CLINICAL STUDY PROTOCOL

A randomised, single blind, placebo controlled, phase 1 trial to evaluate the safety, tolerability, pharmacokinetic and pharmacodynamic activity of Ruxolitinib when co-administered with artemether-lumefantrine in healthy participants

Protocol Number:	MMV_Ruxolitinib_19_01
Investigational Product/s:	Ruxolitinib Phosphate (Jakavi®) Artemether-lumefantrine (Riamet®)
Principal Investigator:	Dr Paul Griffin Q-Pharm Pty Ltd Level 5, 300C Herston Rd Herston Qld 4006, Australia Tel: +61 (0)7 3845 3636
Local Sponsor/CRO:	Southern Star Research (SSR) 1 Merriwa St Gordon, NSW 2072 Australia
Global Sponsor:	Medicines for Malaria Venture (MMV) 20, Route de Pré-Bois 1215 Geneva 15 Switzerland
Date and Version:	Version 4.0, 07 September 2020

Confidentiality Statement

1 PROTOCOL SUMMARY

1.1 PROTOCOL SYNOPSIS

Protocol Title	A randomised, single blind, placebo controlled, phase 1 trial to evaluate the safety, tolerability, pharmacokinetic and pharmacodynamic activity of Ruxolitinib when co-administered with artemether-lumefantrine in healthy participants.
IND Number	N/A
Protocol Number	MMV_Ruxolitinib_19_01
Local Sponsor	Southern Star Research Pty Ltd
Global Sponsor	Medicines for Malaria Venture
Principal Investigator	Dr Paul Griffin, Q-Pharm Pty Ltd
Phase of Development	Phase 1
Number of Study Sites	1
Treatment Groups	**Group 1a** (sentinel group): Oral administration of: • 20 mg/120 mg artemether-lumefantrine (AL) + 20 mg ruxolitinib phosphate (Rux) or • 20 mg/120 mg AL + placebo; Rux or placebo administered 2 hours after AL administration, twice daily (b.i.d) for 3 consecutive days (6 doses in total). The Principal Investigator, Sponsor Medical Director and study Medical Monitor (at a minimum) will review safety and tolerability data up to and including Day 8 from Group 1a before deciding if Group 1b can proceed. The dose of 20 mg Rux will not be altered. **Group 1b**: Oral administration of: • 20 mg/120 mg AL + 20 mg Rux or 20 mg/120 mg AL + placebo; • Rux or placebo administered 2 hours after AL administration, twice daily (b.i.d) for 3 consecutive days (6 doses in total)
Participant Population	Healthy male and female adult volunteers that meet all eligibility criteria will be enrolled: 2 groups each to be enrolled sequentially. Group 1a will be a sentinel group of 2 participants, and Group 1b will be composed of 6 participants; randomised (single blinded; treatment allocation concealed to participants but not Investigator) to be administered either AL + Ruxolitinib or AL + placebo.
Investigational Medicinal Products (IMP)	• Artemether-lumefantrine (AL), 20mg/120mg tablets administered orally 2 hours prior to: • Ruxolitinib phosphate (Rux), 20 mg tablet administered orally or placebo capsule administered orally.
Duration of Study	Approximately 2 months for each participant - screening period of up to 28 days and 28 days on-study, with total of up to 56 days.
Objectives	**Primary Objective:** To assess the safety and tolerability of 3-day b.i.d dosing of AL+Rux and AL+placebo. **Secondary Objectives:**

	1. To assess the effect of AL+Rux and AL+placebo on pSTAT3 inhibition. 2. To characterise the PK profiles of artemether and its major metabolite dihydroartemisinin [DHA], lumefantrine, and Rux. 3. To assess additional safety variables (tympanic body temperature and respiration rate)
Endpoints	**Primary Endpoint:** 1. Incidence, severity, and relationship of observed and self-reported AEs up to 28 days after AL+Rux and AL+placebo administration in all participants by treatment regimen. 2. Clinical laboratory evaluations including haematology, biochemistry and urinalysis. 3. Vital signs (blood pressure and heart rate). 4. 12-lead standard ECG: QT, QTcB and QTcF; HR, PR, QRS. **Secondary Endpoints:** 1. pSTAT3 inhibition ex-vivo on whole blood cells by: • pSTAT3 levels pre- and post- AL+Rux administration intra participant and, • pSTAT3 levels per treatment regimen post AL+Rux and post AL+placebo administration 2. PK parameters of artemether, DHA, lumefantrine, and Rux using non-compartmental methods: AUC_{last}, $AUC_{0-\infty}$, AUC_{0-8}, AUC_{60-72}, C_{max} (first and last dose), t_{max} (first and last dose), elimination half-life ($t_{½}$), t_{lag}, C_{168h} (for lumefantrine only), CL/F, Vz/F and λz in all participants. 3. Safety variables tympanic body temperature (°Celcius) and respiratory rate.
Study Description	This is a randomised, single-blinded, placebo-controlled, single centre, phase 1 trial. Eight healthy males or females, aged between 18-55 years old, who meet all of the inclusion criteria and none of the exclusion criteria, will be enrolled. The study will be composed of 2 groups to be enrolled sequentially. • Group 1a (sentinel group): two participants will be randomised single-blinded such that one participant will receive AL+Rux and the other participant will receive AL+placebo. After review of the safety and tolerability data up to and including Day 8 from Group 1a by the Principal Investigator, the Sponsor Medical Director and the study Medical Monitor (at a minimum), a decision to proceed with Group 1b will be made. • Group 1b will be composed of 6 participants, to be randomised single-blinded such that five participants will receive AL+Rux, and one participant will receive AL+placebo. Participants will be admitted to the clinical trial unit on Day -1 and will be administered an oral dose of AL+Rux or AL+placebo separated by 2 hours (with Rux or placebo administered 2 hours after AL) b.i.d commencing on Day 1 (AL at t = 0, 8, 24, 36, 48 and 60 hours). Participants will be confined to the clinical trial unit for at least 78 hours after first dose IMP administration for blood sampling for PK and PD (pSTAT3) and close safety and tolerability monitoring. After discharge from the clinical trial unit, participants will be followed via outpatient care visits for safety assessments, pSTAT sampling (day 8) and PK blood sampling up to day 29.
Sample Size	Eight healthy males or females, aged between 18 and 55 years old, who meet all of the inclusion criteria and none of the exclusion criteria will be enrolled. As the combination of Rux+AL has not been tested in humans before, a first-in-human approach of eight participants (with ratio active to placebo of 3:1)

	has been selected. Additional reserve volunteers will be consented and screened to be available on Day 1 in the event of withdrawal of participants prior to first-dose IMP administration.
Inclusion Criteria	Potential participants must fulfil all of the following inclusion criteria to be eligible to participate in the study and throughout the study: 1. Male or female (non-pregnant, non-lactating) aged 18 to 55 years inclusive. 2. Contactable and available for the duration of the trial and for up to two weeks following the EOS visit. 3. Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18 to 32 kg/m2 (inclusive) at Screening and Day -1. BMI is an estimate of body weight adjusted for height. It is calculated by dividing the weight in kilograms by the square of the height in metres. **Health status** 4. Certified as healthy by a comprehensive clinical assessment (detailed medical history, full physical examination and special investigations). 5. Vital signs measured after 5 min in the supine position: • Systolic blood pressure (SBP) - 90–140 mmHg, • Diastolic blood pressure (DBP) - 40–90 mmHg, • Heart rate (HR) 40–100 bpm. 6. ECG parameters for both males and females: QT ≤ 500 msec, QTcF ≤450 msec, QTcB ≤450 msec; PR interval ≤210 msec. 7. Heterosexual female participants of childbearing potential who have, or may have, male sexual partners during the course of the study should be using an insertable (implant or IUD), injectable, transdermal or combination oral contraceptive approved by the TGA combined with a barrier contraceptive from the time of informed consent until 30 days after EOS. Abstinent female participants must agree to start a double method if they start a sexual relationship with a male during the trial until 30 days after EOS. Female participants must not be planning in vitro fertilisation within the required contraception period. Women of non-childbearing potential who will not require contraception during the trial are defined as: surgically sterile (tubal ligation is not considered surgically sterile), post-menopausal (spontaneous amenorrhoea for ≥12 months, or spontaneous amenorrhoea for 6-12 months and follicle-stimulating hormone (FSH) ≥40 IU/mL; either should be together with the absence of oral contraceptive use for >12 months). Male participants who have, or may have female sexual partners during the course of the study must agree to use a double method of contraception including condom plus diaphragm, or condom plus stable insertable (implant or IUD), injectable, transdermal or combination oral contraceptive by the female partner, from the time of informed consent through to 90 days after EOS. Abstinent male participants must agree to start a double method if they begin a sexual relationship with a female during the trial, and through to 90 days after EOS. Male participants with female partners that are surgically sterile or post-menopausal, or male participants who have undergone sterilisation and have had testing to confirm the success of the sterilisation, may also be included and will not be required to use above described methods of contraception. In addition, all male participants must agree to not donate sperm for at least 90 days after EOS. **Regulations** 8. Completion of the written informed consent process prior to undertaking any trial-related procedure.

	9. Must be willing and able to communicate and participate in the whole trial. 10. Agree to adhere to Lifestyle Considerations (see Section 4.3.3) throughout trial duration and be willing to consume 250 mL full-fat milk with each dose of AL.
Exclusion Criteria	If any of the following exclusion criteria apply, the potential participant will not be permitted to participate or remain in the study: **Medical history and clinical status** 1. Known hypersensitivity to ruxolitinib, artesunate or any of its excipients, artemether, lumefantrine or other artemisinin derivatives, proguanil/atovaquone, primaquine, or 4-aminoquinolines. 2. Haematology, biochemistry or urinalysis results that are abnormal/outside of the laboratory normal reference ranges AND are either: • Considered clinically signficant by the Principal Investigator or delegate; OR • Considered not clinically significant by the Principal Investigator or delegate BUT ARE ALSO outside of Sponsor-approved clinically acceptable laboratory ranges in Appendix 1. NOTE: Participants are not excluded if abnormal/out of laboratory normal reference range results are considered not clinically significant by the Principal Investigator or delegate AND are within the ranges specified in Appendix 1. 3. Platelets < $200x10^9$/L at Screening or prior to IMP administration is exclusionary. One re-test is permitted if original test result does not reflect the assumed medical status of the individual. 4. Participation in any other investigational product trial within 5 half-lives or 12 weeks preceding IMP administration, whichever is longer, or in the exclusion period of a previous trial according to applicable regulations. 5. Symptomatic postural hypotension at screening and pre-first dose of IMP on Day 1 (confirmed on two consecutive readings), irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 2–3 min when changing from supine to standing position. 6. History or presence of diagnosed (by an allergist/immunologist) or treated (by a physician) food or known drug allergies, or any history of anaphylaxis or other severe allergic reactions including face, mouth, or throat swelling or any difficulty breathing. Participants with seasonal allergies/hay fever or allergy to animals or house dust mite that are untreated and asymptomatic at the time of dosing can be enrolled in the trial. 7. History of convulsion (including drug or vaccine-induced episodes). A medical history of a single febrile convulsion during childhood is not an exclusion criterion. 8. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immuno-deficiencies), insulin-dependent and non-insulin-dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma (excluding childhood asthma), epilepsy, or obsessive-compulsive disorder. 9. History of malignancy of any organ system (other than localised and considered cured basal cell carcinoma of the skin or in situ cervical cancer), treated or untreated, within five years of screening, regardless of whether there is no evidence of local recurrence or metastases. 10. Individuals with history of schizophrenia, bipolar disorder psychoses, disorders requiring lithium, attempted or planned suicide, or any other

	severe (disabling) chronic psychiatric diagnosis including generalised anxiety disorder. 11. Individuals who have been hospitalised within five years prior to enrolment for either a psychiatric illness or due to danger to self or others. 12. History of an episode of mild/moderate depression lasting more than 6 months that required pharmacological therapy and/or psychotherapy within the last 5 years; or any episode of major depression. The Beck Depression Inventory (BDI-II) will be used as a validated tool for the assessment of depression at screening. In addition to the conditions listed above, individuals with a score of 20 or more on the BDI-II and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. These individuals will be referred to a general practitioner or medical specialist as appropriate. Individuals with a BDI-II score of 17 to 19 may be enrolled at the discretion of an Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the individual or to the execution of the trial and interpretation of the data gathered. 13. History of recurrent headache (e.g. tension-type, cluster, or migraine) with a frequency of ≥2 episodes per month on average and severe enough to require medical therapy, during the 2 years preceding screening. 14. Acute illness within the 4 weeks prior to screening and prior to IMP administration. 15. Significant inter-current disease of any type, in particular liver, renal, cardiac, pulmonary, neurologic, rheumatologic, or autoimmune disease by history, physical examination, and/or laboratory studies including urinalysis. 16. Individual has a clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion (e.g. gastrectomy, cholecystectomy, diarrhoea) or known lactose/dairy intolerance. 17. Participation in any research trial involving blood sampling (more than 300 mL/unit of blood) within one month prior to IMP administration, or blood donation to Australian Red Cross Blood Service (Blood Service) or other blood bank during the 8 weeks prior to IMP administration. 18. Medical requirement for intravenous immunoglobulin or blood transfusions. 19. History or presence of alcohol abuse (alcohol consumption more than 40 g/4 units/4 standard drinks per day), or drug habituation, or any prior intravenous usage of an illicit substance. 20. Any individual who has ever smoked >1 pack of cigarettes per day for >10 years, or who currently (within 14 days prior to Screening or prior to IMP administration smokes >5 cigarettes/day. 21. Female who is breastfeeding. 22. Any vaccination within the last 28 days prior to screening or prior to IMP administration. 23. Prior to screening or IMP administration: any systemic or inhaled corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past three months. Any topical, nasal or ophthalmic corticosteroids within the past 2 weeks. Any individual currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past year. 24. Use of antidepressant medication in the past 12 months prior to screening or prior to IMP administration. 25. Use of any other medication except contraceptives (including herbal, vitamin supplement, OTC or prescription) within 14 days or five half-

	lives (whichever is longest) prior to IMP administration. Participants are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the trial. 26. Cardiac/QT risk: • Family history of sudden death or of congenital prolongation of the QT, QTcF, or QTcB interval or known congenital prolongation of the QT, QTcF, or QTcB interval or any clinical condition known to prolong the QT, QTcF, or QTcB interval. • History of symptomatic cardiac arrhythmias or with clinically relevant bradycardia. • Electrolyte disturbances, particularly hypokalaemia, hypocalcaemia, or hypomagnesaemia. • ECG abnormalities in the standard 12-lead ECG (at screening or prior to IMP administration) which in the opinion of an Investigator is clinically relevant or will interfere with the ECG analyses. **General conditions** 27. Any individual who, in the judgement of an Investigator, is likely to be non-compliant during the trial, or is unable to cooperate because of a language problem or poor mental development. 28. Any individual for whom study participation would pose an additional safety risk as assessed by the Principal Investigator. 29. Any individual who is an Investigator, research assistant, pharmacist, trial coordinator, or other staff thereof, directly involved in conducting the trial. 30. Any individual without good peripheral venous access. **Biological status** 31. Positive result on any of the following tests: hepatitis B surface antigen (HBs Ag), anti-hepatitis B core antibodies (anti-HBc Ab), anti-hepatitis C virus (anti-HCV) antibodies, anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti-HIV2 Ab). 32. Recent herpes zoster infection (within the previous 6 months) as determined by clinical history. 33. Positive result for *M. tuberculosis* infection by QuantiFERON-TB Gold assay. 34. Positive urine drug test for any drug listed in Section 7.4.5. Any individual testing positive for acetaminophen (paracetamol) at screening and/or pre-dose may still be eligible for trial participation at the discretion of the Principal Investigator or delegate. 35. Positive alcohol breath test.
Statistical Analysis	• Four analysis datasets will be used for study analyses: Full analysis set (FAS), Safety Set, PK set and pSTAT3 set. • The FAS will be used to list and summarise subject disposition, demographics and baseline characteristics, protocol deviations and treatment exposure. • Safety and tolerability will be assessed by clinical review of all AEs, vital signs, 12-lead ECG, haematology, biochemistry, urinalysis and physical examination results. All descriptive statistics will be evaluated using the Safety Set. • PK parameters of artemether, DHA, lumefantrine and Rux will be estimated using non-compartmental methods from plasma concentration-time data. For calculation of descriptive statistics of plasma concentrations, values below the lower limit of quantitation will be set to zero. PK analyses will be conducted using the PK Set. • Levels of pSTAT3 will be expressed as a percentage and summarised by percentage inhibition pre- and post- IMP administration and as a change in percentage between pre- and post- IMP administration. The following analyses will be conducted: within-participant comparison of each participant before AL+Rux or AL+placebo administration, and 12 hours

	after first administration of AL; between-participants comparison of participants who receive AL+Rux and participants who receive AL+placebo. Analyses of pSTAT3 levels will be conducted using the pSTAT3 Set.

1.2 SCHEMA

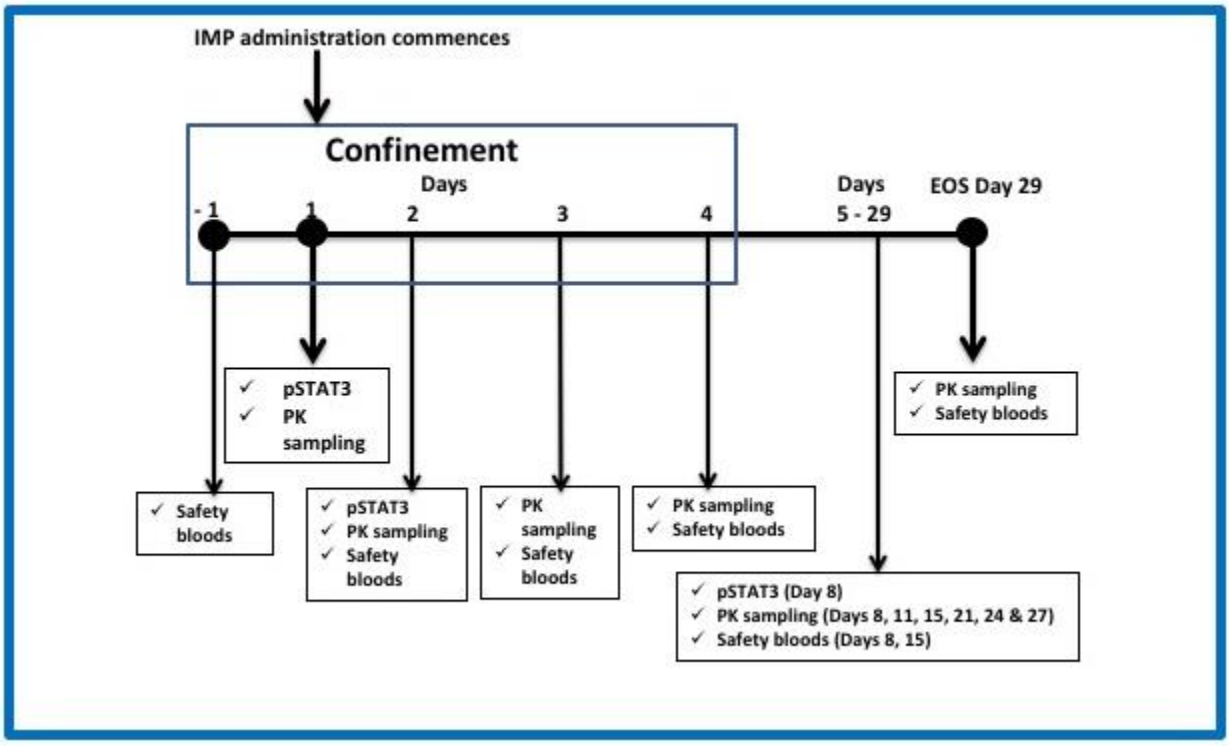

Figure 1: Schema

1.3 SCHEDULE OF ACTIVITIES

Table 1: Schedule of Activities - Overview

Part 1 Activity/Procedure	**Screening/Eligibility/ Safety**		**Treatment and Confinement**	**Outpatient Monitoring**	**EOS/ET**
	Day-28 to Day -1	**Day -1[a]**	**Day 1 to Day 4[a]**	**Day 5 to Day 29[a]**	**Day 29[a]**
Overnight fast of ≥8 h required	X	X	X	X Days 8 & 15	X
Informed consent, BDI-II & demographics	X				
Medical history, eligibility & prior/concomitant medications; recreational drug, alcohol and tobacco use (history and current)	X	X	X pre-dose		
Confinement at clinical unit		X	X		
Discharge from clinical unit			X 78 h post-first dose IMP		
Outpatient visit to clinical unit	X	X		X	X
Urine drug screen & alcohol breath test	X	X			
Full physical examination	X				X
Abbreviated physical examination		X			
Symptom-directed physical examination if clinically indicated			X during & prior to discharge	X	
Vital signs (BP & HR)	X*b*	X*b*	X*c*	X*d*	X*d*
Tympanic Body Temperature (°C) & RR (supine)	X	X	X 4 h & every 24 h post-first dose IMP	X	X
Height (cm) and weight (kg)	X	X weight only			
12-lead ECG	X		X pre-dose, 24, 48, 72 h post-first dose IMP	X Days 8 & 15	X
Urinalysis	X	X*e*	X 24, 48, 72 h post-first dose IMP	X Days 8 & 15	X
Intravenous cannulation		X or Day 1	X or Day -1		
Haematology	X	X*e*	X 24, 48, 72 h post-first dose IMP	X Days 8 & 15	X
Fasting Biochemistry (may include serum β-hCG, FSH [post-menopausal female participants only], and lipids)	X incl. β-hCG (all females), FSH, & lipids, blood group, Rh(D) (fasting)	X*e*	X 24, 48, 72 h post-first dose IMP	X Days 8 & 15	X incl. β-hCG (WOCBP only)

Part 1 Activity/Procedure	**Screening/Eligibility/ Safety**		**Treatment and Confinement**	**Outpatient Monitoring**	**EOS/ET**
	Day-28 to Day -1	**Day -1[a]**	**Day 1 to Day 4[a]**	**Day 5 to Day 29[a]**	**Day 29[a]**
Coagulation profile	X				
Serology	X				
QuantiFERON-TB Gold assay	X				
Urine β-hCG pregnancy test (WOCBP only)		X			
AEs & concomitant medications[f]			X *f*	X	X
AL and Rux PK blood sampling			X*g*	X*h*	X
pSTAT3 blood sampling			X*i*	X*i* Day 8 (168h)	
IMP administration			X*j*		

Abbreviations: AE=adverse event; AL=artemether/lumefantrine (Riamet); BDI-II=Beck Depression Inventory; BP = blood pressure; ECG=electrocardiogram; FSH=follicle stimulating hormone; G6PD=glucose-6-phosphate dehydrogenase; hCG=human chorionic gonadotropin; HR = heart rate; IMP=investigational medical product; inc.=including; PK=pharmacokinetics; pSTAT3=phospho-signal transducer and activator of transcription 3; RR = respiratory rate; Rux=ruxolitinib (Jakavi®); Safety labs=blood collection for haematology and biochemistry; TB=tuberculosis; WOCBP=women of child bearing potential.

a. Day 1 is defined as the day of first dose of IMP (AL at t = 0). Participants will be admitted to the clinical unit on Day -1 for confinement and administration of IMP on Day 1, and will be confined to the unit for at least 78 hours post-first dose IMP. Outpatient visits are scheduled on Days 8 (t = 168h), 11 (t = 240h), 15 (t = 336h), 21 (t = 480h), 24 (t = 552h) and 27 (t = 624h) with EOS on Day 29 (t = 672h). Visit windows for all outpatient visits are as per the PK sampling time windows for those timepoints as described in Section 6.2.
b. Vital signs (blood pressure (BP; systolic and diastolic) and heart rate (HR) at screening and on Day -1: blood pressure and heart rate measured supine after resting in supine position for at least 5 minutes and standing within 2-3 minutes when changing from supine to standing.
c. On Day 1, BP and HR measured pre-first dose (measured supine and standing as per footnote b), and then after participant supine for at least 5 minutes at 4 and 8 hours post-first dose IMP during confinement and then every 8 hours. Pre-first dose results must satisfy relevant inclusion/exclusion criteria.
d. Vital signs (BP and HR) conducted after participant has rested in supine position for at least 5 minutes.

e. Haematology biochemistry and urinalysis results from samples collected on Day -1 must be available for safety review on Day 1 prior to first-dose IMP (AL at t = 0) and must satisfy relevant inclusion/exclusion criteria.
f. Untoward medical events reported between the time of informed consent and first dose of IMP will be recorded as medical history, and must satisfy relevant inclusion/exclusion criteria. Adverse events will be recorded from time of first dose of IMP (AL at t = 0).
g. During confinement, PK blood sampling to be taken at timepoints pre-dose, then at t = 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 62, 64, 72 and 78 hours post commencement of IMP dosing. When sampling coincides with IMP administration, blood samples must be taken prior to IMP. See Section 6.2 for permitted time windows.
h. During outpatient visits, PK blood sampling to be taken on Days 8, 11, 15, 21, 24, 27 and 29 (EOS) at t = 168, 240, 336, 480, 552, 624 and 672 hours post commencement of IMP dosing respectively. See Section 6.2 for permitted time windows.
i. During confinement, pSTAT3 blood sampling to be taken at timepoints pre-dose, then t = 1, 2, 3, 4, 5, 6, 8, and 12 hours post commencement of IMP dosing.When sampling coincides with IMP administration, blood samples must be taken prior to IMP. A pSTAT3 blood sample will also be taken at t = 168 hours post-first dose IMP (AL at t = 0) on Day 8. See Section 6.2 for permitted time windows.
j. Randomised (on Day -1 or Day 1) to be administered either AL+Rux or AL+placebo from Day 1. AL dosing (with 250 mL full-fat milk) at t=0, 8, 24, 36, 48 and 60 hours (see Section 5.1.1 for food and other restrictions). Rux/placebo administered with 250 mL water 2 hours after AL at t = 2, 10, 26, 38, 50 and 62 hours (see Sections 5.1.2 and 5.1.3 for food and other restrictions). All doses to be administered under supervision and at the scheduled timepoints. NOTE: Results of safety laboratory testing (haematology, biochemistry, urinalysis) taken during the Safety/Eligibility visit must be available for review by the Principal Investigator or delegate prior to IMP administration on Day 1. All pre-first dose assessment results must satisfy relevant inclusion/exclusion criteria.

NOTE: This table provides an overview only for activities required from Day 1. See Table 2 for detailed Schedule of Activities including all scheduled timepoints for Day 1 to Day 4 Confinement and IMP Treatment; see Table 3 for detailed Schedule of Activities for Outpatient Visits after discharge from the clinical unit including EOS/ET.

Table 2: Schedule of Activities – Detailed Day 1 to Day 4 Confinement and IMP Treatment

Day[a]	**D1**[a]												**D2**[a]						**D3**[a]						**D4**[a]	
Time (hour)[a]	**Pre-dose**	**0H**	**1H**	**2H**	**3H**	**4H**	**5H**	**6H**	**8H**	**10H**	**12H**	**16H**	**24H**	**26H**	**32H**	**36H**	**38H**	**40H**	**48H**	**50H**	**56H**	**60H**	**62H**	**64H**	**72H**	**78H**
Confinement	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Discharge																										X
Randomization	X[b]																									
IMP[c]																										
IMP - AL		X							X				X			X			X			X				
IMP – Rux/placebo				X						X				X			X			X			X			
Safety[d]																										
Physical exam sympt.		Xe																								Xe
Vital signs[f] (BP & HR)	Xf					X			X			X	X		X			X	X		X			X	X	
Tympanic body temperature (°C) and RR	Xg					Xg							Xg						Xg						Xg	
Standard 12-lead ECG	Xh												Xh						Xh						Xh	
Biochemistry Haematology													Xi						Xi						Xi	
Urinalysis													Xi						Xi						Xi	
Record AE & Concomitant medications[j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PK																										
PK blood samples[k]	X		X	X	X	X	X	X	X		X		X			X			X			X	X	X	X	X

Day[a]	**D1**[a]												**D2**[a]						**D3**[a]						**D4**[a]	
Time (hour)[a]	**Pre-dose**	**0H**	**1H**	**2H**	**3H**	**4H**	**5H**	**6H**	**8H**	**10 H**	**12 H**	**16 H**	**24 H**	**26 H**	**32 H**	**36 H**	**38 H**	**40 H**	**48 H**	**50 H**	**56 H**	**60 H**	**62 H**	**64 H**	**72 H**	**78 H**
PD																										
pSTAT3 sample[/]	X		X	X	X	X	X	X	X		X															

ABBREVIATIONS: abbrev. = abbreviated; AE = adverse event; AL = artemether/lumefantrine (Riamet®); BP = blood pressure; ECG = electrocardiogram; exam = examination; hCG = human chorionic gonadotropin; HR = heart rate; IMP = investigational medicinal product; PD = pharmacodynamics; PK = pharmacokinetics; RR = respiratory rate; Rux = ruxolitinib (Jakavi®); sympt. = symptom directed; WOCBP = woman of child bearing potential.

a. Time (hour) is defined as the time in hours after the first dose of IMP (AL at t = 0 on Day 1). NOTE: Study days are numbered by convention per 24 hour period from t = 0. The actual day of some timepoints in practice may vary depending on the actual time of t = 0 on Day 1. Participants will be admitted to the clinical unit on Day -1 for treatment and confinement, and will not be discharged from the clinical unit until at least 78 hours post-first dose IMP.

b. If not performed on Day -1, participants will be randomized (single-blinded – participant is unaware of treatment allocation) to be administered either AL+Rux or AL+placebo.

c. IMPs will be administered under direct observation at the scheduled timepoints (exact timing to be respected): AL (20 mg artemether, 120 mg lumefantrine per tablet): 4 tablets administered orally with 250 mL full-fat milk at t = 0, 8, 24, 36, 48 and 60 hours (total 24 tablets). Rux (20 mg ruxolitinib per tablet) or placebo capsule: 1 tablet/capsule administered orally with 250 mL water 2 hours after each AL administration at t = 2, 10, 26, 38, 50 and 62 hours (total 6 tablets/capsules). Participants will be blindfolded for Rux/placebo dosing and prevented from witnessing each other's Rux/placebo dosing. Participants should swallow IMP whole without biting or chewing. No food permitted 30 minutes prior to AL dosing, between AL and Rux dosing or 1 hour after Rux dosing. If dosing coincides with other assessments, participant should remain seated for 10 minutes post-dose, then remain semi-recumbent for at least 1 hour. NOTE: Results from biochemistry/haematology/urinalysis taken at eligibility/safety visit must be available and reviewed by the Principal Investigator or delegate prior to first dosing with IMP (AL at t = 0). On Day 1, participants will have fasted for at least 8 hours overnight before breakfast prior to first-dose IMP at t = 0. All pre-first dose assessment results must satisfy relevant inclusion/exclusion criteria.

d. Refer to Safety Section 7 for detailed safety investigations.

e. Symptom-directed physical examination when required if clinically indicated during confinement. Any pre-first dose results must satisfy relevant inclusion/exclusion criteria. Prior to participant discharge, clinical unit staff must clarify with the Principal Investigator or delegate if a symptom directed physical examination is required.

f. Vital signs during confinement (systolic and diastolic blood pressure [mmHg] and heart rate [beats per minute): NOTE: pre-dose within 60 minutes prior to first-dose IMP (AL at t = 0): blood pressure measured after participant supine for at least 5 minutes and then again in standing position within 2-3 minutes of standing (pre-first dose results must satisfy relevant inclusion/exclusion criteria). Then vital signs measured within 15 minutes prior to each subsequent timepoint during confinement after participant supine for at least 5 minutes at: 4 and 8 hours post-first dose IMP, and then every 8 hours during confinement.

g. Respiratory rate (RR: breaths per minute) and tympanic body temperature (ºCelcius) will be measured within 60 minutes prior to first-dose IMP (AL at t = 0; pre-first dose results must satisfy any relevant inclusion/exclusion criteria), and then 15 minutes prior to the subsequent timepoints during confinement. RR will be measured after participant is supine for at least 5 minutes.
h. Standard single 12-lead ECG recorded after supine for at least 5 minutes within 60 minutes prior to first-dose IMP (AL at t = 0; pre-first dose results must satisfy relevant inclusion/exclusion criteria), then within 15 minutes prior to subsequent timepoints at 24, 48 and 72 hours post-first dose IMP (AL at t = 0).
i. Safety laboratory samples (haematology, biochemistry, urinalysis) will be collected 24, 48 and 72 hours post-first dose IMP (within 60 minutes prior to each timepoint – must be collected prior to breakfast). Safety biochemistry laboratory sampling requires fasting for at least 8 hours.
j. Untoward medical events reported before the first dose of IMP will be recorded as medical history and must satisfy relevant inclusion/exclusion criteria. Adverse events and any concomitant medications will be recorded from time of first dose of IMP. AE/concomitant medication timepoint checks: ± 10 minutes for Day 1, then ± 20 minutes for remainder of confinement. AEs/concomitant medications may be reported/ recorded at any time during confinement. See Section 7.6 for further information including SAE/AESI reporting. See Section 4.3.8 for toxicity/stopping rules.
k. PK blood samples will be collected at the timepoints indicated. For timepoints coinciding with IMP administration, blood sampling must occur prior to IMP administration. The exact timing for IMP administration must be respected. See Section 6.2 for permitted time windows.
l. pSTAT3 blood samples will be collected at the timepoints indicated. For timepoints coinciding with IMP administration, blood sampling must occur prior to IMP administration. See Section 6.2 for permitted time windows.

NOTE:

- **When several procedures are scheduled to take place at the same timepoint, the following order is recommended where applicable: urinalysis, ECG, vital signs, blood sampling, drug administration.**
- **Date/time of all assessments/activities must be recorded in source document.**
- **Pre-dose assessments will be conducted within 60 minutes prior to the first dose of IMP (AL at t = 0).**

Table 3: Schedule of Activities – Detailed Days 8 to EOS Outpatient Visits

Day (D)	**D8**	**D11**	**D15**	**D21**	**D24**	**D27**	**EOS D29 (or ET)**
Time (hour)[a]	**168H ± 120 min**	**240H ±24h**	**336H ±24h**	**480H ±24h**	**552H ±24h**	**624H ±24h**	**672H ±24h**
Outpatient visit at clinical site	X	X	X	X	X	X	X
Safety[b]							
Physical exam: Full							X
Physical exam:Symptom-directed	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	
Vital signs (supine)[d]	X	X	X	X	X	X	X
Tympanic body temperature (°C) and RR [d]	X	X	X	X	X	X	X
Standard single 12-lead ECG	X[e]		X[e]				X[e]
Biochemistry, Haematology	X[f]		X[f]				X[f]
Urinalysis	X		X				X
Serum β-hCG (WOCBP only)							X
Record AE and concomitant medications[g]	X	X	X	X	X	X	X
PK							
PK blood samples[h]	X	X	X	X	X	X	X
PD							
pSTAT3 sample	X[i]						

ABBREVIATIONS: AE = adverse event; ECG = electrocardiogram; exam = examination; ET = early termination visit; hCG = human chorionic gonadotropin; PD = pharmacodynamic; PK = pharmacokinetic; WOCBP = woman of child bearing potential.

a. Time (hour) is defined as the time in hours after the first dose of IMP at t=0 on Day 1.
b. Refer to Safety Section 7 for detailed safety investigations. All assessments to be conducted within the same time window permitted for PK sampling (Section 6.2).
c. Symptom-directed physical examination when required if clinically indicated. Clinical unit staff must clarify with the Principal Investigator or delegate if a symptom directed physical examination is required.
d. Vital signs (systolic and diastolic blood pressure [mmHg] and heart rate [beats per minute]) and respiratory rate (breaths per minutes) to be measured after participant supine for at least 5 minutes.
e. Standard single 12-lead ECG recorded after participant supine for at least 5 minutes.
f. Safety biochemistry laboratory sampling on Days 8, 15 and EOS require fasting for at least 8 hours.

g. All adverse events that occur and/or concomitant medications that are administered after discharge from the clinical unit and until EOS will be recorded. See Section 7.6 for further information including SAE/AESI reporting.
h. The exact timing for PK sampling must be respected. See Section 6.2 for permitted time windows. NOTE: permitted outpatient visit windows are with respect to the scheduled PK sampling timepoints in hours post-first dose IMP at t = 0.
i. pSTAT3 sampling: see Section 6.2 for permitted time windows.

NOTE:

- **When several items take place at the same time, the following order is recommended where applicable: urinalysis, ECG, vital signs, blood sampling.**
- **Date/time of all assessments/activities must be recorded in source document.**
- **Time windows for all outpatient assessments are as per PK/PD sampling time windows (**Section 6.2).

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
AESI	Adverse event of special interest
AL	Artemether/lumefantrine (riamet®)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	Amphetamines
ANC	Absolute neutrophil count
APAP	Acetaminophen/paracetamol
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic class
AUC_{last}	Area under the plasma concentration-time curve from time zero to time of last measurable concentration
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero to infinity
BAR	Barbiturates
BDI	Beck depression inventory
β-hCG	B-human chorionic gonadatropin
b.i.d	Twice-daily (medication)
BMI	Body mass index
BZO	Benzodiazepines
C	Drug concentration in the plasma at any given time
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology collaboration
CL/F	Apparent total clearance of the drug from plasma after oral administration
C_{max}	Maximum (peak) plasma drug concentration
CMI	Consumer medication information
COC	Cocaine
CRO	Clinical research organisation
CSR	Clinical study report
CTCAE	Common terminology criteria for adverse events
DBP	Diastolic blood pressure
DHA	Dihydroartemisinin
ECG	Electrocardiograph
ECM	Experimental cerebral malaria

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EOS	End of study
FAS	Full analysis set
FBC	Full blood count
FDA	(USA) Food & Drug Administration
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GMP	Good manufacturing practice
HBc Ab	Hepatitis B core antibodies
HBs Ag	Hepatitis B surface antigen
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HR	Heart rate
HREC	Human research ethics committee
IB	Investigator's brochure
ICH	International conference on harmonisation
IFN	Interferon
IFNAR	IFNα receptor
IL-10	Interleukin 10
IMP	Investigational medicine product
INR	International normalised ratio
IUD	Intrauterine device
IV	Intravenous
JAKs	Janus kinase family of tyrosine kinases
λ_z	Terminal disposition rate constant/terminal rate constant
LAM	List of Approved Medicines
LC-MS/MS	Liquid chromatography-mass spectrometry
LDL	Low density lipoprotein
LFT	Liver function test
mAMP	Methamphetamines
MCC	Microcrystalline cellulose
MCV	Mean corpuscular volume
MDMA	3,4-methylenedioxy-methamphetamine

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

MedDRA	Medical dictionary for regulatory activities
MTD	Methadone
NADH	Nicotinamide adenine dinucleotide
OPI	Opiates
PBMC	Peripheral blood mononuclear cell
PBPK	Physiologically based pharmacokinetic
PCP	Phencyclidine
PD	Pharmacodynamics
PI	Principal investigator
PICF	Participant Information Sheet and Informed Consent Form
PK	Pharmacokinetics
PN	Preferred name
pSTAT3	Phospho-signal transducer and activator of transcription 3
PT	Prothrombin time
qd	Once a day (medication)
RBC	Red blood cell
Rh(D)	Rhesus D antigen
rRNA	Ribosomal ribonucleic acid
Rux	Ruxolitinib (jakavi®)
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SRC	Safety Review Committee
SOA	Schedule of activities
SOC	System organ class
TB	Tuberculosis
TCA	Tricyclic antidepressants
TEAE	Treatment emergent adverse event
Tfh	T follicular helper (cell)
TGA	(Australian) therapeutic goods administration
THC	Tetrahydrocannabinol (cannabis)
TIBC	Total iron binding capacity
$t_{½}$	Elimination half-life
t_{lag}	Lag time
t_{max}	Time to reach maximum (peak) plasma concentration following drug administration

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ULN	Upper limit of normal
V_z/F	Apparent volume of distribution of drug during terminal phase after non-intravenous administration
WBC	White blood cell
WHO	World health organization
WOCBP	Women of childbearing potential

PROTOCOL APPROVAL/ SIGNATURES

I herewith approve the following protocol entitled "**A randomised, single blind, placebo controlled, phase 1 trial to evaluate the safety, tolerability, pharmacokinetic and pharmacodynamic activity of Ruxolitinib when co-administered with artemether-lumefantrine in healthy participants**", Version 4.0 dated 07 September 2020.

SPONSOR SIGNATURE

Signature:	_____
Name:	Dr Farouk Chughlay MD
Role:	Medical and Project Director, Medicines for Malaria Venture
Date:	____/____/2020

DOCUMENT HISTORY	
Document	**History**
Amendment 3	V4.0 07 September 2020
Amendment 2	V3.0 07 August 2020
Amendment 1	V2.0 16 July 2020
Original Protocol	V1.0 18 June 2020

PRINCIPAL INVESTIGATOR SIGNATURE

A randomised, single blind, placebo controlled, phase 1 trial to evaluate the safety, tolerability, pharmacokinetic and pharmacodynamic activity of Ruxolitinib when co-administered with artemether-lumefantrine in healthy participants

MMV_Ruxolitinib_19_01

Version: 4.0

Issue Date: 07 September 2020

Principal Investigator Agreement

I have read the above-mentioned protocol and am aware of my responsibilities as Principal Investigator for this study. As such, I agree to:

- Personally supervise the conduct of this trial;
- Conduct the trial in accordance with International Conference on Harmonization (ICH) E6 Good Clinical Practice: Consolidated Guidance (GCP), applicable regulatory requirements, and the protocol;
- Comply with the procedures for data recording and reporting as required by the regulatory authorities and the Sponsor;
- Permit monitoring, auditing, and inspection of study records as required by ICH GCP;
- Retain the essential clinical study documents as required by ICH GCP and the Sponsor.

Principal Investigator Signature:	
Principal Investigator Name:	Dr Paul Griffin
Date:	____/____/2020

2 INTRODUCTION AND RATIONALE

2.1 DISEASE BACKGROUND

Malaria is a mosquito-borne parasitic infection that is endemic in tropical and sub-tropical countries globally.[1,2] The infective organism is the protozoan *Plasmodia* species, with 5 species known to cause human disease (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*).[1,3] *P. falciparum* is the significant sub-species causing the majority of mortality and morbidity globally.[1] The life cycle of the malaria parasite consists of a liver stage and a blood stage within the human host, the latter being the cause of the clinical symptoms experienced in malaria infection.[4] Malaria is still a major global health issue, and remains a significant health problem in tropical and sub-tropical regions of the world. As such, the World Health Organisation (WHO) has declared malaria a global health priority.[1] According to the WHO 2019 Annual Malaria Report, there were an estimated 228 million cases of malaria globally in 2018 which resulted in 405 000 deaths, and malaria remains responsible for more deaths than any other parasitic disease.[1]

Substantial progress in disease control has been made through vector management, use of insecticide-treated bed nets, and improved diagnosis and drug treatment.[1] Critically, there is no licensed vaccine, and the emergence of artemisinin-resistant parasites in the Greater Mekong Subregion represents a serious threat to malaria treatment and control efforts. A major impediment to malaria eradication currently is poor understanding of host immunity against the *Plasmodium* species. There is a lack of consensus on how to generate long-lasting immunity in malaria endemic areas, and poor performances to date of malaria vaccine candidates in such endemic settings.

Studies performed more than 50 years ago showed anti-parasitic antibodies can protect against clinical malaria and control parasite growth.[2] However, these responses take years to develop in malaria endemic areas, after repeated exposure to malaria parasites.[3] Recent reports also highlight a requirement for antibodies with specific functional properties to mediate immunity.[4] The development of long-lived, anti-parasitic memory B and plasma cells that produce these antibodies requires $CD4_+$ T cell help.[5,6] Specialised $CD4_+$ T follicular helper (Tfh) cells are critical in this process by providing key cognate and soluble signals to B cells.[7,8] Interferon gamma (IFNγ) production by $Tbet_+$ $CD4_+$ T (Th1) cells is also important for generating specific antibody isotypes and activating phagocytic cells for increased antibody-mediated and compliment-mediated removal of parasitised red blood cells, and generating microbiocidal molecules such as reactive oxygen and nitrogen intermediates.[9,10]

However, these anti-parasitic responses are often absent, sub-optimal, or dysfunctional in individuals living in malaria endemic areas.[11-13] The reasons for this are still unclear, but emerging evidence indicates parasite-induced development of atypical B cells, sub-optimal Tfh cells, and Th1 cell responses characterised by autologous interleukin 10 (IL-10) production (type 1 regulatory [Tr1] cell responses), as well as the development of immunoregulatory networks, all contribute to these outcomes.[14-19]

Recently, type I interferons (IFNs) have emerged as important regulators of IL-10 production by Type 1 regulatory T (Tr1) cells. Type I IFNs comprise a large family of cytokines that signal through the common IFNα receptor (IFNAR), consisting of IFNAR1 and IFNAR2 chains. The IFNAR signals via signal transducers and activators of transcription 1 and 2 (STAT-1 and STAT-2) to mediate diverse functions during many infections.[20-22] Polymorphisms in the *IFNAR1* gene have been associated with increased

risk of severe malaria in The Gambia, while nonclinical murine studies identified a type I IFN-dependent transcriptional program associated with the pathogenesis of severe malaria experimental cerebral malaria (ECM).[23-25] Type I IFNs suppress CD4+ T cell-dependent parasite control during experimental blood stage malaria by modulating the function of dendritic cells following *P. berghei* ANKA infection, rather than acting directly on Th1 cells.[26,27] Another trial in mice infected with *P. yoelli* showed that type I IFNs directly promoted the expansion of Tr1 cells.[28]

Significantly, in malaria volunteer infection studies (VIS), type I IFNs produced by several different cell sources were found to be important regulators of developing anti-parasitic immunity. Type I IFNs not only suppressed innate immune cell function and parasitic-specific CD4+ T cell IFNγ production, but also promoted the development of parasitic-specific Tr1 cells.[29] Thus, type I IFNs are key immunomodulatory molecules in humans infected with *P. falciparum*, and targeting this pathway for clinical advantage is a promising strategy to overcome established or developing immunoregulatory networks to improve natural, drug-mediated, or vaccine-induced immunity against malaria.

Ruxolitinib (Jakavi®) blocks type 1 IFN signaling, and is therefore a candidate for investigation in combination with established anti-malarial medications such as Riamet®. If the results of this study provide an acceptable safety, tolerability, pharmacokinetic and pharmacodynamic profile for the combination of ruxolitinib and Riamet®, a future malaria Volunteer Infection Study (VIS) / Controlled Human Malaria Infection (CHMI) study is planned to evaluate the combination's immune enhancing potential in malaria.

Eradication of malaria will not be possible until continuing research in these areas can provide further information.

2.2 INVESTIGATIONAL MEDICINAL PRODUCTS

2.2.1 Ruxolitinib (Jakavi®)

2.2.1.1 Clinical Studies

Ruxolitinib (Rux) is a licensed orally administered small molecule inhibitor of Janus kinase JAK1/2, and has an established safety profile that includes Phase 1 study participants with a relatively short elimination half-life (~3 hours). Rux is registered for the treatment of intermediate or high-risk myelofibrosis in adults, and has also been safely and effectively used in children with type I interferonopathy.[30]

The Janus kinase family of tyrosine kinases (JAKs) are intimately associated with cytokine receptors such as the type I IFN receptor. The JAKs become phosphorylated after cytokines bind to these receptors, and in turn create binding sites for STATs. The STATs are then phosphorylated by JAKs, dissociate from the cytokine receptor and translocate to the cell nucleus to drive transcription of target genes.[31] Following the binding of IL-6 to the IL-6 receptor, JAK1 and JAK2 are phosphorylated leading to phosphorylation of STAT3 (pSTAT3). This response in monocytes and T cells can be used to measure the pharmacodynamic (PD) effect of Rux.[32]

Rux is used to treat neoplastic diseases, but has also been used to successfully treat children with a type I interferonopathy.[33-35] In addition, Rux was found to reduce serum type I IFN levels and IFN-inducible gene scores in dermatomyositis patients.[30] Thus, there is a growing body of evidence that Rux may be used to block type I IFN signaling in humans in a range of diseases, including malaria. [30,35]

Rux is also suitable for application in malaria endemic settings either in combination with anti-parasitic drugs (such as Riamet®) and/or malaria vaccines. The short half-life of Rux will minimise risk of adverse outcomes in populations where co-infections with tuberculosis, human immunodeficiency virus (HIV) and other pathogens is relatively common.

2.2.2 Riamet® - Artemether/lumefantrine (AL)

Riamet® is a registered, standard drug widely used for the treatment of malaria, and is a fixed combination of artemether and lumefantrine (AL). Artemether inhibits an essential calcium adenosine triphosphatase, while the exact mechanism of lumefantrine is unknown.

Any impact of AL on Rux activity will be assessed by measuring pSTAT3 inhibition in blood samples taken from participants, as previously described in phase 1 safety studies.[32]

2.2.3 Clinical Safety and Risk Assessment

Individual risks pertaining to Riamet® and Jakavi® have been well researched. The Sponsor conducted physiologically based pharmacokinetic (PBPK) modelling simulations, and concluded that the risk for a clinically relevant drug-drug interaction between the two drugs was remote especially when administering Rux 2 hours after AL.[36] Toxicity rules for IMPs and other trial interventions have been specifically developed to manage the risk of their use in this study (see Section 4.3.8 below). Risk will be minimised through comprehensive screening of participants against robust inclusion and exclusion criteria encompassing screening for infectious diseases including tuberculosis. Participants will be monitored closely throughout the study with clinical laboratory tests, physical examinations, vital signs, and ECG, including a period of confinement at the clinical unit at the time of IMP administration. Adverse Events (AEs) will be recorded throughout the study and the safety of the study will be reviewed by the Principal Investigator, MMV Medical Director and study Medical Monitor after the sentinel group (Group 1a) has completed Day 8.

2.2.3.1 Ruxolitinib

Review of healthy volunteer data emphasised that possible haematological effects including reduction in absolute neutrophil count (ANC) are monitorable, low grade, predictable in terms of timing and grade, directly linked to the effect of the drug. In particular, at the proposed regimen, a decrease in ANC is expected to be transient and due to margination rather than a direct toxic effect.

The safety, tolerability, and pharmacokinetics (PK) of Rux has been studied in healthy volunteers including males and females (including women of child-bearing potential [WOCBP]).[32] The PK, PD, safety, and tolerability of orally administered Rux has been evaluated in 2 double-blind, randomised, and placebo-controlled healthy volunteer studies conducted in the United States of America. The first study enrolled 23 participants and evaluated single ascending doses of 5 to 200 mg and the effect of food. The most frequent AEs reported were headache (13.0% overall); diarrhoea (13.0% overall), and blood sampling catheter site haemorrhage (13.0% overall). There was no dose dependency for the frequency of AEs as a whole or of any particular AE.

The second study enrolled 71 participants and evaluated multiple ascending doses, including both once- and twice-daily dosing for 10 days. Neutropenia of any severity grade was observed in 11.1% of participants at 50 mg qd, 66.6% of participants at 100 mg

qd, 12.5% of participants at 15 mg b.i.d, 33.3% of participants at 25 mg bid, 66.6% of participants at 50 mg b.i.d and 22.2% of placebo participants. These were generally transient and rapidly normalised following the last dose of study medication. One participant who received Rux 50 mg bid discontinued the study due to grade 4 neutropenia. In both these single- and multiple-dose studies, AEs were generally mild to moderate in intensity and resolved quickly. The 1 episode of grade 4 neutropenia in the participant who received 50 mg bid was considered a dose-limiting toxicity.

The safety, tolerability, and PK of Rux was further evaluated in healthy Japanese volunteers.[37] Forty study participants were randomised to receive single (10–100 mg) and multiple (10 and 25 mg every 12 h) doses of Rux or placebo. AEs occurred in 15% of the participants overall; were either grade 1 or 2 in severity and were considered related to study drug. The majority of AEs were observed in participants during treatment with repeated b.i.d doses, during which the observation period was longer, but there was no clear pattern of dose dependency. Grade 2 neutropenia occurred in five (12.5%) Rux treated participants, however decreased neutrophils were also observed in all placebo treated participants. Neutrophil counts returned to normal within 24 h of the last dose for all participants. No dose discontinuations or SAEs occurred, however dose adjustments were made in two Rux b.i.d treated participants due to decreased neutrophil counts on day 5.

In summary, orally administered Rux was considered safe and well tolerated in healthy volunteers, with 25 mg b.i.d and 100 mg qd established as the maximum tolerated doses. There were no apparent differences in the safety or PK of Rux between Japanese and non-Japanese study participants.

Rux has no known contraindications. Detailed information about Jakavi® is available in the Product Information and the Consumer Medical Information (CMI) sheet.

2.2.3.2 Riamet® - Artemether/lumefantrine (AL)

In this study, the dosing regimen for AL will be as recommended for an adult treated for acute uncomplicated malaria in the Product Information and/or CMI. Detailed information about the risks of using Riamet® is also available in the Product Information and/or CMI sheet.

Briefly, adverse effects that have been reported from using AL include: stomach pain, diarrhoea, dizziness, aching muscles/joints, sore throat/cough, nausea/vomiting, headache, difficulty sleeping, tingling, and fever/shivering. Most of these side effects have been mild and transient.

2.3 RATIONALE

2.3.1 Rationale for the Study

The World Health Organization (WHO) estimated 228 million cases of malaria and 405,000 deaths from malaria occurred worldwide in 2018. The WHO African Region carries the highest burden, with 213 million cases (93%) accounting for 94% of all malaria deaths in 2018. Nearly all of the malaria cases (99.7%) in sub-Saharan Africa were caused by the parasite *Plasmodium falciparum*.[1] Current approaches to malaria control are failing, and emerging evidence suggests that both developing and established immunoregulatory pathways impede the acquisition of natural, drug-mediated, or vaccine-induced immunity against malaria. Type I interferons have been identified as important immune regulators in malaria, and are promising targets for improving anti-parasitic immunity.

Ruxolitinib (Jakavi®) has shown immune-enhancing ability when combined with a drug targeted at the pathogen of interest. The purpose of this Phase 1 study is to investigate if co-administration of ruxolitinib and the antimalarial drug Riamet® (artemether-lumefantrine; AL) can be safely conducted in a larger proof of concept trial against *P. falciparum* malaria, and is safe, tolerable, and has any effect on the pharmacodynamics of ruxolitinib.

The study is designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) (pSTAT3) of oral doses of artemether-lumefantrine (AL; 20 mg/120 mg) and Ruxolitinib (Rux; 20 mg), or oral doses of artemether-lumefantrine (AL; 20 mg/120 mg) and placebo. Ruxolitinib or placebo will be administered 2 h after AL (i.e., AL+Rux or AL+placebo) twice daily (b.i.d) for 3 consecutive days in healthy adult volunteers.

2.3.2 Rationale for the Dose

The dose of 20 mg Rux b.i.d. to be used in the study is the standard dose for patients with platelet count >200 x 10^9/L in the Australian Product Information (PI) for Jakavi®. A 3-day 20 mg b.i.d. dosing regimen was considered appropriate for healthy volunteers based on the reported safety of a higher dose of 25 mg b.i.d. over a 10-day period in healthy volunteers in a phase 1 safety trial.[32] No effect of food was found in this trial, and consequently Rux/placebo can be administered without regard to meals.

The co-administration of AL and Rux was chosen to mimic how Rux is likely to be used in field settings with patients receiving treatment for an episode of acute uncomplicated malaria. The 3-day dosing of Rux in combination with AL was based on an immunological rationale for combining the therapies, involving AL-related killing of parasites leading to activation of type 1 IFN pathways to be disrupted by Rux.

The dose regimen for AL to be used in the trial is the standard adult dose as described in the Product Information and/or Consumer Medication Information (CMI) sheet.

3 STUDY OBJECTIVES AND ENDPOINTS

Primary objective	**Primary endpoint**
1. To assess the safety and tolerability of 3-day b.i.d dosing of AL+Rux and AL+placebo.	1. Incidence, severity, and relationship of observed and self-reported AEs up to 28 days after AL+Rux and AL+placebo administration in all participants by treatment regimen. 2. Clinical laboratory evaluations including haematology, biochemistry, and urinalysis. 3. Vital signs (blood pressure and heart rate) 4. 12-lead standard ECG: QT, QTcB and QTcF; HR, PR, QRS.
Secondary objectives	**Secondary endpoints**
1. To assess the effect of AL+Rux and AL+placebo on pSTAT3 inhibition.	1. pSTAT3 inhibition ex-vivo on whole blood cells by: • pSTAT3 levels pre- and post- AL+Rux administration intra participant and, • pSTAT3 levels per treatment regimen post AL+Rux and post AL+placebo administration
2. To characterise the PK profile of artemether and its major metabolite dihydroartemisinin [DHA], lumefantrine, and Rux (part 1 and 2).	2. PK parameters of artemether, DHA, lumefantrine, and Rux using non-compartmental methods: AUC_{last}, $AUC_{0-\infty}$, AUC_{0-8}, AUC_{60-72}, C_{max} (first and last dose), t_{max} (first and last dose), elimination half-life ($t_{½}$), t_{lag}, C_{168h} (for lumefantrine only), CL/F, Vz/F and λz in all participants.
3. To assess additional safety variables (tympanic body temperature and respiration rate)	3. Safety variables tympanic body temperature (°Celcius) and respiratory rate.

4 INVESTIGATIONAL PLAN

4.1 DESCRIPTION OF OVERALL STUDY DESIGN AND PLAN

This study is a randomised, single-blind, placebo-controlled, single centre phase 1 trial designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of combined oral doses of:

- artemether-lumefantrine (AL; 20 mg/120 mg) and Ruxolitinib (Rux; 20 mg), or
- artemether-lumefantrine (AL; 20 mg/120 mg) and placebo.

The study will be composed of 2 groups (Group 1a and Group 1b) to be enrolled sequentially. Group 1a will participate first as the sentinel safety group.

4.1.1 Group 1a (sentinel group)

Group 1a will be composed of 2 participants.

After informed consent, screening and confirmation of eligibility:

- Participants will be admitted to the clinical trial unit on Day -1.
- One participant will be randomised to receive AL+Rux and the other participant will be randomised to receive AL+placebo. Randomisation will be single blinded (treatment allocation will be concealed to the participants but not to the Investigator).
- On Day 1, participants will be administered oral doses of AL, with oral doses of Ruxolitinib or placebo administered 2 hours later twice daily (b.i.d) for 3 consecutive days (AL at t = 0, 8, 24, 36, 48 and 60 hours, Rux at t = 2, 10, 26, 38, 50 and 62 hours).
- Participants will be confined to the clinical trial unit for approximately 4 days (4 nights) from admission on Day -1 for blood sampling for PK and PD (pSTAT3) and safety monitoring.
- After discharge from the clinical trial unit no less than 78 hours post-first dose IMP, participants will be followed up via outpatient monitoring visits for safety assessments, pSTAT3 blood sampling, and PK blood sampling up to the end of study visit (EOS) on Day 29.
- The decision to proceed with Group 1b will be made by the Principal Investigator, Sponsor Medical Director, and the study Medical Monitor (at a minimum) after review of Group 1a's safety and tolerability data up to and including Day 8.

4.1.2 Group 1b

If the Principal Investigator, Sponsor Medical Director and the study Medical Monitor (at a minimum) confirm Group 1b will proceed, it will be composed of 6 participants:

- 5 participants will be randomized to AL+Rux and 1 participant will be randomized to AL+placebo. Randomisation will be single blinded (treatment allocation will be concealed to the participants but not to the Investigator).
- Study participation will otherwise be as described in Section 4.1.1.

4.2 DISCUSSION OF STUDY DESIGN INCLUDING CHOICE OF CONTROL GROUPS

The study is designed specifically to monitor safety and tolerability of the combination of AL+Rux, and to obtain PK and preliminary PD data. Eight participants will be randomised to receive AL+Rux or AL+placebo in a 3:1 ratio overall (1:1 in Group 1a,

and 5:1 in Group 1b). This is typical of first-in-human studies, and this approach is used in this study as the combination of AL+Rux has not been studied previously.

4.3 SELECTION OF STUDY POPULATION

Individuals must fulfil all of the following inclusion criteria and none of the exclusion criteria to be eligible for inclusion in this trial. No exemptions or protocol waivers will be granted.

4.3.1 Inclusion Criteria

INCLUSION CRITERIA

Demography

1. Male or female (non-pregnant, non-lactating) aged 18 to 55 years inclusive.
2. Contactable and available for the duration of the trial and for up to two weeks following the EOS visit.
3. Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18 to 32 kg/m2 (inclusive) at Screening and Day -1. BMI is an estimate of body weight adjusted for height. It is calculated by dividing the weight in kilograms by the square of the height in metres.

Health status

4. Certified as healthy by a comprehensive clinical assessment (detailed medical history, full physical examination and special investigations).
5. Vital signs measured after 5 min in the supine position:
   - Systolic blood pressure (SBP) - 90–140 mmHg,
   - Diastolic blood pressure (DBP) - 40–90 mmHg,
   - Heart rate (HR) 40–100 bpm.
6. ECG parameters for both males and females: QT ≤ 500 msec, QTcF ≤450 msec, QTcB ≤450 msec; PR interval ≤210 msec.
7. Heterosexual female participants of childbearing potential who have, or may have, male sexual partners during the course of the study should be using an insertable (implant or IUD), injectable, transdermal or combination oral contraceptive approved by the TGA combined with a barrier contraceptive from the time of informed consent until 30 days after EOS. Abstinent female participants must agree to start a double method if they start a sexual relationship with a male during the trial until 30 days after EOS. Female participants must not be planning in vitro fertilisation within the required contraception period.

   Women of non-childbearing potential who will not require contraception during the trial are defined as: surgically sterile (tubal ligation is not considered surgically sterile), post-menopausal (spontaneous amenorrhoea for ≥12 months, or spontaneous amenorrhoea for 6-12 months and follicle-stimulating hormone (FSH) ≥40 IU/mL; either should be together with the absence of oral contraceptive use for >12 months).

   Male participants who have, or may have female sexual partners during the course of the study must agree to use a double method of contraception including condom plus diaphragm, or condom plus stable insertable (implant or IUD), injectable, transdermal or combination oral contraceptive by the female partner, from the time of informed consent through to 90 days after EOS. Abstinent male participants must

INCLUSION CRITERIA

agree to start a double method if they begin a sexual relationship with a female during the trial, and through to 90 days after EOS. Male participants with female partners that are surgically sterile or post-menopausal, or male participants who have undergone sterilisation and have had testing to confirm the success of the sterilisation, may also be included and will not be required to use above described methods of contraception.

In addition, all male participants must agree to not donate sperm for at least 90 days after EOS.

Regulations

8. Completion of the written informed consent process prior to undertaking any trial-related procedure.
9. Must be willing and able to communicate and participate in the whole trial.
10. Agree to adhere to Lifestyle Considerations (see Section 4.3.3) throughout trial duration and be willing to consume 250 mL full-fat milk with each dose of AL.

4.3.2 Exclusion Criteria

If any of the following exclusion criteria apply, the individual will not be permitted to participate or remain in the study.

EXCLUSION CRITERIA

Medical history and clinical status

1. Known hypersensitivity to ruxolitinib, artesunate or any of its excipients, artemether, lumefantrine or other artemisinin derivatives, proguanil/atovaquone, primaquine, or 4-aminoquinolines.
2. Haematology, biochemistry or urinalysis results that are abnormal/outside of the laboratory normal reference ranges AND are either:
   - considered clinically significant by the Principal Investigator or delegate; OR
   - considered not clinically significant by the Principal Investigator or delegate BUT ARE ALSO outside of Sponsor-approved clinically acceptable laboratory ranges in Appendix 1.

   NOTE: Participants are not excluded if abnormal/out of laboratory normal reference range results are considered not clinically significant by the Principal Investigator or delegate AND are within the ranges specified in Appendix 1.
3. Platelets < 200 x10^9/L at Screening or prior to IMP administration is exclusionary. One re-test is permitted if original test results does not reflect the assumed medical status of the individual.
4. Participation in any other investigational product trial within 5 half-lives or the 12 weeks preceding IMP administration, whichever is longer, or in the exclusion period of a previous trial according to applicable regulations.
5. Symptomatic postural hypotension at screening and pre-first dose of IMP on Day 1 (confirmed on two consecutive readings), irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 2–3 min when changing from supine to standing position.

EXCLUSION CRITERIA

6. History or presence of diagnosed (by an allergist/immunologist) or treated (by a physician) food or known drug allergies, or any history of anaphylaxis or other severe allergic reactions including face, mouth, or throat swelling or any difficulty breathing. Participants with seasonal allergies/hay fever or allergy to animals or house dust mite that are untreated and asymptomatic at the time of dosing can be enrolled in the trial.
7. History of convulsion (including drug or vaccine-induced episodes). A medical history of a single febrile convulsion during childhood is not an exclusion criterion.
8. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immuno-deficiencies), insulin-dependent and non-insulin-dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma (excluding childhood asthma), epilepsy, or obsessive-compulsive disorder.
9. History of malignancy of any organ system (other than localised and considered cured basal cell carcinoma of the skin or in situ cervical cancer), treated or untreated, within five years of screening, regardless of whether there is no evidence of local recurrence or metastases.
10. Individuals with history of schizophrenia, bipolar disorder psychoses, disorders requiring lithium, attempted or planned suicide, or any other severe (disabling) chronic psychiatric diagnosis including generalised anxiety disorder.
11. Individuals who have been hospitalised within five years prior to enrolment for either a psychiatric illness or due to danger to self or others.
12. History of an episode of mild/moderate depression lasting more than 6 months that required pharmacological therapy and/or psychotherapy within the last 5 years; or any episode of major depression.

    The Beck Depression Inventory (BDI-II) will be used as a validated tool for the assessment of depression at screening. In addition to the conditions listed above, individuals with a score of 20 or more on the BDI-II and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. These individuals will be referred to a general practitioner or medical specialist as appropriate. Individuals with a BDI-II score of 17 to 19 may be enrolled at the discretion of an Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the individual or to the execution of the trial and interpretation of the data gathered.
13. History of recurrent headache (e.g. tension-type, cluster, or migraine) with a frequency of ≥2 episodes per month on average and severe enough to require medical therapy, during the 2 years preceding screening.
14. Acute illness within the 4 weeks prior to screening and prior to IMP administration.
15. Significant inter-current disease of any type, in particular liver, renal, cardiac, pulmonary, neurologic, rheumatologic, or autoimmune disease by history, physical examination, and/or laboratory studies including urinalysis.

EXCLUSION CRITERIA

16. Individual has a clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion (e.g. gastrectomy, cholecystectomy, diarrhoea) or known lactose/dairy intolerance.
17. Participation in any research trial involving blood sampling (more than 300 mL/unit of blood) within one month prior to IMP administration, or blood donation to Australian Red Cross Blood Service (Blood Service) or other blood bank during the 8 weeks prior to IMP administration.
18. Medical requirement for intravenous immunoglobulin or blood transfusions.
19. History or presence of alcohol abuse (alcohol consumption more than 40 g/4 units/4 standard drinks per day), or drug habituation, or any prior intravenous usage of an illicit substance.
20. Any individual who has ever smoked >1 pack of cigarettes per day for >10 years, or who currently (within 14 days prior to Screening or prior to IMP administration smokes >5 cigarettes/day.
21. Female who is breastfeeding.
22. Any vaccination within the last 28 days prior to screening or prior to IMP administration.
23. Prior to screening or IMP administration: any systemic or inhaled corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past three months. Any topical, nasal or ophthalmic corticosteroids within the past 2 weeks. Any individual currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past year.
24. Use of antidepressant medication in the past 12 months prior to screening or prior to IMP administration.
25. Use of any other medication except contraceptives (including herbal, vitamin supplement, OTC or prescription) within 14 days or five half-lives (whichever is longest) prior to IMP administration. Participants are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the trial.
26. Cardiac/QT risk:
    - Family history of sudden death or of congenital prolongation of the QT, QTcF, or QTcB interval or known congenital prolongation of the QT, QTcF, or QTcB interval or any clinical condition known to prolong the QT, QTcF, or QTcB interval.
    - History of symptomatic cardiac arrhythmias or with clinically relevant bradycardia.
    - Electrolyte disturbances, particularly hypokalaemia, hypocalcaemia, or hypomagnesaemia.
    - ECG abnormalities in the standard 12-lead ECG (at screening or prior to IMP administration) which in the opinion of an Investigator is clinically relevant or will interfere with the ECG analyses.

EXCLUSION CRITERIA

General conditions

27. Any individual who, in the judgement of an Investigator, is likely to be non-compliant during the trial, or is unable to cooperate because of a language problem or poor mental development.
28. Any individual for whom study participation would pose an additional safety risk as assessed by the Principal Investigator.
29. Any individual who is an Investigator, research assistant, pharmacist, trial coordinator, or other staff thereof, directly involved in conducting the trial.
30. Any individual without good peripheral venous access.

Biological status

31. Positive result on any of the following tests: hepatitis B surface antigen (HBs Ag), anti-hepatitis B core antibodies (anti-HBc Ab), anti-hepatitis C virus (anti-HCV) antibodies, anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti-HIV2 Ab).
32. Recent herpes zoster infection (within the previous 6 months) as determined by clinical history.
33. Positive result for *M. tuberculosis* infection by QuantiFERON-TB Gold assay.
34. Positive urine drug test for any drug listed in Section 7.4.5. Any individual testing positive for acetaminophen (paracetamol) at screening and/or pre-dose may still be eligible for trial participation at the discretion of the Principal Investigator or delegate.
35. Positive alcohol breath test.

4.3.3 Lifestyle Considerations

While participating in this trial, participants are asked to:

- Refrain from alcohol consumption of more than 40 g/4 units/4 standard drinks per day, and/or drug habituation until the conclusion of the trial, and also:
- Abstain from any alcohol use for the duration of the clinical unit confinement.
- Refrain from tobacco use of more than five cigarettes or equivalent per day until the conclusion of the trial, and also:
- Abstain from any tobacco use for the duration of clinical trial unit confinement.
- Refrain from excessive consumption of beverages or food containing xanthine bases including Red Bull, chocolate, coffee, black tea etc. (more than 400 mg caffeine per day, equivalent to more than 4 cups of coffee per day), and also:
- Abstain from any consumption of beverages or food containing xanthine bases for the duration of clinical trial unit confinement.
- Refrain from consumption of Seville oranges, and grapefruit or grapefruit juice from 7 days prior to IMP dose until the EOS.
- Refrain from consuming poppy seeds one week prior to Screening and for the duration of the study.
- Abstain from strenuous exercise for 24 h before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies.

Clinical study staff will routinely remind participants of these requirements.

4.3.4 Screen Failures

Healthy candidate volunteers who do not fulfil all the inclusion criteria, and/or fulfil any of the exclusion criteria should not be enrolled into the trial without exception. In case of doubt, the Principal Investigator or delegate is to confer with the Medical Monitor for agreement. Waivers for inclusion of volunteers who are not meeting all eligibility criteria will not be granted. If a participant does not meet all selection criteria (is a screen failure) but at some point in the future is expected to meet the eligibility criteria, the participant may be rescreened on 1 occasion only. Participants who are rescreened will undergo the informed consent process, be assigned a new participant number, and then restart a new screening phase.

Unscheduled visits may be planned to assess, confirm, and follow-up on out-of-range clinical laboratory test, vital sign, or ECG values that determine a participant's eligibility. Re-tests are limited to one per parameter (except for urine drug screen), and only if the Principal Investigator considers that the existing test result may not correctly reflect the participant's medical status. The result of the retest must be considered for participant eligibility and must be available prior to Day 1. Reasons for re-testing must be clearly documented. A positive urine drug screen is exclusionary (except if positive for paracetamol only at the discretion of the Principal Investigator or delegate) and no re-testing is permitted. Findings made during unscheduled visits will be documented.

Minimum information to be collected on screen failures include demography, details of the screen failure, eligibility criteria and any SAE, and will NOT be entered into the eCRF.

Participants who fail screening due to an underlying medical condition (acute self-limiting viral infections do not apply) previously unknown to them will be reimbursed for their

time, and provided with the appropriate referrals for guidance and counselling for their condition.

4.3.5 Withdrawal of Participants

Participants are free to withdraw from participation in the trial at any time upon request.

The Sponsor and/or Principal Investigator or delegate may discontinue or withdraw a participant from the trial for the following reasons:

- Pregnancy (participant must be withdrawn from the study)
- Significant trial intervention non-compliance or protocol deviation (to be decided on a case-by-case basis)
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the trial would not be in the best interest of the participant (see also Section 4.3.8 regarding toxicity rules)
- If the participant meets an exclusion criterion (either newly developed or was not previously recognised) that precludes further trial participation
- Participation in any investigational product trial whilst enrolled in this trial (duration up to 12 months)
- If participant was unblinded (on a case by case basis)

The reason for participant discontinuation or withdrawal from the trial will be recorded on the electronic case report form (eCRF) and withdrawn participants will be asked to complete an early termination visit (ET). ET procedures are the same as for EOS (see Section 6.1.5).

If a participant withdraws or is withdrawn from the trial, the Principal Investigator or delegate will inform the Sponsor immediately. If there is a medical reason for withdrawal, the participant will remain under the supervision of the Principal Investigator or delegate until satisfactory health has returned, or medical/clinical care has been transferred to the participant's general practitioner or appropriate hospital consultant.

The Principal Investigator or delegate will make every effort to determine the primary reason for a participant's withdrawal from the trial and record this information in the eCRF. If the participant is withdrawn from the trial procedures or follow-up for any reason, with the participant's permission, medical care will be provided for any SAEs that occurred during participation in the trial until the symptoms of any SAEs are resolved and the participant's condition becomes stable.

4.3.5.1 Replacement of Withdrawn/Discontinued Participants

- Participants who sign the informed consent form and are randomised but do not receive IMP may be replaced. Replacement volunteers are permitted as back-up.
- Participants who sign the informed consent form, and are randomised and receive at least one dose of IMP, and subsequently withdraw, or are withdrawn or discontinued from the trial, may only be replaced after mutual agreement between the Sponsor and the Principal Investigator or delegate. The decision regarding the replacement of participants will be documented.

4.3.6 Lost to Follow-Up

For participants who are lost to follow-up, the Principal Investigator or delegate will demonstrate due diligence by documenting all steps taken to contact the participant (e.g., dates of phone contacts, registered letter, home visit, etc.) in the source documents.

It will be explained to the participants during the consenting process and throughout the duration of the study that they must be readily contactable to the study and clinical trial unit team.

4.3.7 Study Discontinuation

The Sponsor, Principal Investigator, SRC, approving HREC, and regulatory authorities independently reserve the right to discontinue the trial at any time for safety or other reasons. This will be done in consultation with the Sponsor where practical. In the event of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (the Sponsor will notify regulatory authorities as appropriate). After a decision to prematurely terminate the trial or to suspend the trial, the Sponsor and the Principal Investigator will ensure that adequate consideration is given to protecting the participants' interest and safety. The Principal Investigator or delegate must review all participants as soon as practicable and complete all required records.

4.3.8 Trial Intervention/Treatment Discontinuation – Toxicity Rules

In addition to the classic assessment of SAEs (see Section 7.7) and consideration of adverse events of special interest (AESIs – see Section 7.6.5), specific toxicity rules apply for this trial and must be applied when early termination or suspension is considered.

Toxicity rules

When applying toxicity rules, AEs will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) grading system (Version 5.0 Published: November 27, 2017) for standardised recording and reporting. For the purpose of this protocol, "toxicity" refers to the occurrence of any grade 3 or higher grade AE that is related to an IMP (AL, Rux, and Placebo).

General toxicity rules

General toxicity rules refer to all AEs, except for AEs relating to the liver and haematology. General toxicity rules also apply to neutropenia, as it is an identified liability of Rux.

Trial-specific toxicity rules applying to AEs relating to the liver and haematology have also been created for this trial, as the CTCAE grading system may not give clear enough guidance.

4.3.8.1 Individual Toxicity Rules

IMP administration for an individual participant will be stopped if:

- the participant experiences any CTCAE grade 3 (or higher) AE, or an SAE irrespective of severity/CTCAE grade deemed related to the IMP.
- the participant experiences any CTCAE grade 1 or 2 AE **that raises a safety concern** and is deemed related to IMP.

4.3.8.2 Group Toxicity Rules

IMP administration for a group will be stopped if the following occurs in more than one participant:

- any CTCAE grade 3 (or higher) AE, or an SAE deemed related to the IMP irrespective of severity/CTCAE grade.
- any CTCAE grade 1 or 2 AE **that raises a safety concern** and is deemed related to IMP.

The resumption of the trial can only occur after an amendment to the protocol has been approved by the HREC.

4.3.8.3 Special Individual Toxicity Rules

Dosing of IMP in a participant will be suspended if they experience any of the following:

- LFT/Transaminase elevation:
  - ALT or AST value >3× ULN together with:
    - Total bilirubin increase >2× ULN OR
    - INR >1.5 (see Sections 7.4.1.2 and 7.4.1.3).
  - ALT or AST value >8× ULN.
  - ALT or AST value >3× ULN, and with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia > ULN.
- Haematology:
  - Haemoglobin decrease from baseline (Day -1) of 4g/dL (40g/L) or lower.
  - Clinically significant decrease from Day -1 in neutrophil count (grade 3 [i.e. Absolute count < 1 x 10^9/L (1000/mm^3)] or higher).
  - Platelet count drop of >50% from Day -1, or absolute value < 125 x10^9/L. No dose reduction of IMP permitted.

4.3.8.4 Special Group Toxicity Rules

- If one participant fulfils the special individual toxicity rules, and this could be reasonably attributed to IMP, dosing for all participants in the trial will be temporarily suspended and a meeting arranged with the Principal Investigator, Sponsor Medical Director and study Medical Monitor (at a minimum) to decide if dosing will continue for the remaining participants.
- If two or more participants fulfil any of the other special individual toxicity rules, then dosing will be suspended in all participants and can only continue if a meeting with the Principal Investigator, Sponsor Medical Director and study Medical Monitor (at a minimum) is arranged (and a decision made that dosing can continue), and a substantial amendment is made to the protocol which has been reviewed/approved by the HREC.

4.3.9 Emergency Unblinding of Study Participants

- Emergency unblinding is not required for this study, as the Principal Investigator or delegate and Medical Monitor will be aware of each participant's treatment. The study is single-blinded, so that only the participant is unaware of their treatment.

5 TRIAL INTERVENTIONS/TREATMENTS

5.1 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

Participants will be admitted to the clinical unit for oral dosing of the combination of AL and Rux or AL and placebo as applicable. AL will be administered first, and Rux or placebo (where applicable) administered 2 hours later, b.i.d. for 3 consecutive days.

NOTE:

- See Section 6.2 for permitted time windows when IMP administration coincides with PK or PD sampling. Scheduled times of IMP administration should be strictly adhered to and actual times will be documented and entered into the eCRF.
- Participants will be confined to the clinical unit for all doses for a period of at least 78 hours post-first administration of AL.
- Participants will be administered all IMP doses under direct observation of clinical unit staff at the scheduled timepoints.
- Participants will be blindfolded for administration of Rux/placebo, and suitable arrangements made to prevent other participants from witnessing each other's Rux/placebo dosing.
- For the first dose of IMP, and then for all doses that coincide with other assessments: participants will remain seated for 10 minutes, then stay in a semi-recumbent position for the next hour (except during assessments that require lying positions).

5.1.1 Artemether-lumefantrine (Riamet®)

- A course of Riamet® will be administered to all participants.
- Each AL tablet contains 20 mg artemether and 120 mg lumefantrine.
- The standard adult dosing regimen according to the Product Information and/or CMI will be used: 4 tablets administered orally b.i.d over 3 consecutive days (6 doses at t = 0, 8, 24, 36, 48 and 60 hours, total course of 24 tablets).
- Each AL dose will be taken with 250 mL full-fat milk.
- The first AL tablet will be administered under direct observation at time (t) = 0 on Day 1 to **all** Part 1 participants and thereafter under direct observation at t = 8, 24, 36, 48 and 60 hours.
- No food permitted within 30 minutes prior to AL dosing, or between AL and Rux dosing. Meals are not required to be standardized. Times of meals and completion times will be documented.

5.1.2 Ruxolitinib (Jakavi®)

- A course of Rux (Jakavi®) in the form of 20 mg tablets will be administered only to the 6 participants randomised to the active group: 1 participant in Group 1a and 5 participants in Group 1b.
- Participants must be blindfolded for Rux/placebo administration; other participants will be prevented from witnessing dosing.
- The equivalent of the standard adult dosing regimen according to the PI and CMI will be used: 1 tablet (1× 20 mg) administered orally with 250 mL water b.i.d over 3 consecutive days (6 doses, total course of 6 tablets) and will be taken 2 hours after administration of AL. Participants will be instructed to swallow the IMP whole without biting or chewing.
- The first 20 mg dose of Rux will be administered under direct observation at t = 2 hours, and thereafter under direct observation at t = 10, 26, 38, 50 and 62 hours. All doses must be two hours after the corresponding dose of AL.

- No food permitted between AL and Rux, and for at least 1 hour post-Rux. Meals are not required to be standardized. Times of meals and completion times will be documented.

5.1.3 Ruxolitinib Placebo

- A placebo in capsule form will be administered only to the 2 participants randomised to placebo: 1 participant in Group 1a and 1 participant in Group 1b.
- Participants must be blindfolded for Rux/placebo administration; other participants will be prevented from witnessing dosing.
- A course of placebo treatment will be the same dosing regimen and food restrictions as described for Rux in Section 5.1.2. Participants will be instructed to swallow the IMP whole without biting or chewing.
- Refer to the Pharmacy Manual for further information.

5.2 SELECTION OF DOSE IN THE STUDY

See Section 2.3.2.

5.3 SELECTION AND TIMING OF DOSE FOR EACH PARTICIPANT

See Section 5.1.

5.4 DOSE INTERRUPTIONS AND REDUCTIONS

IMP administration may be interrupted/suspended under the conditions outlined above in Section 4.3.8. Otherwise, dose reductions are not permitted for AL or Rux during treatment.

5.5 SUPPLY, PACKAGING AND LABELLING OF STUDY TREATMENTS

The IMPs will be manufactured and packaged according to Good Manufacturing Practice (GMP) and all local regulations, and will be labelled for clinical trial use in accordance with Australian requirements and Annex 13 GMP. Rux (Jakavi®) tablets, placebo capsules and AL (Riamet®) tablets will be supplied to the clinical unit with an acknowledgement of receipt form.

Investigational Medicinal Products

Riamet® (artemether-lumefantrine [AL])

Riamet® tablets (each containing 20 mg artemether/120 mg lumefantrine) will be supplied as yellow, round, flat tablets marked with N/C and a score line on one side and CG on the other side. Each carton contains 24 tablets.

Jakavi® (ruxolitinib [Rux])

Jakavi® tablets (containing 20 mg ruxolitinib) will be supplied as elongated, curved white to almost-white tablets with ‘L20’ on one side and ‘NVR’ on the other. Each pack contains 56 tablets.

Ruxolitinib (Rux) placebo

Placebo capsules will be prepared by PCI Melbourne and filled with microcrystalline cellulose (MCC) only.

PCI Melbourne will fill all placebo capsules into one bulk container. A single panel label will be applied. The Principal Investigator, pharmacist or delegate will dispense the placebo capsules.

5.6 STORAGE OF STUDY TREATMENTS

Prior to dispensing, IMPs must be stored in a secure and locked storage area with limited access, and under monitored, temperature controlled conditions as appropriate.

The Pharmacist or Designee will be responsible for the correct storage and handling of IMPs. Deviations from the storage requirements, including corrective action, must be documented.

All storage requirements are detailed in the product information/CMI.

5.7 ACCOUNTABILITY, RECONCILIATION AND RETURN OF THE STUDY TREATMENTS

The Principal Investigator or delegate will only dispense the IMPs to eligible participants enrolled in this study and must maintain complete and current dispensing and inventory records. All dispensing episodes for IMPs will be documented on the site's Dispensing Logs.

The logs must contain the following information:

- Date of receipt.
- Number of capsules/ tablets / containers received.
- Batch number(s).
- The identification of the participant to whom the capsules / tablets was dispensed.
- The date(s), time and quantity dispensed to the participant.
- The cumulative total of IMPs at site.
- Capsules /tablets / containers damaged, destroyed, or returned.

Supplies of IMPs will be shipped to the clinical unit prior to study start. The Monitor will complete drug accountability during routine monitoring visits.

At the completion or termination of the study, final accountability and reconciliation will be completed and any discrepancies must be investigated and their resolution documented. All full, partially full, and empty containers of IMPs must be returned to the Pharmacy, Manufacturer or Sponsor for destruction, and the appropriate form sent to the Sponsor.

Please refer to the Pharmacy Manual for further details on the storage, handling, and dispensing of IMPs.

5.8 PROHIBITED CONCOMITANT THERAPY

Permitted and prohibited prior and concomitant prescribed medications, over-the-counter medications and supplements are outlined in the inclusion and exclusion criteria in Section 4.3.1. Before use of any non-study medication during the study, the participant should discuss with the Principal Investigator or delegate.

The following medications are prohibited during the study:

- Psychiatric medications including antipsychotics (typical and atypical), antidepressants, mood stabilisers, and anticonvulsants.
- Illicit drugs (amphetamine, cocaine, cannabis etc.).
- Systemic or inhaled corticosteroids.
- Anti-inflammatory drugs (excluding ibuprofen as indicated below)
- Immunomodulators

- Immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression.
- Anticoagulants.
- Any vaccination (live or attenuated).
- In consideration of specific ruxolitinib and Riamet® interactions:
    - CYP3A4 inhibitors or inducers, such as, but not limited to: boceprevir, clarithromycin, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, saquinavir, telaprevir, telithromycin, voriconazole, avasimibe, carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St.John's wort (Hypericum perforatum), ciprofloxacin, erythromycin, amprenavir, atazanavir, diltiazem, cimetidine, oxcarbazepine, barbiturates, efavirenz, nevirapine, pioglitazone, troglitazone, corticosteroids by the systemic route, grapefruit or grapefruit-containing products;
    - Dual inhibitors of CYP2C9 and CYP3A4 enzymes (e.g. fluconazole).
- In consideration of specific Riamet® interactions:
    - Interaction with drugs that are known to prolong the QT, QTcF and/or QTcB interval: antiarrhythmics of classes IA and III, neuroleptics and antidepressant agents, certain antibiotics including some agents of the following classes: macrolides, fluoroquinolones, imidazole, and triazole antifungal agents, certain non-sedating antihistamines (terfenadine, astemizole), cisapride, flecainide;
    - Interaction with drugs metabolized by CYP2D6 since lumefantrine was found to inhibit CYP2D6 in vitro: (e.g. neuroleptics, metoprolol, and tricyclic antidepressants such as imipramine, amitriptyline, clomipramine) is contraindicated;
    - Antimalarials such as mefloquine and quinine;
    - Drugs that have a mixed inhibitory/induction effect on CYP3A4, especially anti-retroviral drugs such as HIV protease inhibitors (Lopinavir/ ritonavir) and non-nucleoside reverse transcriptase inhibitors (Efavirenz and Nevirapine).

If a medication or treatment is administered that is in breach of these restrictions, the study Medical Monitor must be promptly notified in order to assess the participant's eligibility for continued study participation.

5.9 PERMITTED CONCOMITANT MEDICATION

The participant should discuss use of any medication during the study with the Principal Investigator or delegate.

- The Principal Investigator or delegate may permit the use of ibuprofen up to 1.8 g/day or paracetamol up to 1.2 g/day for the treatment of headache or other pain if required. Ibuprofen is the preferred treatment for headache or pain.

5.10 TRIAL INTERVENTION/TREATMENT COMPLIANCE

Participants will be confined to the clinical unit to be administered all doses of IMP under direct observation, and compliance will be confirmed and documented by clinical unit staff.

5.11 MEASURES TO MINIMIZE BIAS

5.11.1 Randomisation Procedures

Randomisation procedures will be as described in the Randomisation Plan and Randomisation Procedure.

A randomisation number will be allocated to each participant as per the randomisation schedule before the administration of IMPs (AL+Rux or AL+placebo).

Overall, randomisation will be in a ration of 3:1 (active:placebo):

- Participants in Group 1a will be randomised to a treatment allocation (AL+Rux or AL+placebo) in a ratio of 1:1. The randomisation will be single blinded (treatment known to Investigator but not participant).
- Participants in Group 1b will be randomised to a treatment allocation (AL+Rux or AL+placebo) in a ratio of 5:1. The randomisation will be single blinded (treatment known to Investigator but not participant).

5.11.2 Blinding and Unblinding

The study will be single-blinded, with only the participant unaware of their treatment allocation. The single-blind design is aimed at optimising participant safety by allowing the Principal Investigator or delegate to rapidly detect and manage potential safety concerns that may disproportionately arise in the combination AL+Rux arm.

See Section 4.3.9. To maintain the blind for the participant, all source documentation will be prepared as blinded, participants will be blind-folded for administration of Rux/placebo and arrangements made to ensure participants cannot witness each other's Rux/placebo dosing. A participant's treatment allocation should not be unblinded to them except if required in a medical emergency.

6 STUDY PROCEDURES AND ASSESSMENTS

6.1 STUDY CONDUCT SCHEDULE

The study will be conducted according to the Schedule of Activities (see Section 1.3: Table 1, Table 2 and Table 3) and as outlined in this section. See Section 6.2 for permitted time windows for pharmacodynamic and PK sampling and Sections 6.3 and 6.4 for details on PD and PK sampling respectively. See Section 7 for details of safety assessments.

6.1.1 Screening Days -28 to Day -1

See Schedule of Activities Table 1. The potential participant will be asked to attend a screening visit having fasted for at least 8 hours for screening biochemistry testing. The initial Screening visit will be prior to and separate from the safety/eligibility visit on Day -1.

Procedures at Screening:

- Obtain signed informed consent prior to any study-related procedures or assessments.
- Medical history
- Current and prior medications, including details of recreational drug, alcohol and tobacco use
- Demographics
- Body measurements (weight and height)

- Alcohol breath test
- Collect urine for:
    - Urine drug screen
    - Urinalysis
- Full physical examination
- Beck Depression Inventory (BDI-II)
- Vital signs (supine and standing blood pressure and heart rate)
- Tympanic body temperature and respiratory rate
- 12-lead ECG
- Collect blood samples for:
    - Haematology
    - Biochemistry (including lipids for Screening visit only)
    - Serology
    - Coagulation
    - QuantiFERON-TB Gold assay (tuberculosis testing)
    - Serum β-hCG pregnancy test for all females.
    - Follicle stimulating hormone (FSH) for postmenopausal females.
- Verify eligibility.

6.1.1.1 Review of screening/eligibility laboratory testing

- If results are normal/within the laboratory normal reference ranges, the participant may be included (if all inclusion criteria fulfilled and no other exclusion criteria are met).
- If results are abnormal or outside of the laboratory normal reference ranges, then clinical significance must be assigned by the Principal Investigator or delegate:
    - If clinically significant, the participant is excluded as per Exclusion criterion #2.
    - If not clinically significant, the parameter(s) must be checked against Appendix 1: Clinically Acceptable Ranges for Clinically Important Study Inclusion/EXCLUSION Laboratory Tests:
        - If outside the ranges in Appendix 1, the participant is excluded as per Exclusion criterion #2.
        - If within the ranges in Appendix 1, the participant may be included (if all inclusion criteria fulfilled and no other exclusion criteria are met).
- Retesting of any screening parameters (except urine drug screen) is permitted once only if the original result did not reflect the expected medical condition of the participant.
    - If a participant's urine drug screen returns a positive result (except for paracetamol as per Exclusion criterion #34), no retest is permitted and the participant is excluded.
- The results of any retests must be considered for participant eligibility and must be available prior to first-dose of IMP. Findings made during unscheduled visits or testing should be reported in the source document and eCRF.

6.1.2 Eligibility/Safety/Admission Day -1

See Schedule of Activities Table 1. Participants will be requested to attend the clinical unit to confirm eligibility and to be admitted to the clinical unit on Day -1 after fasting for

at least 8 hours. Reserve participants will also attend. This will be in addition to the Screening visit and should not be combined.

Procedures on Day -1 for safety, to confirm eligibility and for admission:

- Review medical history and prior/concomitant medications since screening visit. Record any medically untoward events between Screening and this visit as medical history.
- Conduct alcohol breath test.
- Collect urine for:
    - Urinalysis. Results must be available and reviewed as described in Section 6.1.1.1 prior to IMP administration on Day 1.
    - Urine drug screen
    - Urine β-hCG pregnancy test for WOCBP
- Participants may be cannulated with indwelling intravenous (IV) cannula either on Day -1 or pre-dose on Day 1.
- Collect blood samples for:
    - Haematology
    - Biochemistry.
    - NOTE: Results must be available and reviewed prior to IMP administration on Day 1 as described in Section 6.1.1.1.
- Abbreviated physical examiniation including weight (kg).
- Vital signs (supine and standing blood pressure and heart rate)
- Tympanic body temperature and respiratory rate
- Randomisation may be conducted on Day -1 or Day 1 pre-first dose.

6.1.3 Days 1-4 Treatment with IMP and Confinement

See Schedule of Activities Table 2. Participants will fast overnight at the clinical unit for at least 8 hours. Eligibility must be confirmed prior to IMP dosing.

NOTE: when several procedures are scheduled to take place at the same timepoint, the following order is recommended where applicable: urine collection, ECG, vital signs, blood sampling, IMP administration.

6.1.3.1 Day 1 Pre-first dose IMP

Procedures prior to first dose of IMP (AL at t = 0):

- Confirm eligibility (including review of all Day -1 safety laboratory testing and available Day 1 pre-dose testing)
- Medical history (medically untoward events prior to first dose IMP will be recorded as medical history and must satisfy relevant inclusion/exclusion criteria)
- Concomitant/prior medications
- Vital signs (supine and standing blood pressure and heart rate) within 60 minutes prior to IMP dosing. Pre-dose results must satisfy relevant inclusion/exclusion criteria.
- Tympanic body temperature and respiratory rate within 60 minutes prior to IMP dosing. Pre-dose results must satisfy relevant inclusion/exclusion criteria.
- 12-lead standard ECG. Pre-dose results must satisfy relevant inclusion/exclusion criteria.
- Cannulate with indwelling intravenous (IV) cannula (if not done on Day -1)

  - Collect blood for pre-dose PK sample within 60 minutes prior to IMP dosing (see Section 6.4).
  - Collect blood for pre-dose pSTAT3 sample within 60 minutes prior to IMP dosing
- Randomisation (if not done on Day -1).

6.1.3.2 IMP dosing

Commencing Day 1, AL+Rux or AL+placebo will be administered under observation according to randomisation schedule. See Section 5.1 for details including timing and food/liquids/positioning requirements.

Rux/placebo must be administered 2 hours after the corresponding AL dose.

6.1.3.3 Confinement

Participants will be confined for at least 78 hours post-first dose IMP for safety and tolerability monitoring.

Confinement Procedures:

- Record AEs
- Record concomitant medications
- Collect blood samples for:
  - PK – for timepoints coinciding with IMP administration, PK sampling must occur prior to IMP administration - see Section 6.4 below.
  - pSTAT3 assay. For timepoints, see Section 6.3.1 below.
  - Safety biochemistry and haematology blood sampling within 60 minutes prior to 24, 48 and 72 hours after first dose of IMP (must be before breakfast). Participants will have fasted for at least 8 hours prior to blood sampling.
- Collect urine for urinalysis within 60 minutes prior to 24, 48 and 72 hours after first dose of IMP.
- Vital signs (supine blood pressure and heart rate) at 4 and 8 hours after first dose of IMP and then every 8 hours (within 15 minutes prior to each timepoint).
- Tympanic body temperature and respiratory rate (supine) at 4 hours post-first dose of IMP and then 24, 48 and 72 hours post-first dose IMP (within 15 minutes prior to each timepoint).
- Standard single 12-lead ECG at 24, 48 and 72 hours after first dose of IMP (within 15 minutes prior to each timepoint).
- Symptom-directed physical examination at any time if clinically indicated at the discretion of the Principal Investigator or delegate.

Prior to discharge 78 hours post-first dose IMP:

- Collect blood sample for PK at t = 78 hours timepoint as per Section 6.4
- Record AEs and concomitant medications
- Clinical unit staff will check with the Principal Investigator or delegate to clarify if symptom-directed physical examination required prior to discharge.

6.1.4 Discharge to EOS Outpatient Visits

After discharge from the clinical unit 78 hours post-first dose IMP, participants will attend outpatient visits at the clinical unit scheduled for safety assessments and to ensure blood sampling for PK and pSTAT3 assay will be taken at the required timepoints (see Schedule of Activities Table 3).

Outpatient Visit procedures:

Collect blood for:

- PK samples– see Section 6.4.
- pSTAT3 samples – see Section 6.3.1.

At all visits (time windows for assessments are as permitted for PK sampling in Section 6.2):

- Record AEs
- Record concomitant medications
- Vital signs (supine blood pressure and heart rate only)
- Tympanic body temperature and respiratory rate
- Symptom-directed physical examination if clinically indicated

On Day 8 and Day 15 only (participants should attend having fasted for at least 8 hours):

- Collect blood for:
    - Haematology
    - Biochemistry
- Collect urine for urinalysis
- Standard single 12-lead ECG

6.1.5 End of Study Visit (EOS) Day 29 or Early Termination Visit

Participants will be requested to attend the EOS (or early termination visit if applicable) having fasted for at least 8 hours. EOS assessments must be conducted within the permitted time window for the applicable PK sample (see Section 6.2).

EOS (or Early Termination) procedures:

- Record AEs
- Record concomitant medications
- Full physical examination
- Vital signs (supine blood pressure and heart rate only)
- Tympanic body temperature and respiratory rate
- 12-lead ECG
- Collect blood samples for:
    - PK (if applicable; see Section 6.4)
    - Haematology
    - Biochemistry
    - serum β-hCG pregnancy test for WOCBP
- Collect urine for urinalysis

6.2 PERMITTED TIME WINDOWS FOR SAMPLING

The following time windows will be permitted for blood sampling for PK and pSTAT3 assays:

Time point	**Tolerance window**
Pharmacokinetic/Pharmacodynamic (pSTAT3)	
In confinement	
Pre-first dose IMP	- 60 min to 0 h
1-4 hours inclusive after initial AL administration (T 2 h must be within 2 min pre Rux/placebo dosing)	± 2 min
5-12 hours inclusive after initial AL administration (T 8h must be within 5 min pre AL dosing)	± 5 min
13-78 hours inclusive after initial AL administration (T 24, 36, 48 & 60 h must be within 15 min pre AL dosing) (T 62 h must be within 15 min pre Rux/placebo dosing)	± 15 min
Outpatient Visits	
79-168 hours inclusive (pSTAT3 assay T 168 h)	± 120 min
169-EOS	± 24 h

6.3 PHARMACODYNAMIC ASSESSMENTS

6.3.1 pSTAT3

Blood samples will be collected as described in the Laboratory Manual to determine the levels of pSTAT3 using a validated whole blood enzyme-linked immunosorbent assay (ELISA). See Section 6.2 for permitted time windows.

For timepoints coinciding with IMP administration, blood sampling **MUST** occur **prior** to IMP administration. Exact times of pSTAT3 sampling will be documented and entered into the eCRF.

Blood samples for pSTAT3 assay will be collected at the following times:

- Day 1 prior to commencement of IMP dosing (pre-dose).
- t = 1, 2, 3, 4, 5, 6, 8, 12 hours post commencement of IMP dosing.
- Day 8 at 168 hours post commencement of IMP dosing.

6.4 PHARMACOKINETICS

Plasma concentrations of Rux, AL and artemether metabolite dihydroartemisinin (DHA) will be quantified using a validated method by liquid chromatography-mass spectrometry (LC MS/MS).

Blood sample handling for PK analysis is described in the Laboratory Manual. Blood samples will be collected either by direct venepuncture or via an indwelling cannula inserted in a forearm vein. See Section 6.2 for permitted time windows. Exact times of PK sampling will be documented and entered into the eCRF.

Blood samples for PK will be collected at the following times:

- Day 1 prior to commencement of IMP dosing (pre-dose).
- t = 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 62, 64, 72 and 78 hours post commencement of IMP dosing.
    - For timepoints coinciding with IMP administration, blood sampling **MUST** occur **prior** to IMP administration.
- Days 8, 11, 15, 21, 24 and 27 at t = 168, 240, 336, 480, 552 and 624 hours post commencement of IMP dosing (±120 min for t = 168 hours, ± 24 hours for later timepoints).
- Day 29 at t = 672 hours (± 24 hours).
- Unscheduled PK sample will be taken if ALT or AST value >2x ULN as described in Section 7.4.1.2.

7 SAFETY MONITORING

- Safety will be assessed during the study through physical examination, urinalysis, vital signs, ECG, clinical laboratory tests, and continuous AE reporting, as outlined in the Schedules of Activities (Section 1.3).
- Protocol waivers or exemptions are not permitted.
- Clinical significance of any out-of-range or abnormal result or finding must be recorded by the Principal Investigator or delegate. After first-dose of IMP, any clinically significant observation for safety monitoring (physical examination, vital signs, ECG, clinical laboratory tests) as determined by the Principal Investigator or delegate must be documented as an AE (see Section 7.6.1).
- See Section 6.1.1.1 for review of results for Screening/eligibility purposes.
- All assessments may be performed at extra unscheduled times if deemed necessary by the Principal Investigator or delegate to ensure participant safety. The Medical Monitor may be contacted if required.
- NOTE: when several procedures are scheduled to take place at the same timepoint, the following order is recommended where applicable: urine collection, ECG, vital signs, blood sampling, IMP administration.

7.1 PHYSICAL EXAMINATION

7.1.1 Full Physical Examination

A full physical examination will include assessments of general appearance; head, neck (including thyroid), ears, nose and throat; heart/circulation; chest; lungs; abdomen; skin, and a neurological examination.

A full physical examination will be performed at Screening and at the EOS.

7.1.2 Abbreviated Physical Examination

An abbreviated physical examination will include heart/circulation, chest, lungs, skin, and abdomen, and will be performed on Day -1 on admission to the clinical unit. This will include re-checking the weight (kg) of participants.

7.1.3 Symptom Directed Physical Examination

A symptom-directed physical examination may be performed at any time during the study if clinically indicated, and body systems to be reviewed will be only as clinically indicated. Clinical unit study staff must ensure a study doctor is contacted if there are symptoms that require physical examination, including prior to discharge from the clinical unit after confinement.

7.2 VITAL SIGNS AND OTHER BODY MEASUREMENTS

The normal ranges for vital signs, body temperature and respiratory rate once on trial are:

Parameter	Range
Systolic blood pressure	90-140 mmHg
Diastolic blood pressure	50-90 mmHg
Heart rate	50-100 bpm
Tympanic body temperature	35.0-37.5°C
Respiratory rate	10-25 breaths/min

If results are out of these ranges, the Principal Investigator or delegate must assess, assign and document clinical significance (see Section 7.6.1).

7.2.1 Vital signs

Vital signs include systolic and diastolic blood pressure and heart rate. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) will be measured in millimeters of mercury (mmHg) and heart rate in beats per minute.

Blood pressure and heart rate will be measured after the participant has rested in the supine position for at least 5 minutes and then in the standing position within 2–3 minutes when changing from the supine to standing position at Screening, Day -1 and Day 1 within 60 minutes of the first dose of IMP. All pre-first dose results including Day 1 pre-first dose results must satisfy relevant inclusion/exclusion criteria.

At all other timepoints, vital signs will be measured after the participant has rested in a supine position for at least 5 minutes: during confinement at 4 and 8 hours post-first dose of IMP and then every 8 hours (within 15 minutes prior to each timepoint), and at every outpatient visit after discharge from the clinical unit and EOS (time windows as per PK sampling in Section 6.2).

7.2.2 Other Body Measurements – Respiratory rate and Temperature

Respiratory rate (breaths per minute) and tympanic body temperature (°Celcius) will be measured at Screening, Day -1, Day 1 pre-dose (within 60 minutes), at 4, 24, 48 and 72 hours post-first dose of IMP (within 15 minutes prior to timepoint), then at every outpatient visit after discharge from the clinical unit and EOS (time windows as per PK sampling in Section 6.2). Day 1 pre-first dose results must satisfy any relevant inclusion/exclusion criteria.

7.2.3 Other Body Measurements – Weight and Height

Weight (kg) and height (cm) will be measured at Screening, and weight (kg) will be re-checked on Day -1.

7.3 12-LEAD ECGS

Single, standard 12-lead ECGs will be recorded after the participant has rested supine for at least 5 minutes. ECG tracings will be retained and labelled as per standard procedures at the clinical unit and recorded in the eCRF. The Principal Investigator or delegate will sign and date each ECG as evidence of their review.

ECGs will be taken at Screening, within 60 minutes of the first dose of IMP on Day 1, then 24, 48 and 72 hours post-first dose IMP (within 15 minutes prior to each confinement timepoint), Day 8, Day 15 and at EOS (outpatient time windows as per PK sampling). Day 1 pre-first dose results must satisfy relevant inclusion/exclusion criteria.

Normal ECG ranges once on trial are:

Parameter	Range
PR interval	≤210 msec
QRS	50–120 msec
QTcB/QTcF	≤450 msec

If results are out of these ranges, the Principal Investigator or delegate must assess and assign clinical significance (see Section 7.6.1).

7.4 CLINICAL LABORATORY TESTS

Handling of blood and urine sampling for laboratory safety assessments is described in the Laboratory Manual and/or the preferred Vendor Pathology Laboratory Manual. Biochemistry, haematology and urinalysis will be analysed by the clinical unit's preferred Vendor pathology laboratory. Urine pregnancy testing, urine drug screening and alcohol breath testing will be conducted at the clinical unit.

See Section 6.1.1.1 for review of results for Screening/eligibility purposes.

7.4.1 Biochemistry and Haematology Safety Laboratory Testing

- When biochemistry and haematology safety laboratory tests are scheduled, participants should attend the clinical unit having fasted for at least 8 hours. During confinement, clinical unit staff should ensure participants have fasted for at least 8 hours prior to all scheduled safety laboratory sampling.
- The Principal Investigator or delegate must review all results and assign clinical significance to any results that are abnormal and/or outside the laboratory normal reference ranges:
    - For Screening and eligibility, participants will be **excluded** if they return safety laboratory test results outside of the laboratory normal reference ranges AND the results are:
        - considered not clinically significant by the Principal Investigator **but are also** outside the ranges specified in Appendix 1: Clinically Acceptable Ranges for Clinically Important Study Inclusion/EXCLUSION Laboratory Tests, OR
        - considered clinically significant by the Principal Investigator.
    - After IMP is commenced, results must also be reviewed with reference to the toxicity rules for this study (see Section 4.3.8) and AESIs (see Section 7.6.5). See also Section 7.6.1 for clinically significant results.
- Results of safety laboratory tests conducted on Day -1 must be available and reviewed prior to first administration of IMP on Day 1, and must satisfy relevant inclusion/exclusion criteria.
- Blood samples for haematology and biochemistry laboratory safety assessments will be collected at Screening (including blood group and Rh(D), lipids), Day -1, at 24, 48 and 72 hours after first-dose IMP (within 60 minutes prior to each confinement timepoint), Day 8, Day 15 and EOS (outpatient time windows as per PK sampling in Section 6.2).

7.4.1.1 Haematology Parameters

Haematology: Full blood count (FBC) with differential, white blood cell count (WBC) with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), Mean

corpuscular volume (MCV), red blood cell (RBC) count, haemoglobin, haematocrit, platelet count, red blood cell distribution width, and reticulocyte count. Blood group and Rh(D) tests to be included at Screening only.

- A manual blood smear should be reviewed if there are immature/abnormal cells detected on the automated differential or if an automated differential was not able to be performed.
- Blood will be also be collected for Haematology if ALT or AST >2x ULN as described in Section 7.4.1.2.

7.4.1.2 Biochemistry Parameters

Biochemistry: Albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), Lactate dehydrogenase (LDH), bicarbonate, total and direct bilirubin, urea, calcium, corrected calcium, phosphate, sodium, creatinine, estimated glomerular filtration rate (eGFR; Chronic Kidney Disease Epidemiology collaboration [CKD-EPI equation]), glucose (fasted), potassium, chloride, magnesium, uric acid, total protein, creatine kinase (CK);

- Lipid profile (at Screening only): total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) [participants must fast for at least 8 hours prior]
- If ALT or AST value >2 ULN post-first dose IMP, then:
    - blood samples for the following tests should be collected within 24-48 hours:
        - Coagulation (see Section 7.4.1.3; also Section 7.6.5 and Section 4.3.8.3)
        - Liver panel (ALT, AST, LDH, ALP, GGT, total and direct bilirubin)
        - CK
        - Haematology (see Section 7.4.1.1)
        - Unscheduled PK sample.
    - Collect additional history of potentially hepatoxic concomitant medications/substances (including paracetamol and alcohol) and exercise.

7.4.1.3 Coagulation

Coagulation at Screening only; may also be requested at any time during the study for safety at the discretion of the Principal Investigator or delegate, and must be requested if ALT or AST > 2x ULN as described in Section 7.6.5 and Section 4.3.8.3:

- prothrombin time (PT), activated partial thromboplastin time (APTT), international normalised ratio (INR)

7.4.2 Urinalysis Safety Laboratory Testing

- Urine will be collected to conduct urinalysis at Screening, Day -1 Eligibility, at 24, 48 and 72 hours after first-dose IMP (within 60 minutes prior to each confinement timepoint), Day 8, Day 15 and EOS (with biochemistry and haematology safety blood testing; outpatient time windows as per PK sampling).
    - Dipstick testing on a freshly voided urine sample including tests for proteins, glucose, blood (erythrocytes), leukocytes, ketone bodies, nitrite, bilirubin, urobilinogen, and pH.
    - Quantitative assessment of glucose, protein, creatinine and protein:creatinine ratio for each sample;

- Any urine dipstick test result of more than traces (pH will not trigger quantitative analysis) will trigger a quantitative analysis for all of the following parameters: red blood cells, white blood cells, and bacteria (bacteria not quantitative).
- If available, the quantitative results will be the leading one over the dipstick results for decision making eg, for study inclusion/exclusion or clinical assessment.
- See Section 7.4.1 for information on screening/eligibility review of results.

Urinalysis will be conducted at the clinical unit's preferred Vendor pathology laboratory.

7.4.3 Pregnancy and Follicle Stimulating Hormone Testing

- Follicle Stimulating Hormone (FSH): blood will be collected from postmenopausal females only at Screening.
- Serum β-hCG pregnancy test: blood will be collected from all women during Screening and from WOCBP only at EOS.
- Urine β-hCG pregnancy test: urine will be collected from all WOCBP on Day -1.
  - A positive urine pregnancy test must be confirmed by a serum β-hCG pregnancy test.

7.4.4 Serology and Tuberculosis Testing

- Serology: blood will be collected at screening
  - testing for hepatitis B (HBsAg, anti-HBc [IgG + IgM if IgG is positive]), hepatitis C (anti-HCV), human immunodeficiency virus (HIV) HIV 1/2 (anti-HIV1 and anti-HIV2 Ab)
- Latent *M. tuberculosis* infection testing using Quantiferon-TB gold assay:
  - blood will be collected at Screening;
  - One repeat can be performed for indeterminate result. If repeat result is again indeterminate the individual will not be eligible.

7.4.5 Alcohol and Drug Screening

- Urine will be collected for drug screening at Screening and Day -1, testing for:
  - Acetaminophen/paracetamol (APAP) (not exclusionary)
  - Amphetamines (AMP)
  - Methamphetamines (mAMP)
  - Barbiturates (BAR)
  - Benzodiazepines (BZO)
  - Cocaine (COC)
  - Methadone (MTD)
  - Opiates (OPI)
  - Phencyclidine (PCP)
  - Cannabis (THC)
  - Tricyclic antidepressants (TCA)
  - 3,4-methylenedioxy-methamphetamine (MDMA)
- Alcohol will be tested for by breath test at Screening and on Day -1.

If considered necessary to confirm lifestyle considerations are being adhered to during the study (Section 4.3.3), alcohol and drug screening tests may also be conducted at other

unscheduled timepoints at the discretion of the Principal Investigator or delegate. However, re-tests for screening/eligibility purposes are not permitted.

7.5 BECK DEPRESSION INVENTORY

Originally described by Beck et al (1961), the Beck Depression Inventory (BDI) is a validated objective tool for the assessment of depression.[38] Updated in 1996, the BDI-II is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression, and participants will be required to complete the BDI-II at Screening for for eligibility.[39] The BDI-II takes approximately 10 minutes to complete, although participants require a fifth – sixth grade reading level to adequately understand the questions. A score of ≥20 at screening and/or a response of 1, 2 or 3 for item 9 indicating current suicidal ideation is exclusionary (see exclusion criterion 12 Section 4.3.2). A BDI score of 17 to 19 may be enrolled at the discretion of the Principal Investigator if they do not have a history of the psychiatric conditions mentioned in exclusion criterion 12 (Section 4.3.2) and their mental state is not considered to pose additional risk to the health of the volunteer or the execution of the study and interpretation of the data gathered.

7.6 ADVERSE EVENTS (AES)

The Principal Investigator or delegate and clinical facility staff are responsible for detection, recording, and reporting of events that meet the criteria and definition of various adverse events (AEs) as listed below. Treatment emergent adverse events (TEAEs) will be recorded from the administration of the first dose of IMP on Day 1 (see Schedule of Assessments Section 1.3).

All individual events will be followed until:

- The event is resolved, or
- No further medically relevant information in relation to the event can be expected, and
- the Principal Investigator considers it justifiable to terminate the follow-up.

Events that are unresolved at the time of the participant's last follow-up visit should continue to be followed up by the Principal Investigator for as long as medically indicated or until the participant is referred to a general practitioner or medical specialist as appropriate. The Sponsor retains the right to request additional information for any participant with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Any medically untoward event occurring in participants from the time of consent to the time of administration of IMPs will be considered medical history.

7.6.1 Definitions

An AE is any untoward medical occurrence, i.e., unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease that occurs in a participant during the course of the study, and which does not necessarily have a causal relationship with study treatments or procedures.

AEs include, but are not limited to:

- A new symptom, sign or medical condition.
- A disease or medical condition detected or diagnosed during the course of the study even though it may have been present prior to the start of the study.
- An exacerbation of a pre-existing medical condition/disease.

- An increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Continuous persistent disease or symptoms present at study start that worsen following the start of the study.
- An abnormal assessment (e.g., change on physical examination, ECG finding) if it represents a clinically significant finding that was not present at study start or worsened during the course of the study.
- An abnormal laboratory test result if it represents a clinically significant finding (e.g., CTCAE grade 2 or above), symptomatic or not, which was not present at study start or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study treatment.

Abnormal laboratory findings and other objective assessments should NOT be routinely captured and reported as AEs. However, abnormal laboratory findings or other objective measurements that meet the following criteria should be captured and reported in the AE Section of the eCRF:

- The result meets the criteria for reporting as an SAE (Section 7.7) or AESI (Section 7.6.5);
- The test result is associated with accompanying symptoms, and/or
- It requires additional diagnostic testing or medical/surgical intervention, and/or
- It leads to a change in IMP dosing, or discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
- It is considered by the Principal Investigator (or delegate) or Sponsor to be clinically significant or represent a clinically significant change from baseline.

AEs will not include:

- A medical or surgical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE).
- A pre-existing disease or condition present at the start of the study that does not worsen during the study.
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or social admissions).

7.6.2 Causal Relationship to Investigational Medicinal Product

A causal relationship of any adverse events experienced by participants to either of the IMPs will be determined by reference to the current product information and/or CMI.

If considered related:

- The temporal relationship between the event and the administration of IMPs is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the participant's medical condition, other therapies or accident.

If considered not related:

- The event can be readily explained by other factors such as the participant's underlying medical condition, concomitant therapy or accident and no plausible temporal or biologic relationship exists between the challenge agent and/or IMPs and the event.

7.6.3 Severity Grading of Adverse Events

The severity of AEs will be recorded in accordance with the CTCAE v5.0, published 27 November 2017. This guidance provides a common language to describe levels of severity, to analyse and interpret data, to scale the aggregate AE score, and to articulate the clinical significance of all AEs.

The severity of AEs will be graded as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An AE is defined as 'serious' when it meets one of the pre-defined serious outcomes as described in Section 7.7.1.

7.6.4 Documentation of Adverse Events

When an AE occurs, it is the responsibility of the Principal Investigator or delegate to review all documentation (e.g. progress notes, laboratory, and diagnostics reports) relative to the event. The Principal Investigator or delegate will then record the AE on the AE eCRF. Additional reporting requirements for an AE meeting serious criteria are discussed in Section 7.7.2 below. The Principal Investigator or delegate will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In all cases, when available, the diagnosis should be reported as the event and not the individual signs/symptoms.

The following information should be recorded for all AEs:

- Description
- Dates and times of onset and resolution
- Duration in hours
- Time of onset relative to IMP administration
- Seriousness
- Severity
    - In the source data, the description of the AE will report the various severities observed over time. If the severity of an AE increases, separate AEs per severity grading will be recorded into the eCRF.
    - If the AE resolves and then reoccurs, then two AEs will be reported.
- Action taken in response to the AE regarding IMP:
    - Dose not changed
    - Drug interrupted
    - Drug withdrawn
    - Not applicable
    - Unknown
- Outcome of AE:

  - Recovered/resolved, or
  - Recovering/resolving, or
  - Not recovered/not resolved, or
  - Recovered with sequelae/resolved with sequelae
  - Fatal
  - Unknown
- Relationship to the trial treatments or procedures (causality assessment of related or not related), or any other treatment or procedure conducted during the trial.

7.6.5 Adverse Events of Special Interest

An adverse event of special interest (AESI) (serious or non-serious) is one of scientific and medical concern specific to the Sponsor's product or programme, for which ongoing monitoring and rapid communication by the PI to the Sponsor is appropriate. Such an event may require further investigation in order to characterise and understand it.

The pharmacovigilance provider (Prime Vigilance) must be notified of all AESIs (serious and non-serious) within 24 hours of the site becoming aware of the event. The notification should be via a Serious Adverse Event Report Form (marked as 'AE of special interest').

Follow-up information will be submitted in a timely fashion as further information become available.

Hepatic AEs of Special Interest

- Any ALT or AST above 5×ULN,
- An elevation in bilirubin 2×ULN,
- Any AST or ALT above 2×ULN and:
  - Total Bilirubin Level (TBL) >1.5×ULN OR
  - INR >1.4 (see Section 7.4.1.2),
- Any AST or ALT above 2×ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).

Cardiac AEs of Special Interest

- QTcB or QTcF at any time >480 msec,
- Bundle branch block (except right bundle branch block that was present prior to IMP administration),
- Any arrhythmia, except:
  - Sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,
  - sinus tachycardia that is clinically asymptomatic, and associated with a body temperature >38.0°C, and not associated with any other relevant ECG abnormalities,
  - Respiratory sinus arrhythmia,
  - Wandering atrial pacemaker,
  - Isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.

Haematological AEs of Special Interest

- Absolute neutrophil count <0.5 × 10^9/L,
- Platelet count <125 × 10^9/L.

Dermatological AEs of Special Interest

Clinical signs of possible cutaneous adverse reactions such as:

- Dermatitis,
- Rash, including erythematous, macular, papular, maculopapular, pruritic, pustular, and vesicular.

If one of these cutaneous reaction is observed and when feasible, pictures of the lesions should be obtained.

Dermatological AEs need not be reported as AESIs if clearly unrelated to IMP (e.g. rash from cannula dressing or ECG dots).

7.7 SERIOUS ADVERSE EVENTS (SAES)

7.7.1 Definitions of Serious Adverse Events (SAEs)

An SAE is any AE that:

- Results in death, or
- Is life-threatening, or
- Requires inpatient hospitalization or prolongation of existing hospitalization, or
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Constitutes a possible Hy's Law case:
    - Hy's Law case is defined as a subject with any value of alanine or aspartate aminotransferase greater than 3×ULN together with an increase in total bilirubin to a value greater than 2×ULN and not associated to an alkaline phosphatase value greater than 2×ULN (FDA Guidance on Drug Induced Liver Injury: Premarketing Clinical Evaluation [2009]).

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

The term 'life-threatening' in the definition of 'serious' refers to an event/reaction in which the subject was at immediate risk of death at the time of the event/reaction; it does not refer to an event/reaction, which hypothetically might have caused death, if it were more severe.

Any planned procedures that require admission to hospital for compliance with this protocol as well as any planned elective procedures are not considered SAEs (unless the underlying condition has worsened or the procedure results in a worsening of the subject's condition).

7.7.2 Reporting for Serious Adverse Events (24 hours)

SAEs must be followed until their signs and symptoms have remitted or stabilized, until the participant is lost to follow-up, or until the End of Study Visit, whichever occurs last.

AEs meeting the serious criteria MUST be reported promptly to the pharmacovigilance provider (Prime Vigilance) and as per the Safety Management Plan within 24 hours of the site becoming aware of the event:

SAE Reporting:
PrimeVigilance

Email: MMV@primevigilance.com
Fax: +44 (0) 800 471 5694

Any copies of participant's medical records provided for SAE reporting must have all participant identifiers redacted before submission.

The SAE report form will always be completed as thoroughly as possible with all available details of the event and signed by the Principal Investigator or delegate. If the Principal Investigator or delegate does not have all information regarding an SAE, he/she will not wait to receive additional information before reporting the event. A follow-up SAE report should be completed within 14 days, or if there is no new information the SAE report form should be updated when additional information is received.

The Principal Investigator or delegate will always provide an assessment of causality at the time of the initial report as described in Section 7.6.2.

Email transmission of the SAE report form is the preferred method to transmit this information to Prime Vigilance. In rare circumstances notification by telephone is acceptable, with a copy of the SAE report form sent by overnight mail.

Initial notification via the telephone does not replace the need for the Principal Investigator or delegate to complete and sign the SAE report form within the outlined time frames. The Sponsor will provide a list of project contacts for SAE receipt, telephone numbers, and mailing addresses

The Principal Investigator or delegate, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the HREC.

7.7.3 Pregnancy

Pregnancy itself is not defined as an AE/SAE. Any complication or termination of pregnancy for medical reasons are to be reported as an AE/SAE. Spontaneous abortion, still birth or congenital anomaly must be reported as an SAE.

Any WOCBP (Woman of Child-Bearing Potential) enrolled in the study who becomes pregnant during the study or within 30 days after EOS should be followed through delivery or termination of the pregnancy. The Investigator will collect pregnancy information and report to the Sponsor within 24 hours of becoming aware of a participant's pregnancy. Follow-up will generally not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Once pregnancy is confirmed, pregnant female participants will be immediately withdrawn from the study as outlined in Section 4.3.5.

Where possible, the Investigator will also attempt to collect and report information on pregnancy outcomes of female partners of any male participants who were administered IMP in this study, if the female partner became pregnant during the study or within 90 days after EOS. Appropriate signed informed consent will be required directly from the pregnant female partner to obtain and report this information. Any participant's female partner who becomes pregnant during the study should be followed through delivery or termination of the pregnancy.

7.8 SAFETY REVIEW COMMITTEE (SRC)

The Principal Investigator, Sponsor Medical Director and study Medical Monitor (at a minimum) will review the sentinel group (Group 1a) safety information up to and including Day 8 before deciding if the remainder of the cohort (Group 1b) should proceed.

8 STATISTICAL ANALYSES

8.1 GENERAL APPROACH

The following sections describe the statistical analysis as it is foreseen during the planning phase of trial. A detailed Statistical Analysis Plan (SAP) will be finalised and approved prior to database lock and will provide details of the safety analyses to be performed as well as the format of listings and tables to be provided for completion of the clinical study report (CSR). Any deviations from the SAP will be described and justified in the final CSR. Additional analytical plans will be prepared to detail the PK and PD analyses.

Statistical analysis will be performed using SAS® software version 9.4 (or higher) (SAS Institute Inc., Cary, NC, USA).

The general analytical approach for all safety endpoints will be descriptive in nature. Unless otherwise stated, the following statistical approaches will be taken:

Continuous variables:	Descriptive statistics will include the number of non-missing values, mean, standard deviation (SD), median, minimum, and maximum. The minimum and maximum values will be presented to the same number of decimal places as recorded in the raw data; mean, median and SD will be presented to one more decimal place than the raw data.
Categorical variables:	Descriptive statistics will include frequency counts and percentages per category. Percentages will be rounded to one decimal place, with the denominator being the number of participants in the relevant population with non-missing data.
Imputation:	No missing data will be imputed.
Confidence intervals (CIs):	If required, CIs will be two sided and will use 95% confidence levels. Any analyses requiring significance testing will use a two-sided test at the 5% significance level.
Baseline:	Baseline will be defined as the last available assessment prior to the first IMP administration.
Unscheduled assessments:	Unscheduled visits will be excluded from visit-based summary tables.
Early termination visits:	Assessments conducted at Early Termination will be excluded from visit-based summary tables.

8.2 SAMPLE SIZE

Eight healthy males or females, aged between 18 and 55 years old, who meet all of the inclusion criteria and none of the exclusion criteria will be enrolled in two sequential groups of 2 participants (Group 1a) and 6 participants (Group 1b).

The 2 participants in Group 1a will serve as sentinels for Group 1b.

As the combination of Rux+AL has not been tested previously, a first-in-human approach of a sample size of 8 participants has been selected.

8.3 ANALYSIS SETS AND SUB-SETS

8.3.1 Analysis Sets

In the first instance, four (4) analysis datasets will be used for study analyses: Full Analysis Set (FAS), Safety Set, PK Set, and pSTAT3 Set.

Additional analysis populations may be defined in the SAP.

The number of participants in each analysis set will be summarised, with a corresponding listing.

8.3.1.1 Full Analysis Set

The Full Analysis Set (FAS) will consist of all enrolled participants. The FAS will be used to assess all participant disposition, baseline, demographic, treatment exposure and protocol deviation data.

8.3.1.2 Safety Set

The Safety Set will include all enrolled participants who received at least one dose (full or partial) of IMP. The Safety Set will be used to assess all safety data.

8.3.1.3 PK Set

The PK Set will include all enrolled participants who received at least one dose AL, Rux, and/or placebo and have sufficient samples for analysis. The PK Set will be used to assess all PK data.

8.3.1.4 pSTAT3 Set

The pSTAT3 Set will include all enrolled participants who received at least one dose (full or partial) of IMP, a valid baseline pSTAT3 result and at least one valid post-baseline pSTAT3 result. The pSTAT3 Set will be used to assess all PD data.

8.3.2 Sub-Sets

Participants will be analysed in the following treatment groups:

- AL+Rux (n=6; 1 participant from Group 1a + 5 participants from Group 1b)
- AL+placebo (n=2; 1 participant from Group 1a and 1 participant from Group 1b)

Unless otherwise stated, summary tables will be presented by treatment group and overall.

8.4 PARTICIPANT DISPOSITION

A listing of participant disposition will present participant dates of informed consent, randomisation, key visits as well as study completion details. Early termination data, including the reason for early termination will be listed in an additional listing.

A participant disposition summary table will present, at a minimum, the number of participants who completed the study per protocol and the number of participants who discontinued classified by reason for early termination.

8.5 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

All descriptive statistics for demographic and baseline characteristic parameters will be evaluated using the Full Analysis Set.

8.5.1 Demographics

Demographic data will be listed for all enrolled participants and summarised by treatment arm and overall.

8.5.2 Medical History

All medical history data will be listed, grouped by participant.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedRA) and summarised by system organ class (SOC) and preferred term (PT).

8.5.3 Prior Medications

Prior medications will be listed for all enrolled participants. Prior medications are defined as any medication that is started before administration of IMP, regardless of when it ended. If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before IMP or concomitantly, it will be considered as prior and concomitant.

8.5.4 Eligibility

Eligibility will be listed for all enrolled participants, including findings for drug and alcohol screening.

8.6 PROTOCOL DEVIATIONS

All protocol deviations will be listed. A protocol deviation summary table will present the total number of protocol deviations as well as the number of participants who reported at least one protocol deviation, broken down by deviation type including (but not limited to):

- Informed consent
- Eligibility
- Visit not done
- Visit performed out of window
- Study procedure not done
- Study procedure done out of window
- Safety reporting
- Investigational Product
- Privacy and Data Protection
- Concomitant Medication
- Other

8.7 TREATMENT EXPOSURE

Participant exposure to all protocol-specified treatments will be listed and summarised.

8.8 SAFETY (PRIMARY ENDPOINT)

8.8.1 Safety Endpoints

Safety and tolerability will be assessed by clinical review of the following parameters:

- AEs (including SAEs and AESIs)
- Vital signs and other body measurements including respiratory rate and body temperature
- 12-lead ECG
- Haematology, chemistry, coagulation and urinalysis
- Physical examination

All descriptive statistics for safety parameters will be evaluated using the Safety Set.

8.8.2 Adverse Events

All AE data will be listed for each participant, including severity, relationship to IMPs, relationship to non-IMP protocol-specific treatments, outcome and actions taken. In addition, listings of AEs leading to discontinuation of the study, SAEs and deaths, will be provided as applicable.

All AE summaries will be restricted to TEAEs only, where a TEAE is defined as an AE that commences on, or after, the first administration of IMP. TEAEs without an onset date or time will be defined as treatment emergent except if an incomplete date (e.g., month and year) clearly indicates that the event started prior to first administration of IMP, or if the AE stop date indicates that the event started and/or stopped prior to the first administration of IMP.

All reported TEAEs, including SAEs and AESIs, will be mapped to standard MedDRA coding terms and grouped by SOC and PT.

At a minimum, the following AE summary tables will be provided:

- Overall summary of TEAEs
- All TEAEs
- TEAEs by severity
- TEAEs by relationship (separate tables for each relationship type assessed)
- Serious TEAEs
- TEAEs leading to study withdrawal
- TEAEs of Special Interest (AESIs)

For the summaries of TEAEs, participants who experience the same AE (in terms of the MedDRA preferred term) more than once will only be counted once.

8.8.3 Concomitant Medications

Medications used in this study will be coded using the World Health Organisation Drug Dictionary Enhanced (WHO-DDE) and/or the World Health Organisation Drug Global (WHODrug Global).

Concomitant medications are defined as medications continued or newly received at or after administration of IMP, through to the End of Study visit.

If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before initial treatment or concomitantly, it will be considered as prior and concomitant.

Concomitant medications will be summarised by Anatomical Therapeutic Class (ATC) and preferred name (PN). The summary tables will show the number and percentage of participants taking each medication by ATC and PN.

Participants who take the same medication (in terms of the ATC and PN) more than once will only be counted once for that medication.

8.8.4 Laboratory Findings

Laboratory parameters will be listed by participant and visit, including:

- haematology,
- biochemistry,
- coagulation,
- urinalysis,
- serology,
- FSH, and
- pregnancy test.

All laboratory findings will be listed with flags for values outside the laboratory normal reference ranges and clinical significance status.

Haematology, biochemistry and continuous urinalysis laboratory data will be summarised for each scheduled visit, including observed values and absolute change from each baseline. Categorical urinalysis results will be summarised for each scheduled visit using frequency tabulations.

Any available microscopic urinalysis will be listed.

8.8.5 Physical Examination

Physical examination parameters will be listed for all participants and visits.

8.8.6 Vital Signs and other Body Measurements.

Vital sign parameters and other body measurements will be listed for all participants and visits, with three separate listings:

- Vital signs
- Respiratory rate and temperature
- Height and weight

Observed values, as well as absolute changes from each baseline, will be summarised descriptively vital signs, respiratory rate and temperature by scheduled visit, with two separate tables:

- Vital signs
- Respiratory rate and temperature

8.8.7 12-Lead ECG

ECG parameters will be listed by participants and visits.

Observed values, as well as absolute changes from each baseline, will be summarised descriptively for all ECG parameters by visit.

Safety analysis of ECGs will include in particular the number of participants during the study with:

- QTcF and/or QTcB prolongation of more than 30 msec and 60 msec; and/or

- QTcF and/or QTcB > 450 msec.

8.8.8 Beck Depression Inventory

Beck Depression Inventory (BDI-II) data will be listed for all participants.

8.9 PHARMACOKINETIC ENDPOINTS

8.9.1 Pharmacokinetic parameters

Secondary endpoint PK parameters of artemether, DHA, lumefantrine, and Rux will be estimated using non-compartmental methods from plasma concentration-time data:

- AUC_{last}
- $AUC_{0-\infty}$
- AUC_{0-8}
- AUC_{60-72}
- C_{max} (first and last dose)
- t_{max} (first and last dose)
- Elimination $t_{½}$
- t_{lag}
- C_{168h} (for lumefantrine only)
- CL/F
- V_z/F
- λ_z

For calculation of descriptive statistics of plasma concentrations, values below the lower limit of quantitation will be set to zero.

PK parameters will be determined using STATA® (version 14.0 or higher).

8.10 PHARMACODYNAMIC ENDPOINTS

8.10.1 pSTAT3 levels

The secondary endpoint pSTAT3 inhibition ex-vivo on whole blood cells will be assessed using a validated ELISA. pSTAT3 inhibition will be expressed as a percentage and summarised by percentage inhibition pre and post IMP administration and as change in percentage between pre and post IMP administration. The following analyses will be conducted:

- Within-participant comparison of each participant before AL+Rux or AL+placebo administration, and 12 hours after first administration of AL.
- Between-participants comparison of participants who receive AL+Rux and participants who receive AL+placebo

8.11 INTERIM ANALYSES

This trial has no formal interim analyses other than review of safety and tolerability as described in Section 7.8.

9 STUDY ADMINISTRATION

9.1 ETHICAL CONSIDERATIONS

9.1.1 Ethical Principles

This clinical study was designed and shall be implemented and reported in accordance with the Declaration of Helsinki, the Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) dated 09 November 2016 and the National Statement on Ethical Conduct in Human Research, (2007 – updated 2018).

9.1.2 Informed Consent

Eligible participants may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the participant source documents and a copy of the signed patient information and consent form provided to the participant.

9.1.3 Investigator and Human Research Ethics Committee (HREC) Responsibilities

Before initiating a trial, the investigator/institution should obtain approval/favorable opinion from the Human Research Ethics Committee (HREC) for the trial protocol, written informed consent form, consent form updates, participant recruitment procedures (e.g., advertisements) and any other written information to be provided to participants.

Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to the Sponsor appointed monitors, auditors, Quality Assurance representatives, HREC representatives, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform the Sponsor immediately that this request has been made.

9.2 PROTOCOL ADHERENCE

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Additional assessments required to ensure safety of participants should be administered as deemed necessary on a case by case basis. In this instance the Sponsor medical monitor must be advised before or as soon as possible after such assessments are conducted.

Under no circumstances should an investigator collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs. Investigators ascertain they will apply due diligence to avoid protocol deviations. If an investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the Sponsor and approved by the HREC it cannot be implemented. All significant protocol deviations will be recorded and reported in the CSR.

9.3 PROTOCOL AMENDMENTS

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor and the HREC prior to implementation. Only amendments that are intended to eliminate an apparent immediate hazard to participants

may be implemented immediately provided the Sponsors medical monitor is notified as soon as possible after the event and the reviewing HREC is subsequently notified. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any participant included in this study, even if this action represents a deviation from the protocol. In such cases, the Medical Monitor and Sponsor must be notified immediately.

9.4 DATA HANDLING AND RECORD KEEPING

Participants will be assigned a unique identifier when participating in the study, and any participant datasets or records that are transferred to the Sponsor or CRO will only contain this identifier. Any other identifiable information about the participant will not be transferred. The Principal Investigator will ensure procedures are in place to appropriately protect the confidentiality of the participant records and data, including adequate safe guards for digital/computer access. The participants will be informed that their personal study-related data will be used by the Sponsor and that their medical records may be examined by auditors and regulatory agencies.

Study-related participant data will be entered into electronic case report forms (CRFs), except for data that may be transmitted to the Sponsor or CRO electronically (such as laboratory data). The Principal Investigator is responsible for ensuring that accurate source documents for all data entered into the CRF are maintained at the study site.

All study documents including source documents and signed PICFs must be retained by the Principal Investigator for at least 15 years and according to local regulatory requirements. No study documents may be destroyed or transferred during the retention period without the Sponsor being directly notified in writing.

9.5 QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor or CRO maintains responsibility for quality assurance of the data and for data management, and retains accountability for actions delegated to other parties (including CRO). Study monitors appointed by the Sponsor or CRO will conduct ongoing visits to the study sites (and/or remote monitoring according to Sponsor or CRO standard operating procedures (SOPs) if required due to COVID-19 restrictions) to confirm the CRF data is accurate according to source documents and complete, and that the study is being appropriately conducted according to the protocol, ICH GCP and local regulatory requirements. Details may also be provided in the Monitoring Plan.

9.6 PUBLICATION POLICY

Neither the complete nor partial results of the study achieved under this protocol, nor any of the information provided by the Sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study Sponsor. Any Investigator involved with this study is obligated to provide the Sponsor with complete study results and all data derived from the study.

Results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results.

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11 APPENDICES

APPENDIX 1: CLINICALLY ACCEPTABLE RANGES FOR CLINICALLY IMPORTANT STUDY INCLUSION/EXCLUSION LABORATORY TESTS

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Test	Acceptable Inclusion Range	
	Low	High
Sodium	130 mmol/L	150 mmol/L
Potassium	3.0 mmol/L	5.5 mmol/L
Chloride	0.95 x LLN	1.05 x ULN
Magnesium	0.95 x LLN	1.05 x ULN
Calcium (Corrected)	0.95 x LLN	1.05 x ULN
Phosphate	0.95 x LLN	1.05 x ULN
Glucose Fasted	N/A	1.0 x ULN
Urea	N/A	1.75 x ULN
Creatinine	N/A	1.0 x ULN
eGFR (CKD-EPI equation)	> 59 ml/min/1.73m^2	N/A
Creatine kinase	N/A	< 2 x ULN
Uric acid	N/A	1.1 x ULN
Total Protein	≥ 0.85 x LLN	≤ 1.25 x ULN
Albumin	≥ 0.85 x LLN	≤ 1.25 x ULN
Total Bilirubin	N/A	1.25 x ULN
Direct Bilirubin	N/A	1.25 x ULN
ALP	N/A	1.5 x ULN
AST	N/A	1 x ULN
ALT	N/A	1 x ULN
GGT	N/A	1.5 x ULN

Test	**Acceptable Inclusion Range**	
	Low	**High**
Lactate Dehydrogenase	0.9 x LLN	1.1 x ULN
Total cholesterol	N/A	1.2 x ULN
HDL	> 1.1 mmol/L	N/A
LDL	N/A	< 3.34 mmol/L
Triglycerides	NA	1.2 x ULN
Prothrombin time INR and APTT	1.0 x LLN	1.0 x ULN
Haemoglobin	0.9 x LLN	1.1.x ULN
Red blood count	0.9 x LLN	1.1 x ULN
Reticulocytes	0.9 x LLN	1.1 x ULN
MCV	0.9 x LLN	1.1 x ULN
Platelets	> 200 $x10^9$/L	1.1 x ULN
White Blood Cells	0.9 x LLN	1.1 x ULN
Neutrophils	1.0 x LLN	1.0x ULN
Lymphocytes	1.0 x LLN	1.0 x ULN
Monocytes	N/A	1.2 x ULN
Eosinophils	N/A	1.0 x ULN
Basophils	N/A	2.0 x ULN
Urinalysis		
Glucose (quantitative)	N/A	≤ 1.9 mmol/L
Protein (quantitative)	N/A	< 1+
Red Blood Cells (quantitative)	N/A	<10* x 10^6/L
White Blood Cells (quantitative)	N/A	<10 x 10^6/L
Bacteria (any analysis)	N/A	< 1+
Ketones (dipstick)	N/A	< 1+

Test	**Acceptable Inclusion Range**	
	Low	**High**
Nitrate (dipstick)	N/A	Negative
Urobilinogen (dipstick)	N/A	< 1+
Bilirubin (dipstick)	N/A	< 1+

ABBREVIATIONS: ALP = alkaline phosphatase; ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; CKD-EPI = Chronic Kidney Disease Epidemiology collaboration; eGFR = estimated glomerular filtration rate; GGT = gamma-glutamyl transferase; HDL = high density lipoprotein; INR = international normalised ratio; LDL = low density lipoprotein; LLN = lower limit of normal; MCV = mean corpuscular volume; N/A = not applicable; ULN = upper limit of normal.

*A result $\geq 10 \times 10^6$/L is acceptable for female participants currently menstruating.