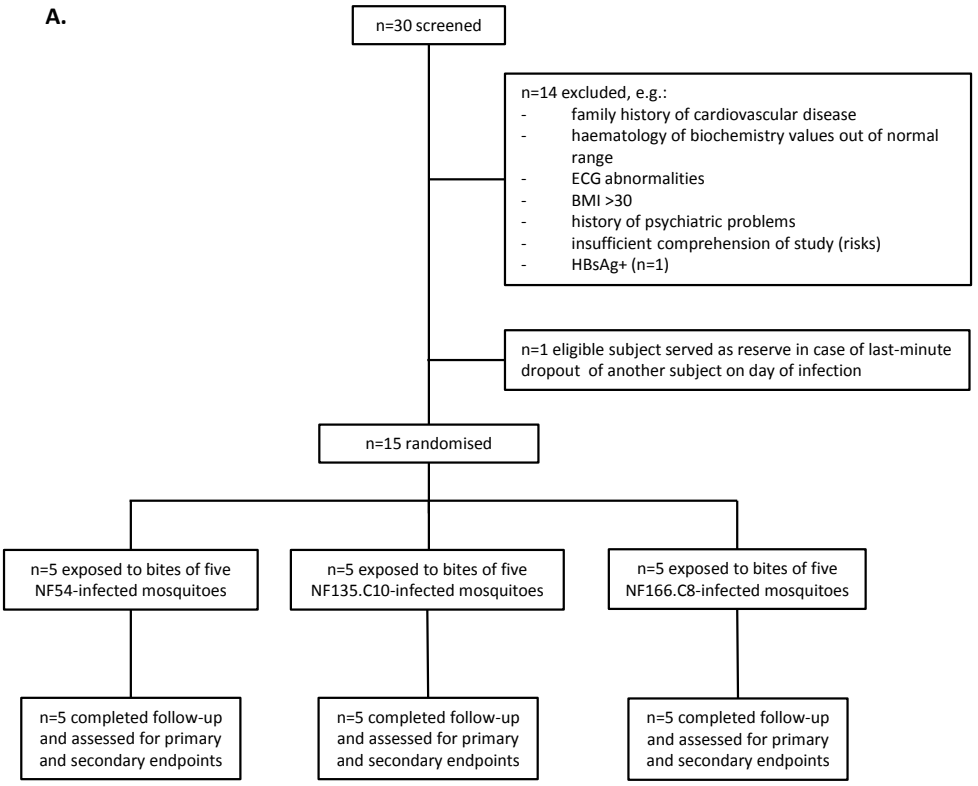
This PDF file includes:

A.


n=30 screened
n=14 excluded, e.g.:
- family history of cardiovascular disease
- haematology of biochemistry values out of normal range
- ECG abnormalities
- BMI >30
- history of psychiatric problems
- insufficient comprehension of study (risks)
- HBsAg+ (n=1)
n=1 eligible subject served as reserve in case of last-minute dropout of another subject on day of infection
n=15 randomised
n=5 exposed to bites of five NF54-infected mosquitoes
n=5 exposed to bites of five NF135.C10-infected mosquitoes
n=5 exposed to bites of five NF166.C8-infected mosquitoes
n=5 completed follow-up and assessed for primary and secondary endpoints
n=5 completed follow-up and assessed for primary and secondary endpoints
n=5 completed follow-up and assessed for primary and secondary endpoints

B.

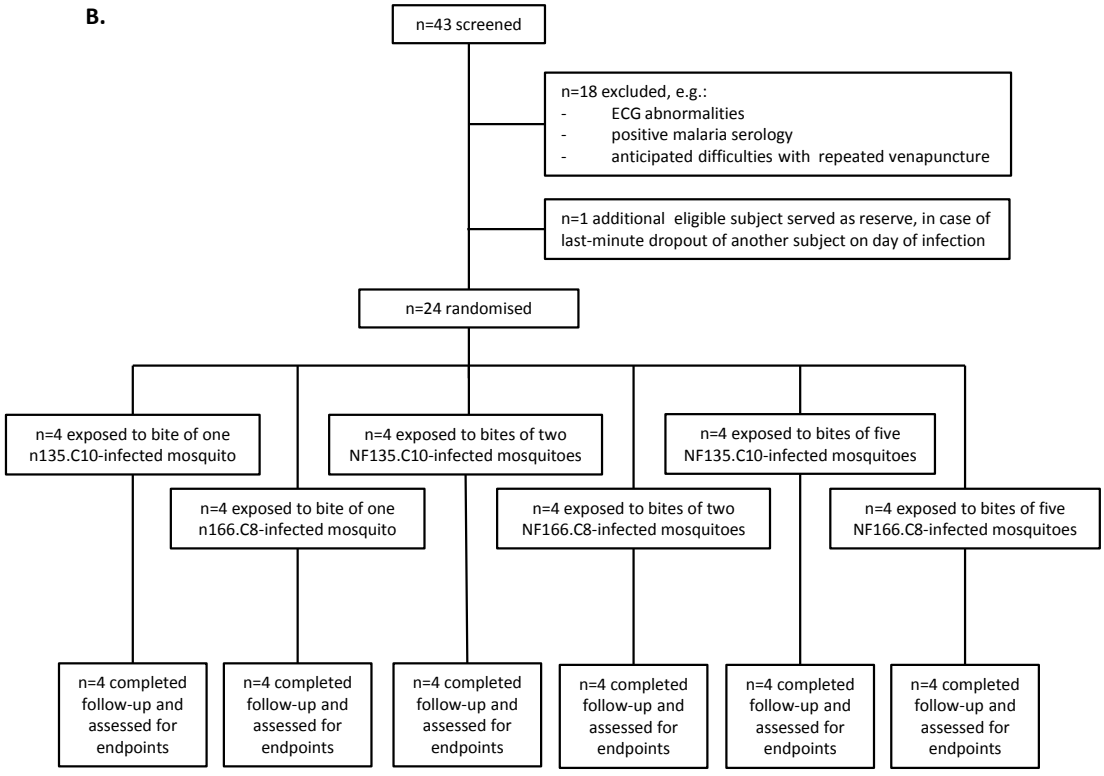

n=43 screened
n=18 excluded, e.g.:
- ECG abnormalities
- positive malaria serology
- anticipated difficulties with repeated venapuncture
n=1 additional eligible subject served as reserve, in case of last-minute dropout of another subject on day of infection
n=24 randomised
n=4 exposed to bite of one n135.C10-infected mosquito
n=4 exposed to bites of two NF135.C10-infected mosquitoes
n=4 exposed to bites of five NF135.C10-infected mosquitoes
n=4 exposed to bite of one n166.C8-infected mosquito
n=4 exposed to bites of two NF166.C8-infected mosquitoes
n=4 exposed to bites of five NF166.C8-infected mosquitoes
n=4 completed follow-up and assessed for endpoints
n=4 completed follow-up and assessed for endpoints
n=4 completed follow-up and assessed for endpoints
n=4 completed follow-up and assessed for endpoints
n=4 completed follow-up and assessed for endpoints
n=4 completed follow-up and assessed for endpoints

Fig. S1. Study flow charts.

A. CHMI-a (recruitment started August 2012, completed September 2012; day of infection 25-09-2012; final day of follow-up 30-10-2012); **B.** CHMI-b (recruitment started August 2014, completed October 2014; day of infection 07-10-2014; final day of follow-up 11-11-2014). Note: for comparison with CHMI-a, only the results of the two CHMI-b groups exposed to the bites of five infectious mosquitoes are presented in this paper.

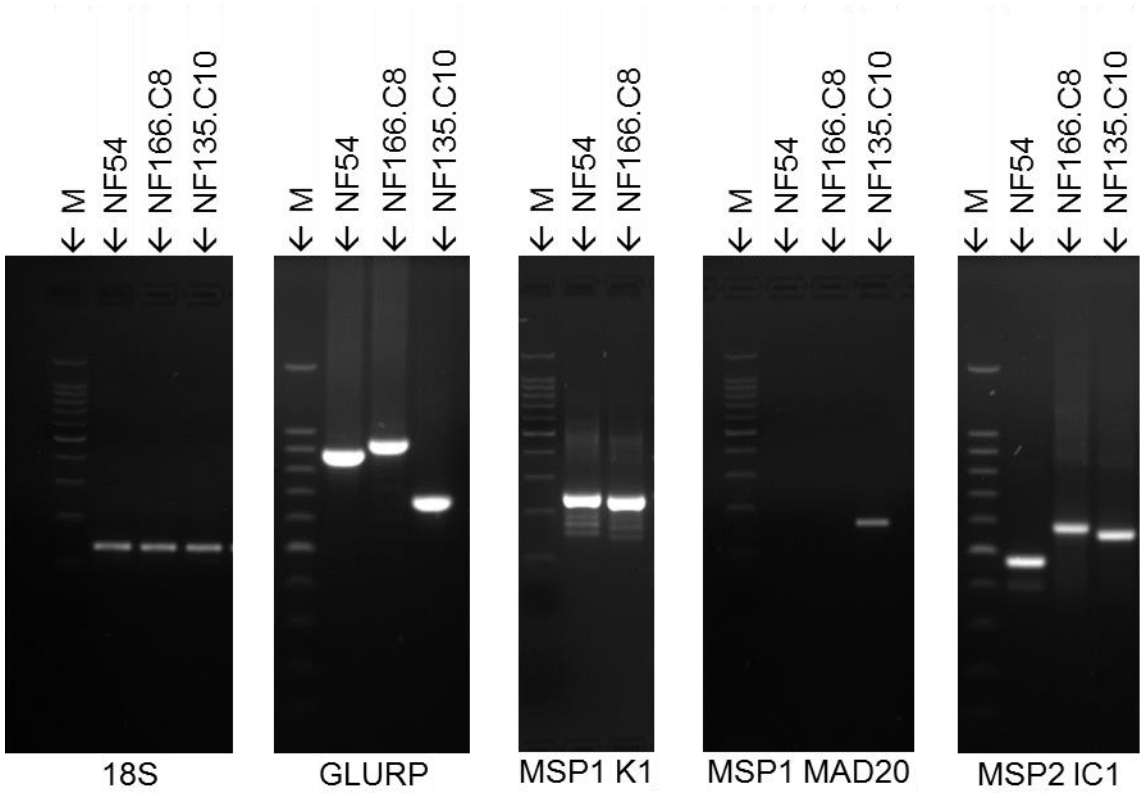


Fig. S2. Genetic characterization of *P. falciparum* isolates.

PCRs for 18S rDNA, GLURP, the K1, MAD20 & R033 allelic variants of MSP1 and the IC1 & FC27 variants of MSP2 were performed on the *P. falciparum* isolates NF54, NF166.C8 & NF135.C10. Note: NF54 and NF166.C8 failed to produce any MSP1 MAD20 band. M – 100bp marker.

table S1. Study demographics

	NF54	**NF135.C10**	**NF166.C8**
CHMI-a			
gender (male / total)	5 / 5	2 /5	2 / 5
age (median [range])	19 [18-21]	22 [18-27]	24 [18-35]
CHMI-b[1]			
gender (male / total)		0 / 4	3 / 4
age (median [range])		22.5 [19-27]	24 [19-25]

[1]Data only shown for the two CHMI-b groups exposed to bites of five infectious mosquitoes.

Table S2. Characteristics of *P. falciparum* isolates used in CHMI studies.

		NF54		**NF135.C10**		**NF166.C8**
Country of origin	Netherlands (West Africa)		Cambodia		Guinea	
Year of isolation	1979		1993		2010	
Drug sensitivity (IC_{50}):						
Chloroquine (nM)		8.8		96		9.9
Mefloquine (nM)		19		37		11
Atovaquone (nM)		1.2		0.94		0.37
Proguanil (µM)		0.57		169		0.29
Dihydroartemisinin (nM)		0.36		0.19		0.05
Lumefantrine (nM)		94		122		49
Parasite batches used in individual CHMIs:	CHMI-a	CHMI-b	CHMI-a	CHMI-b	CHMI-a	CHMI-b
No. sub-cultures since thawing of Master Cell Bank aliquot	23	-	17	21	18	17
proportion of oocyst-infected mosquitoes	10/10	-	10/10	10/10	10/10	10/10
mean oocyst count/mosquito (n=10)	20.1	-	15.6	32.5	15.7	15.2
proportion of sporozoite-infectious mosquitoes	9/10	-	9/10	10/10	10/10	10/10
mean sporozoite count/mosquito (n=10)	$101x10^3$	-	$69x10^3$	$69x10^3$	$40x10^3$	$51x10^3$

Table S3. Overview of all comparable CHMI studies performed at our center.

Study# (*ref#*)	Year	Treatment threshold[1]	Number of subjects			Mosquito salivary gland sporozoite load		
			NF54	NF135.C10	NF166.C8	NF54	NF135.C10	NF166.C8
1[2] (*31*)	2000	TBS (NS)	5	-	-	N/A	N/A	N/A
2[2] (*31*)	2001	TBS (NS)	5	-	-	N/A	N/A	N/A
3[2] ((*31*)	2002	TBS (NS)	5	-	-	N/A	N/A	N/A
4[2] (*31*)	2003	TBS (NS)	5	-	-	N/A	N/A	N/A
5 (*32*)	2007	TBS	5	-	-	31500	-	-
6[3]	2008	TBS	18	-	-	72800	-	-
7 (*33*)	2009	TBS	5	-	-	88000	-	-
8 (*9*)	2010	TBS	4	3	-	69000	12500	
9 (*34*)	2011	TBS	5	-	-	79500	-	-
10 (*19*)	2011	TBS	5	-	-	100000	-	-
11 (*27*)	2012	TBS	4	-	-	98250	-	-
12 (CHMI-a)[4]	**2012**	**TBS**	**5**	**5**	**5**	**101250**	**69000**	**40000**
13 (*35*)	2012	TBS	-	5	-	-	38250	-
14 (*36*)	2012	TBS	5	-	-	75800	-	-
15 (*36*)	2013	qPCR	4	-	-	98000	-	-
16 (CHMI-b)[4]	**2014**	**qPCR**	**-**	**4**	**4**	**-**	**69000**	**51000**
17	2015	qPCR	5	-	-	74000	-	-
18[5]	2015	qPCR	5	5	5	26500/ 44300	18000/ 23500	59500/ 17000
Total			**90**	**22**	**14**			

[1]In initial studies at our centre, subjects were treated upon positive thick blood smear (TBS), whereas in more recent studies treatment was initiated upon positive quantitative PCR (qPCR). The lower detection threshold of the latter results in shorter pre-patent periods, lower parasitaemia at time of treatment and hence improved tolerability & safety for study subjects. In the very earliest studies, the methodology for performing thick blood smear analysis was not yet standardised (NS), which can result in subtly different detection thresholds between subjects and between studies. For meta-analysis of pre-patent periods and of highest parasite densities (usually at time of treatment), we have thus limited comparisons to those 10 studies (#5-14, including n=56 NF54, n=13 NF135.C10 and n=5 NF166.C8 subjects) in which standardised thick blood smears were used to initiate treatment. For meta-analysis of time to first qPCR positivity and magnitude of the first wave of parasitaemia to emerge from the liver, all 18 studies are included.
[2]In the very earliest studies at our centre, subjects were exposed to the bites of between 4-7 *P. falciparum* NF54-infected mosquitoes; in all subsequent studies listed, all subjects were exposed to the bites of *precisely* 5 mosquitoes infected with the given isolate. No statistically significant difference was observed in the height of the first wave of parasitaemia in these first four studies compared to all later studies involving NF54.
[3]Subjects were immunised with a candidate malaria vaccine prior to CHMI, but where wholly unprotected against challenge.
[4]Studies for which directly-linked *in vitro* data on sporozoite invasion are available (boldface), as presented in this manuscript.
[5]Subjects were infected in two groups, for which two separate batches of *P. falciparum*-infected mosquitoes were used.

Table S4. Adverse events by severity grade during CHMI-a and CHMI-b studies.

table S4a. Adverse events by severity grade during CHMI-a

	NF54 (n=5)			NF135.C10 (n=5)			NF166.C8 (n=5)		
	Subjects[1]	Duration[2]	Grade[3]	Subjects[1]	Duration[2]	Grade[3]	Subjects[1]	Duration[2]	Grade[3]
All adverse events									
Fever[4]	4	2.0 [0.2-2.8]	2 [2-2]	3	1.4 [0.4-3.2]	2 [1-2]	3	2.8 [0.6-6.8]	3 [1-3]
Headache	5	3.8 [0.3-7.8]	1 [1-2]	5	1.8 [0.1-5.3]	2 [1-2]	5	4.3 [1.6-7.3]	2 [1-2]
Malaise/fatigue[5]	5	**5.3 [3.0-7.8]**	2 [1-3]	5	**2.7 [1.3-4.6]**	2 [1-3]	5	**2.7 [1.6-4.0]**	2 [1-3]
Myalgia	3	3.5 [1.6-5.9]	1 [1-2]	5	2.2 [1.0-4.0]	1 [1-2]	3	1.7 [0.8-3.3]	1 [1-2]
Arthralgia	-	-	-	1	4.1 .	1 .	-	-	-
Nausea[6]	2	1.3 [1.0-1.1]	1.5 [1-2]	3	1.1 [0.0-3.0]	1 [1-2]	2	1.1 [0.1-2.2]	1.5 [1-2]
Chills/rigors	3	1.4 [0.7-2.6]	2 [1-2]	3	0.6 [0.1-1.1]	1 [1-1]	2	0.9 [0.2-1.7]	1.5 [1-2]
Diarrhoea[6]	3	1.3 [0.0-3.9]	1 [1-1]	2	0.4 [0.0-0.8]	1 [1-1]	1	0.0 .	2 .
Abdominal pain[6]	3	2.4 [0.2-4.9]	2 [1-2]	1	0.3 .	1 .	3	0.9 [0.1-1.5]	1 [1-2]
Unsolicited[7]	4 (6)	3.0 [1.4-6.9]	1 [1-2]	2 (2)	2.0 [0.0-4.0]	1.5 [1-2]	5 (9)	4.8 [0.0-29]	1 [1-1]
Any[8]	5 (35)	3.0 [0.0-7.8]	1 [1-3]	5 (30)	1.8 [0.0-5.3]	1 [1-3]	5 (33)	3.0 [0.0-29]	1 [1-3]
Grade 3 AEs									
Fever[4]	-	-		-	-		2	3.9 [1.1-6.8]	
Malaise/fatigue	2	6.0 [5.0-7.0]		1	3.0 .		1	4.4 .	
Any[8]	2 (2)	6.0 [5.0-7.0]		1 (1)	3.0 .		3 (3)	4.0 [1.1-6.8]	

table S4b. Adverse events by severity grade during CHMI-b[9]

	NF135.C10 (n=4)			NF166.C8 (n=4)		
	Subjects[1]	Duration[2]	Grade[3]	Subjects[1]	Duration[2]	Grade[3]
All adverse events						
Fever	4	0.7 [0.2-1.9]	2 [1-3]	1	1.0 .	2 .
Headache	3	1.3 [0.0-3.6]	1 [1-2]	3	1.8 [0.3-2.9]	2 [1-3]
Malaise/fatigue	4	2.7 [1.4-3.6]	1 [1-2]	3	3.9 [0.7-8.3]	2 [1-2]
Myalgia	3	1.9 [1.0-3.3]	1 [1-2]	2	0.7 [0.6-0.7]	1 [1-1]
Arthralgia	1	1.0 .	1 .	-	-	-
Nausea[6]	4	0.7 [0.0-1.6]	1 [1-2]	1	0.2 .	1 .
Chills/rigors	3	1.3 [0.8-2.0]	2 [1-2]	3	0.7 [0.1-1.3]	1 [1-2]
Diarrhoea	-	-	-	-	-	-
Abdominal pain[6]	2	1.1 [0.1-2.1]	1 [1-1]	1	1.4 .	1 .
Unsolicited[7]	2 (2)	4.0 .	1 [1-1]	2 (4)	2.4 [0.0-8.3]	1 [1-2]
Any[8]	4 (27)	1.4 [0.0-4.0]	1 [1-3]	4 (18)	1.8 [0.0-8.3]	1 [1-3]
Grade 3 AEs						
Fever	1	1.9 .		1	2.9 .	
Any[8]	1 (1)	1.9 .		1 (1)	2.9 .	

[1]Number of subjects per group experiencing adverse event. Multiple similar events/episodes per subject were counted as one, with cumulative duration and maximum gradation recorded. For Unsolicited and Any adverse events, total numbers of events/episodes per group are shown in parentheses.
[2]In days; data represent mean [range] per group.
[3]AE gradation: 1=mild, 2=moderate, 3=severe; Fever gradation respectively: 37.5-38.0ºC, 38.0-39.0ºC, >39.0Cº; data represent medians [range].

[4]Two NF135.C10 subjects and two NF166.C8 subjects (those with grade 3 symptoms) held persisting fever across the third and final day atovaquone-proguanil treatment, which subsequently resolved spontaneously.
[5]Mean difference in duration of malaise/fatigue: 2.6 [95% CI 0.0 to 5.1] days between NF54 and NF135.C10 ($p<0.05$); 2.6 [95% CI 0.0 to 5.1] days between NF54 and NF166.C8 ($p<0.05$); 0.0 [95% CI -2.6 to 2.5] days between NF135.C10 and NF166.C8 ($p>0.05$); all comparisons by 1-way ANOVA/Tukey's post-hoc test.
[6]Gastro-intestinal complaints, including nausea (but not vomiting), diarrhoea and abdominal pain were mainly experienced following intake of atovaquone-proguanil.
[7]Unsollicited adverse events in CHMI-a included 5 episodes of common cold, 2 of tender submandibular swelling, 1 of cold sore, 2 of anorexia, 1 of gastric reflux, 2 of syncope, 1 of hyperventilation syndrome, 1 of blurry vision, 1 of mild oedema orbitae and 1 of lumbago. In CHMI-b, there was 1 episode of atypical chest pain, 1 of dizziness, 1 of syncope, 1 of a minor transport accident, 1 of influenza-like illness (ongoing at the final day of follow-up) and 1 subject developed haematomas in both anticubital fossae.
[8]No other statistically significant difference in frequency, duration or gradation of any adverse events was observed between the three groups.
[9]Data only shown for the two CHMI-b groups exposed to bites of five infectious mosquitoes.

Table S5. Inclusion and exclusion criteria for CHMI studies.

CHMI-a	**Inclusion criteria**
1.	Males and females aged 18-35 years
2.	In general good health based on history, physical examination and basic haematology and biochemistry
3.	Negative pregnancy test for females
4.	Use of adequate contraception for females
5.	Signed informed consent, based on a thorough understanding of the concept and procedures of the study
6.	Volunteer agrees to allow informing his/her general practitioner about participation and agrees to sign a request for medical information from the GP concerning any contra-indications for participation in the study
7.	Willingness to undergo a Pf sporozoite challenge
8.	Agreement to stay in a hotel close to the trial center during part of the study (day 5 until three days post-treatment)
9.	Reachable (24/7) by mobile telephone during the whole study period
10.	Available to attend all study visits
11.	Agreement to refrain from blood donation to Sanquin or for other purposes, during the course of the study and thereafter following Sanquin guidelines.
12.	Willingness to undergo an HIV, HBV and HCV screening test
13.	Negative urine toxicology screening test at the screening visit and on the day before challenge
14.	Willingness to take a curative regimen of Malarone®
	Exclusion criteria
1.	History of malaria
2.	Plans to travel outside of the Netherlands during the study period
3.	Previous participation in any malaria vaccine study and/or positive serology for *P. falciparum*
4.	Symptoms, physical signs or laboratory values suggestive of systemic disorders, including but not limited to renal, hepatic, cardiovascular, pulmonary, skin, immunodeficiency, psychiatric and other conditions, which could compromise the health of the volunteer during the study or interfere with the interpretation of the study results
5.	History of diabetes mellitus or cancer (except basal cell carcinoma of the skin)
6.	Clinically significant ECG abnormalities at screening, or history of arrhythmia's or prolonged QT-interval
7.	Positive family history of cardiac disease in 1st or 2nd degree relatives < 50 years old
8.	An estimated ten year risk of fatal cardiovascular disease of ≥5%, as estimated by the Systematic Coronary Risk Evaluation (SCORE) system
9.	Body Mass Index (BMI) below 18 or above 30 kg/m2
10.	Any clinically significant deviation from the normal range in haematological or biochemical blood tests or urine analysis
11.	Positive HIV, HBV or HCV screening tests
12.	Participation in any other clinical study within 30 days prior to the onset of the study or during the study period
13.	Pregnant or lactating women
14.	Volunteers unable to give written informed consent
15.	Volunteers unable to be closely followed during the study period for social, geographic or psychological reasons
16.	Previous history of drug or alcohol abuse interfering with normal social function in the period of one year prior to study onset
17.	A history of psychiatric disease or convulsions
18.	Known hypersensitivity to anti-malarial drugs
19.	History of severe reactions or allergy to mosquito bites
20.	The use of chronic immunosuppressive drugs, antibiotics, or other immune modifying drugs within three months prior to study onset (inhaled and topical corticosteroids are allowed) or during the study period
21.	Contra-indications for Malarone® use, including treatment taken by the volunteers that interferes with Malarone®
22.	Any confirmed or suspected immunosuppressive or immunodeficient condition, including asplenia
23.	Co-workers of the departments of Medical Microbiology of the UMC St Radboud, the department of Internal Medicine of the Havenziekenhuis or the department of Medical Microbiology & Infectious Diseases of the Erasmus MC
24.	A history of sickle cell, thalasaemia trait or G6PD deficiency
CHMI-b	**Inclusion criteria**
1.	Subject is aged ≥ 18 and ≤ 35 years and in good health.
2.	Subject has adequate understanding of the procedures of the study and agrees to abide strictly thereby.
3.	Subject is able to communicate well with the investigator, is available to attend all study visits, lives in proximity to the trial centre (<10 km) or (if >10km) is willing to stay in a hotel close to the trial centre during part of the study (day 5 post-infection until three days post-treatment). Furthermore the subject will remain within the Netherlands during the study period and is reachable (24/7) by mobile telephone throughout the entire study period.
4.	Subject agrees to inform his/her general practitioner and (if applicable) medical specialist about participation in the study and to sign a request to release by the GP any relevant medical information concerning possible contra-indications for participation in the study.
5.	Subject agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period and for a defined period thereafter according to current Sanquin guidelines.
6.	For female subjects: subject agrees to use adequate contraception and not to breastfeed for the duration of study.
7.	Subject has signed informed consent.
	Exclusion criteria
1.	Any history, or evidence at screening, of clinically significant symptoms, physical signs or abnormal laboratory values

	suggestive of systemic conditions, such as cardiovascular, pulmonary, renal, hepatic, neurological, dermatological, endocrine, malignant, haematological, infectious, immunodeficient, psychiatric and other disorders, which could compromise the health of the volunteer during the study or interfere with the interpretation of the study results. These include, but are not limited to, any of the following:
1.1	Body weight <50 kg or Body Mass Index (BMI) <18.0 or >30.0 kg/m2 at screening
1.2	A heightened risk of cardiovascular disease, defined as: an estimated ten year risk of fatal cardiovascular disease of ≥5% at screening, as determined by the Systematic Coronary Risk Evaluation (SCORE); history, or evidence at screening, of clinically significant arrhythmia's, prolonged QT-interval or other clinically relevant ECG abnormalities; or a positive family history of cardiac events in 1st or 2nd degree relatives <50 years old.
1.3	Functional asplenia, sickle cell trait/disease, thalassaemia trait/disease or G6PD deficiency.
1.4	History of epilepsy in the period of five years prior to study onset, even if no longer on medication.
1.5	Positive HIV, HBV or HCV screening tests.
1.6	Chronic use of i) immunosuppressive drugs, ii) antibiotics, iii) or other immune modifying drugs within three months prior to study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period.
1.7	History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years
1.8	Any history of treatment for severe psychiatric disease by a psychiatrist in the past year.
1.9	History of drug or alcohol abuse interfering with normal social function in the period of one year prior to study onset, or positive urine toxicology test for cocaine or amphetamines at screening or prior to infection.
2.	For female subjects: positive urine pregnancy test at screening or prior to infection.
3.	Any history of malaria, positive serology for *P. falciparum*, or previous participation in any malaria (vaccine) study.
4.	Known hypersensitivity to or contra-indications (including co-medication) for use of atovaquone-proguanil (Malarone®) or artemether-lumefantrine (Riamet®), or history of severe (allergic) reactions to mosquito bites.
5.	Receipt of any vaccinations in the 3 months prior to the start of the study or plans to receive any other vaccinations during the study period or up to 8 weeks thereafter.
6.	Participation in any other clinical study in the 30 days prior to the start of the study or during the study period.
7.	Being an employee or student of the department of Medical Microbiology of the Radboudumc, the department of Internal Medicine or Laboratory of the Havenziekenhuis or the department of Medical Microbiology & Infectious Diseases of the Erasmus MC.
8.	Any other condition or situation that would, in the opinion of the investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.