Study Acronym: LEISH2a

A phase IIa safety study to assess the safety and immunogenicity of a new Leishmania vaccine candidate ChAd63-KH

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Protocol date: **May 2018**

Sponsor: University of York

Authorised by:		**Role:**	Date:
Name	Ahmed Musa	Chief Investigator	
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Signature			27/09/18
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Modification History

Version	Date	Author(s) and details of changes from previous version
V1.0		
V1.1	02-09-2016	Lacey and Wiggins - removal of HLA test, insertion of RDT for malaria
V1.2	03-10-2016	Lacey and Wiggins – insertion of contacts for SAEs, pregnancy and serious breaches. Addition of definition of malnutrition to exclusion criteria.
V1.3	09-12-2016	Wiggins – change CRO representative from Hany Robin to Ali Nakuzi. Kaye, Lacey and Musa - Reduction of time interval between screening and dosing after DSMB and Sponsor approval. Change of source data definition to hospital notes. Minor adjustment to dosing preparations. Removal of reference to “Consent form 2”. Revisions to toxicity table to match study population parameters. Clarification of blood tests required (Section 7.0, p.26).
V1.4		Lacey and Wiggins – clarification of screening and dosing timelines (Section 7.2), and re-screening blood tests (Section 6.5). Removal of biochemistry and haematology tests (Section 7.2) at Day 21 and 42, which had been left in the text in error, to ensure consistency with Schedule of Visits (Table 2). Wiggins – change in Section 15.0 from Rhian Gabe to Ada Keding.
V1.5		Lacey – changes to offer standard PKDL treatment at either D42 or D90, according to patient preference, to those subjects without sufficient clinical improvement, and to add a final day 120 outpatient visit. Wiggins – change ULN <40 to ULN <60 for ALT in Laboratory Parameters table to correspond with biochemistry analyser operating procedures. Wiggins – change of named CRA/monitor to generic post.
V2.0		
V3.0		

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IMP Manufacture	Advent, Via Pontina, Pomezia, Rome, Italy Email: stefania.dimarco@reithera.com
Funding	Wellcome Trust Gibbs Building 215 Euston Road London NW1 2BE UK

Clinical Site and Participating Laboratories

All trial visits will take place at the Centre for Tropical Medicine, Dooka. All haematological and biochemical screening and safety tests will be conducted at the Centre for Tropical Medicine, Dooka. Samples for immunological assays will be processed and stored initially in Dooka, and then transported to the Institute of Endemic Diseases, Khartoum. Some samples will be subsequently shipped to the Centre for Immunology and Infection, York.

Definitions used in this protocol:

Chief Investigator (CI): Takes ultimate responsibility for the design, conduct, analysis and reporting of a clinical study or trial.

Principal Investigator (PI): Directly assists the CI in delivery of the trial objectives, and may take the lead role in the event of temporary unavailability of the CI.

Trial Physician: Responsible for the day-to-day running of the trial, and reports to the CI and PI

Clinical Investigator: A member of the clinical team with designated responsibilities, who advises and supports the Chief Investigator.

Project Lead: The Wellcome Trust Translational Award principal investigator

Laboratory Investigator: An individual member of the laboratory team with designated responsibilities, who in terms of the clinical trial is under the overall supervision of the Chief Investigator and the Project Lead.

Statistical Investigator: An individual member of the statistical team with designated responsibilities, who in terms of the clinical trial is under the overall supervision of the Chief Investigator and the Project Lead.

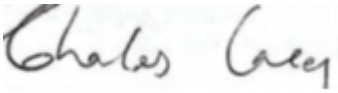
Protocol Signature Page

The signature below confirms agreement by the individual authorised by the Sponsors and responsible for signing the clinical trial agreement that study LEISH2a will be conducted in accordance with this protocol and GCP and ICH guidelines Any amendments to this protocol that have a direct influence on the volunteers in the trial will be approved by the relevant ethics committees before implementation.

I, the Chief Investigator, agree to allow sponsor monitor and auditors, full access to all medical records at the research facility for volunteers screened or enrolled in the study.

I agree to maintain all study documentation until the Sponsor consents to disposal of files in writing.

I have read and understood the information in the Investigator's Brochure including the potential risks and side effects of the product and will ensure that all colleagues and employees assisting in the conduct of the study are informed about the obligations incurred by their involvement in the study.



25 May 2018

Chief Investigator	Investigator Signature	Date
Professor Ahmed Musa	Professor Charles Lacey	

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, and members of the Research Ethics Committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Professor Ahmed Musa and Professor Charles Lacey.

SAE & SUSAR NOTIFICATION

In the case of an SAE or SUSAR complete the report form and notify ClinServ and the Sponsor within 24 hours

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Contents

ABBREVIATIONS

AE	Adverse Event
ADL	Activities of Daily Living
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
AMA	Atypical Membrane Antigen
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
ChAd	Chimpanzee Adenovirus
ChAd63	Chimpanzee Adenovirus 63
CI	Chief Investigator
CII	Centre for Immunology & Infection, York
CL	Cutaneous Leishmaniasis
CRF	Case Report Form
CS	Circumsporozoite
CTIMP	Clinical trial of Investigational Medicinal Product
CTSC	Clinical Trial Steering Committee
CTL	Cytotoxic T Lymphocytes
BNF	British National Formulary
DALY	Disability-adjusted life year
DNA	Deoxyribonucleic acid
DSMB	Data Safety and Monitoring Board
ELISPOT	Enzyme-linked immunosorbent spot
EMU	Experimental Medicine Unit
FBC	Full Blood Count
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
GP	General Practitioner
GUM	Genitourinary Medicine
HASPB	Hydrophilic Acylated Surface Protein B
HBsAg	Hepatitis B Surface Antigen
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HuAd	Human Adenovirus
HYMS	Hull York Medical School
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IDRI	Infectious Diseases Research Institute
IEND	Institute of Endemic Diseases, Khartoum
IFN	Interferon

IL	Interleukin
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IVDU	Intravenous Drug User
KH	Kinteoplastid Membrane Protein 11 + Hydrophilic Acylated Surface Protein B
KMP-11	Kinetoplastid Membrane Protein 11
LFT	Liver Function Test
LLN	Lower Limit of Normal
LST	Leishmania skin test
ME-TRAP	Multiple Epitope Thrombospondin Related Adhesion Protein
MHRA	Medicines and Healthcare Products Regulatory Agency
ml	millilitre
MSP	Merozoite Surface Protein 1
MVA	Modified Vaccinia Virus Ankara
NHS	National Health Service
NIH	National Institute of Health
OTC	Over the Counter
Pfu	Plaque Forming Units
PI	Principle Investigator
PKDL	Post kala azar dermal leishmaniasis
R&D	Research and Development
RDT	Rapid Diagnostic Test
REC	Research Ethics Committee
rVV	Recombinant vaccinia virus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	Standard of Care treatment for PKDL
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TaV	Thosea asigna virus
Th	T helper
TLR	Toll Like Receptor
TMG	Trial Management Group
TOPS	The Over Volunteering Prevention System
TSF	Trial Site File
U&E	Urea and Electrolytes
UK	United Kingdom
ULN	Upper Limit of Normal
UNICEF	United Nations Children's Fund
US	United States
VL	Visceral Leishmaniasis
vp	virus particles
WBTP	Whole blood transcriptomic profiling
WHO	World Health Organisation
YTU	York Trial Unit
µg	microgram

1.0 STUDY SUMMARY

Title	A phase IIa study to assess the safety and immunogenicity of a candidate Leishmania vaccine ChAd63-KH
Trial Centre	Professor El-Hassan's Centre for Tropical Medicine, Dooka, Gedarif State, Sudan
Trial Identifier	LEISH2a
Clinical Phase	IIa
Design	Open label three stage therapeutic study
Population	Adults, male and female, aged 18 – 50 with persistent PKDL; adolescents 12-17 with persistent PKDL
Sample Size	24 Volunteers (16 adults, 8 adolescents)
Follow-up duration	120 days from dosing visit
Planned Trial Period	14 months
Primary Objective	To assess the safety of a new candidate Leishmania vaccine ChAd63-KH in patients with persistent PKDL. The first eight adult volunteers will receive $1x10^{10}$vp and the subsequent eights adult volunteers will receive $7.5\ x10^{10}$vp. Doses will be administered at a single time point. Adolescents will be vaccinated with either $1x10^{10}$vp or $7.5\ x10^{10}$vp, to be determined by evaluation of all available data after DSMB & CTSC review.
Secondary Objectives	1. To compare the humoral and cellular immune responses generated by the candidate vaccine in patients with persistent PKDL. 2. To observe any clinical changes in the cutaneous PKDL disease over a 90 day period following vaccination
Investigational Products ChAd63-KH	
Form	Liquid
Dose	ChAd63-KH, 1st phase $1x10^{10}$ vp, 2nd phase $7.5x10^{10}$ vp, 3rd phase to be determined
Route	Intramuscular injection in the deltoid region

2.0 BACKGROUND AND RATIONALE

2.1 The need for a therapeutic vaccine against VL / PKDL

Human visceral leishmaniasis (VL), also known as kala azar, is a neglected tropical disease. With 95% of cases occurring in India, Bangladesh, Nepal, the Sudan and Brazil, VL is a disease of the poor[1]. With an estimated 40,000 or more deaths annually, mostly children and young adults, VL ranks second only to malaria amongst parasitic infections for mortality, and as measured by DALYs lost, it ranks in the top ten infectious diseases globally. In some developing country regions, there is epidemiologic concurrence, and clinical interactions between VL and HIV, and in East Africa, co-infection rates of 34% have been reported[2]. During VL and after treatment, post kala azar dermal leishmaniasis (PKDL) may develop. PKDL is a complex, chronic and disfiguring skin disease that manifests as abundant "nodular", "papular" or hypo-pigmented "macular" skin lesions. Granulomas with few parasites, residual parasite antigen and mononuclear cell inflammation are the main histopathological features. PKDL significantly affects quality of life, often mistaken for leprosy. PKDL patients are also reservoirs for VL transmission. In Sudan, PKDL occurs in ~30-60% of patients cured from VL. Spontaneous healing within 6 months is the rule, but in ~15% of patients, mostly children, lesions persist. Treatment for PKDL in Sudan requires hospitalisation (Ambisome, 50mg/kg/20 days), with significant costs for healthcare providers, patients and households[3-6].

The potential emergence of resistance to existing anti-leishmanial drugs in East Africa is a significant threat, as is the apparent poor efficacy in East Africa of drugs currently effectively deployed in SE Asia. Affordable second line treatments are often not available, and no single new therapies are in phase 2 or phase 3 trials, making the development of new preventative and/or therapeutic measures a major international research priority. World Health Assembly resolution EB118.R3 (Geneva 05/07) 'calls on partner bodies… to accelerate research on, and development of, leishmaniasis vaccines'.

A therapeutic vaccine would have wide-ranging direct and indirect healthcare benefits that could be realized rapidly (UNICEF / UNDP / World Bank/WHO (TDR) and IDRI Consultation Report [3,5]. For PKDL patients, an effective therapeutic vaccination used alone would have very significant clinical benefits, reducing the need for extensive hospitalization and chemotherapy. Combining vaccine with drug (immuno-chemotherapy) might significantly increase the effective life of new drugs, with lower dose / abbreviated regimens helping to limit the emergence of drug resistance. Finally, the capacity development accompanying therapeutic trials and their simplicity in design makes such trials an important stepping stone in leishmaniasis elimination programmes. Prior human trials of combined immuno-chemotherapy of PKDL provided optimism that therapeutic vaccines against leishmaniasis can be developed [3].

No effective vaccine has yet been developed for VL / PKDL despite significant research efforts. Unsuccessful prophylactic vaccine trials in humans have employed crude antigen mixtures, autoclaved parasites or defined antigens chosen for their abundance and/or immunogenicity during natural infection and have adopted vaccination regimens designed to target $CD4^+$ T cells. In contrast, and based on the importance of $CD8^+$ T cells for protection against leishmaniasis and a range of other intracellular pathogens, we have sought to develop a novel therapeutic vaccine for VL / PKDL, biased towards the induction of $CD8^+$ T cell responses.

We have recently completed a successful first-in-human clinical trial of a new therapeutic vaccine for VL / PKDL (ChAd63-KH; see section 2.7). This trial demonstrated safety of ChAd63-KH in healthy UK adult volunteers and immunogenicity against the two *Leishmania* antigens on par with that seen to other vaccine candidate antigens in clinical development for other diseases (e.g. malaria, HCV, Ebola). Following external peer review of the data generated during LEISH1, we have been awarded further Wellcome Trust funding to progress this vaccine into Phase II clinical trials in patients with PKDL.

2.2 Lifecycle of the *Leishmania* parasite

The life cycle of the *Leishmania* parasite is shown below in Figure 1.0[4]

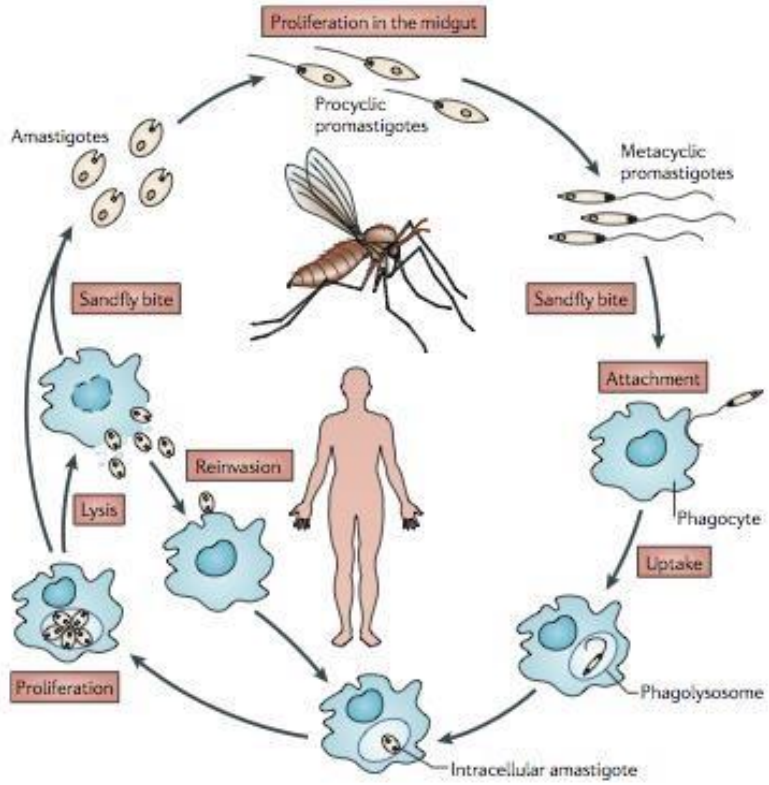

Fig. 1.0 Life cycle of the Leishmania parasite

The intracellular habitat of *Leishmania* parasites for almost the entire life cycle in man means that cell mediated immune mechanisms rather than antibody play the major role in determining natural host resistance. The induction of cellular immunity is therefore the primary goal of vaccination.

2.3 Rationale for a therapeutic $CD8^+$ T cell-inducing vaccine against leishmaniasis

Over the past two decades, prophylactic vaccination for leishmaniasis has been focused on eliciting $CD4^+$ Th1 T cells, rooted in the Th1/Th2 paradigm established with experimental models of cutaneous leishmaniasis. Despite success in rodent models, limited immunogenicity and/or negligible protection characterizes the outcome of most *Leishmania* vaccine trials conducted in primates or man[7]. Regulatory T cells, largely functioning through production of IL-10, appear to block effective responses to prophylactic vaccination[8]. In addition, new evidence suggests that current models for evaluating prophylactic vaccines may be inappropriate, lacking the essential factors associated with sandfly transmission that facilitate parasite establishment[9]. Although progress is being made to overcome some of

these obstacles (e.g. with new adjuvants and immunomodulators, including TLR agonists[10, 11] the clinical evaluation of prophylactic vaccines for CL, and more so for VL, will continue to pose significant practical difficulties.

Our alternative approach, of inducing and expanding host $CD8^+$ T cell responses, is rooted in a long history of research. Our work, along with that of others, has demonstrated the importance of $CD8^+$ T cells for primary resistance, resistance to re-challenge and vaccine induced resistance against experimental infection with *L. infantum* and *L. donovani*, the causative agents of VL / PKDL[12]. Following prophylactic vaccination against experimental VL, $CD8^+$ T cells are the major correlate of protection and, where tested, essential for efficacy. For example, the protection against *L. donovani* induced by both a kinetoplastid membrane protein-11 (KMP-11) -DNA vaccine and a hydrophilic acylated surface protein B1 (HASPB) protein vaccine was associated with strong antigen-specific CTL responses and IFN-γ generation[13-15]. Clinical studies also increasingly point to a host protective role for $CD8^+$ T cells. The frequency of activated $CD8^+$ T cells is substantially increased in asymptomatic and treated VL patients, compared to untreated controls, and *Leishmania* specific $CD8^+$ T cells have been identified as an important contributor to anti-leishmanial immunity in asymptomatic VL patients[16]. Direct evidence that a therapeutic vaccine targeting $CD8^+$ T cells may be both beneficial and achievable in VL stems from three sets of observations. First, we have directly demonstrated therapeutic vaccination in experimental models of VL, dependent upon induction of $CD8^+$ T cells[17]. Second, using a model of adoptive cellular immunotherapy, we have shown that CTL, as well as central and effector memory $CD8^+$ T cells can be re-activated in mice with ongoing VL, leading to reduced parasite burden[18]. Third, the pathology associated with established experimental VL is similar to that observed in human disease. Recent studies confirm the similarity of regulatory T cell responses in human and murine VL[19] and by adoptive transfer, we have shown that in spite of disruption to the splenic microenvironment, the capacity to prime $CD8^+$ T cells remains intact[18]. Collectively, therefore, these data demonstrate that both priming of naïve $CD8^+$ T cells and the activation of pre-existing effector/memory $CD8^+$ T cell responses can occur in the face of disease-associated pathology and of pre-existing, IL-10-mediated immune regulation. When present at an appropriate frequency, antigen specific $CD8^+$ T cells can thus provide therapeutic benefit. The challenge for therapeutic vaccination, therefore, is to similarly enhance $CD8^+$ T cell frequencies in man, breaking or bypassing any pre-existing regulatory control of $CD8^+$ T cell function. Viral vectors such as Adenovirus or MVA have been shown in several clinical settings to be the carriers of choice to induce $CD8^+$ T cells[20-22]. Currently available viruses can accommodate more foreign DNA than the sequences encoding either or both vaccine antigens proposed here.

PKDL is a human disease with no animal models, and hypotheses concerning pathogenesis have relied on clinical observations [reviewed in [4]]. Approximately 70% of PKDL patients in Sudan respond in the Leishmanin skin test and produce high levels of IFNγ but low levels of IL-10. These patients have a favourable prognosis, and either heal spontaneously or respond better to chemotherapy[5, 23]. In contrast, LST-negative patients with high IL-10 and low IFNγ do not heal spontaneously and are refractory to sodium stibogluconate. CD8+ T cells and Treg cells have also been detected PKDL lesions in Sudan, and Treg are seen in blood, declining sharply after successful treatment or spontaneous healing (El Hassan et al. unpublished data). Treg cells are able to suppress cytotoxic CD8+ cell function (through IL-10 and TGFβ), and may therefore prevent CD8+ T cells from eliminating *Leishmania* antigen-containing macrophages and epithelioid cells in the lesions. Consistent with this view, anergic CD8+ T cells with abundant PD1 mRNA and low expression of perforin and

Granzyme B are found in dermal lesions during Indian PKDL, alongside alternatively activated macrophages ([24] and Chatterjee, unpublished). CD8+ T cell anergy and / or exhaustion is also observed in VL, reinforcing the view that CD8+ T cells play a pivotal role in control of infection.

2.4 HASPB and KMP11 (KH) as vaccine antigens

We identified and selected two antigens (HASPB and KMP11) for which experimental protection data were most compelling and which were weakly if at all recognized by $CD8^+$ T cells during experimental or human VL[25]. HASPB is known to feature stretches of 11-14 amino acid-long repeats, and so to minimize any impact of isolate-specific variation in the repeat regions, we designed a synthetic HASPB gene comprising the conserved N and C termini flanking 10 repeats from the 17 repeats identified to date. These have been arranged to preserve their native order and re-iteration as observed in multiple Indian field isolates, and to maintain natural protein length. This unique gene was termed HASPB consensus. KMP11 is highly conserved. Both proteins were expressed from a single vector using the 2A sequence from TaV virus (http://www.st-andrews.ac.uk/ryanlab/2A_2Alike.pdf). During translation, the polyprotein is processed at the 2A sequence resulting in each protein being expressed as a single cleavage product. 2A sequences have been approved for use in human gene therapy by the FDA[26] but had not previously been used in human vaccines. Our pre-clinical and clinical data indicates that this novel approach has been highly successful.

To evaluate the efficacy of a pre-clinical adenoviral vectored polyprotein vaccine (termed here HuAd5-KH) as a stand-alone therapy, we evaluated parasite burden in the spleens of infected mice. The spleen is relatively refractory to drug treatment providing a severe therapeutic challenge, even for drugs established in the clinic. For example, administration of sodium stibogluconate (300mg/kg i.v) results in only 11-52% suppression of parasite burden and multiple doses at 8mg/kg of Ambisome (liposomal amphotericin B) cannot achieve greater than 80% efficacy [27, 28]. Hence, we set our criteria for effective single dose therapeutic vaccination as a suppression of splenic parasite growth of > 50%. The therapeutic efficacy of 10^9 pfu HuAd5-KH administered as a single dose i.d. was 66 ± 8% ($p=0.001$ vs. unvaccinated mice), with a response rate of 91% (21/23 animals tested). HuAd5-KH thus constitutes an effective stand-alone therapeutic intervention in this experimental model of infection. Vaccination also induced potent CD8+ T cell responses against multiple epitopes within the KH polypeptides[25].

2.5 Simian adenoviruses

The prevalence of immunity to human adenovirus prompted the consideration of simian adenoviruses as vectors for clinical studies. They exhibit hexon structures that are highly related to those of human adenoviruses (fig 2.0). Indeed, ChAd63 hexons are most similar in sequence to HuAd4 hexons previously used by the US military in mass vaccination campaigns where over 2 million adults received serially passaged adenovirus and which showed good safety and efficacy data[29]. Hexons are the major capsid proteins in adenoviruses; they are potently immunogenic and the main target of neutralising antibodies [30]. In chimpanzee adenoviruses the E1 locus can be deleted to render viruses replication deficient and allow transcomplementation on an E1 HuAd5 complementing cell line[31]. An additional attractive feature of the system is that the lack of sequence homology between HuAd5 and simian adenoviruses at the E1 flanking sequence prevents homologous recombination and production of replication competent virus.

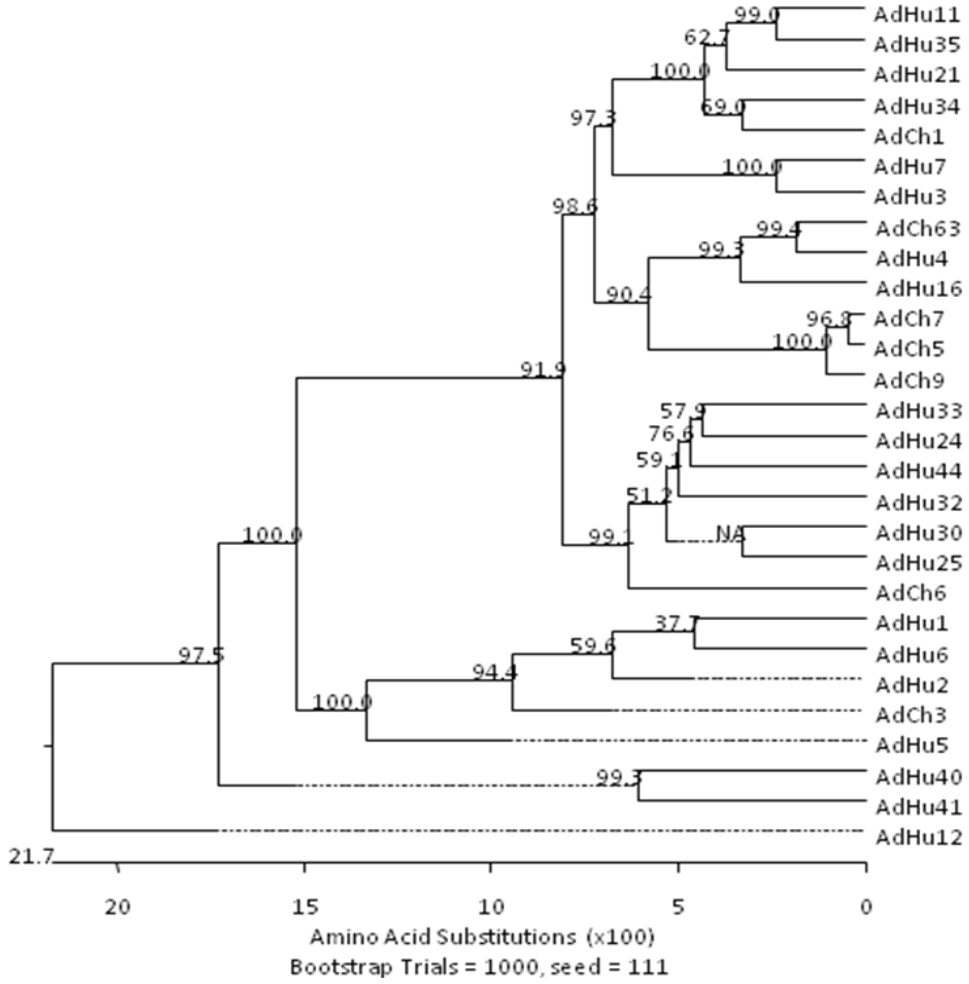

Fig 2.0: Phylogenetic of human and relationship chimpanzee adenovirus hexons

Simian adenoviruses are not known to cause pathological illness in humans and the prevalence of antibodies to chimpanzee adenoviruses is less than 5% in humans residing in the US. Early murine work using chimpanzee adenovirus 68, ChAd68, expressing *gag* of HIV-1 showed, that ChAd68 was as effective at generating a transgene product specific $CD8^+$ T cell response, with approximately 20% of all splenic $CD8^+$ being gag specific, in comparison with HuAd5 (9%) and poxvirus (<5%)[32]. In the same study, pre-exposure to HuAd5 abolished any protection offered by immunisation with HuAd5 but only slightly reduced that elicited by ChAd68. In a recent study in Kenya, 23% of children (aged 1-6 years) had high-titre neutralising antibodies to HuAd5, whilst only 4% had high-titre neutralising antibodies to ChAd63. Immunity to both vectors was age-dependent.

2.6 Human Clinical Trials with ChAd63

Since 2007 ChAd63 has been in use in a number of malaria vaccine trials conducted by three separate Chief Investigators at Oxford University. As of 2014, 566 healthy adults, 72 children and infants and 43 patients with chronic Hepatitis C infection have received at least one dose of ChAd63 incorporating a variety of vaccine inserts (Table 1.0, personal communication Stefano Colloca, Okairos, Italy).

Table 1.0: ChAd Based Clinical Trial Information

trial code (EudraCT number)	Phas e	antigen and Ad vector	dose range (Ad)	dose range (MVA)	location	n.volunteers	volunteer category	status
HCV001 (2007-004259-12)	I	HCV (NS3-5b) ChAd3, Ad6	$5x10^8$ to $7.5x10^{10}$ vp	n.a.	UK	41	healthy adults	completed
HCV002 (2008-006127-32)	I	HCV (NS3-5b) ChAd3, Ad6	$5x10^8$ to $2.5x10^{10}$ vp	n.a.	UK	32	HCV chronically infected patients (with or without concomitant IFN/RBV)	ongoing
HCV003 (2009-018260-10)	I	HCV (NS3-5b) ChAd3, MVA	$2z.5x10^{10}$ vp	$2x10^8$pfu	UK	15 11	healthy adults HCV chronically infected patients (with or without concomitant IFN/RBV)	ongoing
HCV005	I/II	HCV (NS3-5b) ChAd3, MVA	$2.5x10^{10}$ vp	$2x10^8$pfu	USA	175	Healthy intravenous drug users (IVDU)	ongoing
HIV-CORE002	I	HIVconsv ChAd63, MVA, DNA	$5x10^9$ to $5x10^{10}$ vp	$2x10^8$pfu	UK	26	Healthy adults	completed
VAC033 (2006-005966-37)	I	Malaria (METRAP) ChAd63, MVA	$1x10^8$ to $1x10^{11}$ vp	$2x10^8$pfu	UK	54	healthy adults	completed
MAL034 (2008-006804-46)	I/IIa	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$vp	$2x10^8$pfu	UK	43	healthy adults	completed
VAC036 (2007-004567-21)	I	Malaria (AMA1) ChAd63, MVA	$5x10^9$ to $5x10^{10}$ vp	$2.5x10^8$pfu	UK	16	healthy adults	completed
VAC037 (2009-012591-27)	I/IIa	Malaria (MSP1) ChAd63, MVA	$5x10^9$ to $5x10^{10}$ vp	$5x10^8$pfu	UK	16	healthy adults	completed
VAC038	I	Malaria (CS) ChAd63, MVA	$5x10^9$ to $5x10^{10}$	$2x10^8$pfu	Ireland	24	healthy adults	ongoing
VAC039 (2010-018341-56)	I/IIa	Malaria (AMA1, MSP1, METRAP) ChAd63, MVA	$5x10^{10}$vp	$1.25x10^8$ to $2x10^8$pfu	UK	38	healthy adults	completed
VAC040	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$2x10^8$pfu	Kenya	30	healthy adults (semi-immune)	completed
VAC041	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$1x10^8$ to $2x10^8$pfu	Gambia	16 24	healthy adults (semi-immune) children (2-6 years of age)	completed
VAC042	I	Malaria (METRAP) ChAd63, MVA	$1x10^{10}$ to $5x10^{10}$ vp	$1x10^8$ to $2x10^8$pfu	Kenya	24 (5-12mo) 24 (10 weeks)	Infants (2-12 months)	completed
VAC043 (2010-023824-26)	I	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$vp	$2x10^8$pfu	UK	42	healthy adults	ongoing
VAC044 (2010-024166-22)	I	Malaria (AMA1) ChAd63, MVA	$5x10^{10}$vp	$1.25x10^8$ pfu	UK	30	healthy adults	ongoing
VAC045	I/IIa	Malaria (METRAP, CS) ChAd63, MVA	$5x10^{10}$vp	$2x10^8$pfu	UK	30	healthy adults	completed
VAC046	IIb	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$vp	$2x10^8$pfu	Kenya	60	healthy adults (semi-immune)	ongoing
VAC047	IIb	Malaria (METRAP) ChAd63, MVA	$5x10^{10}$vp	$2x10^8$pfu	Senegal	60	healthy adults (semi-immune)	ongoing
LEISH1 2012-0005596-14	I	Leishmania (KMP11 + HASPB1, KMP11; ChAd63	$1x10^{10}$; $7.5x10^{10}$	NA	UK	20	Healthy adults	completed

Published data on completion of the trials is available for many of these trials using multiple different vaccine inserts - MSP1[33], AMA1[34] and ME-TRAP[35]. There were no unexpected or serious adverse events recorded in any of these trials. Most recently, ChAds have formed the basis for the development of new Ebola vaccines[36,37].

In the first of these of trials 16 participants received the vaccine ChAd63-MSP1. There were no unexpected or serious adverse events. 97% of the local and systemic AE's were mild in severity and all AE's completely resolved. The remainder of the AE's were recorded as moderate and were headache and pain at the site of the injection. The moderate AE's were all recorded in the higher dose arm of the study.

In the trial using the vaccine ChAd63-AMA 69% of the adverse events were recorded as mild and all adverse events completely resolved. 12 out of 16 participants experienced one or more local adverse events related to the vaccine and all were mild except 3 cases of arm pain (one case severe and two moderate), one moderate case of arm swelling and one moderate case of erythema. All moderate and severe adverse events occurred in the higher dose trial arm.

The final trial using the ChAd63 ME-TRAP insert recruited 54 participants of which 22 participants received intramuscular, and 32 intradermal vaccine. Over 90% of adverse events were mild in nature and the incidence of local adverse events was lower in those receiving intramuscular ChAd63.

2.7 LEISH1, a first-in-human trial of ChAd63-KH

Funded by the Wellcome Trust, we developed ChAd63-KH for human use, in collaboration with Okairos srl. The Investigational Medical Product was manufactured to cGMP by Advent Srl, Pomezia, Italy (395 vials at 7.5x1010 vp/ml; vp/ifu ratio = 115; 0.65ml per vial). MHRA/REC approval for a UK first-in-human clinical trial was granted in early 2013 and an open label Phase I dose escalation study was conducted at the York Clinical Research Facility (LEISH1; EudraCT No: 2014-0005596-14). 20 healthy UK adult subjects were enrolled and vaccinated with either $1x10^{10}$ (n=5) or $7.5x10^{10}$ vp (n=15) of ChAd63-KH. There were no early terminations of follow up and all subjects completed through to Day 90 post vaccination. Local and systemic AEs were of limited to Grade 1 or Grade 2, and none were categorized as serious. There were no SUSARs or SAEs. Three of five (60%) subjects had at least one local AE in the low dose group, compared to 11 of 15 (73%) in the high dose group. In total, 35 local adverse events (median = 1 event per patient) were reported. These were largely injection site reactions. Twenty-five events (71%) applied to the vaccinated arm, and only these events were defined as at least possibly related to vaccination. In addition, 4 of 5 (80%) subjects had at least one systemic AE in the low dose group, compared to 13 of 15 (87%) subjects in the high dose group. In total, 64 systemic adverse events (median = 3 events per patient) were reported, 28 of which (44%) were defined as at least possibly related to vaccination. Transient lymphopenia (>25% of baseline) was observed at 24h in 14/15 high dose subjects, compared to a moderate transient lymphopenia (<25% of baseline) in 2/5 low dose subjects and 1/15 high dose subjects. Full details of AEs reported in this trial are available in the LEISH1 end of trial report.

Immunogenicity measured by CD8+ T cell production of IFNγ (ELISPOT) was observed in 5/5 and 11/15 subjects in the low and high dose arms respectively. Immune response followed a similar kinetic as seen in other vaccination studies (with expansion and contraction phases) and most subjects responded to greater than one of the peptide pools used to span the entire KH antigen. Whole blood transciptomic profiling (WBTP) was used to analyze the innate immune response to vaccination and discover underlying mechanisms associated with dose dependent immunogenicity of this vaccine.

2.7 Dose justification for LEISH2a

Neither ChAd63-KH nor any other ChAd vectored vaccine has been administered previously to patients with PKDL. We have therefore elected to commence with the lowest of these doses (1 x 10^{10}vp in 1 ml) and following satisfactory review of safety data and adverse events by the DSMB, we will increase the dose to 7.5x10^{10}vp which is the maximum concentration obtainable in 1ml (the maximum desirable amount to be injected into the deltoid) from the produced vaccine.

3.0 OBJECTIVES

3.1 Primary Objective

To assess the safety of a new candidate Leishmania vaccine ChAd63-KH in patients with persistent PKDL. The first eight adult volunteers will receive 1x10^{10}vp the subsequent eight adult volunteers will receive 7.5 x10^{10}vp. Eight adolescents will be vaccinated with a single dose selected by review of all available clinical data. Doses will be administered at a single time point to patients under hospital care. DSMB will review data between dose escalation and before vaccinating adolescents.

The specific end points for safety will include the exclusion of reversion to VL which will be specifically monitored for and assessed according to criteria detailed in section 8.1. Safety will be measured by assessing adverse event data collected through history, clinical examination, blood tests and if necessary tissue specific parasitological confirmation.

3.2 Secondary Objectives

A. To compare the humoral and cellular immune responses generated by the candidate vaccine in patients with persistent PKDL.

The specific immunological end points will be measures of T cell, B cell and innate immunity induced by the vaccine e.g. as measured by ELISPOT, flow cytometry, ELISA and/or transcriptomics

B. To observe any clinical changes in the cutaneous PKDL disease over a 42 day period following vaccination.

The severity, extent and distribution of the PKDL disease will be measured by the following standardized grading system, and image capture.

Grading system for PKDL:

1. Scattered maculopapular or nodular lesions, mainly around the mouth

2. Dense maculopapular or nodular rash covering most of the face and extending to chest, back, upper arms and legs
3. Dense maculopapular or nodular rash covering most of the body, including hands and feet.

4.0 INVESTIGATIONAL PRODUCT

4.1 Vaccine

The first 8 adult volunteers will receive a single intramuscular dose of ChAd63-KH $1x10^{10}$ vp and the subsequent 8 adult volunteers will receive a single intramuscular dose of ChAd63-KH $7.5x10^{10}$ vp. The 8 adolescents will receive either 1 or $7.5x10^{10}$ vp based on evaluation of all available clinical data.
The vaccine is manufactured by Advent, Via Pontina, Pomezia, Rome, Italy.

4.2 Product and administration

The IMP will be stored in below -60°c.
Prior to administration the IMP will be removed from dry ice and allowed to defrost to room temperature. Within one hour of the IMP being removed from the freezer it will be administered to the volunteer. The low dose IMP (ChAd63-KH $1x10^{10}$vp) will be diluted with NaCl 0.9% according the pharmacy worksheet. The date and time of administration will be recorded in the CRF.

The dose volume will be injected into the deltoid muscle of the upper arm using a 21-23 gauge needle long enough to reach deep into the muscle. The needle will be inserted at an angle of approximately 90° to the skin. Volunteers receiving the vaccine will be asked which arm they would like to be injected. Full in-patient clinical care will be available during and immediately after vaccination.

The injection site will be covered with a light dressing. This will be removed before the volunteer leaves hospital.

4.3 Accountability for used and unused supplies

The CI must ensure that all IMP supplies are kept in a secure area accessible only to authorised individuals and maintained in temperature-controlled storage as per the IB.

Upon receipt of the vaccine, a designated member of staff will check shipping conditions, organise storage and acknowledge receipt to the supplier, according to the defined SOP. The IMP will be managed and handled in accordance with the relevant SOPs.

A record must be kept of all IMP used during the trial. This will include the description (lot numbers and expiry dates) and quantity of IMP received at trial site and date of receipt, as well as a record of when (date of administration) and to whom (volunteer number) it was dispensed.

At the end of the trial, IMP accountability will be checked by the member of the research team and trial monitor. The Sponsor and the CI will retain copies of the complete IMP accountability records.

All supplies (used and unused) will be retained at the trial sites until the Sponsor gives instructions for their return/destruction as specified in the technical agreement.

4.4 Non-trial treatment

Volunteers must not have had non-trial immunisation with live attenuated vaccines from 60 days prior to their screening assessment and other vaccines from 14 days prior to their screening assessment. Volunteers must also agree to avoid all non-trial immunisation in the 14 days following vaccination with the trial vaccine.

All concomitant medication will be recorded in CRF including any dispensed by a clinical investigator in the management of adverse events.

5.0 TRIAL DESIGN

5.1 Study Centre

The clinical study will be carried out at Professor El-Hassan's Centre for Tropical Medicine, Dooka

5.2 Study Design

This is an open label phase IIa study of a candidate Leishmania vaccine in patients with persistent PKDL.

5.3 Study Volunteers

There will be two study groups, one consisting of 16 adult patients (male and female volunteers aged 18 to 50 years) and one consisting of 8 adolescents (12-17 male and female). All subjects will be willing and able to adhere to the trial conditions, methodology and to give written informed consent. For more details refer to section 6.0. All subjects will be hospitalized 1 day prior to vaccination and will remain in hospital until d7 post vaccination.

5.4 Trial Intervention

The first 8 adult volunteers will receive a single intramuscular dose of ChAd63-KH, $1x10^{10}$ vp and the subsequent 8 adult volunteers will receive a single intramuscular dose of ChAd63-KH $7.5x10^{10}$ vp. The 8 adolescents will receive one or other dose after consideration of all data by the DSMB. There is no control or placebo arm to the trial.

5.5 Primary and Secondary Endpoints

The primary endpoints for safety and reactogenicity will be actively and passively collected data on adverse events, and the exclusion of reversion to VL. The secondary endpoints for immunogenicity will be markers of humoral and cell-mediated immunity. The endpoints for severity of PKDL will be measured using a grading system.
Data on safety will be solicited on questioning and on examination of participants. Data on laboratory events will be collected through routine testing or in response to a clinical event.

5.6 First Volunteer

The first volunteer (receiving ChAd63–KH $1x10^{10}$ vp) will be vaccinated and monitored over the subsequent 7 days under hospital care and then as an outpatient until d90 post

vaccination. Clinical data up to day 21 post vaccination will be reviewed by the TMG to assess reversion to VL (diagnostic criteria: fever, splenomegaly and/or hepatomegaly and/or lymphadenopathy with pancytopenia, drop in serum albumin, loss of weight with demonstrable LD bodies in the lymph nodes or bone marrow aspirate, negative blood film for malaria, and exclusion of other febrile illnesses). In the absence of any safety concerns the subsequent seven volunteers will be vaccinated with the same dose. No more than 2 volunteers will be vaccinated on any given day and no volunteers will be vaccinated simultaneously.

The first DSMB meeting after the trial starts will be planned to take place after the first eight volunteers have received the vaccine and have attended their Day 21 safety visit.
After this meeting the first volunteer receiving ChAd63–KH $7.5x10^{10}$ vp will be vaccinated. The first volunteer receiving ChAd63–KH $7.5x10^{10}$ vp will follow the same safety procedure as above before the further 7 volunteers receive ChAd63-KH $7.5x10^{10}$ vp.

5.7 Duration of Study
All volunteers will attend for a screening assessment up to 28 days prior to enrolment. The follow up visits are as described in section 7 with a final visit 120 days after vaccination.

5.8 Definition of the Start and End of the Trial
The start of the trial is defined as the date of the screening visit of the first volunteer. The end of the trial is defined as the date of the last day 120 visit of the last volunteer.

5.9 Trial Procedures
All trial procedures will be carried out according to the appropriate SOPs.

6.0 RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

6.1 Number and Source of Volunteers
We aim to recruit 24 patents with persistent PKDL for the study. Volunteers will be recruited by active case detection.

6.2 Inclusion and Exclusion Criteria
This study will be conducted in patients who meet the following inclusion and exclusion criteria.

Inclusion Criteria

Adults
The volunteer must be:

- Aged 18 to 50 years on the day of screening
- Females must be unmarried, single, or widowed
- Willing and able to give written informed consent

Adolescents

- Aged 12 to 17 years on the day of screening
- Female adolescents must be unmarried

- Written informed consent must be obtained from a parent

All Participants

- Uncomplicated PKDL of > 6 month's duration
- Available for the duration of the study
- In otherwise good health as determined by medical history, physical examination, results of screening tests and the clinical judgment of a medically qualified Clinical Investigator
- Negative for malaria on blood smear
- Judged, in the opinion of a medically qualified Clinical Investigator, to be able and likely to comply with all study requirements as set out in the protocol
- Willing to undergo screening for HIV, Hepatitis B and Hepatitis C
- For females only, willing to undergo urinary pregnancy tests on the day of screening, on the day of vaccination (prior to vaccination) and 7 and 42 days after vaccination.

Exclusion Criteria

The volunteer may not enter the study if any of the following apply:

- Has mucosal or conjunctival PKDL
- Has had treatment for PKDL within 21 days
- Is negative for antibodies in the RK39 strip test
- Receipt of a live attenuated vaccine within 60 days or other vaccine within 14 days of screening
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine or a history of severe or multiple allergies to drugs or pharmaceutical agents
- Any history of severe local or general reaction to vaccination as defined as ○ Local: extensive, indurated redness and swelling involving most of the antero-lateral thigh or the major circumference of the arm, not resolving within 72 hours
    - ○ General: fever ≥ 39.5°C within 48 hours, anaphylaxis, bronchospasm, laryngeal oedema, collapse, convulsions or encephalopathy within 48 hours
- Females – pregnancy, less than 12 weeks postpartum, lactating or willingness/intention to become pregnant during the study and for 3 months following vaccination.
- Seropositive for hepatitis B surface antigen (HBsAg) or Hepatitis C (antibodies to HCV)
- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months
- Tuberculosis, leprosy, or malnutrition (malnutrition in adults defined as a BMI <18.5, and in adolescents (12-17yrs) as a Z score cut-off value of <-2 SD).
- Any other significant disease, disorder or finding, which, in the opinion of a medically qualified Clinical Investigator, may either put the volunteer at risk because of participation in the study, or may influence the result of the study, or the volunteer's ability to participate in the study

- Unlikely to comply with the study protocol

6.3 Screening Procedures and Investigations

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The following general eligibility questions will be discussed prior to conducting informed consent:

- Age
- Availability for the duration of the study
- General health state
- Allergy status
- History of Leishmaniasis infection
- Contact details

At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The Chief Investigator (or a study physician in accordance with the delegation log) has both an ethical and a legal responsibility to ensure that each volunteer being considered for inclusion in the study is given a full explanation of the study. A check of eligibility will be conducted and any questions about the study will be answered. If the volunteer is still willing and interested they will be asked to sign and date three copies of Consent Form 1 - one for the volunteer to keep, one to be stored in the volunteer's case file and one to be placed in the Trial Site File.

To ensure informed consent, the following information will be provided by a member of the study team

- Pre-HIV test risk assessment and discussion
- That it is unknown whether or not the study vaccine will cure them from their Leishmania infection, and that subsequent drug treatment will be given as required, either at d42 post vaccination, or, if the patient wishes for any reason to delay standard of care treatment, at the penultimate follow-up visit at D90.
- The importance of continued follow up in the study if the volunteer is enrolled into the study and receives the vaccine to monitor any unforeseen events will be stressed

After informed consent has been obtained, assessments and investigations will be undertaken according to the schedule. These include;

- Medical history,
- Vaccination history
- General Examination
- Collection of urine for urinalysis and, if female, a pregnancy test
- Blood samples for routine laboratory investigations (haematology and biochemistry) and the blood borne viruses Hepatitis B, Hepatitis C and HIV.
- Sample for InBios Kalaazar Detect (Leishmania antibody test)

A record of the above procedures and any findings will be entered into the Case Report Form (CRF).

Each volunteer who enters the trial by signing a copy of the consent form will be assigned an Identification Number. These numbers will not be reassigned. A screening log of screened volunteers with their identification number and demographic will be maintained

and kept in the in the trial site file to track volunteers who have been screened for the study. If the volunteer is not enrolled into the dosing part of the trial, study staff will document the reason for not enrolling in the screening log.

A photograph will be taken for identification purposes and will be placed in the volunteer's file only. Photographic records of lesions pre and post vaccination will also form part of the clinical file. Any photographs of the whole face will have the eyes blacked out.

Following the screening visit a medically qualified Clinical Investigator will review the results of each volunteer to determine if they are eligible for further involvement in the study. The Chief Investigator (or a medically qualified clinical investigator in accordance with the delegation log) must confirm eligibility for each volunteer based on the screening assessment. This must be documented in the CRF.

6.4 Eligibility and Screening failures

A medically qualified Clinical Investigator must confirm eligibility for each volunteer based on the screening procedures, including findings from clinical histories, examinations, laboratory results and GP report. This must be documented in the CRF. If the volunteer is deemed to be eligible the dosing visit can be arranged.

If for any reason the volunteer is considered a screen failure the volunteer will be contacted by a Clinical Investigator, notified of all of their results and the reason for the screen failure. Volunteers failing screening will be treated with SOC.

6.5 Re-Screen

If the investigator believes that one or more of the laboratory screening test results are an anomaly and temporary (as described in MHRA Good Clinical Practice guidelines; 1st Edition 2012, Section 11.4.3 - page 377), then a re-screen may be carried out.
The reason for carrying out a re-screening must be clearly documented in the CRF and subjects may only be re-screened once.
If, following a re-screen, any laboratory parameter falls outside of the inclusion range for the study then the subject will be excluded from the study and no further re-screening should take place.
If a re-screening takes place due to out-of-range results in haematology, biochemistry and urinanalysis, **all** these tests must be repeated, not simply the out-of range result.

6.6 Pregnancy

If a volunteer is found to have a positive pregnancy test at the screening visit or prior to receipt of the vaccination they will be immediately considered a screen failure. The volunteer will be given the result by a Clinical Investigator

If a female volunteer become pregnant during the 90 days after vaccine administration, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. The pregnancy will be reported to the Sponsor in accordance with the SOP for Research Related Adverse Event Reporting Procedure.

6.7 Procedure for follow-up of adverse events and pregnancy

The Clinical Investigators will make every effort to monitor all adverse events, regardless of severity, until resolution or stabilisation, in order to report this on the CRF during the trial.

After the database is locked and the trial is closed, any additional information about adverse events or pregnancy that comes to the attention of a Clinical Investigator will be reported by email to the Chief Investigator and the Sponsor.

7.0 TREATMENT OF TRIAL VOLUNTEERS

7.1 Study procedures

Procedures will be performed on the visit time points indicated in the schedule of procedures. Additional procedures or laboratory tests may be performed, at the discretion of a Clinical Investigator if clinically required.

Observations which will be documented are blood pressure, pulse rate and temperature

Blood tests will be drawn for the following laboratory tests:

- Haematology: Haemoglobin, White blood cells, Neutrophils, Lymphocytes, Platelets
- Biochemistry: Creatinine, ALT, AST, Albumin, Total Bilirubin, Direct Bilirubin
- Blood Borne Virus Screen: Hepatitis B surface antigen, HIV antibodies, Hepatitis C antibodies,
- Inbios Kalaazar Detect (RK39 Leishmania antibody test) will be conducted at the trial site.
- Immunology: KMP HASPB antibodies, microarray bloods and cellular responses
- RDT for Malaria parasite at day 0. If positive appropriate treatment will be given.

Urinalysis dipstick will be conducted at the trial site for the presence of leucocytes, protein and blood. In female volunteer's urine will be tested for beta-human chorionic gonadotrophic (HCG) at screening, at the vaccination visit (prior to vaccination), at visit 5 and at the final study visit.

General examination which will be performed will include cardiovascular, respiratory, neurological, inspection of administration site and palpation of axillary and cervical lymph nodes.
Abdominal examination with emphasis on spleen and liver size below costal margins at screening and follow up visits will be performed and recorded

Height and Weight will be measured at the screening visit and at visit 8.

7.2 Schedule of Visits

The schedules of visits are presented below in Table 2.0.

Volunteers will be required to stay in hospital for 7 days and be monitored as an outpatient up to 42-days post vaccination. Time 0 will start on the day of vaccination. Volunteers with remaining PKDL at D42 will be asked if they wish to treated at that visit, and if so, admitted to hospital for SOC (usually 20 days). If they would prefer to delay their treatment until D90 they will have this option, and then be admitted at D90 for SOC. A final outpatient visit will be made at day 120 post vaccination.

Visit 1: Screening and Enrolment
All potential volunteers will have a screening visit which can take place between 1 to 28 days before the vaccination. Informed consent will be undertaken at the screening visit before any screening procedures. The screening procedures will be undertaken as indicated in the schedule below. Volunteers will be considered enrolled once informed consent has been taken.

If participants test positive for malaria at screening, then they will be treated with SOC and can be re-screened after 7 days.

Visit 2: Vaccination
The eligibility of the volunteer will be reviewed following the screening assessment once all of the results from the screening visit have been considered. If eligible, a day 0 visit will be scheduled for the volunteer to receive the vaccination. A further assessment of eligibility will be conducted on the day of vaccination prior to the volunteer receiving the vaccine, Repeat blood tests (and pregnancy tests for females) are **only** not required if the vaccination visit takes place **within 24 hours** of the **initial screening visit or repeat screening visit**. The importance of continuing to attend follow up visits after receipt of the vaccine to monitor any unforeseen events will again be stressed to the volunteer. Blood will also be taken for exploratory immunology analysis as detailed in Table 2.0.

The vaccination will be into the deltoid muscle of an arm of the volunteers choosing. Following vaccination the area of skin injected will be covered with a small light dressing and this will be removed after at least one hour. The dressing will be disposed of in line with local recommendations.

All volunteers will be issued with a study volunteer identity card and encouraged to contact the research team if there are any problems.

Volunteers will be required to stay in hospital for the first 7 days post vaccination. During this time, observations will be performed 10 minutes, 60 minutes and 120 minutes and then at 24 hrs, 3 days & 7 days after vaccination.

Outpatient Follow Up Visits
Further visits will take place at 21, 42, 90 and 120 days after vaccination. These will be compliant with the protocol if they take place +/-3 days (d21 & 42) or +/-10 days (day 90) either side of the target date determined by the date of vaccination.

Volunteers will be assessed for local and systemic adverse events using a focused history, physical examination and malaria blood tests. Blood will also be taken for exploratory immunology analysis as detailed in Table 2.0.

Treatment of PKDL as Standard of Care

The PKDL rash will be assessed at Visit 7, 42 days after vaccination, in the same manner as at previous visits. If there is a <75% improvement in the degree of PKDL then the patient will be offered standard PKDL treatment at no cost of 20 days liposomal Amphotericin B. If there is a 75-90% improvement in the degree of PKDL then the CI in consultation with the patient will decide on conservative treatment or Amphotericin B. In general, with >90% improvement then no further intervention will be recommended. If the patients wish to delay this treatment until D90, they will have the option to do so.

Visit 10 Follow-up

The last trial follow up visit will be at day 120 +/- 10 days either side of the target date determined by the date of vaccination.

The volunteers will be assessed for local and systemic adverse events using a focused history, physical examination and routine haematology and biochemistry blood tests. A urine sample will be taken for urinalysis from all volunteers.

Table 2.0: Schedule of Visits

	Screening	Vaccination[d]	In patient monitoring	In patient monitoring	In patient monitoring	Outpatient Follow up	Outpatient Follow up	Outpatient D90	Outpatient D120
Visit / Observation Number	1	2	3	4	5	6	7	8	9
Timeline (Days)	-	0*	1	3	7	21	42	90	120
Window	-28 to -1 days	-	+/-3 hours	+/- 1 days	+/- 1 days	+/- 3 days	+/- 3 days	+/- 10 days	+/- 10 days
Vaccination		X							
Medical History/Adverse event reporting[1]	X	X	X	X	X	X	X	X	X
General Examination, vital signs	X	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X
Height and weight	X								X
Examination of administration site		X[b]	X	X	X				
PKDL examination, grading & recording	X	X	X	X	X	X	X	X	X
Urinalysis	X	X						X	X
Urinary pregnancy test (females only)	X	X[d]			X			X	X
Haematology & Biochemistry (5ml)	X	X[d]	X		X			X	X
Blood Borne Virus Screen(2.5ml)	X						X		
InBios Kalaazar Detect (Leishmania antibody test) (1ml)	X								

Microarray bloods (2.5ml)		X[c]	X	X	X				
Cellular Responses (10ml)		X				X (15ml)	X	X	
Serum (2.5ml)		X					X		
Malaria RDT (0.5ml)	X					X	X	X	X
Blood Volume Per Visit	9.0ml	20.0ml	7.5ml	2.5ml	7.5ml	15.5ml	15.5ml	15.5ml	5.5 ml

* admission to hospital day before vaccination day

[a] Full general examination only if required

[b] At vaccination, examination of administration site and recording of observations will be done at 10 minutes, 60 minutes and 120 minutes post vaccination.

[c] Microarray blood sampling will be performed pre-vaccination.

[d.] Repeat haematology and biochemistry, as well as repeat urine pregnancy test if female, do not need to be performed **if vaccination is within 24 hours** of the initial or repeat screening tests**.**

7.3 Additional Visits

Additional visits and assessments may be required to evaluate an adverse event and/or to identify a diagnosis. As per normal SOC, a biopsy might be necessary to provide a differential diagnosis of macular lesions, at some stage of the study. These visits and assessments are compatible with the protocol.

8.0 ASSESSMENT OF SAFETY

The Clinical Investigators are responsible for the accurate recording of all AEs, SAEs, & SUSARs. The Clinical Investigators are also responsible for reporting of all SAEs and SUSARs to the CRO, ClinServ, and the Sponsor, the University of York, within 24 hours as detailed in 8.4.

For all adverse events, the Clinical Investigators will take appropriate action to ensure the safety of all volunteers and staff in the study. All adverse event reporting will be carried out in accordance with the Study-Specific Adverse Event Reporting SOP.

8.1 Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer who has consented and is participating in a clinical trial of an investigational medicinal product (in this case the study vaccine), including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease occurring during such a clinical trial of an investigation medicinal product whether or not considered related to the IMP.

Adverse Drug Reaction (ADR)

An ADR is any untoward or unintended response in a volunteer to an investigational medicinal product which is related to any dose administered to that volunteer. This means that a causal relationship between the study medication and an AE is at least a reasonable possibility.

Unexpected Adverse Reaction

An unexpected adverse reaction is where the nature or severity is not consistent with the Investigator's Brochure.

Serious Adverse Event (SAE) or Adverse Drug Reaction

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the vaccine.

- Death (i.e. results in death from any cause at any time)
- Life-threatening event (i.e the volunteer was, in the view of a clinical investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.

- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.
- Congenital anomaly or birth defect.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an AE that is both unexpected and serious and thought to be related to the investigational product. There are no serious adverse reactions expected in this study and so any such event would be unexpected.

Adverse Drug Reactions

Various local and systemic adverse events are known to be associated with licensed vaccines and are referred to as “solicited adverse events”. These include disturbances in routine laboratory parameters and are described in the table below.

Information on solicited adverse events will be collected on the day of vaccination and at follow up visits as defined in the schedule. AEs will be recorded by hospital staff through direct questioning and examination. Events will be graded according to the toxicity table in Appendix 1. In addition systemic laboratory adverse events will be collected through routine laboratory testing according to the schedule. These will be recorded on the standard laboratory report The information obtained will be recorded in the volunteers file.

Reversion to Visceral Leishmaniasis

There is a possibility that the leishmania vaccine administration might cause ‘reversion’ of the cutaneous leishmanial disease into visceral disease. This possibility is judged by the investigators to be remote. Nevertheless, such a development would constitute a serious safety issue. Therefore, we wish to institute very rapid review of the first vaccine recipients to exclude this possibility. The criteria for such ‘visceralisation’ are described above in section 5.6 (p.21), and will be judged by the Clinical Investigators at the trial site. We will organise Trial Management Group (TMG) teleconferences as soon as subject 1, and then subsequently subject 3, have reached 21 days post-vaccination to review the clinical data and exclude the possibility of visceralisation. A TMG could be organized at any other time for similar considerations. If the consensus view of the TMG was that the clinical data supported the possibility of visceralisation, this would require the CI to immediately seek the formal opinion of the DSMB.

Table 3.0: Solicited Adverse Events

Site	Adverse Event
Local - at the injection site	Pain
	Discomfort
	Redness
	Swelling (soft)
	Induration (hard)
	Warmth
	Itch
	Blisters or scaling
Systemic	Fever
	Chills
	Malaise
	Myalgia
	Headache
	Nausea
	Splenomegaly
	Hepatomegaly

8.2 Causality Assessment

For every AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by a Clinical Investigator. An interpretation of the causal relationship of the intervention to the AE in question will be made using clinical judgment, based on the type of event; the relationship of the event to the time of vaccine administration and alternative causes such as intercurrent or underlying illness and concomitant therapies. The Investigator will also consult the Investigator Brochure.

Table 4.0: Guidelines for assessing the relationship of vaccine administration to an AE

0	No Relationship	Adverse events that can be clearly explained by extraneous causes and for which there is no plausible association with study product. Or adverse events for which there is no temporal relationship
1	Unlikely	Adverse events that may be temporally linked but which are more likely to be due to other causes than the study product
2	Possible	Adverse event that could equally well be explained by the study product or other causes, which are usually temporarily linked. Or of a similar pattern of response to that seen with other vaccines
3	Probable	Adverse events that are temporarily linked and for which the study product is the more likely explanation than other causes. Or known pattern of response seen with other vaccines
4	Definite	Adverse events that are temporarily linked and for which the study product is the most likely explanation. Or known pattern of response seen with other vaccines

8.3 Reporting Procedures for All Adverse Events

All adverse events occurring during the study observed by a Clinical Investigator or reported by the participant whether or not attributed to study medication will be reported in the CRF. The severity of clinical and laboratory adverse events will be assessed according to the AE grading located in the appendix section of the protocol.

8.3.1 Follow up

All adverse events that result in a participant's withdrawal from the study or that are present at the end of the study, will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned.

8.3.2 Ongoing and end of trial reporting

At the conclusion of the study all AE's/AR's must be subject to statistical analysis and that analysis and subsequent conclusions will be included in the final study report.

One year following the granting of a Clinical Trials Authorisation, and thereafter annually, the CI will send an Annual Safety Report to the:

- Sponsor
- Relevant Sudanese competent regulatory authorities (Sudanese National medicines and Poisons Board)
- Research ethics committee that granted approval

8.4 Reporting Procedures for Serious Adverse Events and SUSARs

The Investigator will complete an SAE report form and notify ClinServ and the Sponsor immediately (within 24 hours) of becoming aware of an SAE or SUSAR. ClinServ will assess such an adverse event, seek further clarification from the clinical investigators if necessary, and report

to the Sudan National Medicines and Poisons Board within 48 hours of the occurrence of the event.

8.5 Withdrawal of Volunteers

A volunteer has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. A Clinical Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. If withdrawal is due to an adverse event, appropriate follow-up visits or medical care will be arranged until the adverse event has resolved or stabilised. If a volunteer is considered to have failed the screening assessment or withdraws from the study at any time, either by choice or on the recommendation of clinical personnel, data and samples collected up to that point will remain available for analysis as part of the study.

If a volunteer who has withdrawn from the study requests for their existing, un-analysed samples to be destroyed or for their data to not be included in reports/ statistical analyses, the CI will take responsibility for ensuring that appropriate action is taken.

There will be no replacement of volunteers withdrawn from the trial.

8.6 Pregnancy Reporting

If a female volunteer becomes pregnant during the 90 days after vaccine administration, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. The pregnancy will be reported to the Sponsor in accordance with SOP for Research Related Adverse Event Reporting Procedure

8.7 Temporarily Interruption or Discontinuation of the Study

Definitions:

"Discontinuation of vaccination" is the permanent withholding of further vaccinations from all or some study groups in the trial.

"Interruption of vaccination" is the temporary withholding of further vaccinations.

"Dose escalation" refers to the decision to progress from low dose to a higher dose. This progression is from one dose level to another.

"Dose limiting toxicities" refer to AEs that preclude further dose escalation or discontinuation of vaccination.

Safety Review Prior to Dose Escalation

The DSMB will meet before the trial starts and agree Terms of Reference.
The first meeting after the trial starts will be planned to take place after the first eight adult volunteers have received the vaccine and have attended their day 21 safety visit.

All Adverse Events will be reported to the Sponsor after the first eight adult volunteers have attended their day 7 and 42 safety visits.

Once dose escalation has occurred the frequency of adverse events reporting will be agreed by the DMSB at the dose escalation meeting. Serious Adverse Events will continue to be reported as soon as possible, and not exceeding 24 hours, after the event, following the SAE procedure described in section 8.4. Immediate reporting will allow the Sponsor to take appropriate measures to address any potential new risks.

Clinical Criteria for Interruption or Discontinuation of Vaccination

If any of the following occur, interruption or discontinuation of all further vaccination will take place and the Sponsor will be notified within 24 hours.

(1) Death in any subject in which the cause of death is judged to be possibly, probably or definitely related to study vaccine
(2) The occurrence in any subject of an anaphylactic reaction to study vaccine
(3) If 2 or more participants experience an unexplained, unexpected grade 3 or 4 clinical or laboratory event (confirmed on attendance or repeat testing) that has not resolved within 72 hours and considered possibly, probably or definitely related to vaccine.
(4) Evidence of reversion to visceral leishmaniasis that is regarded as clinically conclusive in 1 or more subjects
(5) Evidence of worsening of the PKDL disease at 21 days post-vaccine that is regarded as clinically conclusive in 2 or more subjects

The Sponsor will notify the CTSC and the CTSC and / or the Sponsor will determine whether or not to call an unscheduled meeting of the DSMB to review the safety data, and whether or not to hold further immunisations until this has taken place.

If an unscheduled DSMB is warranted, the Sponsor will be informed and the DSMB asked to make a recommendation to the Chief Investigator and the Sponsor about continuing further immunisations.

If the study is halted or stopped for a reason involving risk to a volunteer's health or safety then an Urgent Safety Measure will be implemented in accordance with the Safety Reporting SOP. If the study is halted or stopped for any other reason the CI or Sponsor representative will notify the National Medicines and Poisons Board not later than 15 days from the date of the halting of the study in accordance with Safety Reporting SOP.

Restarting the study will be a substantial amendment.

8.8 Sponsor

The Sponsor(s) reserves the right to terminate the study at any time.

9.0 STATISTICS

9.1 Sample Size

This is an observational safety study of a first-in-patient Leishmania vaccine, where 24 volunteers will be vaccinated with ChAd63-KH.

For LEISH2a, results will be presented for all patients, those administered the low dose and those administered the higher dose by age group (adult or adolescent). There is no intention to directly compare these groups against a non-vaccinated control group. Whilst comparisons between the different dose and age groups may be made, there is no aim to show statistically significant differences from any such comparisons.

As this is the first time this has been administered to humans with ongoing persistent PKDL this sample size should allow initial assessment of safety outcomes and determination of the magnitude of the outcome measures, rather than aiming to obtain statistical significance. For these reasons no formal statistical assessment has been conducted in terms of sample size. The number of participants in each part of the study is typical for early vaccine studies and is considered sufficient to achieve the objectives of the study.

9.2 Statistical Analysis Plan (SAP)

An SAP will be produced by the study statistician before any analysis of the study data.

10.0 MANAGEMENT OF DATA, SAMPLES AND TRIAL PROCEDURES

10.1 Source Data and Case Report Forms (CRFs)

Consenting volunteers will be allocated a CRF, however, the hospital notes will also act as the source data. The CRF will hold personal identifiable information on the volunteer, including name, address, and date of birth and volunteer trial number. The volunteer's file will be held at the clinical site in a secure location. Permission will be obtained as part of the informed consent process to allow the research team and other responsible individuals access to the volunteers' trial records.

Data collected directly from the volunteer or from medical examinations will be entered directly into the CRF. All laboratory reports will be filed in the CRF after review and sign off by a medically qualified Clinical Investigator. Data collected at the clinical site will be transcribed directly onto case report forms. The type of data to be recorded in the CRF will be in line with the details provided in the study schedule section. Appendix 6 provides details of what constitutes source data in this trial. CRF's will be identified with volunteer trial number and initials. No personal identifiable information will be sent outside of the hospital.

The CRO will undertake data management responsibilities as delegated by the University of York, which include the provision of study database, data entry and validation procedures. The CRO will work with the trial team to draft the CRFs. Local appropriately trained staff (named on a delegation log) will complete and send CRFs to the CRO for entry into the study database according to the study SOPs.

10.2a Screening Log

A screening log containing hospital numbers, name, date of birth and whether the volunteer was enrolled into the dosing part of the study will be kept in the trial site file which will be kept in a secure location at the trial site, with access restricted to study staff and monitors only.

10.2b Volunteer Log

A volunteer log containing hospital numbers, trial number, name, date of birth and whether the volunteer was enrolled into the dosing part of the study will be kept in the trial site file which will be kept in a secure location at the trial site, with access restricted to study staff and monitors only.

10.3 Access to Data

The investigators will maintain appropriate medical and research records for this trial. The Chief Investigator, co-investigators and clinical research nurses will have access to records. The investigators will permit authorised representatives of the sponsor(s), and regulatory agencies to

examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

10.4 Data Protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the sponsor.

10.5 Archiving of Data

All study documents will be securely stored for a minimum of 15 years after the close of the trial in accordance with SOP on Archiving of Research Study Documents.

10.6 Confidentiality

Volunteers will be identified only by their trial volunteer number, initials and date of birth on any documentation or samples that leave the trial site. No personal identifiable data will be stored with external organisations. Volunteers will not be identifiable in any study report or publication.

10.7 Management of Biological Samples

Blood samples taken at the screening visit for routine laboratory parameters and blood borne viruses as well as routine safety parameters during the trial will be tested at the Dooka clinical laboratory. These samples will be identified by the volunteer's trial number, date of birth and initials as required the laboratory.

10.8 End of the Study

The end of the study is defined in section 5.8. The Chief Investigator will notify the National Medicines and Poisons Board of the end of the trial within 90 days of its completion or within 15 days if the study is terminated early.

10.9 Risk Assessment

A full trial risk assessment will be carried out in accordance with the Sponsor's SOP.

10.10 Trial Monitoring

The conduct of the trial will be monitored by the CRO. Monitoring will be undertaken at regular intervals by suitably qualified and trained personnel in accordance with the SOP entitled "Monitoring of LEISH 2a". The monitoring plan will be approved by the CTSC.

10.11 Trial Management

The Sponsor delegates certain roles and responsibilities to the CI and CRO, as detailed in the SOP entitled "Delegation of Tasks for LEISH 2a".

A Clinical Trial Steering Committee (CTSC) will be formed comprising the CI, other Clinical Investigators based at the study site, an external independent expert and members nominated by the Wellcome Trust. The CTSC will be responsible for monitoring the progress of the trial and approving the protocol and any changes. It will meet at least once every 3 months during the course of the trial. Records of these meeting will be maintained.

The CTSC will provide feedback to the DSMC.

A Trial Management Group (TMG) will be formed comprising the CI, other Clinical Investigators based at the study site, the UoY Clinical Investigator, the Project Lead, the CRO, with others co-opted as necessary. The TMG will meet by teleconference every month, unless a CTSC is scheduled.

10.12 Data and Safety Monitoring Board

The Sponsor will appoint an independent Data Safety Monitoring Board which will perform the functions of data and safety monitoring. Numbers and membership of the DSMB will be decided by the Sponsor compliant with Welcome Trust recommendations. A DSMB Charter will be drawn up with the advice and agreement of DSMB members. The DSMB will agree the Charter and their terms of reference before commencement of the trial.

11.0 ETHICS

11.1 Declaration of Helsinki

The Investigators will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki 2008.

11.2 ICH Guidelines for Good Clinical Practice

The Investigators will ensure that this study is conducted in full conformity with relevant regulations and with the ICH guidelines for GCP (CPMP/ICH/135/95) July 1996.

11.3 Research Ethics Committee (REC)

A copy of the protocol, informed consent forms, any other written volunteer information and the advertising material will be submitted to the University of Khartoum and the University of York Ethics Committees for approval.

11.4 Volunteer Confidentiality

No material will be kept on file that refers to the study volunteer by their full name other than in source documentation kept at the trial site. The confidentiality of volunteers will be respected and maintained at all times.

The volunteer's trial number only will identify study reports. CRF's, associated database records and blood samples will only be identified by the volunteer's trial number and date of birth.

11.5 Risks

The Investigators will ensure that the dignity, rights, safety and well-being of volunteers are given priority at all times.

A risk assessment has been performed to assess the risks and benefits of trial participation to the individual volunteer safety, as well as the risks that underlie the validity of the trial results with respect to safety and immunogenicity outcome measurements. The outcome of this assessment has been used to guide the development of procedures with respect to informed consent, confidentiality, trial monitoring and audit.

Relevant departments will receive the protocol and information relating to the IMP prior to the study commencing in accordance with the SOP on Dosing Day Procedure

A GM risk assessment has also been undertaken and reviewed by the GM safety committee at the University of York. This has informed the development of procedures for handling, storing and disposing of the vaccine.

11.6 Benefits

As this is a phase IIa study in patients there may be direct clinical benefit to the volunteers. However, the results of the study will mainly provide safety information which will help in the development of an effective Leishmania vaccine.

Volunteers will receive a thorough medical examination. In the event of any abnormal findings, volunteers will be advised of the best course of action and referred on to the appropriate clinician where appropriate.

11.7 HIV Testing

Volunteers will be required to undergo HIV testing as part of the eligibility screening assessment. Volunteers will receive pre-test counselling in line with current local practice.

11.8 Reimbursement

Volunteers will be compensated for their time. Details of compensation are provided in section 14.

12.0 REGULATORY AND GOVERNANCE ISSUES

12.1 Required approvals

Current regulatory frameworks for this phase IIa trial require that the following approvals and registration must be obtained before commencement of the trial:

Clintrials.gov Registration
Ethical approval from University of Khartoum, Sudan
Regulatory approval from National Medicines and Poisons Board, Sudan
Ethical Approval from University of York, UK

12.2 Amendments

Both substantial and non-substantial amendments will be dealt with according to the SOP on making Amendments to the LEISH 2a study.

12.2 GCP, GLP and GMP Compliance

All clinical staff will receive GCP training at the Site Initiation Visit. The study will be monitored and audited in line with GCP requirements and all adverse events will be recorded and reported.

All trial supplies will be manufactured to EU Good Manufacturing Practice (GMP) standards, as detailed in the Investigator's Brochure and IMP Dossier.
All laboratory procedures will be undertaken in line with Good Laboratory Practice (GLP) standards.

13.0 INDEMNITY

University of York will act as Sponsor for the project.

13.1 Negligent Harm
Legal liability insurance need details for York and Sudan staff indemnity insurance.

13.2 Non-Negligent Harm
Cover for non-negligent harm (no fault compensation) will be provided for trial participants.

14.0 FINANCE

14.1 Financing
The study will be funded by a Translation Award from the Wellcome Trust

14.2 Reimbursement for Volunteers
Volunteers will be compensated for their time and for the inconvenience caused by procedures at the following visits:

Screening Visit
Vaccination visit
Safety visits
Follow up visits

Treatment and investigations will be provided free of charge for those who need it.

15.0 PUBLICATION

The results of the study will be analysed and prepared in a study report for publication in a peer reviewed professional journal. The Chief Investigator, Principal Investigator, Professor Charles Lacey, Professor Paul Kaye and the Statistical Investigator, Ada Keding, will form the basis of the writing group. Authorship will reflect work done by the Investigators.

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Appendix 1: **Grading of Clinical and Laboratory Adverse Events**

Based on systems in use at the MRC CTU and NIH Division of AIDS (unless stated otherwise).

Abbreviations:
ULN Upper Limit of Normal
LLN Lower Limit of Normal
ADL Activities of Daily Living
OTC Over the Counter

The tables below define clinically significant abnormal pathology results which are classed as AE's. Any results that are outside of the normal range for the laboratory at local hospital but do not meet the criteria for being classed as an AE will not be classed as such. For events or results that are not specified in the tables, a decision will be made by the CI or a trial physician, as delegated by the CI, as to whether the result is clinically significant. Only those events or results that are deemed clinically significant will be classed as AE's.

For clinical events not specified in the tables below the following grading will be applied:

- **Grade 1** (mild): Awareness of sign or symptom by tolerated with minimal or no interference with ADL's. Transient (< 48 hours). May be relieved by OTC medication by no other medical intervention required.

- **Grade 2** (moderate): Notable symptoms resulting in greater than minimal interference with ADL's. No or minimal medical intervention required.

- **Grade 3** (severe): Symptoms causing significant incapacity resulting in bed rest or activity reduced by >50% of usual level and/or unable to work. Medical intervention required.

- **Grade 4** (extreme / life-threatening): Significant medical intervention / therapy required. Hospitalisation required to prevent permanent impairment or death.

Laboratory Parameters

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Extreme
Haematology				
Haemoglobin	11.0 - 10.0 g/dL	<10.0 - 8.0 g/dL	<8.0 – 6.5 g/dL	<6.5g/L
White Blood Count Upper	10.0–14.99 $x10^9$/L	15.0-19.9 $x10^9$/L	20.0-24.99 $x10^9$/L	>25.0 $x10^9$/L
White Blood Count Lower	<2.49 – 2.0 x 10^9/L	< 1.99 – 1.5 $x10^9$/L	<1.49 - 1.0 $x10^9$/L	< 1.0 $x10^9$/L
Neutrophils	0.8 – 0.99 $x10^9$/L	0.65-0.79$x10^9$/L	0.5-0.64 $x10^9$/L	< 0.5 $x10^9$/L
Lymphocytes	0.6 – 0.65 x 10^9/L	0.5 – 0.59 x 10^9/L	0.35 – 0.49 x 10^9/L	<0.35 x 10^9/L
Platelets decreased	150.0 - 75.0 $x10^9$/L	<75.0 -50.0$x10^9$/L	<50.0-25.0 $x10^9$/L	< 25.0 $x10^9$/L
Platelets elevated	450 -555 x 10^9/L	555 – 600 x 10^9/L	>600 x 10^9/L	-
Biochemistry				
AST (ULN<38 (M) <31 (F))	>ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 20.0 x ULN	> 20 x ULN
ALT (ULN<60 (M) <32 (F))	>ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0-20.0 x ULN	> 20 x ULN
Bilirubin (total) (ULN <1.0mg/dL)	>ULN -1.5mg/dL x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN
Bilirubin (direct) (ULN <0.3mg/dl)	>ULN -1.5mg/dL x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN
Creatinine (ULN <1.4mg/dL (M) <1.2mg/dL (F))	>ULN - 1.5 x ULN	> 1.5 – 3.0 x ULN	> 3.0 – 6.0 x ULN	> 6.0 x ULN
Albumin (LLN 3g/dL)	<3-2.5 g/dL	<2.5-2.0 g/dL	<2.0-1.5 g/dL	<1.5g/dL

SOLICITED VACCINE REACTIONS

GENERAL				
Fever Oral>12 hours	37.7 - 38.9°C (100.0 – 101.5°F)	39.0 – 39.7°C (101.6 – 102.9°F)	39.8 – 40.5°C (103 - 105°F)	>40.5°C (105°F) OR max temp of >105°F
Chills/rigors	Mild hot/cold flush requires blanket or occasional aspirin/paracetamol	Limiting daily activity >6 hours, or need regular aspirin/paracetamol	Uncontrollable shaking, treatment from doctor needed	Hospitalisation
Malaise/abnormal tiredness	Normal activity reduced – not bad enough to go to bed	Fatigue such that ½ day in bed for 1 or 2 days	Fatigue such that in bed all day or ½ day for more than 2 days	Hospitalisation
General (all over) muscle aches and pains	No limitation of activity	Muscle tenderness, aches/pains limiting activity e.g. difficulty climbing stairs	Severe limitation e.g. can't climb stairs	Hospitalisation
Headache	No treatment or responds to paracetamol like treatment	Regular paracetamol like treatment needed	Regular strong painkillers needed	Hospitalisation
Nausea	Intake maintained	Intake reduced less than 3 days	Minimal intake 3 days or more	Hospitalisation
Immediate reactions (within 6 hours of injection)			Laryngeal oedema insufficient to require intubation; diarrhoea insufficient to require IV fluids, or asthma insufficient to require hospitalisation OR Urticaria, angiooedema OR Generalised pruritus	Anaphylactic shock
CUTANEOUS				
Discomfort/pain in injected muscle (including ache) or overlying skin	Mild itch or ache that responds to paracetamol like treatment, if needed	Pain requiring regular paracetamol like treatment	Pain requiring regular strong painkillers	Hospitalisation

Erythema at injection site	Erythema up to and including 50% of baseline arm circumference OR Symptoms of irritation	Erythema greater than 50% of the arm circumference at baseline With or without Symptoms of	Erythema greater than 50% of the arm circumference at baseline AND symptoms of irritation requiring repeated medication	Hospitalisation
	that are easily tolerated and do not require repeated medication OR Both	irritation that do not require repeated medication OR Symptoms of irritation that require repeated medication AND erythema up to and including 50%		
Blistering or ulceration at injection site	Fluid filled vesicles or superficial disruption of epithelium covering an area < 1cm	Fluid filled vesicles or superficial disruption of epithelium, area 1 - 2cm OR Blood filled vesicles OR Full thickness disruption of epithelium healed within 2 weeks	Full thickness disruption of epithelium not healed within 2 weeks	**Necrosis**
Swelling at injection site	Soft swelling – local Swelling <25% of arm	Soft swelling – local Swelling 25-50%of arm	Soft swelling – local Swelling >50% of arm Or Induration/hardened swelling (when considered by the clinician to be associated with a process arising in the muscle)	

Appendix 2: Source Data Definition

Type of Data	Source Document
Informed consent	Paper copy in TSF
Relevant Medical History and Current Medical Conditions	CRF and medical notes
Physical Examination and Observations	CRF
Concurrent medication	CRF
Clinical Trial History	CRF
Fulfilment of eligibility criteria	CRF
Demographics	CRF
Clinical Laboratory Reports – Haematology, Biochemistry, HIV,	Printed report form, kept in CRF
Urinalysis, Urine pregnancy tests	CRF
Time of study vaccine administration in the clinical unit	CRF
Date/time of immunogenicity sampling	CRF
Date of visits/examinations	CRF
Drug accountability	IMP log
Adverse events	CRF
Protocol Deviations	CRF and file notes in TSF
Withdrawal	CRF

Appendix 3: Grading System for Reporting of AEs By Hospital Staff

Trial Number :

Date of Vaccination:

Guide for grading reactions

	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 (extreme)
GENERAL				
Chills/shaking	*Mild hot/cold flush requires blanket or occasional aspirin/paracetamol*	*Limiting daily activity more than 6 hours, or need regular aspirin/paracetamol*	*Uncontrollable shaking, treatment from doctor needed*	*Requires a stay in hospital*
Malaise/abnormal tiredness	*Normal activity reduced – not bad enough to go to bed*	*Fatigue such that ½ day in bed for 1 or 2 days*	*Fatigue such that in bed all day or ½ day for more than 2 days*	*Requires a stay in hospital*
General (all over) muscle aches and pains	*No limitation of activity*	*Muscle tenderness, aches/pains limiting activity e.g. difficulty climbing stairs*	*Severe limitation e.g. can't climb stairs*	*Requires a stay in hospital*
Headache	*No treatment or responds to paracetamol like treatment*	*Regular paracetamol like treatment needed*	*Regular strong painkillers needed*	*Requires a stay in hospital*
Nausea	*Able to eat & drink normally*	*Eating & drinking reduced less than 3 days*	*Eating & drinking very little for 3 days or more*	*Requires a stay in hospital*
SKIN				
Discomfort/pain in injected muscle (including ache) or overlying skin	*Mild itch or ache that responds to paracetamol like treatment, if needed*	*Pain requiring regular paracetamol like treatment*	*Pain requiring regular strong painkillers*	*Requires a stay in hospital*

Appendix 4: Sample IMP Label

FOR CLINICAL TRIAL USE ONLY

LEISH2a TRIAL

Sponsor: University of York

Investigator: Prof A. Musa

Eudract no: 2016-000369-22 **Vol:**
0.65ml

ChAd63-KH 7.5x10^{10} vp/ml suspension for injection

For Intramuscular injection only

Batch no:

Expiry/Re-test Date :

Store at or below -60°C

Subject no.

Appendix 5: Low Dose Preparation Procedure and Worksheet

LEISH2a TRIAL

Procedure for preparing doses for administration of ChAd63-KH 1 x 10^{10} vp in 1mL Intra-Muscular Vaccine Low Dose

REQUIRED MATERIALS AND EQUIPMENT:

- WORKSHEET: Intra-Muscular Vaccine - **Low Dose ChAd63-KH 1 x 10^{10} vp /mL**
- 1 x 0.65mL vial containing ChAd63-KH vaccine 7.5 x 10^{10} vp /mL
- Sodium Chloride 0.9% solution; sterile, unopened for injection
- 2 x pairs non sterile gloves
- 2 x 23G ('blue') needle (BD Microlance)
- 1 3mL syringe
- Sharps Disposal bin
- Disposable Apron
- Disposable Tray
- Microbiological safety cabinet (Class II)

PROCEDURE:

This procedure must be followed by the two people preparing and checking the dose for administration:

- 1st person: will prepare the dose and complete each stage of the worksheet, **(Parts A to G)**
- 2nd person: will check all stages of dose preparation and countersign the worksheet, **(Parts A to G)**

1. Check the prescription to ensure the correct **Low dose** of Vaccine is prescribed **(ChAd63-KH 1 x 10^{10} vp)** and complete **Part A** of worksheet
2. Wear pair of disposable gloves
3. **Part B**: Remove 1 x vial ChAd63-KH 7.5 x10^{10} vp /mL vaccine from the dry ice Allow to thaw by standing the vial on the bench-top at room temperature
4. Complete the label on the ChAd63-KH 7.5 x10^{10} vp /mL vial with the subject ID, site ID, CI name (Professor A. Musa) and preparation date
5. Gently re-suspend vaccine by slowly agitating vial with fingertips
6. Ensure the ChAd63-KH 7.5 x 10^{10} vp /mL vaccine is fully defrosted, before drawing up
7. **Part C:** Add 23G needle to the 3.0mL dosing syringe and draw up 0.3mL ChAd63-KH 7.5 x10^{10} vp /mL vaccine, (containing 2.25 x 10^{10} vp)
8. Draw up 1.95mL saline into the same syringe to a final volume of 2.25mL. Discard remainder of saline bottle.
   This gives a concentration of 2.25 x 10^{10} vp in 2.25mL - equivalent to 1 x 10^{10} vp in 1mL
9. **Part D**: Draw back air to 3mL mark and invert gently 10x to mix.
10. **Part E**: Discard needle and replace with new 23G needle.

11. Expel any air bubbles from the dosing syringe and discard to waste leaving 1mL for injection **(final dosing volume = 1mL)**
12. Before administration confirm the subject's name and subject number both correspond with vaccine dose to be administered (as indicated on the subject prescription/administration record)
13. **Part F**: Inject 1mL into the deltoid muscle of the upper arm. Apply a small occlusive dressing over the injection site
14. **Part G**: Check the syringe to make sure all the contents have been administered
15. Place all the used equipment from above and the 3mL syringe containing the remains of the diluted vaccine into the **sharps bin**
16. Retain the part used vial of ChAd63-KH 7.5 $x10^{10}$ vp /mL for reconciliation with IMP accountability logs
17. Dispose of any other equipment as per waste management policy

Checked and authorised by	
Date	

LEISH2a TRIAL

Worksheet for preparing doses for administration of ChAd63-KH 1 x 10^{10} vp in 1mL Intra-Muscular Vaccine Low Dose

PART A

DATE OF VACCINE RECONSTITUTION:	
TRIAL SITE:	**PROFESSOR EL-HASSAN CENTRE FOR TROPICAL MEDICINE, DOOKA**
Subject Name:	Subject Trial Number:
Subject Hospital Number:	Subject Allergies: (or add None)

VACCINE COMPONENTS:

	Concentration of vial	Volume in vial	Batch Number	Expiry Date	Signature	Checked by:
ChAd63-KH	**7.5 x 10^{10} vp/mL**	0.65mL				
Sodium Chloride	**0.9%**	>10mL				

PART B	**Prepared by: (signature)**	**Checked by: (signature)**
Time vial of ChAd63-KH 7.5 x 10^{10} vp /mL removed from dry ice: _____		
Time vial of ChAd63-KH 7.5 x 10^{10} vp /mL defrosted and ready for reconstitution: _____		

PART C

C1 Volume check: **0.3mL** ChAd63-KH 7.5 x 10^{10} vp /mL vaccine		
C2 Volume check: **1.95mL** Sodium Chloride 0.9% solution:		

PART D

Time reconstitution completed: _____		

Low Dose Preparation Procedure and Worksheet cont.

PART E

1 x 1mL dose ChAd63-KH 1 x 10^{10} vp /mL drawn up for administration:		

PART F

Time vaccine administered (within 1 hour of removal from dry ice):_____		

PART G

Subject received the correct and complete immunisation		

Appendix 6: High Dose Preparation Procedure and Worksheet

LEISH2a TRIAL

Procedure for preparing doses for administration of ChAd63-KH 7.5 x 10^{10} vp/mL Intra-Muscular Vaccine <u>High Dose</u>

REQUIRED MATERIALS AND EQUIPMENT:

- Worksheet: Intra-Muscular Vaccine – **High Dose: ChAd63-KH 7.5 x 10^{10} vp/mL**
- 2 x 0.65mL vials containing ChAd63-KH 7.5x10^{10} vp /mL
- 2 x pairs Non sterile gloves
- 1 3.0mL syringe1 x 23G ('blue') needle (BD Microlance)
- Sharps disposal bin
- Disposable Apron
- Disposable Tray
- Microbiological safety cabinet (Class II)

PROCEDURE:

This procedure must be followed by the two people preparing and checking the dose for administration:

1st person: will prepare the dose and complete each stage of the worksheet
2nd person: will check all stages of dose preparation and countersign the worksheet

1. Check the prescription to ensure the correct dose of Vaccine is prescribed (ChAd63-KH 7.5x10^{10} vp/mL) and complete **Part A** of the worksheet
2. Wear pair of disposable gloves
3. **Part B**: Remove 2 x vials ChAd63-KH 7.5 x10^{10} vp /mL vaccine from the freezer. Allow to thaw by standing the vials on a bench-top at room temperature
4. Complete the labels on the ChAd63-KH 7.5 x10^{10} vp /mL vials with the subject ID, site ID, CI name (Professor A. Musa) and preparation date
5. Gently re-suspend vaccine by slowly agitating vial with fingertips
6. Ensure the ChAd63-KH 7.5 x10^{10} vp /mL vaccine is fully defrosted, before drawing up
7. **Part C**: Add 23G needle to the **3.0mL Dosing Syringe** and draw up the entire contents of the first vial of ChAd63-KH 7.5 x10^{10} vp /mL
8. Do NOT remove the needle from the syringe
9. From the second vial of ChAd63-KH 7.5 x10^{10} vp /mL withdraw enough vaccine to make a volume of 1.1mL
10. Expel any air bubbles back into the vial (keep volume of 1.1mL vaccine in syringe)
11. Discard needle and replace with new 23G needle.

High Dose Preparation Procedure and Worksheet cont.

12. Expel any air bubbles from the dosing syringe and discard to waste leaving 1mL for injection **(final dosing volume = 1mL)**

13. Before administration confirm the subjects name and subject number both correspond with vaccine dose to be administered (as indicated on the subject prescription/administration record)
14. **Part D**: Inject 1mL into the deltoid muscle of the upper arm. Apply a small occlusive dressing over the injection site
15. **Part E**: Check the syringe to make sure all the contents have been administered
16. Place all the used equipment from above into the **sharps bin**
17. Retain the used and part used vials of ChAd63-KH 7.5 $x10^{10}$ vp /mL for reconciliation with IMP accountability logs

Dispose of any other equipment as per waste management policy

Checked and authorised by	
Date	

LEISH2a TRIAL

Worksheet for preparing doses for administration of ChAd63-KH 7.5 x 10^{10} vp in 1mL Intra-Muscular Vaccine High Dose

PART A

DATE OF VACCINE RECONSTITUTION:	
TRIAL SITE:	**PROFESSOR EL-HASSAN CENTRE FOR TROPICAL MEDICINE, DOOKA**

Subject Name:	Subject Trial Number:
Subjects Hospital Number:	Subject Allergies: (or add None)

VACCINE COMPONENTS:

	Concentration of vial	Volume in vial	Batch Number	Expiry Date	Signature	Checked by:
ChAd63-KH	**7.5 x 10^{10} vp/mL**	0.65mL				
ChAd63-KH	**7.5 x 10^{10} vp/mL**	0.65mL				

PART B	**Prepared by: (signature)**	**Checked by: (signature)**
Time 2 x vials of ChAd63-KH 7.5 x 10^{10} vp/mL removed from dry ice: _____		
Time 2 x vials of ChAd63-KH 7.5 x 10^{10} vp/mL defrosted and ready for reconstitution: _____		

PART C

C1 Volume check: **1mL** ChAd63-KH 7.5 x 10^{10} vp/mL vaccine (1 x 1mL dose ChAd63-KH 7.5 x 10^{10} vp/mL drawn up for administration)		

PART D

Time vaccine administered (within 1 hour of removal from dry ice):_____		

PART E

Subject received the correct and complete immunisation		