ChAd63 CS and MVA CS, novel malaria vaccine candidates

Eoghan de Barra
Royal College of Surgeons in Ireland, edebarra@rcsi.ie

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ChAd63 CS and MVA CS, novel malaria vaccine candidates

Eoghan de Barra MB BCh BAO (NUI) MRCPI
Department of International Health and Tropical Medicine
Royal College of Surgeons in Ireland

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Supervisor:
Professor Samuel McConkey

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Department of International Health and Tropical Medicine, Royal College of Surgeons in Ireland
Clinical Research Centre, Beaumont Hospital, Dublin 9
Ireland

The Jenner Institute, Old Road Campus Research Building, Roosevelt Drive
Oxford OX3 7DQ, United Kingdom

Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ
United Kingdom
Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree Doctor of Medicine (MD) is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original and, to the best of my knowledge, does not breach copyright law and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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VAC038, phase I study – regulatory submissions and amendments to study protocol. Liaison between sponsor and Independent ethics committee and Irish Medicines Board. Liaison with Environmental Protection Agency. Site lead for all inspections of regulatory agencies. Recruitment of volunteers, screening and enrolment. Preparation of laboratory at the Clinical Research Centre (CRC), Royal College of Surgeons in Ireland, Beaumont hospital including ordering all hardware and consumables. Development of standard operating procedures specific to the site and trial for conduct of the trial. Vaccine receipt, storage and administration. Supervision of clinical trial nursing staff in co-ordination of all follow up visits and reviewing all adverse events and queries. All cellular immunology, including ELISpot, which was performed on fresh volunteer samples in the CRC and Intracellular cytokine staining, which was performed on frozen samples at the Jenner Institute, University of Oxford. Conduct of the clinical trial, liaising with sponsor, ethics, regulatory agencies, data safety monitoring board and media. Collection and analysis of clinical and immunological data. Completion of all study
reports, updates to Investigator brochures for relevant products. Authorship of the peer reviewed publication on the phase one study. Presentations at international meetings and on invitation of the funder.

VAC045, the phase two study with Controlled Human Malaria Infection (CHMI) was carried out at Imperial College, London and at University of Oxford, UK – one of the study clinicians, during the malaria challenge and the intensive follow up period thereafter, when volunteers are reviewed daily and assessed for malaria clinically and with blood film and PCR. Clinical conduct of the study, recording of adverse events, collection of data and determination of endpoints in line with the study protocol. Co-author of the peer reviewed publication on the phase two study.

VAC052 – a phase two study of vaccine efficacy using CHMI. Carried out at Imperial College London and University of Oxford, UK. One of the study clinicians, during the malaria challenge and the intensive follow up period thereafter, when volunteers are reviewed daily and assessed for malaria clinically and with blood film and PCR. Clinical conduct of the study, recording of adverse events, collection of data and determination of endpoints in line with the study protocol. Co-author for presentation at International meeting. Peer reviewed publication pending.
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Abbreviations

ChAd63  Chimpanzee adenovirus 63
AdHu   Human adenovirus
AdHu5  Human adenovirus serotype 5
AE     Adverse event
AMA1   Apical membrane antigen 1
CCVTM  Centre for Clinical Vaccinology and Tropical Medicine
CBF    Clinical Bio manufacturing Facility
CRF    Case Report Form or Clinical Research Facility
CS or CSP Circumsporozoite protein
ELISpot Enzyme-linked immunospot
FBC    Full blood count
GCP    Good Clinical Practice
GIA    Growth Inhibition Assay
GMO    Genetically modified organism
HBsAg  Hepatitis B Surface Antigen
HCG    Human Chorionic Gonadotrophin
HCV    Hepatitis C virus
HIV    Human immunodeficiency virus
HLA    Human leukocyte antigen
IDT    Impfstoffwerk Dessau-Tornau
REC    Independent Research Ethics Committee
LSM    Local safety monitor
ME-TRAP Multiple epitopes and thrombospondin related adhesion protein
MSP1   Merozoite Surface Protein 1
MVA    Modified vaccinia virus Ankara
pfu    Plaque forming unit
PMR    Parasite Multiplication Rate
REC    Research Ethics Committee
SAE    Serious adverse event
SOP    Standard Operating Procedure
SUSAR  Suspected unexpected serious adverse reaction
µg     microgram
vp     viral particle
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*Plasmodium falciparum* (*P. falciparum*) malaria remains a significant cause of mortality and morbidity throughout the world. Development of an effective vaccine would be key intervention to reduce the considerable social and economic impact of malaria. Following review of previous work on malaria vaccines, we conducted a Phase Ia, non-randomized clinical trial in 24 healthy, malaria-naïve adults of the chimpanzee adenovirus 63 (ChAd63) and modified vaccinia virus Ankara (MVA) replication-deficient viral vectored vaccines encoding the circumsporozoite protein (CS) of *P. falciparum*. ChAd63-MVA CS administered in a heterologous prime-boost regime was shown to be safe and immunogenic, inducing high-level T cell responses to CS. With a priming ChAd63 CS dose of $5 \times 10^9$ vp responses peaked at a mean of 1947 SFC/million Peripheral Blood Mononuclear Cells (PBMC) (median 1524) measured by ELISpot 7 days after the MVA boost and showed a mixed CD4+ / CD8+ phenotype. With a higher priming dose of ChAd63 CS dose $5 \times 10^{10}$ vp T cell responses did not increase (mean 1659 SFC/million PBMC, median 1049). Serum IgG responses to CS were modest and peaked at day 14 post ChAd63 CS (median antibody concentration for all groups at day 14 of 1.3µg/ml (range 0 – 11.9), but persisted throughout late follow up (day 140 median antibody concentration groups 1B & 2B 0.9µg/ml (range 0 – 4.7). ChAd63-MVA is a safe and immunogenic delivery platform for the CS antigen in humans which warrants efficacy testing. Use of ChAd63-MVA viral vector, expressing various antigens, is a promising heterologous prime-boost vaccine strategy that could be applied to numerous other diseases where both cellular and humoral immune responses are required for protection. Both vaccines proceeded on to phase IIa controlled human malaria infection challenge and showed some protection. The overall project to produce an effective malaria vaccine continues. This work was a step along the path to that goal.
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Professor Sam McConkey at RCSI had a dream of bringing innovative vaccine research to Ireland and many years of planning preceded my involvement in the project. I thank him for the preparation and for somehow trusting me to deliver on executing the clinical trial. He was also the first consultant physician to entrust his clinical responsibilities wholly to me. It was a privilege and an important step towards being an independent specialist.

Though this project was challenging and required courage, my effort pales compared to that of those who placed their trust in me. The history of science is full of colourful heroes, but in the modern age of regulated clinical trials, the volunteer is the true hero.
1 Introduction

1.1 Malaria

Malaria is the most important parasitic disease of humans. Malaria has influenced the outcomes of wars, the failures of empires and colonialism and continues to hamper the development of many countries where it is still endemic. Indeed malaria has shaped not only human history, but our very evolution. The “malaria hypothesis,” links high frequencies of certain human genetic polymorphisms, especially those affecting red blood cells (RBCs), to selective pressure of malaria because they have protected against the effects of malarial infections. A greater number of identified human genetic polymorphisms meet some or all of the expectations of the malaria hypothesis than can be attributed to selection under any other single agent. The variety and geographic spread of malaria-protective polymorphisms suggests that humans had already spread across the world before human malaria did.

Figure 1.1 Phylogeny of the malaria parasites of humans and of some other related malaria parasite species.

“All the infections that the sun sucks up/ From bogs, fens, flats, on Prosper fall and make him / By inch-meal a disease!” - William Shakespeare (1564–1616), The Tempest (Act II, Scene II)
Recorded human history of malaria stretches back some 5,000 years. The *Nei Ching* (The Canon of Medicine) from 4,700 years ago, apparently refers to repeated paroxysmal fevers, tertian and quartan, associated with enlarged spleens and a tendency to epidemic occurrence.[7] The non-lethal nature, relapses and epidemic tendency suggest that *Plasmodium vivax* was the pathogen. Egyptian mummies with splenomegaly are suspected to have had malaria. Malaria antigens have been detected in skin and lung samples from mummies dated 3200 to 1304 B.C.[8] Cuneiform script written on clay tablets found in the library of Ashurbanipal (7th century B.C.) mention deadly periodic fevers, suggesting that more than 2,500 years ago, the birthplace of civilisation between the Tigris and Euphrates Rivers was malarious. [9] Texts of the Vedic period of Indian history (1500 to 800 B.C.) refer to autumnal fevers as the “king of diseases” and describe splenomegaly, suggesting that India was malarious at this time. By the sixth century B.C. malaria appears in Greek poems, Homer’s Iliad amongst others. About 300 years later, the physician Hippocrates discussed tertian and quartan fevers in his *Book of Epidemics*. Hippocrates did observe the relationship with marshes and a seasonality, but believed the disease to be due to drinking stagnant marsh water.[10] Descriptions of a malignant tertian fever being absent from Hippocrates writings leads to the belief that *Plasmodium falciparum* was a rare or non-existent pathogen in Europe at this time. Roman times saw populations grow and more favourable conditions for “Roman fever”, which became known as *Mal’aria*, meaning “bad air” in Italian. During the middle ages malaria appears to have gradually spread, extending to temperate regions of Asia and Europe. In Europe reaching as far north as England[11]. There is little evidence of malaria in the pre-Colombian New World. It would appear that it was imported with the conquistadors and the mass movements of people from West Africa[12]. Through explorations of Jesuit priests, the bark of the Cinchona tree provided quinine and relief for those that get this scarce resource but malaria was still thought to be caused by miasma, or “bad air” rather than an infectious parasite[13].

On 6 November 1880, Alphonse Laveran observed a male gametocyte in a blood smear from an Algerian patient with malaria[14]. This marked the start of the scientific identification of the malaria parasite. In 1899 the British Medical Journal carried a brief report on correspondence from Ronald Ross, a military physician working in India, which set out the role of mosquitoes in the transmission of avian malaria and by implication, human malaria[15] He was rewarded with the 1902 Nobel prize for this discovery. William George McCallum confirmed plasmodial exflagellation as a process of sexual reproduction in 1897 [16, 17] and Batistta Grassi et al. confirmed anopheline mosquitoes as the vector of human malaria in 1900[18], thus completing the puzzle of the complex lifecycle of this organism.
Malaria is caused by protozoan parasites of the genus Plasmodium. There are five described species that infect humans, *Plasmodium falciparum, vivax, ovale, malariae* and *knowlesi*, and many others that infect animal hosts. The life cycle is complex, involving a female anopheline mosquito and a vertebrate host. An anopheline mosquito, infected with malaria, bites a vertebrate in order to get a blood meal to support her developing eggs. In the process, motile infectious forms of malaria called sporozoites are passed from mosquito into the vertebrate host. The sporozoites migrate through the dermal tissues and enter the blood circulation. In the liver the sporozoites enter hepatocytes, where they increase in number via asexual reproduction. They then erupt from the hepatocytes as merozoites that go on to infect erythrocytes, where further asexual reproduction takes place, resulting in increased numbers of merozoites and destruction of the erythrocyte. Some merozoites develop into gametocytes and can be taken into the gut of a biting mosquito where male and female gametocytes fuse to form ookinetes. These eventually mature to a motile zygote, which migrates to the salivary gland of the mosquito ready to be injected into the next host.

**Figure 1.2 Malaria lifecycle.** The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease.
The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal. The parasites’ multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito’s stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito’s salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle. www.CDC.gov/malaria.

1.1.1 Malaria epidemiology

By the mid-19th century malaria was present in every continent as shown in Figure 1.1.1 above. Currently P. falciparum accounts for the majority of malaria deaths, but this was likely not always the case, with repeated and untreated infections with P. vivax and P. malariae likely to have contributed significantly particularly in Asia. It is estimated that malaria was a factor in 2-5% of all deaths on the planet during the 20th century. Given the regional differences in malaria and human populations, malaria was probably the number one killer in many regions of the world early in the 20th century.
Figure 1.4 Malaria mortality in the 20th century. A shows the total number of deaths due to malaria per year and B the number of malaria deaths per 10,000 population per year; in Europe and North America (⧫—⧫); the Caribbean and Central and South America (▪——▪); sub-Saharan Africa (●——●); China and Northeast Asia (X—X); the Middle East, South Asia, and the Western Pacific (▴——▴); and worldwide (⧫---⧫).[6]

The 20th century saw a great reduction in the human impact of malaria, but this has not been evenly distributed in all regions. Figure 1.4 shows mortality statistics at the beginning and end of the 20th century, the increase in total deaths in Africa and modest decrease in deaths per 10,000 population is noteworthy. There were large reductions in the number of malaria cases and deaths between 2000 and 2015. In 2000, it was estimated that there were 262 million cases of malaria globally (range: 205–316 million), leading to 839,000 deaths (range: 653,000–1.1 million). By 2015, it was estimated that the number of malaria cases had decreased to 214 million (range: 149–303 million), and the number of deaths to 438,000 (range: 236,000–635,000).[19] Thus malaria continues to inflict a considerable toll on much of the world’s population and negatively impacts on economic development in many countries [4]. Regions of the world affected by malaria are not homogeneous, the level of endemicity of malaria varies greatly and gives rise to different epidemiological patterns. The endemicity of malaria is best defined by the entomological inoculation rate (EIR), or number of infectious mosquito bites received per person per year, but is defined traditionally in terms of the spleen to parasite rates in children aged between 2 and 9 years[1].

Hypoendemic: Unstable transmission; under 5 children spleen rate 0 - <10%, parasitaemia 0 - <10%. There is only periodic transmission following unusual rainfall with severe clinical outbreaks in children and adults and mortality is high in all population sectors.
Mesoendemic: unstable transmission; under 5 years old spleen rate >20 <50%, parasitaemia <20%. Transmission is seasonal under normal rainfall conditions. Cerebral malaria is common, but infection tolerated is well in adults. There is some clinical impact seen in all age groups.

Hyperendemic: under 5 years old spleen rate >50 <70%, < 5 years old parasitaemia >50 <70%. Transmission is seasonal but intense.

Holoendemic: under 5 year old spleen rate >75%, parasitaemia 60-70%. Mortality is highest in the 1st and 2nd year of life and transmission is stable, continuing year round.

These patterns highlight the protective effect that repeated exposures to malaria has. In a holoendemic area, provided a child can survive the first few years of life, immunity to malaria can be lifelong. Naturally acquired immunity to falciparum malaria protects millions of people routinely exposed to Plasmodium falciparum infection from severe disease and death, but there remains no detailed understanding about how this protection works.[20]

Figure 1.5 Percentage of population at risk by country. WHO 2013. http://www.who.int/

Target 6C of 2000 Millennium Development Goals called for halting and beginning to reverse the global incidence of malaria by 2015. The report shows — unquestionably — that this target has been achieved. Fifty-seven countries have reduced their malaria cases by 75%, in line with the World Health Assembly’s target for 2015.[19]
1.1.2 Malaria control measures

The first large scale malaria control project was to protect workers constructing the Panama canal. In 1904, William Gorgas began the planning and subsequent implementation of a programme of malaria control. Many of the measures also sought to control Yellow fever. All water pools within 200 yards of villages were drained and bush or grass within the same perimeter was cut. Undrainable water was treated with oil to kill mosquito larvae. If oil was insufficient, larvical agents created for the purpose were used. Quinine was provided freely to all workers. Widespread screening of buildings and quarters against mosquitos was carried out and collectors were hired to gather adult mosquitos from houses during the daytime. The result of this malaria programme was eradication of yellow fever and a dramatic decrease in malaria deaths. The death rate due to malaria in employees dropped from 11.59 per 1,000 in November 1906 to 1.23 per 1,000 in December 1909. It reduced the deaths from malaria in the total population from a maximum of 16.21 per 1,000 in July 1906 to 2.58 per 1,000 in December 1909. Among the work force, the percentage of employees hospitalized due to malaria was 9.6% in December 1905, 5.7% in 1906, 1.8% in 1907, 3.0% in 1908, and 1.6% in 1909.[21]

Armed with increasing knowledge and experience in implementing control measures and with the discovery of Dichloro-diphenyl-trichloroethane (DDT), a potent residual insecticide, the end of the second world war saw malaria eradication becoming a reality. By 1951 malaria was eliminated from the United States. In 1955 the WHO submitted a malaria eradication proposal. Results were promising with intense insecticide spraying and drug administration, but infection rates climbed soon after intensive phases were completed and many countries were not even included. In the years that followed the WHO has continued to recommend a range of measures aimed at reducing the burden of infection. These include: prevention through
mosquito vector control using long-lasting insecticidal bed-nets and, in some settings, indoor residual spraying with insecticides; seasonal malaria chemoprevention in specific settings; intermittent preventive treatment for infants and during pregnancy; prompt diagnostic testing; and treatment of confirmed cases with effective anti-malarial medicines. These measures have dramatically lowered malaria disease burden in many African settings over the years. The number of deaths from malaria having fallen by 60% globally since 2000.[22]

This impressive decline in malaria morbidity and mortality is adding to renewed optimism that more can be done and the dream of malaria eradication might one day be realised. The development of an effective vaccine is seen a key component. The current leading vaccine, known as RTS,S has been developed by GlaxoSmithKline in collaboration with Walter Reed Army Institute of Research. The current WHO position states that, “RTS,S is being considered as a complementary intervention, i.e. any use of RTS,S would be in addition to use of the existing non-vaccine malaria preventive measures described,” and that the “need for high quality, safe and effective drugs to treat malaria will continue regardless of any deployment of a first-generation malaria vaccine.”[22]

1.2 Vaccination

Vaccination is defined by the WHO as the administration of agent-specific, but relatively harmless, antigenic components that in vaccinated individuals can induce protective immunity against the corresponding infectious agent. In practice, the terms “vaccination” and “immunization” are often used interchangeably. Whether it was Buddhist nuns in China crushing scabs or fleas from smallpox survivors, perhaps as far back as the 7th Century, or Brahmin holy men in India introducing dried pus from smallpox survivors in the 16th Century, immunization has long been recognised as a tool in the control of infectious diseases. In 1721 Lady Mary Wortley Montagu, wife of the then British ambassador to the Ottoman empire, brought the practice of variolation (inoculation of dried material from the pustules of smallpox survivors under the skin of others to provide protection) back to England. Edward Jenner attained a special place in the history of vaccination for his 1796 work using cowpox to immunize individuals against smallpox. However, effective inoculation against smallpox had gone on for centuries before his time, across many continents, incorporating varying approaches.[23] Indeed in 1774, farmer Benjamin Jesty exposed his daughters to cowpox when his rural English community was threatened with smallpox, having observed the protection that milkmaids enjoyed. Nevertheless, Jenner’s contribution in refining the process and perhaps more importantly championing the cause, was significant. 1885 saw Louis Pasteur’s rabies
vaccine and over the subsequent 40 years vaccines against diphtheria, tetanus, anthrax, typhoid and tuberculosis were developed. This coincided with the emerging field of bacteriology. These vaccines were developed on a microscopic level. Pathogens were isolated, inactivated or attenuated and then used as inoculations.

In many instances, the existence of an effective vaccine alone did not result in a large scale impact on public health. In 1900 in the United States of America, 21,064 cases of smallpox were reported and 894 patients died.[24] Only during the 20th Century did the routine practice of vaccinating large populations develop and targeted campaigns against specific diseases were launched globally. Large, international, co-ordinated vaccination campaigns were required to eradicate smallpox from the world. This was finally achieved in 1979. Smallpox remains the only pathogen of humans that has been eradicated. Vaccination has led to control of diphtheria, tetanus, yellow fever, pertussis, *Haemophilus influenzae* type b, poliomyelitis, measles, mumps, rubella, typhoid and rabies through the mid-20th Century. Latterly and into the 21st Century, vaccines against meningococcal and pneumococcal disease, hepatitis A and B, influenza, Japanese encephalitis and Rotavirus have reduced the morbidity and mortality attributable to each pathogen. Vaccination campaigns against hepatitis B in Taiwan and parts of West Africa in the 1980s were targeting a reduction in hepatocellular and more recently 2006 saw the licensure of the first vaccine against Human Papilloma Virus (HPV), a vaccine aimed at preventing cancer, HPV being causative in cervical cancer. Vaccines have shifted the balance of power in favour of the host, but effective vaccines for many diseases remain elusive. In addition to this, there continues to be a need to improve current vaccines and vaccine uptake. Pathogen factors such as antigenic drift, multiple serotypes and short duration of efficacy and human population factors, such as immunocompromise due to extremes of age or medications, continue to pose scientific technical challenges. In addition there is an ongoing battle for public opinion to keep vaccination rates high.

*Figure 1.7 Challenges of modern vaccine development.* [25]
Early vaccines were used empirically without detailed understanding of the immunological processes involved. Essentially vaccines act by stimulating the innate immune response and activating antigen presenting cells (APC). This induces an immune response to that selected pathogen. The protective efficacy of a given vaccine will depend in part on the choice of antigen, its mode of presentation to APCs and immune-stimulatory effect of the compound / platform.

Figure 1.8 Innate and adaptive immunity overview. [26]

Early vaccines were ‘whole cell’ attenuated forms of the pathogen and were generally quite immunogenic. The same immunogenic potential also led to more side effects impacting on tolerability. Subunit or purified antigens, utilise small targeted antigens, which, though better tolerated, produce a less immunogenic response. (Figure 1.9 below).
One approach to enhance the immunogenicity of vaccines, is the use of adjuvants. Adjuvants are substances added to vaccines to enhance the immunogenicity of highly purified antigens. Early adjuvants included alum or oil emulsions, indeed for almost 70 years alum or AlOH, which comes in various forms, remained the pre-eminent vaccine adjuvant despite the lack of clear understanding as to how it works.[27] Adjuvant development has become far more sophisticated as understanding of the immunological processes influencing vaccine function has advanced. Numerous approaches are under development; liposomes protect antigens and deliver them to APCs; immune stimulating complexes are ring-like structures containing cholesterol, phosphatidylcholine and saponins that interact with dendritic cells and enhance presentation; montanide are oil-based formulations that give rise to a depot at the inoculation site; Polycationic peptides enhance cellular uptake of proteins or bacterial DNA by cells; triple combination adjuvant – include a variety of molecules including polymers designed to activate APCs; adjuvant systems – as developed by GlaxoSmithKline (GSK) are various combinations of classical adjuvants and immunostimulators specifically targeted to specific pathogens and populations, they include AS03 (H5N1 vaccine) and AS04 (HPV vaccine, Cervarix®) and AS02 and AS01 used in the RTS,S vaccine; nanoemulsions, one is licensed and several have progressed to human clinical trials.[28]

Live attenuated vaccines do not need adjuvants. They stimulate sufficient immune response, by way of a mild infection, to result in lasting immunity without causing disease. This approach does not work where natural infection does not result in immunity or where manufacturing a
safe and stable attenuated form of the pathogen is not possible. An alternative approach to enhance immunogenicity is the use of relatively benign, but immunostimulatory viruses, to deliver the antigen. Viruses, such as adenoviruses which can be genetically modified to produce a specific antigen, produce a significant immunogenic response.

1.3 Vaccination against Malaria

For more than 40 years it has been known that it is possible to achieve high-level, sustained, protective immunity against the pre-erythrocytic stages of *P. falciparum* infection through immunization with the bites of >1000 infected, irradiated mosquitoes. [29-32] But this has not been considered a practical approach to mass immunization. One group, Sanaria, continue to champion this approach with a whole cell vaccine. Production of the whole cell vaccine requires teams of technicians dissecting mosquito salivary glands to recover sporozoites, followed by irradiation and storage in liquid nitrogen. To date immunity is only reliable with 4 intravenous injections of $2.7 \times 10^5$ sporozoites.[33, 34] The challenges in this approach are many. Infected mosquitos need to be bred and skilled technicians have to individually dissect them to isolate sporozoites. This is not a process that could be easily scaled up to the large volumes required for a vaccine to be available for millions across the globe. Additionally this approach requires a liquid nitrogen cold chain all the way to the vaccinee. This would pose financial and logistical difficulties for the tropical areas targeted that might be insurmountable. Finally intravenous administration carries its own challenges and risks, especially given the target population is infants. The time and technical skills required, along with risks of adverse events, all increase with intravenous over intramuscular injections. All this means that the whole sporozoite vaccine approach is unlikely to be the solution.

Up to 2015 there was no licensed vaccine against malaria. Indeed there was no licensed vaccine against any protozoan parasite. Their lifecycles are complex, have multiple stages, express a variety of antigens and have mechanisms to evade the human immune system. RTS,S/AS01 is a malaria vaccine that has received a positive opinion from the European Medicines Agency (EMA) for its quality, safety and efficacy under article 58, which allows the EMA to give a scientific opinion about products intended exclusively for markets outside of the European Union. RTS,S, was developed by a partnership between the Walter Reed Army Institute of Research and GlaxoSmithKline (GSK) supported by the Gates Foundation. It is a vaccine construct of the hepatitis B vaccine and the circumsporozoite protein of *Plasmodium falciparum* which has shown a field efficacy of between 33 and 50%[35]. The vaccine requires
administration of four doses, the first three at monthly intervals, and the fourth given 18 months after the third dose. Despite RTS,S not fulfilling all of the goals set out by the WHO Department of Immunization, Vaccines and Biologicals [36], the WHO’s Strategic Advisory Group of Experts on Immunization (SAGE) and the Malaria Policy Advisory Committee (MPAC) met in October 2015 and jointly recommended pilot projects to understand how to best use a vaccine that protects against malaria in young children.

For many years the evidence suggested that antibodies against the major sporozoite surface antigen, the circumsporozoite protein (CS), were responsible for protection and this formed the basis of the design of the RTS,S vaccine.[37] However, based on data from murine adoptive transfer experiments and human trials it now seems that CD8+ T cells specific for parasite-derived peptide/class I MHC molecule complexes on the surface of infected hepatocytes are the primary immune effectors[38-45]. Thus the goal in malaria vaccine development is a vaccine that induces both humoral and cell-mediated immune responses resulting in memory T and B cells that are specific for epitopes derived from parasite proteins. Initially, it was thought that cytolysis of the infected hepatocyte by parasite-specific CD8+ T cells was the primary effector mechanism, but recent data suggest that the elimination of the infected hepatocytes is mediated by interferon-gamma (IFN-γ) released by CD8+ T cells [46].

Targets and approaches malaria vaccines take many forms as outlined in Figure 1.10 below. Broadly vaccines are intended to act either pre-erythrocytic / hepatic, during the blood-stage or are designed to block transmission by impacting on gametocytes.
Figure 1.10 Life cycle of the malaria parasite illustrating the various stages that are relevant to vaccine design. These are (1) the anopheline mosquito vector, used in experimental protocols to immunize with irradiated sporozoites administered by mosquito bite; (2) the sporozoite, the target of several vaccines, including RTS,S; (3) the liver-stage, usually targeted by vectored vaccines; (4) the blood stage, usually targeted by protein in adjuvant vaccine candidates. Merozoite antigens have been most often included in blood-stage vaccines; (5) the gametocyte which along with the ookinete, formed after fertilization in the mosquito midgut, is the source of parasite antigens used in sexual-stage transmission-blocking vaccines. Pre-erythrocytic vaccines, which target the sporozoite and the liver-stage parasite are intended to prevent infection as well as disease while blood-stage vaccines are intended to prevent clinical illness and death. [47]

Circumsporozoite Protein (CSP)

The structural and functional properties of CSP were defined in the 1980s by the study of the mechanisms of the protective immunity induced by immunisation of rodents, monkeys and humans with sporozoites attenuated by irradiation[48]. Irradiated sporozoites invade hepatocytes but their further development is arrested. Protection appears to be dependent on the persistence of these arrested forms in the liver. Protection on sporozoite challenge in humans can be achieved following multiple, repeated bites from irradiated mosquitoes infected with \textit{P. falciparum}, and this protection has been shown to be mediated by antibodies generated to CSP.[32] CSP is expressed by sporozoites and liver schizonts and plays a key role in the attachment phase of sporozoite invasion into hepatocytes.[48] Sporozoites travel from skin to liver within minutes, allowing minimal time for anti-CSP antibodies to target sporozoites and facilitate their destruction prior to hepatocyte invasion. Thus a vaccine based on antibodies alone may fail to achieve complete protection against \textit{P. falciparum} sporozoites. The longer phase of replication within the hepatocytes would appear to offer an opportunity for cell mediated immunity to identify and destroy infected hepatocytes. Liver stage antigens need to
be processed and presented on the surface of infected hepatocytes for T cell recognition. CSP also appears to be the dominant antigen expressed during the hepatic stage of malaria and has been recovered from the cytoplasm of infected hepatocytes, confirming the importance of CSP as a target antigen in liver stage immunity.\[48\] Sporozoites also secrete CSP while traversing the endothelium. Thus, CD4+ T cells may recognize processed CSP on the plasma membrane of non-parenchymal liver cells, such as Kupffer cells or dendritic cells. If T cell recognition is followed by release of cytokines such as interferon-γ in the proximity of the infected hepatocyte, then the liver stage development will be inhibited. Indeed a mouse model using *P. berghei* has shown CS protein vaccination to provide 100% sterile protection from challenge with *P. berghei* parasites\[49\] supporting its potential as an effective antigen in humans. Any potential candidate antigen needs to be considered for its conservation across strains or types within the target species. There is considerable naturally occurring diversity of CSP and the potential impacts of heterologous infection have long been considered with early investigations suggesting that there might be an impact.\[35, 50\] The challenge strain used in clinically assessing antigens utilises a heterologous strain as was done in this work, the intention being to assess the efficacy cross-strain, or broadly neutralising protection of the candidate vaccine against what is a diverse pathogen.

The structures of CSP genes from human, monkey, rodent and avian malarias all have a common basic structure. Indeed the CS protein of *Plasmodium falciparum* and the CS protein of a simian malaria parasite, *Plasmodium knowlesi*, have two regions of homology, one region contains 12 of 13 identical amino acids. Within the nucleotide sequence of this region, 25 of 27 nucleotides are conserved. The conservation of these regions in parasites widely separated in evolution suggests that they may have a function such as binding to liver cells and may represent an invariant target for immunity.\[51\] At the C-terminus there is a thrombospondin type I repeat domain that is shared with many host proteins which encodes a glycosylphosphatidylinositol (GPI)-anchor sequence and the N-terminus contains a few conserved amino acids. The middle third contains the immunodominant B cell epitopes (NANP), recognised by neutralising antibodies obtained from immunization with either irradiated sporozoites or genetically attenuated sporozoites.\[52\]

As mentioned earlier, a mouse model using *P. berghei* has shown CS protein vaccination to provide 100% sterile protection from challenge with *P. berghei* parasites. This work was done during the 1990s by Schneider and colleagues when they conducted a large study on the immunogenicity and protective efficacy of numerous antigen delivery systems utilizing a mouse model. They investigated prime-boost immunization strategies with combinations of various recombinant vaccinia virus strains and plasmid DNA. Modified vaccinia virus Ankara (MVA)
was found to be the most efficacious. MVA was developed for the eradication of Smallpox, but is a promising tool with which to combat other infections. MVA is a vaccina virus strain derived by 500 passages in chick embryo fibroblasts (CEFs) of material from a pox lesion on a horse in Ankara, Turkey [53]. It is highly attenuated but provides protective immunity against veterinary orthopoxvirus infections. MVA was used in the final stages of the smallpox eradication campaign. It was administered to more than 120000 humans in Southern Germany and Turkey. No significant side effects were recorded, despite the deliberate vaccination of high risk groups such as young, old or eczematous patients[54-56]. MVA contains six large genomic deletions compared with parental virus, replicates well in CEFs, but poorly in most mammalian cells. Blanchard et al investigated MVA in detail during the 1990s exploring its further potential as a human vaccine. They reported that MVA lacked functional receptors for TNF, IFN-γ, IFN-α/β and CC chemokines, but expresses the IL-1βR, suggesting suitable safety and immunologic profiles[57]. They went on to show that MVA undergoes limited safety and replication in transformed human cells such as TK−143B and HeLa cells, but not in primary human fibroblasts[57], further supporting its safety as a human vaccine. This work was followed by demonstrations of the enhanced immunity and indeed protective efficacy provided by vaccines utilising boosting with MVA[38]. Utilising MVA, Schneider and colleagues were able to show protective efficacy against sporozoite challenge in both BALB/c and C57BL/6 mice. Importantly the specific order of immunization was essential for protection, MVA following plasmid DNA. None of the mice that received MVA followed by DNA were protected[38]. Thus the role of MVA as a boosting agent was established. Antigens used were, CSP alone, thrombospondin related adhesion protein (TRAP) alone and a combination of both. CSP alone yielded 100% protection as did the combination of CSP and TRAP[38]. This preliminary mouse data suggested that a prime-boost platform, utilising MVA boosting and expressing CSP offered the promise of an effective human vaccine. The next steps involved the optimisation of the delivery platform and using it to deliver the novel circumsporozoite protein.

**A novel Circumsporozoite Protein**

Following the observation of poor immunogenicity of the standard full length CSP insert used in previous vectors in clinical trials[58-61] a novel CS antigen was designed for use in the ChAd63-MVA CS vaccine. One of the main differences was to omit the extreme C-terminus of the protein that encodes the GPI-anchor sequence. It had been shown by other groups that the GPI anchor interfered with total circumsporozoite protein production, cellular distribution, antigen processing and secretion, leading to less effective antigen presentation and its omission in vaccination with adenoviral vectors yielded an increase in interferon-gamma producing T-cells.[62]
ChAd63 – MVA, optimal delivery platform

Researchers at the University of Oxford have been working for over 16 years to develop a pre-erythrocytic *P. falciparum* malaria vaccine using the sporozoite and liver stage antigen multiple epitopes and thrombospondin related adhesion protein (ME-TRAP). This antigen contains a fusion protein of multiple epitopes (ME: a string of 20 epitopes, mainly CD8+ T cell epitopes from pre-erythrocytic antigens) and the *P. falciparum* pre-erythrocytic antigen thrombospondin-related adhesion protein (TRAP).[63] Multiple vectors for this antigen have been clinically tested including DNA, fowl pox (FP) and modified vaccinia virus Ankara (MVA), however T cell immunogenicity and clinical efficacy has been limited.[58, 64, 65] More recently, heterologous prime boost with Chimpanzee adenovirus 63 (ChAd63) and MVA, both expressing ME-TRAP, has been shown to be the most immunogenic regimen to date, inducing more than 2400 IFNγ producing T cells per million peripheral blood mononuclear cells (PBMCs) after the boosting vaccination.[66-68] This heterologous prime-boost regime with the viral vectors ChAd63 and MVA has been shown to induce the highest T cell responses in humans of any vaccine platform, as well as strong antibody responses.[47, 69, 70] Simian adenoviruses are not known to cause pathology or illness in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the USA.[71] In equatorial Africa prevalence is higher. A recent study in Kenya showed 4% of children to have high neutralising antibodies to ChAd63.[72] The presence of pre-existing antibodies to adenoviral vectors has been an issue with human adenoviral vectors. However, data from the Phase IIb efficacy study of ChAd63-MVA ME-TRAP showed no correlation between neutralising antibodies to ChAd63 in volunteers prior to vaccination with their subsequent T cell count post MVA boost, suggesting that even if neutralising antibodies exist they may not limit immunogenicity.[73] Several antigens have been trialled using this platform [66, 68, 74] including ME-TRAP, which has shown sterile protection in 21% of malaria-naïve volunteers in controlled human malaria infection (CHMI)[73]. In this study we combined this platform with a novel CSP antigen.

ChAd63 – MVA safety data.

As stated above simian adenoviruses are not known to cause pathology or illness in humans. The pursuit of these vectors was prompted by concerns over the impact of high levels of pre-existing anti-vector immunity in the target populations. Prevalence of antibodies in Southern and West African populations to AdHu5, a widely used human adenoviral vector, have been in the region of 80%.[75] As already stated previously existing antibodies to ChAd63 has been reported in only about 4% of Kenyan children and 5% of the US population.
**Pre-clinical**

The ChAd63 vector is replication deficient as the essential E1 gene region has been deleted and the virus only propagates in cells expressing E1 functions. This means the virus will not replicate in human cells within the body. ChAd63 CS has been shown to be immunogenic in Balb/C mice. Distribution studies in mice showed no evidence of replication of the virus or presence of disseminated infection 1 week after intradermal (ID) and intramuscular injections using similar ChAd63 vaccines (expressing ME-TRAP or the blood stage malaria antigen merozoite surface protein 1 (MSP1)). A distribution study was therefore not thought to be necessary for ChAd63 CS. Previous toxicology studies in mice showed no evidence of systemic ChAd63-related toxicity after administration (either intradermal or intramuscular). A low level of local irritation at the site of administration after intradermal administration is normally noted in these toxicology studies. A similar toxicology study was conducted with Balb/C mice being vaccinated with ChAd63 CS and boosted with MVA CS 14 days later. Treatment with the vaccine ChAd63 CS followed by MVA CS was well tolerated and was not associated with any adverse effects.

**Clinical expressing other antigens**

ChAd63 expressing various antigens has been administered to over 400 individuals including children and has demonstrated an excellent safety profile. Multiple studies have shown $5 \times 10^{10}$ vp ChAd63 to be the optimal dose.[47, 66, 67, 73, 74] With this platform the immune system is primed with a simian adenovirus expressing an antigen and then boosted 8 weeks later with MVA expressing the same antigen.

**Malaria vaccine development – general considerations**

In bringing any medicinal product from basic science through to regular use in humans there is a long and rigorous process. During development researchers need to be aware of the practical endpoint application of the product. The development of an effective vaccine against malaria is a key goal of the WHO. To guide research work on vaccines against malaria, the WHO has published the Preferred Product Characteristics (PPC). This provides clear targets in terms of target population, methods of assessment and practical feasibility of use.

The briefly summarized WHO Preferred Product Characteristics (PPC) for malaria vaccines[36] are as follows:

1. **Vaccines preventing malaria disease**
Target groups:
- Populations living in areas with significant malaria transmission
- Protection of primigravid women through immunization of women of childbearing age.
- Non-immune individuals moving to become resident in malaria-endemic areas.
- Non-immune individuals who are visiting or temporarily employed in malaria endemic areas.
- Mass immunization campaigns, in addition to the use of vaccine in routine immunization schedules, may have a role for highly effective disease-reducing vaccines as a way of rapidly protecting susceptible populations and achieving accelerated disease control.
- Endpoints and case definitions for evaluating disease-reducing malaria vaccines
- Sets out trial designs and endpoints.[76]

Follow up efficacy at one and two years.
- The safety and reactogenicity of the vaccine is comparable to or better than WHO recommended vaccines in use in low and middle-income countries. Data should allow assessment of deferred increases in morbidity as vaccine-induced immunity wanes. For vaccines within the Expanded Programme of Immunization (EPI) schedule, absence of clinically important interference with EPI vaccines will have to be documented.
- The vaccine should reduce incidence of all clinical malaria episodes by at least 75% for no less than one year and preferably at least two years. Booster doses should be required no more frequently than annually. The duration of protection is as important as the short-term efficacy for the primary target group of children under the age of 5 years in medium to high transmission malaria endemic countries. Thus, the initial efficacy and duration of protection will be considered together.

2. **Vaccines to reduce and interrupt malaria transmission**
- Reducing transmission or preventing re-introduction through periodic mass preventive campaigns.
- Managing outbreaks through reactive campaigns to stop transmission

3. **Considerations on Programmatic suitability**
- New vaccines should have presentation and packaging consideration early to assess operational impact on existing immunization programmes.
1.4 Clinical trials process - Phase II and III studies.

The process of licensing a product for use in humans should be performed in accordance with the standards of Good Clinical Practice (GCP), which are agreed by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The conduct of trials and decision on approval is regulated by national authorities and ethics committees. Approval must be sought in advance of each stage. Phase I studies involve a small group of humans (20-30), often including the first human to receive a product with the objective of evaluating safety. Phase II aims to establish efficacy, usually in comparison with a placebo, again with small numbers or 20 to 30 individuals using the disease or a disease model. Phase III aims to confirm the findings of safety and efficacy seen in phase II in a larger population.

As already mentioned there are numerous potential targets for malaria vaccines. Indeed new targets are constantly being discovered. The WHO maintains a table of all products progressing through clinical trials, see Figure 1.11. Phase II malaria vaccine trials can be IIa, controlled human malaria infection (CHMI) or IIb, field efficacy. To date, only RTS,S AS01 has progressed to Phase III trial, with field efficacy being studied in infants and young children in endemic areas.

Figure 1.11 The WHO rainbow table of malaria vaccine projects (March 2016)
Intentionally infecting humans with malaria is not new. The Austrian physician Julius Wagner-Jauregg won the Nobel Prize in Physiology or Medicine in 1927, "for his discovery of the therapeutic value of malaria inoculation in the treatment of dementia paralytica"[77]. Malaria infection was achieved both by direct inoculation of parasitized red cells and via the bite of infected mosquitoes[78]. In the 1970s Clyde and Reickmann, working independently but using similar techniques, demonstrated protection from *P. falciparum* malaria in malaria naïve volunteers who had previously been administered irradiated sporozoites via mosquito bite[30, 79]. By the end of the 1970s methods for continuous culturing of human malaria were established in Groningen, Netherlands [80]. In 1986 at Walter Reid Army Institute of Research the first modern CHMI took place[81]. CHMI became a viable methodology for rapidly assessing candidate vaccines[82, 83] and consensus on standard design and conduct was achieved[84].

This thesis describes the progression of ChAd63 – MVA CS candidate malaria vaccines, through a phase I trial for assessment of safety and immunogenicity and on into a phase IIa trial of clinical efficacy by way of CHMI, in the pursuit of a safe and effective malaria vaccine.
2 Materials and methods

This chapter will describe the procedures and processes followed in the course of this research. Firstly the regulatory requirements and documentation that must be in place prior to commencement of a clinical trial of this nature will be described. Secondly the conduct of the trial will be described including the definitions used. The laboratory experiments will then be described in detail. Finally the Controlled Human Malaria Infection study will be described.

2.1 Clinical trial authorisation procedure

This work involved the first in human administration of a medicinal product so consequently there were several regulatory requirements. Firstly the trial (VAC038) was registered on the European Medicines Agency database (EudraCT 2011-001875-38). A study of this type, involving novel agents, had never been performed in Ireland before. In addition it has been a number of years since any phase I trials involving healthy volunteers had been carried out in Ireland. Given the lack of a recent track record of the regulatory agencies in Ireland, it was decided to apply for all relevant regulatory approvals in the UK in parallel as a back up to the plans in Ireland.

Regulatory approval for the study was granted by the UK Medicine and Healthcare Regulatory Authority (MHRA) (ref. 21584/0285/001-000) and by the Irish Medicines Board (CT number CT 900/516/2, Case number 2107330). Ethical approval was granted by the National Research Ethics Committee – South Central – Oxford A in the UK (ref. 11/SC/0289) and by the Research Ethics Committee of Beaumont Hospital in Ireland (ref. 11/58). Vaccine use was authorized by the Environmental Protection Agency (EPA) of Ireland (Reference number G0451-01) and by the Genetic Modification Committee of the Oxford Radcliffe Hospitals trust (Ref. GM462.11.63). All participants gave written informed consent prior to any study procedure being undertaken. The study was conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. The Local Safety Committee provided safety oversight and GCP compliance was independently monitored by an external organization (Appledown Clinical Research Ltd, Great Missenden, UK). Though approval in one European Union (EU) member state may now confer clinical trials authorisation in another EU member state, this was not the case at inception of this trial or during its lifetime. Details of the regulatory process are contained in the results section, 3.1 Regulatory approval for conduct of the clinical trial, VAC038.
2.1.1 Good Clinical Practice
The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) brings together the regulatory authorities and pharmaceutical industry in order to achieve greater harmonisation to ensure that safe, effective, and high quality medicines are developed and registered in the most resource-efficient manner. Compliance with standards set out by Good Clinical Practice (GCP) is a requirement of most regulatory authorities. All staff involved in running a clinical trial must be trained in GCP. In this study this was achieved through recognised local and online courses. Roles and responsibilities are laid out in GCP. Key areas are discussed in the following section.

2.1.2 Sponsor
The University of Oxford acted as sponsor for this trial. The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

2.1.3 Funder
This work was funded by a grant from the European Vaccine Initiative (EVI). EVI is an agency that provides a mechanism to facilitate concerted interaction between an European Community (EC) core activity and European Union (EU) Member States' investments and to accelerate the process of bringing promising research results, i.e., experimental vaccines for diseases of poverty, via limited industrial production to clinical evaluation in European volunteers; and subsequent clinical evaluation in close collaboration with clinical research networks in disease endemic areas.

2.2 Documentation
A range of defined documentation was generated in order to comply with the requirements of the various regulatory bodies. These must all be submitted to the Independent Ethics Committee (IEC) and medicines regulator for review when seeking approval to conduct a trial. Copies can be found in the Appendix.

2.2.1 Protocol
The protocol is a document that describes how a clinical trial will be conducted (the objective(s), design, methodology, statistical considerations and organization of a clinical trial,) and ensures the safety of the trial subjects and integrity of the data collected.
2.2.2 Investigator's brochure
The Investigator's Brochure (IB) is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects. Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol, such as the dose, dose frequency/interval, methods of administration and for safety monitoring procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical trial. The IB must be updated annually whilst trials including that product are still underway.

2.2.3 Volunteer information sheet
This is a requirement for seeking informed consent. Essentially it is a document written in plain language that explains the purpose of the study, the risks and benefits of participation and procedures of the study.

2.2.4 Consent forms
Key items of the study are listed to be initialled by the volunteer and a copy of the volunteer information sheet should be attached. A separate request form is required where any DNA material is being studied. This was performed for this study.

2.2.5 Advertisement
Copies of all advertising materials to be used must be submitted to the regulatory authorities. For VAC038 we submitted copies of posters, fliers and wording of radio and email advertisements.

2.2.6 Case report form (CRF)
This is a tool for documenting information gathered during the course of a clinical trial. Standardised CRF's were generated to maximise clear concise data collection and also to ensure procedures, such as vaccination, were performed as per protocol.

2.2.7 Diary cards
Diary cards detail information that volunteers should record during the study. They are reviewed periodically during the trial and the information forms a major part of the safety data gathered.

2.2.8 Clinical study plan
This is not a GCP or regulatory requirement, but is a very useful document. It details the day to day steps in the running of a clinical trial, particularly the management of trial volunteers and biological samples. It offers a quick reference to contact information and SOPs. It does not supersede the protocol.

2.2.9 Development of Standard Operating Procedures (SOP)

Standard Operating Procedures (SOPs) are uniformly written procedures, with detailed instructions on routine operations, processes and practices to be followed within an organisation. Many of the SOPs needed for this study already existed in the RCSI Clinical Research Centre and were referenced. Some study specific SOPs were created. All SOPs used are listed in a training matrix, which matches SOPs, personnel and training required/completed. All SOPs relevant to the conduct of VAC038 are listed below.

RCSI Clinic Research Centre SOPs

1. SOP 1 Writing and Reviewing SOPs.
2. SOP 2 Application to conduct research in the CRC
3. SOP 3 Ethics Committee Approval pt 1
4. SOP 3 Ethics Committee Approval pt 2
5. SOP 4 Budget Proposals
6. SOP 5 Investigator Contracts
7. SOP 6 CRC Study File
8. SOP 7 Allocation of space & resources
9. SOP 8 Insurance & Indemnity (no form)
10. SOP 8 Insurance & Indemnity
11. SOP 9 Study set-up & continuing review
12. SOP 10 Admission to CRC
13. SOP 11 Consenting Procedure
14. SOP 12 Collecting, handling, storing and transport of biological specimens
15. SOP 13 Management of a patient whose health deteriorates while in the CRC 2011
16. SOP 13 Management of patient whose health deteriorates while in the CRC
17. SOP 14 Handling, storage and administration of medicinal products
18. SOP 15 Protocol Compliance
19. SOP 16 Transport of biological specimens by road from the CRC to external laboratories
20. SOP 17 Data Protection
21. SOP 18 Archiving clinical research documents
22. SOP 19 Audit of clinical trials
23. SOP 20 Documenting & reporting Adverse events & Serious adverse events
24. SOP 21 Lone working in the CRC
25. SOP 22 Instructions for Invoicing & Non-payment of invoices
26. SOP 23 Service of equipment
27. SOP 24 Bookings rooms in the CRC
28. SOP 25 Terms & Conditions for use of Consultation rooms, day ward & boardroom in the CRC
29. SOP 26 Investigator File
30. SOP 31 Operating instructions for use of centrifuge
31. SOP 32 Use of & temperature monitoring of fridge, -20, -80 freezer
32. SOP 34 Use of ECG machine
33. SOP 35 Safety checks
34. SOP 41 Planning & recording of training for staff
35. SOP 51 Instructions for using Photocopier
36. SOP 52 Instructions for using fax machine

RCSI Vaccine study specific SOPs (All written by Eoghan de Barra)
1. RCSI Vaccine study SOP 1- Access to the CRC
2. RCSI Vaccine study SOP 2 - Training of staff with responsibilities relating to the handling of GMO
3. RCSI Vaccine study SOP 3 - Emergency response in the event of unintentional GMO release
4. RCSI Vaccine study SOP 4 - Reporting of incidents involving GMOs
5. RCSI SOP Vaccine storage and accountability 2.0
6. RCSI SOP Vaccination 2.0
7. RCSI Vaccine study SOP 7 - Autoclave operation, deactivation of GM waste
8. RCSI Vaccine study SOP 8 - Malaria vaccine trial sample handling V1.0
9. RCSI Vaccine study SOP 9 – Accurate timekeeping in VAC038
10. RCSI Vaccine study SOP10 - Venepuncture

Jenner Institute, University of Oxford SOPs
1. SOP Vaccine Dilutions (MC012F1)
2. SOP VC004 Safety Reporting
3. ML002 v2.0 Malaria Peripheral Blood Mononuclear Cells (PBMC) Separation & Freeze
4. ML003 v2.0 PBMC Thawing
5. ML005 v2.0 Malaria Lab Data Handling
2.3  **Clinical Trials Approval (CTA)**
This was a multicentre study. Approvals were sought in parallel in Ireland and in the UK, partly as this was the first time such a study, using viral vectored vaccines was proposed to be carried out in Ireland and to have the UK site prepared in case there were regulatory blocks in Ireland. Applications were therefore lodged in Ireland with the Irish Medicines Board (IMB) and in the UK with the Medicines Healthcare products Regulatory Agency (MHRA).

2.3.1  **Independent Ethics committee (IEC)**
Ethics approval was sought from the Beaumont Hospital Research Ethics committee in Ireland and the National Research Ethics Service committee South Central – Oxford A in the UK.

2.3.2  **Genetically Modified Organism (GMO) release**
Directive 2001/18/EC of the European Parliament regulates the deliberate release of GMOs within the European Union (EU). A detailed risk assessment must be carried out by the national regulatory authority prior to granting of approval for deliberate release permission. A Genetically Modified Organism (GMO) is defined as an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Thus, the vaccines under investigation were subject to this directive.

2.3.3  **Local Safety Committee / GMO committee**
Research involving the use of a GMO must have the approval of the facilities’ local safety and GMO committee to ensure local procedures and measures are in place to safely handle the material and any incidents that might arise following its use. An application to the RCSI local safety committee was made.

2.4  **Volunteer motivation and experience study**
A separate ethics approval was sought and granted to anonymously gather data on the motivation and experiences of volunteers in this clinical trial. Ethics approval was granted by Beaumont Hospital Research Ethic Committee. All volunteers who had been screened were invited to complete an anonymous online questionnaire adapted from a previously published and validated questionnaire.[85]
2.5 Volunteer motivation and experience questionnaire

This questionnaire was designed to look at why healthy volunteers participate in phase I studies, and to find out about their experience of participating in such studies.

There are three parts to the questionnaire. The first part of the questionnaire asked about socio-economic details, the second sought to explore motivation for participation in a phase I study and the third part the experience of being involved in a phase I study. The full questionnaire can be found in appendix 9.1.

2.6 Vaccine production, shipping and storage

2.6.1 Vaccine production

Vaccines for human use must be produced under Good Manufacturing Practice conditions. Good manufacturing practice (GMP) is the minimum standard that a medicines manufacturer must meet in their production processes. Products must: be of consistent high quality; be appropriate to their intended use; meet the requirements of the marketing authorisation (MA) or product specification. Before a batch can be released for use it must be certified by the Qualified Person (QP). The QP is also responsible for approving the product for use post shipping.

2.6.2 ChAd63 CS and MVA CS Vaccines

Previous vectored vaccines expressing the entire CS construct (CS) have been evaluated in Oxford, demonstrating only modest (400 – 1000 IFN-γ spot-forming units (SFU) per million PBMC) T cell immunogenicity and efficacy on sporozoite challenge.[59, 86] The poor immunogenicity of the standard full length CSP insert used in previous vectors in clinical trials (CSO)[58, 59, 61], suggest that there may be an important difference in the intrinsic immunogenicity of the previously tested CSO insert compared to the ME-TRAP insert. Using information from multiple sources[62, 87, 88], the team based at the Jenner Institute, University of Oxford, designed a novel CS antigen, to be used in this study, which omits the extreme C-terminus of the protein that encodes the GPI-anchor sequence. The CS insert is a codon-optimised cDNA encoding a C-terminally truncated Plasmodium falciparum CS protein. Compared to wild-type, the expressed protein lacks the C-terminal 14 amino acids, in order to inactivate the GPI-anchor signal sequence. It also has a reduced number of NANP repeats. The expression of CS in ChAd63 is controlled by a CMV promoter and BGH polyA signal. The CMV promoter and BGH polyA are in ChAd63. The modified vaccinia virus Ankara (MVA)
vaccine consists of the attenuated vaccinia virus with expression of the DNA sequence encoding the Malaria CS antigen regulated by the vaccinia P7.5 early/late promoter. The region including CS and the P7.5 promoter is flanked by sequence from the vaccinia thymidine kinase (TK) locus to allow insertion into the vaccinia genome at this locus. The recombinant vaccine consists of MVA expressing a codon-optimised partial sequence from the *P. falciparum* liver-stage antigen circumsporozoite protein. The sequence of the CS insert in this vaccine contains from N- to C- terminus: the leader sequence from human tissue plasminogen activator (tPA) followed in-frame by the sequences encoding the central repeat region and the majority of the C terminus of circumsporozoite protein. The DNA sequence coding for the CS antigen insert was synthesized by GeneArt GmbH (Regensburg, Germany) using codons most often used in mammals to encode the sequence. Generation of the recombinant vectors has been previously described [67]. Briefly, ChAd63 CS was generated in suspension PC92-GMP cells and purified by caesium chloride density-gradient centrifugation. They were manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility, University of Oxford in the case of ChAd63 CS and by IDT Biologika, Rossau, Germany in the case of MVA CS. MVA CS was generated in chicken embryo fibroblasts (CEFs) and purified by sucrose density-gradient centrifugation. Both Investigational Medicinal Products (IMPs) were manufactured and released in accordance with the European Clinical Trials Directive (2004). Each vaccine lot underwent comprehensive quality control analysis to ensure that the purity, identity and integrity of the virus met pre-defined specifications. Vaccine lots were stored at the clinical site in a temperature-monitored freezer. The immuno-potency of the ChAd CS and MVA CS vaccines was confirmed by immunogenicity evaluation in mice. To ensure on-going stability for both vaccines, MVA CS was tested regularly by titration on CEFs in addition to mouse potency testing and ChAd63 CS was tested by hexon immunostaining for infectious units.

**Table 2.1 ChAd63 CS Batch Analysis Data** - comprehensive quality control analysis to ensure that the purity, identity and integrity of the virus met pre-defined specifications prior to approval for human use.

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Slightly opaque solution essentially free from visible particulates / particle free</td>
</tr>
<tr>
<td>Aggregrates*</td>
<td>Report result</td>
</tr>
<tr>
<td>Sterility</td>
<td>Complies Ph Eur.</td>
</tr>
<tr>
<td>Test</td>
<td>Specification</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Endotoxin (EU/mL)</td>
<td>&lt; 1.0 EU/mL</td>
</tr>
<tr>
<td>Osmolality (mOsmol/kg)</td>
<td>As buffer A438&lt;br&gt;(394-420 mOsmol/kg)</td>
</tr>
<tr>
<td>pH</td>
<td>pH 6.5 – 6.8</td>
</tr>
<tr>
<td>Concentration (vp/mL)</td>
<td>&gt; 1.1 x 10¹¹ vp/mL</td>
</tr>
<tr>
<td>Infectivity* (ifu/mL)</td>
<td>Report result</td>
</tr>
<tr>
<td>Ratio of Viral Particle Concentration to Infectious Vector Titre</td>
<td>&lt; 200:1</td>
</tr>
<tr>
<td>$A_{260}/A_{280}$ ratio</td>
<td>1.2 – 1.4</td>
</tr>
<tr>
<td>Identity (PCR)</td>
<td>Positive for CS</td>
</tr>
<tr>
<td>Absence of previous CBF product by identity PCR</td>
<td>Negative by identity PCR</td>
</tr>
<tr>
<td>Insert sequence Integrity check</td>
<td>Similar to reference sequence</td>
</tr>
<tr>
<td>Genome Integrity*</td>
<td>Restriction digest pattern matches expected band pattern</td>
</tr>
<tr>
<td>Residual Host Cell Protein (ng/mL)</td>
<td>Not more than 200 ng/mL</td>
</tr>
<tr>
<td>Residual Benzonase (ng/mL)</td>
<td>Not more than 50 ng/mL</td>
</tr>
<tr>
<td>Residual Host cell DNA (ng/mL)</td>
<td>Not more than 10 ng/dose</td>
</tr>
<tr>
<td>Residual Caesium chloride</td>
<td>Not more than 300 μg/mL</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>Report result for information only</td>
</tr>
<tr>
<td>Abnormal Toxicity Test</td>
<td>Complies Ph. Eur.</td>
</tr>
<tr>
<td>Extended <em>in vitro</em> Assay for the Detection of Viral Contaminants</td>
<td>No evidence of viral contamination</td>
</tr>
<tr>
<td>using 3 Detector Cell Lines</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Specification</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Complies Ph Eur.</td>
</tr>
<tr>
<td>Test for the Presence of Unapparent Viruses using Suckling Mice, Adult Mice, Guinea Pigs and Embryonated Eggs</td>
<td>No evidence of viral contamination</td>
</tr>
<tr>
<td>Detection of Replication Competent Adenoviruses</td>
<td>Less than 1 per $3 \times 10^{10}$ viral particles</td>
</tr>
<tr>
<td>Detection of Adeno Associated Virus</td>
<td>Negative</td>
</tr>
<tr>
<td>FPERT</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 2.2 ChAd63 CS Tox batch data – sample batch analysis data for ChAd63 CS.

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility check</td>
<td>Pass</td>
<td>Pass</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>$&lt; 1.0$ EU/mL</td>
<td>$&lt; 0.200$ EU/mL</td>
</tr>
<tr>
<td>Virus particle number</td>
<td>$&gt;1.1 \times 10^{11}$ VP/mL</td>
<td>$1.26 \times 10^{11}$ VP/mL</td>
</tr>
<tr>
<td>$A_{260}/A_{280}$ ratio</td>
<td>$1.2 – 1.4$</td>
<td>1.29</td>
</tr>
<tr>
<td>Virus Infectivity</td>
<td>Report result</td>
<td>$1.43 \times 10^{9}$ pfu/mL</td>
</tr>
<tr>
<td>P:I ratio</td>
<td>Report result</td>
<td>1:72</td>
</tr>
<tr>
<td>Identity check</td>
<td>Positive for CS</td>
<td>Positive for CS</td>
</tr>
</tbody>
</table>

2.6.3 Vaccine shipping and storage

Vials of ChAd63 CS and MVA CS were stored between −70°C and −90°C. All movements of the study vaccines were documented. Vaccines were shipped via World Courier Ltd in accordance with international shipping regulations with temperature logger devices. Cold chain was confirmed before any vials were permitted for use. Vaccine accountability, storage, shipment and handling was in accordance with local Standard Operating Procedures (SOP) and regulatory requirements.
2.7 Recruitment of volunteers

2.7.1 Volunteer screening study

Much of the success of a clinical trial relies on the ability of a site to recruit. No trial activity, including advertising for volunteers may commence in advance of clinical trials authorisation. Thus this may give rise to delays in overall timelines. In order to address this and to assess feasibility of conducting a phase I study in Ireland, ethics approval was sought, and granted, for a volunteer screening study. This approval allowed for advertising and screening of potential volunteers, who could then be contacted and screened for the actual trial, when approvals were in place.

2.7.2 Advertising and communications

A variety of approaches were employed to recruit volunteers for screening. All forms of advertising had to be approved by the IEC and could only be used when clinical trials authorisation was approved. Posters, radio, newspaper and email advertisements were submitted for approval. The relative value of these approaches and word of mouth is presented in the results section, 3.2.2.

2.7.3 Screening and enrolment

The study was conducted at the Clinical Research Centre, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland. Healthy, malaria-naïve males and non-pregnant females aged 18-50 were invited to participate in the study. There was no selection of volunteers on the basis of pre-existing neutralizing antibodies (NAb) to the ChAd63 vector prior to enrolment. Volunteers were given study details initially on the telephone and volunteer information sheets were forwarded for review. A minimum period of 48 hours was allowed to elapse prior to consent being sought at the screening visit.

2.7.4 Inclusion and Exclusion Criteria

Inclusion Criteria

The volunteer must satisfy all the following criteria to be eligible for the study:

- Healthy adults aged 18 to 50 years
- Able and willing (in the Investigator’s opinion) to comply with all study requirements
- Willing to allow the investigators to discuss the volunteer’s medical history with their General Practitioner
• Women only: Must practice continuous effective contraception for the duration of the study.

• Agreement to refrain from blood donation during the course of the study and for 6 months after the end of their involvement in the study.

• Written informed consent.

Exclusion Criteria

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

• History of clinical *P. falciparum* malaria

• Travel to a malaria endemic region during the study period or within the preceding six months with a significant risk of malaria exposure.

• Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period.

• Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data.

• Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.

• Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)

• Pregnancy, breast feeding or intention to become pregnant during the study

• History of allergic disease or reactions likely to be exacerbated by any component of the vaccine e.g. egg products, Kathon.

• History of clinically significant contact dermatitis.

• Any history of anaphylaxis post vaccination or any serious reaction following vaccination.

• History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).

• History of migraine headache.

• History of serious psychiatric condition that may affect participation in the study.

• Any other serious chronic illness requiring hospital specialist supervision. Use of regular medications such as anti-hypertensives would not necessarily result in exclusion.
• Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week or Carbohydrate Deficient Transferrin (CDT) >3%.
• Suspected or known injecting drug abuse in the 5 years preceding enrolment.
• Suspected or known use of opiates, cocaine, amphetamines, benzodiazepines or marijuana.
• Seropositive for hepatitis B surface antigen (HBsAg).
• Seropositive for hepatitis C virus (antibodies to HCV).
• Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination.
• Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
• Being in a particularly dependent relationship with the investigator by way of occupation or otherwise, which in the investigators’ opinion places the volunteer in a vulnerable population.

Re-vaccination exclusion criteria
The following adverse events (AEs) associated with vaccine immunisation constitute absolute contraindications to further administration of an IMP to a volunteer. If any of these events occur during the study, the subject will be withdrawn from the trial and followed up by the clinical team or their GP until resolution or stabilisation of the event;
• Anaphylactic reaction following administration of vaccine
• Any serious reaction following vaccination
• Pregnancy

2.8 Conduct of clinical trial (GCP and requirements of IMB/EPA/ethics)

2.8.1 First in human clinical trials
Guidelines on the requirements for first-in-man clinical trials for potential high risk medicinal products were published in 2007 by the European Medicines Agency (EMEA), the overall regulatory of medicinal products within the EU. (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002989.pdf). To minimise risk several key aspects of the trial design should be evaluated and guide the choice of: study population; first dose; number of subjects per dose increment (cohort); interval between dosing subjects within the same cohort; dose escalation increments;
transition to next dose cohort; stopping rules; defining responsibilities for decisions with respect to subject dosing and dose escalation. All of these aspects were built into the trial design and protocol for this study.

### 2.8.2 Study design

The objective of the study was to assess the reactogenicity and immunogenicity of ChAd63 CS at two doses, $5 \times 10^9$ virus particles (vp) and $5 \times 10^{10}$ vp, administered alone and in heterologous prime boost with MVA CS $2 \times 10^8$ plaque forming units (pfu) in healthy malaria-naïve adults. This was a phase Ia open-label, non-randomized malaria vaccine trial. The clinical trial protocol is in the appendix, see Protocol S1, Checklist S1 and Supplementary Information S1. The sample size ($n = 24$) was chosen to allow determination of the magnitude of the primary outcome measures, especially of serious and severe AEs, rather than assessment of statistically significant differences between groups. Allocation to study groups (Table 2.3) occurred at screening based on volunteers’ availability. Twelve volunteers were vaccinated intramuscularly (IM) with $5 \times 10^9$ vp ChAd63 CS (in 0.9% NaCl and administered in 350µL) (groups 1A & 1B). Eight of these volunteers were subsequently vaccinated 56 days later with $2 \times 10^8$ pfu MVA CS IM, undiluted and administered in 340µL (group 1B). Another twelve volunteers were vaccinated IM with $5 \times 10^{10}$ vp ChAd63 CS undiluted and administered in 350µL (group 2A & 2B) and eight of these were subsequently vaccinated 56 days with $2 \times 10^8$ pfu MVA CS undiluted and administered in 340µL. All vaccines were administered in the deltoid region, with volunteers in groups 1B & 2B receiving ChAd63 CS and MVA CS in alternating arms.

**Table 2.3:** Overview of trial groups: All vaccinations are intramuscular.

<table>
<thead>
<tr>
<th>Group Number</th>
<th>No. of volunteers</th>
<th>ChAd63 CS Day 0</th>
<th>MVA CS Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>4</td>
<td>$5 \times 10^9$ vp</td>
<td>-</td>
</tr>
<tr>
<td>1B</td>
<td>8</td>
<td>$5 \times 10^9$ vp</td>
<td>$2 \times 10^8$ pfu</td>
</tr>
<tr>
<td>2A</td>
<td>4</td>
<td>$5 \times 10^{10}$ vp</td>
<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>8</td>
<td>$5 \times 10^{10}$ vp</td>
<td>$2 \times 10^8$ pfu</td>
</tr>
</tbody>
</table>
Volunteers attended clinical follow-up at days 1, 14, 28, 56 and 90 following ChAd63 CS immunization in groups 1A and 2A and at days 1, 14, 28, 56, 57, 63, 84 and 140 following ChAd63-CS immunization in groups 1B and 2B. Safety assessments, including blood sampling for safety and immunology analysis at these visits were conducted as described in Tables 2.4.2 and 2.4.3 below. Participants were given a diary in which to record AEs. A time window ranging between 1 and 14 days was allowed for vaccination and follow-up visits.

2.8.3 Vaccination and clinical review schedule
The first volunteer to receive each vaccine at each dose was vaccinated alone and observed in clinic for 12 hours. They were then reviewed again in clinic 24 hours post vaccination. When 72 hours had elapsed and in the absence of safety concerns, volunteers 2 and 3 were vaccinated with that vaccine and dose. Once 72 hours had elapsed, and in the absence of safety concerns, other volunteers were administered the vaccine at the same dose. Apart from the first volunteer to receive each vaccine at a particular dose, all volunteers were observed in clinic for 30 minutes after each immunization. Prior to dose escalation of the ChAd63 CS vaccine from $5 \times 10^9$ vp to $5 \times 10^{10}$ vp, the independent data safety monitoring board reviewed and approved a report of all safety data collected from volunteers up to 14 days after receiving $5 \times 10^9$ vp ChAd63 CS.

Volunteers were given a digital thermometer, injection site reaction measurement tool and symptom diary card to record their daily temperature, injection site reactions and solicited systemic AEs for 14 days following vaccination with ChAd63 CS and 7 days following vaccination with MVA CS. Local and systemic reactogenicity was evaluated at subsequent clinic visits and graded for severity, outcome and association to vaccination as per the criteria outlined in Tables S1, S2 and S3. Blood was sampled at all visits post vaccination except days 1 and 57, and the full blood count with differential, platelet count and serum biochemistry (including electrolytes, urea, creatinine, bilirubin, alanine aminotransferase, alkaline phosphatase and albumin) measured.
The following two tables, 2.4 and 2.5 summarise the vaccination, clinical review and laboratory analyses schedules.

**Table 2.4: Schedule of clinical reviews for Groups 1A & 2A**

**Timeline of visits Groups 1A and 2A**

<table>
<thead>
<tr>
<th>Review No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline (days)</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>28</td>
<td>56</td>
<td>90</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Window (days)</td>
<td>-90</td>
<td><strong>±2</strong></td>
<td>±7</td>
<td>±7</td>
<td>±14</td>
<td>+14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion / Exclusion criteria</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
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</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
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<tr>
<td>Urinalysis</td>
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</tr>
<tr>
<td>β-HCG urine test</td>
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<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Review contraindications</td>
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<tr>
<td>Vital signs</td>
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<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
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<tr>
<td>--------------------------------</td>
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<td>---</td>
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<td>---</td>
<td></td>
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</tr>
<tr>
<td><strong>Local &amp; systemic AEs assessed</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td><strong>Diary cards provided</strong></td>
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<td>X</td>
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<td><strong>Diary cards collected</strong></td>
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<td>X</td>
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<tr>
<td><strong>HLA typing (mL)</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>HBV,HCV,HIV (mL)</strong></td>
<td>5</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Haematology (mL)</strong></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em><em>Biochemistry</em> (mL)</em>*</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate Deficient Transferrin</strong></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exploratory immunology</strong></td>
<td>70</td>
<td>13</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood volume per visit (mL)</strong></td>
<td>16</td>
<td>74</td>
<td>13</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cumulative blood volume (mL)</strong></td>
<td>16</td>
<td>90</td>
<td>103</td>
<td>179</td>
<td>255</td>
<td>331</td>
<td>407</td>
<td></td>
</tr>
</tbody>
</table>

**S** = screening visit, **V** = vaccination visit, *(x)* = If necessary (Windows refer to time since last visit)

* Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests. **Window: -12 hours/+48 hours. † can be via telephone*
### Table 2.5: Schedule of clinical reviews for Groups 1B & 2B

**Timeline of visits Groups 1B and 2B**

<table>
<thead>
<tr>
<th>Review No.</th>
<th>S</th>
<th>D0</th>
<th>D1</th>
<th>D14</th>
<th>D28</th>
<th>D56</th>
<th>D57</th>
<th>D63</th>
<th>D84</th>
<th>D140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline (days)</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>28</td>
<td>56</td>
<td>57</td>
<td>63</td>
<td>84</td>
<td>140</td>
<td>180</td>
</tr>
</tbody>
</table>
| Window (days) | -90 | **±2** | ±7 | **±2** | ±7 | **±2** | ±7 | ±14 | +14 | \n
| Inclusion / Exclusion criteria | X | X | X | X | X | X | X | X | X | X |
| Informed consent | X | | | | | | | | | |
| Medical History | X | (x) | (x) | (x) | (x) | (x) | (x) | (x) | (x) | (x) |
| Physical Examination | X | (x) | (x) | (x) | (x) | (x) | (x) | (x) | (x) | (x) |
| Urinalysis | X | | | | | | | | | |
| β-HCG urine test | X | X | X | | | | | | | |
| Review contraindications | X | X | X | | | | | | | |
| Vaccination | X | X | | | | | | | | |
| Vital signs | X | X | X | (x) | (x) | X | X | (x) | (x) | (x) |
| Local & systemic AEs assessed | X | X | X | X | X | X | X | X | X | X |
Diary cards provided | X | X | X
---|---|---|---
Diary cards collected |  | X | X
HLA typing (mL) | 4 |  |  |  |
HBV,HCV,HIV (mL) | 5 |  |  |  |  |  |
Haematology (mL) | 2 | 2 | 2 | 2 | 2 | 2 |
Biochemistry* (mL) | 4 | 4 | 4 | 4 | 4 | 4 |
Carbohydrate Deficient Transferrin | 5 |  |  |  |  |  |
Exploratory immunology | 70 | 13 | 70 | 70 | 70 | 70 | 70 | 70 |
Blood volume per visit (mL) | 16 | 74 | 13 | 76 | 76 | 76 | 76 | 76 |
Cumulative blood volume (mL) | 16 | 90 | 103 | 179 | 255 | 331 | 344 | 420 | 496 | 572 |

S = screening visit, V = vaccination visit, (x) = If necessary (Windows refer to time since last visit)  
* Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests. **Window: -12 hours/ +48 hours. † can be via telephone

2.9 Assessment of scientific objectives

2.9.1 Primary evaluation criteria

Safety of the vaccine regimens was assessed by analysing actively and passively collected data on AEs from diary cards, clinical review of volunteers and laboratory measurements.

2.9.2 Secondary evaluation criteria

Immunological assays were conducted according to the procedures established in the test laboratories. The following parameters were considered evidence of the impact of vaccination in inducing malaria-specific immune responses.

(A) Interferon gamma CS peptide ELISpot.
(B) Flow cytometry to measure T cell responses to CS
(C) Antibody response. ELISA was used to assess the levels of anti-CS antibodies.

For full details of methodology see section 2.11.5 ELISpot and 2.11.6 Intracellular cytokine staining and 2.11.6 Antibody immunology.

2.9.3 Data Safety Monitor Board (DSMB)
An independent board of infectious diseases physicians experienced in clinical trials were invited to form the DSMB for the trial. They were provided with the current protocol and updated on trial progress. In accordance with the EMEA guidelines and in compliance with the clinical trial authorisation provided by the IMB, the DSMB was required to review the safety data of the volunteers who received the lower dose ChAd63 CS vaccine, and give a positive opinion, before any volunteers could receive the higher dose vaccine.

2.9.4 Interim safety notifications and reports
Specific requirements were made by the IMB with regards to safety and progress. Firstly they were to be notified as soon as the first three volunteers had been vaccinated. They were also notified immediately when once all volunteers in group 1A and 1B (ChAd63 CS low dose) had been vaccinated. A report detailing all adverse events observed in these 12 volunteers was forwarded to the DSMB as outlined above in 2.4.5. A further interim report, for the IMB, was provided once three months had elapsed after the vaccination of the first volunteer. Other notifications including the end of study report were produced in accordance with GCP guidelines and regulatory requirements.

2.9.5 Adverse event recording and reporting
All AEs that occurred during the study observed by the investigator or reported by the volunteer were recorded in the Case Report Form (CRF). AEs that were present at the end of the study were followed up (with the volunteer’s permission) until a satisfactory resolution or stabilisation occurred.

The severity of clinical and laboratory adverse events and their relationship to vaccination were assessed according to the scales in Tables 2.6, 2.7 and 2.8 and Appendix 9.2 and 9.3. These scales are recommended by the Brighton collaboration (https://brightoncollaboration.org), a collaborative research network with a mission ‘to enhance the science of vaccine research by providing standardized, validated, and objective methods for monitoring safety profiles and benefit to risk ratios of vaccines.’ These scales are endorsed by the WHO.
Laboratory values which fell outside the reference range of the local laboratory processing samples were deemed laboratory abnormalities. These abnormalities were assessed by the trial clinician (Eoghan de Barra). If deemed an untoward medical occurrence, this abnormality was documented as an AE and ascribed a severity grading as per predefined table - Severity grading criteria for laboratory abnormalities (appendix 9.2). Laboratory abnormalities that were not considered AEs were collated and included in the end of study report.

Table 2.6 Assessment of severity of AEs.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of the indicated symptom</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>Awareness of a symptom but the symptom is easily tolerated</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Discomfort enough to cause interference with usual activity</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Incapacitating; unable to perform usual activities; requires absenteeism or bed rest</td>
</tr>
</tbody>
</table>
Table 2.7 Assessment of severity of local AEs.

(A) Swelling & Erythema

<table>
<thead>
<tr>
<th>Grade</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1 - 50</td>
</tr>
<tr>
<td>2</td>
<td>&gt;50 – 100</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

(B) Pain

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No pain at all</td>
</tr>
<tr>
<td>1</td>
<td>Painful to touch, no restriction in movement of arms, able to work, drive,</td>
</tr>
<tr>
<td></td>
<td>carry heavy objects as normal</td>
</tr>
<tr>
<td>2</td>
<td>Painful when limb is moved</td>
</tr>
<tr>
<td></td>
<td>*(i.e. restriction in range of movement in arm, difficulty in carrying</td>
</tr>
<tr>
<td></td>
<td>objects)*</td>
</tr>
<tr>
<td>3</td>
<td>Severe pain at rest</td>
</tr>
<tr>
<td></td>
<td><em>(i.e. unable to use arm due to pain.)</em></td>
</tr>
</tbody>
</table>

Table 2.8 Assessment of relationship to vaccination.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Relationship</td>
<td>No temporal relationship to study product <em>and</em> Alternate aetiology (clinical state, environmental or other interventions); <em>and</em> Does not follow known pattern of response to study product</td>
</tr>
<tr>
<td>1</td>
<td>Possible</td>
<td>Reasonable temporal relationship to study product; <em>or</em> Event not readily produced by clinical state, environmental or other interventions; <em>or</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Similar pattern of response to that seen with other vaccines</td>
</tr>
<tr>
<td>2</td>
<td>Probable</td>
<td>Reasonable temporal relationship to study product; <em>and</em> Event not readily produced by clinical state, environment, or other interventions <em>or</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Known pattern of response seen with other vaccines</td>
</tr>
<tr>
<td>3</td>
<td>Definite</td>
<td>Reasonable temporal relationship to study product; <em>and</em> Event not readily produced by clinical state, environment, or other interventions <em>and</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Known pattern of response seen with other vaccines</td>
</tr>
</tbody>
</table>

2.10 Laboratory materials
### Table 2.9 General reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Company</th>
<th>Cat No</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI</td>
<td>Sigma</td>
<td>R0883</td>
</tr>
<tr>
<td>Penicillin/Streptomycin</td>
<td>GibcoBRL/ Invitrogen</td>
<td>15140-122</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>Gibco</td>
<td>25030-24</td>
</tr>
<tr>
<td>FCS</td>
<td>Biotest</td>
<td>S1810</td>
</tr>
<tr>
<td>PBS with Tween-20</td>
<td>Sigma</td>
<td>P3563</td>
</tr>
<tr>
<td>Leucosep tubes</td>
<td>Greiner</td>
<td>227209</td>
</tr>
<tr>
<td>0.22 µm filters for serum PVDF membrane</td>
<td>Fisher Scientific</td>
<td>FDR-120-060Y</td>
</tr>
<tr>
<td>Casyton counting buffer</td>
<td>Sedna Scientific</td>
<td>43003</td>
</tr>
<tr>
<td>Casyton tubes</td>
<td>Sedna Scientific</td>
<td>43001</td>
</tr>
<tr>
<td>DMSO</td>
<td>Sigma</td>
<td>D2650</td>
</tr>
<tr>
<td>Cryobabies labels</td>
<td>Jencons</td>
<td></td>
</tr>
<tr>
<td>Mr. Frosty cryocontainer</td>
<td>Fisher</td>
<td>CRY-120-01OT</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>BDH</td>
<td>296946H</td>
</tr>
<tr>
<td>Lymphoprep</td>
<td>Axis Shield</td>
<td>1114545</td>
</tr>
<tr>
<td>Cryovials</td>
<td>Nunc</td>
<td>CRY-960-130J</td>
</tr>
<tr>
<td>PHA-L</td>
<td>Sigma</td>
<td>L41444</td>
</tr>
<tr>
<td>PBS</td>
<td>Sigma</td>
<td>3813</td>
</tr>
<tr>
<td>Plate sealers – Titre tops</td>
<td>Scientific laboratory supplies</td>
<td>PJB-720-030M</td>
</tr>
<tr>
<td>U-bottom 96 well plates</td>
<td>VWR</td>
<td>402030716</td>
</tr>
<tr>
<td>ELISpot plates (Multiscreen-IP)</td>
<td>Millipore</td>
<td>MAIPS4510</td>
</tr>
<tr>
<td>Carbonate buffer capsules</td>
<td>Sigma</td>
<td>C-3041</td>
</tr>
<tr>
<td>IFN antibody catcher (1-DIK)</td>
<td>Mabtech</td>
<td>3420-2A</td>
</tr>
<tr>
<td>IFN antibody detector (7-1B6-Biotin)</td>
<td>Mabtech</td>
<td>3420-2A</td>
</tr>
<tr>
<td>Strepavidin-ALP</td>
<td>Mabtech</td>
<td>3420-2A</td>
</tr>
<tr>
<td>BCIP/NBT plus</td>
<td>Moss Inc/ Europa Bioproducts</td>
<td>NBTH-1000</td>
</tr>
<tr>
<td>Benzonase</td>
<td>Novagen</td>
<td>70664-3</td>
</tr>
</tbody>
</table>
### Table 2.10  ICS reagents / FACS antibodies

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-CD28 and anti-CD49d at 1µg/ml</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>5µl/ml of CD107a-PeCy5</td>
<td>eBioscience</td>
</tr>
<tr>
<td>Staphylococcal enterotoxin B</td>
<td>Sigma</td>
</tr>
<tr>
<td>Brefeldin A</td>
<td>Sigma</td>
</tr>
<tr>
<td>Monensin (GolgiStop)</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>dead cell discrimination dye VIVID, 1/80</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>CD4-APC (1/20)</td>
<td>eBioscience</td>
</tr>
<tr>
<td>CD14- and CD19-Pacific Blue (both 1/50)</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD3-PeCy5 (part A, 1/20)</td>
<td>eBioscience</td>
</tr>
<tr>
<td>CD3-Alexa Fluor 700 (part B, 1/100,</td>
<td>eBioscience</td>
</tr>
<tr>
<td>CD8-APC-Alexa Fluor 780 (1/50) and IFN-γ-FITC (1/50), IL-2-PE (1/100)</td>
<td>eBioscience</td>
</tr>
</tbody>
</table>

### 2.11 Laboratory methods

#### 2.11.1 Screening and safety bloods

Blood samples were collected into lithium heparin-treated, citrate or whole blood vacutainer blood collection systems (Becton Dickinson, UK). Samples were labelled, processed as per Table 2.11 below, and dispatched with appropriate request forms to Claymon Bionmis laboratories. Results were returned both electronically and in paper form.
Table 2.11  Screening samples

<table>
<thead>
<tr>
<th></th>
<th>Bottle</th>
<th>Processing</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FBC</strong></td>
<td>5ml EDTA</td>
<td>None</td>
<td>Biomnis</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>5ml Serum</td>
<td>Centrifuge*</td>
<td>Biomnis</td>
</tr>
<tr>
<td><strong>Carbohydrate Deficient Transferrin</strong></td>
<td>5ml Serum +4°C</td>
<td>Centrifuge*</td>
<td>Biomnis</td>
</tr>
<tr>
<td><strong>HIV, HBV &amp; HCV</strong></td>
<td>2ml Serum</td>
<td>None</td>
<td>Biomnis</td>
</tr>
<tr>
<td><strong>Urine toxicology</strong></td>
<td>Universal container</td>
<td>None</td>
<td>Biomnis</td>
</tr>
<tr>
<td><strong>Urinalysis</strong></td>
<td>Universal container</td>
<td>In house</td>
<td>CRC - clinic</td>
</tr>
<tr>
<td><strong>Urine Pregnancy</strong></td>
<td>Universal container</td>
<td>In house</td>
<td>CRC - clinic</td>
</tr>
</tbody>
</table>

*Centrifuge bottle @ 3000 RPM for 15 minutes within 2 hours of collection

2.11.2  Human leucocyte antigen typing

All volunteers enrolled into the trial had blood samples collected immediately prior to first vaccination event for HLA typing, 4ml citrate tube. These samples were processed at the Immunology laboratory, Beaumont hospital. Results of HLA A, B and C types for each volunteer were returned.

2.11.3  Cellular immunology

Interferon gamma CS peptide Enzyme-Linked ImmunoSpot (ELISpot) and flow cytometry were used to measure T cell responses to CS and were considered evidence of the impact of vaccination in inducing malaria-specific immune responses. The ELISpot assay is a widely used method for monitoring cellular immune responses in humans and other animals. The ELISpot assay is a proven means for monitoring cell-mediated immunity, being able to detect antigen specific T cells within a population of PBMCs. Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to assess the levels of anti-CS antibodies. Cellular immunology was investigated as per Tables 2.4: Schedule of clinical reviews for Groups 1A & 2A and Table 2.5: Schedule of clinical reviews for Groups 1B & 2B.
2.11.4 Peptide pools

CSP overlapping peptides (NEO Peptide, Cambridge, MA, USA), 15 amino acids (aa) in length and overlapping by 10 aa spanning the entire CSP insert, were reconstituted in 100% DMSO at 50-200 mg/mL and combined into various pools for ELISpot and flow cytometry assays. The composition of peptide pools containing 2 to 15 peptides is shown below (Table 2.12). Lyophilised peptides were stored frozen at -80°C and not used if defrosted and refrozen more than three times.
Table 2.12 Peptide pools use in ELISpot analysis.

<table>
<thead>
<tr>
<th>Mimotope Order</th>
<th>PEPTIDE</th>
<th>SEQUENCE</th>
<th>MG</th>
<th>SOLVENT</th>
<th>VOLUME (µL)</th>
<th>MG/ML</th>
<th>Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CSN1</td>
<td>MRKAILSVSFLFV</td>
<td>2.4</td>
<td>DMSO</td>
<td>24</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>CSN2</td>
<td>ILSSFLFVEALFQ</td>
<td>2.3</td>
<td>DMSO</td>
<td>23</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>CSN3</td>
<td>SFLEALFQEQCQY</td>
<td>2.8</td>
<td>DMSO</td>
<td>28</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>CSN4</td>
<td>EALFQEQYGSGSNN</td>
<td>3</td>
<td>DMSO</td>
<td>30</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>CSN5</td>
<td>EYCGQSGSNTRVNL</td>
<td>2.3</td>
<td>DMSO</td>
<td>23</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>CSN6</td>
<td>GSSSNTRVLNELYNDV</td>
<td>2.7</td>
<td>DMSO</td>
<td>27</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>CSN7</td>
<td>TRVLNELYNDVAGTN</td>
<td>2</td>
<td>DMSO</td>
<td>20</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>CSN8</td>
<td>ELNYDAGTNLVNEL</td>
<td>2.6</td>
<td>DMSO</td>
<td>26</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>CSN9</td>
<td>NACTVNLNELEMMNY</td>
<td>2.6</td>
<td>DMSO</td>
<td>26</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>CSN10</td>
<td>LYNELEMNYYGKQEN</td>
<td>2</td>
<td>DMSO</td>
<td>20</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>CSN11</td>
<td>EMNYGYKQENWYSLK</td>
<td>2.6</td>
<td>DMSO</td>
<td>26</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>CSN12</td>
<td>GKOENWYSLKNSRS</td>
<td>2.7</td>
<td>DMSO</td>
<td>27</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>CSN13</td>
<td>WYSLKNNSRSRLGEND</td>
<td>2.6</td>
<td>DMSO</td>
<td>26</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>14</td>
<td>CSN14</td>
<td>KNSRLGENDGNINE</td>
<td>2.8</td>
<td>DMSO</td>
<td>28</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>CSN15</td>
<td>LGENDDGNEDNEKL</td>
<td>2.8</td>
<td>DMSO</td>
<td>28</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>CSN16</td>
<td>DGNENDEKLRKPPK</td>
<td>2.8</td>
<td>DMSO</td>
<td>28</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>17</td>
<td>CSN17</td>
<td>DNEKLRKPKHKLKQ</td>
<td>2.7</td>
<td>DMSO</td>
<td>27</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>18</td>
<td>CSN18</td>
<td>RKPKHKLQKPADGN</td>
<td>2.5</td>
<td>DMSO</td>
<td>25</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>19</td>
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2.11.5 ELISpot methodology

ELISpot coating buffer was prepared by dissolving one carbonate-bicarbonate buffer capsule (Sigma) in 100 ml deionised water. 96 well ELISpot plates (Millipore) were coated in a Class II microbiological safety cabinet (MSC). A coating solution was made by adding 10 µl of catcher antibody (1-D1K, Mabtech) per ml of ELISpot coating buffer resulting in a concentration of 10 µg/ml. 50 µl per well of the coating solution was added to each well of the ELISpot plate. Plates were then stored at room temperature (RT) for 3-8 hours or at +4°C for 8-48 hours.

Blood samples were collected into lithium heparin-treated vacutainer blood collection systems (Becton Dickinson, UK). PBMCs were isolated and used within 6 h as follows; 15ml of Lymphoprep was pipetted into a 50-ml Leucosep tube (Greiner), and centrifuged at 1000 x g for 1 min to get the Lymphoprep below the porous filter disc. Tubes and lids were labelled with the subject number.

Mr Frosty Cryocontainers (Fisher) were cooled to 4ºC in preparation for freezing of excess cells and the end of the ELISpot preparation.

R0 (500ml RPMI +5ml Pen/strep + 5ml L-glutamine) and R10 (500ml RPMI +5ml Pen/strep + 5ml L-glutamine + 50ml filtered FCS) were prepared.

The ELISpot plates were blocked by flicking off the coating solution, and washing the plates 3 times with 100 µl per well of sterile phosphate buffered saline (PBS) (Sigma) using a multichannel pipette. The wells were then blocked with 100 µl per well of R10 and kept at room temperature for 1-8 hours or at +4°C for 8-48 h. The peptide plates were thawed in the MSC. 15-30 ml heparinised blood was poured into a Leucosep tube (each 50-ml blood sample is divided into two Leucosep tubes) and centrifuged at 1000 x g for 13 minutes at RT without brake. The excess plasma was removed with a transfer pipette then the PBMCs were collected from the interface, and placed into a new, labelled 50-ml falcon tube. This amounted to approximately 10ml of buffycoat for each volunteer sample at each timepoint. The tubes were topped up to 45 ml with R0, and spun at 620 xg for 5 minutes at RT. The cell pellet was flicked to resuspend it, and the cells from one donor were pooled; 30 ml of R0 was added to cells in one of the falcon tubes and then transferred to the other tube. With all cells now in one tube it was spun at 620 xg for 5 minutes at RT. The pellet was resuspended in 10 ml R10 for counting. Cells were then counted using a haemocytometer and resuspended to form a concentration of 200,000 PBMCs per 50 µl of R10.
The blocking solution was flicked off from plate, and peptides (9 pools as per Table 2.12 above) and other stimulants (negative control, R0, PHA, SEB, FEC) were added to the wells by transferring 50 μl of solution from the stimulant plate to the corresponding wells on the ELISpot plate with a multichannel pipette. 9 peptide pools were used. Details are available in Figure 2.1. Negative control was 50 μl of R10. Three separate positive controls were used; Lectin from *Phaseolus vulgaris* (red kidney bean) final concentration 10μg/mL (PHA); Staphylococcal enterotoxin B from *Staphylococcus aureus* (final concentration 0.02μg/mL) (SEB); FEC (pool of peptides from influenza, Epstein Barr virus and cytomegalovirus, final concentration 10μg/mL, Neo peptide). 50 μl of cell suspension was added to the appropriate wells on the plate (ie. 200,000 PBMC per well).

The plate was incubated for 18-20 hours at +37°C, 5% CO2 in a tissue culture incubator.

The remaining cells were spun in the 50-ml falcon at 620 xg 5 min at RT, then resuspend up to a maximum volume of 5 ml FCS (i.e. 0.5 ml of FCS per vial to be frozen, ensuring to always have at least 5 million cells per vial). The cells were placed on ice for 30 minutes. Equal volumes of ice-cold 20% DMSO in FCS was added to the cell/FCS suspension. The vial contents was mixed by pipetting gently up and down. Cell suspensions were aliquoted into labelled cryovials (1 ml per vial) and placed immediately into the Mr Frosty containers. The container was placed in a –80°C freezer for at least 24 hours. Thereafter the cryovials were transferred to liquid nitrogen storage.

Following incubation the cell suspension was flicked off into a container of Virkon® (DuPont), and the plate was washed 6 times with PBS-Tween, flicking off the washing solution and blotting onto absorbent paper between washes. The detector antibody (7-B6-1-Biotin) (Mabtech) was diluted to 1:1000 in PBS resulting in a concentration of 1μg/ml, and 50 μl added to each well. The plate was incubated for 2-4 hours at RT. The detector antibody was flicked off and the plate was washed 6 times with PBS-Tween, flicking off the washing solution and blot onto absorbent paper between washes.

Streptavidin-ALP (SA-ALP) (Mabtech) was diluted 1:1000 in PBS, and 50 μl added to each well. The plate was incubated for 1-2 hours at RT. The SA-ALP was flicked off and the plate was washed 6 times with PBS-Tween, flicking off the washing solution and blotting onto absorbent paper between washes. 50μl of developer (BCIP/NBT(Plus), solution. Europa bioproducts) was added per well and allowed to develop for approximately 5-15 minutes. The developing reagent was flicked off into the sink. Plates were washed in tapwater, wash backing removed and dried overnight on paper towels. Spots were counted using an ELISpot counter.
(Autoimmun Diagnostika (AID), Germany). Results are expressed as IFN-γ spot-forming units (SFU) per million PBMC. Background responses in unstimulated control wells were almost always less than 20 spots, and were subtracted from those measured in peptide-stimulated wells.

**Figure 2.1 ELISPOT plate layout.** All wells were in duplicate. This represents well layout for one blood sample for one volunteer at one timepoint. ELISpots were processed on a 96 well plate, just 32 wells are shown here.
Table 2.13  Immunology bloods peri-vaccination

Day 0 – Pre Vaccination

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Day 1 – post vaccination

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2.11.6 Intracellular cytokine staining (ICS)

Cytokine secretion by PBMC was assayed by intracellular cytokine staining (ICS) followed by flow cytometry. Excess cells from each Ex-vivo ELISpot run were frozen in FCS containing 10% DMSO and stored in liquid nitrogen. Frozen cells were thawed in a water bath at 37°C and immediately transferred into warm R10 medium. Cells were washed twice in warm R10, resuspended in R10 containing 25U/mL benzonase (Sigma) and incubated for 4 hours at 37°C, 5% CO₂ in a humidified incubator. Live cells were counted by trypan blue staining, washed and resuspended in fresh R10 and used in the assay.

Responses were assessed by 7 colour flow cytometry by stimulating aliquots of 1x10⁶ cells in 1ml of medium containing anti-CD28 and anti-CD49d at 1µg/ml (Becton Dickinson) and 5µl/ml of CD107a-PeCy5 (eBioscience) with either no antigen, a pool of all 55 peptides spanning peptides 1 through 43 of the CSP antigen (2µg/ml) (see peptide pools used in ELISpot Table 2.12) or a positive control, Staphylococcal enterotoxin B (Sigma, 1µg/ml), in 5ml polystyrene FACS tubes for 18 hours. Brefeldin A (Sigma) and Monensin (GolgiStop, BD Biosciences), both at 1µg/ml, were added for the last 16 hours. Cells were incubated with a dead cell discrimination dye (VIVID, 1/80, Invitrogen), and then surface stained at 4°C with CD4-APC (1/20, eBioscience) or CD4-Qdot 625 (1/50, Invitrogen), CD14- and CD19-Pacific Blue (both 1/50, Becton Dickinson). After permeabilisation, intracellular staining was performed at room temperature with CD3-PeCy5 (part A, 1/20, eBioscience) or CD3-Alexa Fluor 700 (part B, 1/100, eBioscience) plus CD8-APC-Alexa Fluor 780 (1/50) and IFN-γ-FTTC (1/50), IL-2-PE (1/100) and TNFα-Pe-Cy7 (1/50, all eBioscience) and fixed in 1% paraformaldehyde. Acquisition was performed on the day of staining on a BD LSRII; at least 500,000 events were collected per sample. Data was prepared and analysis performed using FlowJo v8.8.6 (Treestar Inc.), Pestle v1.6 and Spice v5.05 (Mario Roederer, Vaccine Research Centre, NIAID, NIH). Dead cells (Vivid+), monocytes (CD14+), and B cells (CD19+) were excluded from the analysis. A time gate was first evaluated, and then cells were gated on lymphocytes, singlets, live CD3+, CD8+ or CD4+ (excluding double-positives), and then IFN-γ and combinations of markers. A sample gating strategy is provided in Figure 4.5. Responses were determined after subtraction of the response in the unstimulated control for each sample. Pie charts were created using absolute measures with a threshold of 0.001%. MFI (Mean Fluorescence Intensity) was calculated using the geometric mean of the cytokine-positive population and iMFI (integrated MFI) represents the integration of the frequency with the geometric mean of the cytokine-secreting population, giving a measure of the total amount of cytokine production.
2.11.7 Antibody immunology

22.5ml of whole blood was collected in 10ml plain vacutainer blood collection systems (Becton Dickinson, UK). For serum preparation, untreated blood samples were stored at 4°C and then the clotted blood was centrifuged for 5 min (1000 xg). Serum was stored at -80°C.

Total IgG ELISA and Immunofluorescence assay (IFA)

(These assays were performed by the staff of Walter Reid Army Institute of Research (WRAIR), USA).

Enzyme Linked Immunosorbent Assay (ELISA) methodology as follows; PfCSP ELISA: ELISA 96-well plates were coated with a synthetic peptide (Eurogentec) based on the repeat region of the PfCSP with the amino acid sequence CS(NANP)6C. The peptide was coated at a concentration of 0.2 μg/mL in a volume of 100 μL per well. Plates were placed inside a humidity chamber and incubated overnight (16 - 20 h) at 22°C. Plates were washed four times with 1xPBS (pH 7.4) containing 0.5% Tween-20 and blocked with 0.5% casein blocking buffer (Sigma) for 1 h at 22°C. Plates were washed four times and serially diluted samples were added and incubated at 22°C for 2 h. After washing four times, peroxidase labelled goat anti-human IgG (KPL) was added at a dilution of 1:4,000 and incubated at 22°C for 1 h. After washing four times, ABTS Peroxidase substrate (KPL) was added for development and incubated for 1h at 22°C. The data were collected using Softmax Pro GXP, data were fit to a 4-parameter logistic curve, and the serum dilution at which the optical density was 1.0 (OD 1.0) calculated. To serve as a positive control, serum obtained from a volunteer participating in a phase I/IIa challenge trial of R32NS181 formulated with alum was used. The individual had anti-PfCSP antibodies but was not protected (personal communication from WRAIR serology laboratory). Samples were considered positive if the difference between the post-immunization OD 1.0 and the pre-immunization OD 1.0 (net OD 1.0) was > 50 and the ratio of post-immunization OD 1.0 to pre-immunization OD 1.0 (ratio) was > 2. For example, if the OD 1.0 was 150 post-immunization and 50 pre-immunization, the net OD 1.0 would be 100, and the ratio of OD 1.0 post-immunization to OD 1.0 pre-immunization would be 3. This would be considered positive.

Preparation of Sporozoite Slides

NF54 mosquitoes were dissected. The thorax was cut at the scutum. The separated heads and the anterior portions of the scutum were kept while the remaining portions of the thorax and abdomen were discarded. The sporozoites were isolated using Ozaki tubes and then purified using a DEAE column. Sporozoites were counted using a hemocytometer. A sporozoite
suspension of 5,000 sporozoites/well was added to each well. Slides were dried overnight. The slides were then wrapped in aluminum foil and placed in a desiccator at -20°C.

**Immunofluorescence assay (IFA)**

The negative control was obtained from a pool of normal rhesus sera and was diluted at 1:50 in PBS+1% BSA. The positive control was obtained from a subject immunized with NANP repeat peptide and also diluted at 1:50 in PBS +1% BSA. Slides were thawed for 20 minutes at room temperature and then blocked with 16 µL of PBS +1% BSA. Then, 15 µL of each sample was added to each well and incubated for 1 hour in a humidity chamber. The slides were then washed 3 times for 2 minutes in 1XPBS. Next, 16 µL of the secondary antibody, goat anti-rhesus IgG (H+L) FITC (Cat# 6200-02, SouthernBiotech) diluted to 1:2000 with PBS+1% BSA was added to each well. The slides were incubated for 1 hour, then were washed 3 times for 2 minutes in 1XPBS. Finally, 1 µL of Vectashield with DAPI (Cat# H-1200, Vector laboratories) was added to each well and the slide was covered with a cover slip. The slide was covered with aluminum foil until read.

**Image Analysis**

Slides were read using an Olympus AX80 Provis fluorescent microscope with a 40x objective, exposure time of 1/3.5 seconds, an isotropic signal of 400 and at 1360x1024 resolution. The well was first scanned then a representative photograph was taken. Images were then analyzed using Image-Pro Plus, MediaCybernetics utilizing a software macro designed such that sporozoites were identified based on size and dimensions ignoring luminous artifacts. Sporozoites were manually selected until all sporozoites were analyzed (a minimum of three). The luminosity for each parasite was automatically averaged and reported, maintaining a CV% less than 20%. Titre cut offs were determined based on the last dilution that gave a positive luminosity (Mean+2SD of the negative control). For IFA, analytic endpoint titre was the last dilution with measured lumens > lower limit of detection (LLD), Calculated endpoint titre was the extrapolated dilution where the lumens > LLD , BLD= Below the limit of detection in this assay (500) vs <500. Lumen = the international system of units (SI) unit of luminous flux.

**2.12 Quality control**

10% of all ELISpot plates underwent a quality control (QC) procedure performed by the Jenner Institute, University of Oxford. This involved review of time lines of cell processing, visual inspection of plate, review of raw data output and validation of positive and negative controls. Positive controls were deemed valid if there were at least 200 spots in one of the
positive control wells (PHA, SEB or FEC). Two negative control wells containing only medium and PBMC where required on each plate. In order to pass QC the mean count of these two wells had to be less than 20 spots per well.

2.13 Controlled human malaria infection

Two phase IIa trials were undertaken assessing the ChAd63 – MVA CS vaccine. I screened and enrolled volunteers into these studies at the University of Oxford, assisted in the malaria infection at Imperial College, London and operated as one of the clinical trial physicians during the intensive follow up period when volunteers were being assessed twice daily for malaria. I collected data and supervised the blood smear readers as part of a team of trial physicians. Unlike in the phase I trial, I did not perform the cellular immunology, nor did I perform antibody assays or the malaria qPCR. These were performed by the team of scientists at the Jenner Institute.

VAC045

This study was conducted at the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford (Oxford, United Kingdom), and at the National Institute for Health Research (NIHR) Wellcome Trust Clinical Research Facility, part of the University of Southampton and University Hospital Southampton National Health Service (NHS) Foundation Trust (Southampton, United Kingdom). The challenge procedure was performed as previously described[89], using 5 infectious bites from *P. falciparum* strain 3D7–infected *Anopheles stephensi* mosquitoes. This took place at the Alexander Fleming Building, Imperial College (London, United Kingdom), and mosquitoes were supplied by the Department of Entomology, Walter Reed Army Institute of Research (WRAIR; Washington, DC). Healthy, malaria-naïve men and non-pregnant women aged 18–45 years were invited to participate in the study. All volunteers gave written informed consent prior to participation, and the study was conducted according to the principles of the Declaration of Helsinki and in accordance with good clinical practice. There was no selection of volunteers on the basis of pre-existing neutralizing antibodies to the ChAd63 vector before enrolment.

Summary of conduct and methodology of Controlled Human Malaria Infection (CHMI) study:

Following a collaborative consensus process involving investigators from the US Military Malaria Vaccine Program (USMMVP), Sanaria, University of Maryland, University of Oxford,
Although there remain minor differences between centres in follow-up procedures in CHMI trial conduct, there is consensus on the following key points.

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination and a pregnancy test for female volunteers.

- Follow-up visits should be scheduled at least once daily, but may increase in frequency to two or three times daily, starting at day 5-7 post-challenge. At all visits volunteers should be questioned about the occurrence of adverse events and use of medication.

- In the event that a volunteer does not attend for a scheduled follow-up visit it is imperative that investigators find that volunteer as quickly as possible and assess them for patent parasitaemia and clinical malaria. Should the volunteer withdraw consent from further follow-up prior to receipt of antimalarial drugs, it may be appropriate to withdraw the volunteer from the trial protocol and administer a course of antimalarial chemotherapy under close supervision.

- Grading and reporting of adverse events should be performed using international and local guidelines. It should be noted that the occurrence of a low frequency of grade 3 severe adverse events, of short duration, and with no long-term sequelae, is not unexpected in CHMI studies. A minority of those challenged are known to experience grade 3 systemic adverse events and this fact should be included in the informed consent form.

- Vital signs should be recorded at least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.

- It is critical that every volunteer must receive every dose of anti-malarial therapy. In some settings fully directly observed treatment will be essential. Where directly observed treatment is not used, investigators must follow volunteers closely to ensure compliance with the treatment regimen.

- After challenge, all volunteers should be followed until they have completely finished anti-malaria treatment.

- Volunteers should be evaluated at least two weeks after finishing treatment.

- A local safety monitor and an independent safety monitoring committee should be established to act as independent experts in evaluating adverse events. The safety monitor or monitoring committee may advise the investigators on initiating antimalarial treatment for a specific volunteer or volunteer group. While safety monitoring committees are not a
requirement for phase I trials, they should be considered a requirement for CHMI trials which have an efficacy component and which have major potential safety concerns.

VAC045 CHMI Inclusion and Exclusion Criteria

Inclusion Criteria:

- Healthy adults aged 18 to 45 years.
- Able and willing (in the Investigator’s opinion) to comply with all study requirements.
- Willing to allow the investigators to discuss the volunteer’s medical history with their General Practitioner.
- Women only: Must practice continuous effective contraception for the duration of the study.
- Agreement to refrain from blood donation during the course of the study and for at least 3 years after the end of their involvement in the study.
- Written informed consent to participate in the trial.
- Reachable (24/7) by mobile phone during the period between CHMI and completion of antimalarial treatment.
- Willingness to take a curative anti-malaria regimen following CHMI.
- For volunteers not living in Oxford: agreement to stay in a hotel room close to the trial centre during a part of the study (from at least day 6.5 post mosquito bite until antimalarial treatment is completed).
- Answer all questions on the informed consent quiz correctly.

Exclusion Criteria:

- History of clinical malaria (any species).
- Travel to a malaria endemic region during the study period or within the preceding six months with significant risk of malaria exposure.
- Use of systemic antibiotics with known antimalarial activity within 30 days of CHMI (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin)
- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days)
immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).
• Use of immunoglobulins or blood products within 3 months prior to enrolment.
• History of allergic disease or reactions likely to be exacerbated by any component of the vaccine (e.g. egg products, Kathon) or malaria infection.
• Any history of anaphylaxis post vaccination.
• History of clinically significant contact dermatitis.
• History of sickle cell anaemia, sickle cell trait, thalassemia or thalassemia trait or any haematological condition that could affect susceptibility to malaria infection.
• Pregnancy, lactation or intention to become pregnant during the study.
• Contraindications to the use of all three proposed anti-malarial medications; Malarone®, Riamet® and Chloroquine.
• History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
• History of serious psychiatric condition that may affect participation in the study.
• Any other serious chronic illness requiring hospital specialist supervision.
• Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week.
• Suspected or known injecting drug abuse in the 5 years preceding enrolment.
• Seropositive for hepatitis B surface antigen (HBsAg).
• Seropositive for hepatitis C virus (antibodies to HCV) with positive PCR for hepatitis C at screening.
• An estimated, ten year risk of fatal cardiovascular disease of ≥5%, as estimated by the Systematic Coronary Risk Evaluation (SCORE) system.76
• Positive family history in 1st and 2nd degree relatives < 50 years old for cardiac disease.
• Volunteers unable to be closely followed for social, geographic or psychological reasons.
• Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination.
• Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
Table 2.14: Overview of trial groups in VAC045: All vaccinations are intramuscular.*No more than six control volunteers will be enrolled for any one challenge time point.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of volunteers</th>
<th>Day 0</th>
<th>Day 56</th>
<th>Day 76</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>ChAd63 CS 5 x 10^10 vp IM</td>
<td>MVA CS 2 x 10^8 pfu IM</td>
<td>CHMI</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>ChAd63 ME-TRAP 5 x 10^10 vp IM</td>
<td>MVA ME-TRAP 2 x 10^8 pfu IM</td>
<td>CHMI</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>CHMI</td>
</tr>
</tbody>
</table>

15 volunteers in Group 1 were vaccinated with ChAd63 CS 5 x 10^10 viral particles (vp) (undiluted and administered in 354µL) followed 8 weeks later by MVA CS 2 x 10^8 plaque forming units (pfu) (undiluted and administered in 339µL). 15 volunteers in Group 2 were vaccinated with ChAd63 ME-TRAP 5 x 10^10 vp (undiluted and administered in 347µL) followed 8 weeks later by MVA ME-TRAP 2 x 10^8 pfu (undiluted and administered in 274µL).
Table 2.15. Schedule of Clinic Attendances Post CHMI in VAC045: All Volunteers (Windows refer to time since last visit). C = challenge, number thereafter denotes days since exposure to malaria. (x) = If considered necessary, emphasising any acute complaints, * Biochemistry includes Sodium, Potassium, Urea, Creatinine, Albumin & Liver Function Tests. Physical examination includes blood pressure, pulse and temperature. *Blood drawn for explorative immunology on C7 (70mls), C7.5 (5mls), C9 (10mls), C14 (10mls) and C21 (70mls) if persistently slide-negative. If slide-positive, 5mls of blood will be drawn at diagnosis (but not if diagnosed on C7.5, C9, C14 or C21) and at 48 hours post diagnosis and then no further blood drawn for explorative immunology until C35. *Blood will be drawn for haematology & biochemistry on C9 and within 24 hours of diagnosis. If a volunteer is slide positive before C9 then blood was drawn for biochemistry and haematology within 24 hours of diagnosis and then not again until C35. £ Urinary β HCG was performed in female volunteers prior to commencing anti-malaria treatment.

<table>
<thead>
<tr>
<th>Timeline (days in relation to CHMI)</th>
<th>C</th>
<th>C6.5</th>
<th>C7</th>
<th>C7.5-C14</th>
<th>C15-C23</th>
<th>C35</th>
<th>C90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Window (days)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>±5</td>
<td>14</td>
</tr>
<tr>
<td>Medical History</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Clinical Assessment</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Urinary β HCG*</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Observations%</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Medic Alert Card Given to Volunteers</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local &amp; systemic events</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Treatment for Malaria</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick Smear / PCR for malaria</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3 x 14</td>
<td>3 x 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology (mL)</td>
<td></td>
<td></td>
<td></td>
<td>2+</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Biochemistry (mL)*</td>
<td></td>
<td></td>
<td></td>
<td>3+</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Exploratory immunology</td>
<td></td>
<td></td>
<td></td>
<td>70+</td>
<td>25+</td>
<td>75+</td>
<td>70</td>
</tr>
<tr>
<td>Blood volume per visit(s) (mL)</td>
<td>0</td>
<td>3</td>
<td>73</td>
<td>72</td>
<td>102</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Cumulative blood volume (mL) - Groups 1 &amp; 2</td>
<td>489</td>
<td>492</td>
<td>562</td>
<td>634</td>
<td>736</td>
<td>811</td>
<td>886</td>
</tr>
<tr>
<td>Cumulative blood volume (mL) - Group 3</td>
<td>93</td>
<td>96</td>
<td>169</td>
<td>241</td>
<td>343</td>
<td>418</td>
<td>493</td>
</tr>
</tbody>
</table>

2.13.1 Ethical and Regulatory Approval
All necessary approvals for the study were granted by the United Kingdom National Research Ethics Service, Committee South Central–Oxford A (reference 12/SC/0037), and the United Kingdom Medicines and Healthcare Products Regulatory Agency (reference 21584/0293/001-0001). The study was additionally reviewed by the Western Institution Review Board (Seattle,
WA; reference 20120266) at the request of the PATH Malaria Vaccine Initiative and was approved. The Genetically Modified Organisms Safety Committee of the Oxford University Hospitals NHS Trust (reference GM462.11.65) authorized recombinant vaccine use. The trial was registered with ClinicalTrials.gov (reference NCT01623557). The local safety committee provided safety oversight, and good clinical practice compliance was independently monitored by an external organization (Appledown Clinical Research, Great Missenden, United Kingdom).

2.13.2 Funding
This work was supported by the PATH Malaria Vaccine Initiative, the United Kingdom National Institute of Health Research, through the Oxford Biomedical Research Centre (grant A91301, Adult Vaccine), and the Wellcome Trust (grants 084113/Z/07/Z and 45488/Z/05 and grant 097940/Z/11/Z).

2.13.3 Vaccination, safety monitoring and immunological assessment.
All were performed as per the VAC038 study described above in sections 2.7, 2.8.3, 2.9.5 and 2.11.

2.13.4 Clinical Follow-up & Safety Assessment
In addition to the safety and monitoring as was carried out during the phase I study, volunteers in the CHMI were reviewed the day before CHMI (C-1). Local and systemic reactogenicity was evaluated at subsequent clinic visits and graded for severity, outcome and association to vaccination as previously described above. Post CHMI volunteers were reviewed on day 6 post CHMI in the evening (C+6.5) and then twice a day, morning and evening between C+7 and C+14. Undiagnosed volunteers were reviewed once a day in the morning between C+15 and C+21. At each visit, blood was sampled for microscopy & qPCR, physical observations performed and AEs solicited. On diagnosis, volunteers were treated with a 3-day curative course of oral Malarone® where each dose was directly observed in clinic. Volunteers intolerant of Malarone® were prescribed an appropriate alternative (oral Riamet® or Chloroquine). Volunteers were reviewed 24 and 48 hours post diagnosis where blood was sampled for microscopy. Provided these two blood films were negative for parasites, volunteers were not reviewed again in clinic until C+35. If one of these blood films were positive, volunteers continued to be reviewed in clinic at 24-hour intervals until two consecutive blood films were negative. Volunteers were then reviewed at C+35 and C+90 where safety assessments were conducted. Full blood count with differential, platelet count and serum biochemistry (including electrolytes, urea, creatinine, bilirubin, alanine aminotransferase, alkaline phosphatase and albumin) were measured at all visits before CHMI (except days 1 and 57), at visit C+9, within
24 hours of diagnosis, and at visits on C+35 and C+90. Blood was sampled for exploratory immunology studies at all visits before CHMI, C-1, C+7, C+14, C+21 (if persistently slide negative), within 48 hours of diagnosis, C+35 and C+90.

2.13.5 Criteria for Malaria Diagnosis

Diagnosis of malaria following CHMI was defined as positive findings of thick film microscopy, with at least 1 morphologically normal malaria trophozoite seen by ≥1 experienced microscopist. qPCR was simultaneously performed, although all investigators directly involved in clinical management were blinded to these results. For volunteers with positive findings of thick film microscopy but no symptoms consistent with *P. falciparum* infection, investigators were unblinded to the qPCR results, with the volunteer treated only if any preceding samples had >500 parasites/mL. For volunteers with symptoms or signs that, in the opinion of the clinical investigators, likely represented malaria (eg, fever, rigors, or severe symptomatology), despite negative findings of thick film microscopy and no alternative cause, investigators were unblinded to the qPCR results. If any volunteer's preceding samples had >500 parasites/mL, the volunteer was treated for malaria. A vaccinee was classified as a participant who demonstrated a delay to patency/treatment if treatment was started >2 times the standard deviation in days after the mean time to treatment of unvaccinated control volunteers. This corresponds to clearance of an estimated >95% of pre-erythrocytic-stage parasites [36]. Patency refers to the patent period; a period of time in the course of a parasitic disease during which the parasitic organisms can be demonstrated in the body.

2.13.6 Quantitative Real-Time PCR (qPCR) assessment of Pf in blood

qPCR was conducted as previously described. [66] Briefly, blood was filtered to reduce white cell content and DNA was extracted from filtered 0.5mL blood using a Qiagen Blood Mini Kit. 10% of each extraction (total eluate volume = 50μL, with 5μL used per assay) was run in triplicate for qPCR – equivalent to 150μL blood directly assessed. Parasites per mL (p/mL) equivalent mean values were generated by a standard Taqman absolute quantitation, against a defined plasmid standard curve with an ABI StepOne Plus machine and v2.1 software. Default Universal qPCR and QC settings were used apart from the use of 45 cycles and 25μL reaction volume. Based upon results obtained using dilution series of microscopically-counted cultured parasites, this method has a lower limit of quantification (LLQ, defined as %CV<20%) of around 20 parasites/mL blood (p/mL).[90] Counted parasite dilution series results suggest that the lower limit of probable detection (LLD, i.e. a probability of >50% of ≥1 positive result among three replicate PCR reactions) is in the region of 5 p/mL, while samples at 1 p/mL are
consistently negative (24/24 PCR reactions). Positive results in this assay (even at very low level) are thus essentially 100% specific for genuine parasitaemia, with positive results beneath the LLQ likely to signify parasitaemia in the range 2-20p/mL.

2.14 Statistical analysis
Data were analyzed using GraphPad Prism version 5.04 for Windows (GraphPad Software Inc., California, USA). Geometric mean or median responses for each group are described. The Kruskall-Wallis test was used for one-way analysis of variance by ranks. Significance testing of differences between two groups used the two-tailed Mann-Whitney U test or Wilcoxon signed rank test as appropriate. No corrections for multiple hypothesis testing were used. Additionally for the CHMI; parasite densities were log transformed to remove skewness, with 1 added to each value to allow transformation of zero values. Significance testing of differences between groups used either a 2-tailed t test or the 2-tailed Mann–Whitney test (or the Kruskal–Wallis test, for comparisons of >2 groups) for nonparametrically distributed data. Correlations were assessed using the Spearman rank correlation coefficient. Time to treatment was analyzed using Kaplan–Meier survival curves, and between-group comparisons were made using the log-rank test.
3 Regulatory approval, enrolment and volunteer experience

Regulatory approval for conduct of the clinical trial, VAC038.

Clinical trial approval

In April 2011, the Department of International Health and Tropical Medicine at the Royal College of Surgeons in Ireland (RCSI) in collaboration with the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), University of Oxford, UK began the process of gaining regulatory approval for the first phase I trial of such a vaccine in Ireland. The vaccines under investigation, ChAd63 CS and MVA CS, are both genetically engineered, viral vectored vaccines, the ChAd63 being of simian origin. As such, approval of the Environmental Protection Agency (EPA) was required in addition to approval of the IMB and IEC. This was a multicentre study. Approvals were sought in parallel in the UK, partly as this was the first time such a study, using viral vectored vaccines was proposed to be carried out in Ireland and to have the UK site prepared in case there were regulatory blocks in Ireland. Presented below are the key time points and challenges in seeking all of the approvals to conduct this phase I study in Ireland and a comparison with the UK process. Following initial contact with the IMB a pre-submission meeting between the investigators / sponsors and the IMB took place in May 2011. The timeline of 180 days was reduced to 60 days when it was clarified that the study did not constitute a gene therapy/Advanced Therapy Medicinal Product. The initial application was submitted 21st July 2011. On the 29th of August a formal reply was received from the IMB containing 94 queries (8 preclinical, 16 clinical and 70 related to product manufacture). We responded to all queries by the 12th of September. Over the subsequent 10 days further queries were received from the IMB, ultimately totalling 124. We were advised that further queries would be forthcoming and that the IMB would be unable to process them in time for the 3rd October (day 60) timeline. Therefore we withdrew the CTA application. It was re-submitted on the 29th October with 72 additional documents and responses to 8 new queries, plus further pre-clinical data on biodistribution, 3 x biodistribution queries, 4 x Quality clarifications (responded to 22nd Nov). In total 139 queries were answered with provision of >100 supporting documents. On the 5th of December 2011 IMB regulatory approval was received.

In the UK the Medicines and Healthcare products Regulatory Agency (MHRA) approved the trial VAC038 in 26 days, subject to full batch analysis for ChAd63 CS being submitted as a substantial amendment prior to dosing. The MHRA try to perform the initial assessment for applications for phase 1 healthy volunteer studies within 14 days or less. Figure 3.1 below, displays a summary of timelines for regulatory approvals in the UK and Ireland. The obvious difference was mainly due to the number and nature of queries from the IMB which are presented graphically in Figure 3.2.
Figure 3.1 Summary of timelines – regulatory approvals in Ireland and UK, 2011.
A graphic comparing the regulatory approval timelines in Ireland and the UK. Blank sections represent ‘clock stopped’ time when queries or requirements from external agency were outstanding.

Figure 3.2 A summary of the chronology of queries and resubmissions for clinical trials approval (CTA). Green blocks represent ‘clock running’ time, light coloured sections, ‘clock stopped’ time.
Figure 3.3 The nature of all queries sent out by the Irish Medicines Board in response to the CTA submission. The majority were relating to quality and manufacturing processes.

3.1.2 Independent Ethics Committee (IEC)

Ireland:

Initial submission to Beaumont Research Ethics Committee in Dublin took place on the 27th May 2011. A 60 day clock started on the 13th of June 2011, following a meeting of the committee. This was stopped on the 15th July when queries were sent to the investigators; 3 relating to pre-clinical data, 10 relating to the protocol and study design, 7 to safety and 24 relating to the Volunteer Information Leaflet (VIS) and consent form. The Ethics Committee also requested copies of all communications between the investigators and the IMB. Following responses to these queries and further communication from the IMB the clock was restarted 29th September 2011. Favourable opinion with 3 conditions (namely IMB approval) was granted on 25th of October 2011. 59 days of clock, 135 days total.

UK:

Ethics approval in the UK was sought from National Research Ethics Service committee South Central – Oxford A. The committee reviewed the trial at a face to face meeting with the sponsor/investigator on the 5th of August 2011. Approval was obtained on the 12th of August, 42 days following initial submission. It should be noted that this IEC has experience in reviewing many of our phase I clinical trials using viral vectored vaccines.

3.1.3 Genetically Modified Organism (GMO) release
Directive 2001/18/EC of the European Parliament regulates the deliberate release of GMOs within the European Union (EU). Across the EU the national parliaments can interpret directives slightly differently and this is the case in relation to deliberate release regulation of GMOs in Ireland and the UK. As with the other regulatory areas, approval was sought in both jurisdictions.

Ireland:
The Environmental Protection Agency (EPA) is the regulator. The EPA considered administration of the GMO vaccine as a deliberate release, as they considered the volunteers as a part of the wider environment. Thus a formal application for permission was required. Application to the EPA for consent for deliberate release of a GMO was made on 22nd June 2011, confirmation of receipt was received on the 12th of July 2012 and a 90 day clock was commenced. Deliberate release required notification to be posted in the national press and to the local authority where the planned release was to take place. Notices were placed in the national press and individual notifications were sent to the local authority, Fingal County Council. A period of time is required to allow objections to be made, however no objects were lodged with the EPA in relation to this study. Standard Operating Procedures relating to handling and disposal of GMOs were forwarded to the EPA. Consent was obtained on the 29th of September 2011, 98 days from application to consent.

UK:
In the UK the Health and Safety Executive (HSE) and Department for Environment, Food & Rural Affairs, (DEFRA) regulate this area. The HSE consider administration of a GMO vaccine as contained use (Contained Use Regulations 2000). No national or local notification is required in the case of a contained release. A detailed risk assessment was produced for the Oxford site and submitted to the local Genetic Modification Safety Committee. GMO authorisation was then approved within a month.

3.1.4 Regulatory Inspections
3.1.4.1 Funder
Prior to final awarding of the grant funding for this project the funders required an independent inspection to be performed. This was to satisfy them that both facilities and staff were capable of delivering the project safely and competently. This inspection took place on the 19th of August 2011 at the RCSI Clinical Research Centre (CRC). The two suboptimal finds were that the carpet in the clinical areas should be removed and that all clocks should be synchronised. The carpet was removed and a new SOP on timing was written stating that only “smart phone”
times be used for recording timings as these are automatically updated and are thus synchronised.

3.1.4.2 Sponsor

It is a requirement of GCP that the sponsor undertakes a site initiation visit prior to the commencement of any clinical trial activities. Therefore this can only take place when Clinical Trials Authorisation is in place. At this meeting the sponsor clearly sets out to the staff at the trial site how the trial shall be conducted with reference to the protocol. Clinical Trial Authorisation for VAC038 was granted on the 5th of December 2011 and the Site Initiation Visit (SIV) then took place on the 13th of December 2011. The sponsor also undertook a mid-trial quality monitoring visit. The quality manager from the Oxford unit visited the trial site and assessed data collection, laboratory process and data management.

3.1.4.3 Independent monitor

The Sponsor arranged independent monitoring which was carried out by Appledown Clinical Research Ltd. They were present at the SIV and conducted early monitoring following enrolment of the first 3 subjects and quarterly thereafter. Monitoring was also carried out on site prior to the IMB inspection. An end of study monitoring visit was also performed. Monitoring reports were returned to the site and issues raised were addressed.

3.1.4.4 Environmental Protection Agency (EPA)

On Friday the 30th of March 2012 a senior inspector from the EPA carried out an inspection of the clinical trial site. GMO handling and waste disposal were reviewed. A positive opinion was received and trial activities continued. An end of study report, dated the 24th of June 2013, accounting for all GMO material was forwarded to the EPA at the end of the study. This was a legal requirement.

3.1.4.5 Irish Medicines Board

There were a number of conditions attached to the Clinical Trial Authorisation granted by the IMB. One was that the trial would be subject to an early GCP inspection. This inspection took place on 17th, 18th and 19th of July 2012. Two senior inspectors from the IMB spent 3 days at the trial site examining all documentation. In advance of the inspection a dossier was prepared and forwarded to the IMB as per their request:

- SOP Index
- Copies of the following SOPs:
Informed consent procedure.

Monitoring procedures. This would also include any monitoring conventions and/or source data verification guidelines specific to this trial.

Investigational Medicinal Product (IMP) management. This would include any trial specific drug supply plan.

Adverse event and SAE reporting procedures

Medical emergency procedures

Training procedures

Filing of essential documents

- Job descriptions and CVs of key personnel who have worked on the trial from the outset and who will be involved in the inspection
- A line listing of subjects who entered the study at the investigator site in the form of the screening and enrolment log.
- A declaration and account of any SAEs (None had occurred).
- A copy of the current protocol and consent forms
- A copy of the current monitoring plan and interim visit report.
- A copy of the current laboratory manual. A copy of the clinical study plan.
- Copies of advertisements used for the trial
- Contractual agreements between Oxford and the RCSI, Biomnis external lab, Appledown monitors contract and e-mail arrangement with Beaumont immunology lab.
- A list of other departments involved in the trial, Biomnis and the immunology lab (HLA lab).
- Directions to the trial site.

The IMB inspection yielded a report with a number of minor findings and no major findings. The areas with findings were as follows;

- Training: related to specific documentation of training records.
- RCSI CRC Document Management System
- Delegation of Responsibilities and Job Descriptions
- Informed consent records
- Investigator Site File
- Agreements
- Monitoring
- Data Safety Monitoring Board
- Facilities and Equipment
Drug Accountability
Source Data Verification

These were responded to in a letter dated the 24th of September 2012. All findings were explained and actions taken where necessary.

3.1.5 Discussion of regulatory approvals and inspections

There were many novel aspects to this trial from an Irish perspective. It was the first trial involving a GMO viral vectored vaccine in Ireland and posed challenges for the regulator in terms of expertise in assessment. The GMO release approval was relatively straightforward despite the fact that Irish legislation interpreted vaccination as a deliberate release, considering humans as part of the wider environment, whereas in the UK vaccination is considered a contained release. This led to the requirement to place notifications in the national press allowing interested parties to object. Interestingly there were no objections received by the EPA. This is in contrast to the numerous objections that they receive when deliberate release has been sought in the case of planting GMO foodstuffs. All inspections were passed without incident and the study team have since been invited to speak at EPA events outlining our experience and advice to other researchers.

There was a marked difference in the approaches of the UK and Irish independent ethics committees. The UK committee had experience of numerous previous similar vaccine trials. The Irish committee also sought copies of all submission to the IMB. This represented vast amounts of documentation on technology and manufacturing. This could be considered to be beyond the remit of an ethics committee. The committee also sought to exclude the enrolment of ‘medical students, nursing students and anyone else considered to potentially have a dependent relationship with the investigators’. This is in contrast to the UK ethics committee decision where all student volunteers are referred to an independent arbitrator of the university to discuss. Many of the volunteers in the UK studies are medical students. Given the role of the principal investigator within the healthcare system in Ireland, as an educator and clinician the exclusion of students of healthcare subjects from future studies could make recruitment challenging pending the interpretation of the potentially dependent relationship. This should be clarified for future studies.

Clinical Trial Approval (CTA) was the most challenging aspect of the regulatory approval process. The timelines between the two jurisdictions varied greatly. The MHRA (UK) had prior experience of this vector expressing different antigens and had ready access to experts to assess the trial. For the IMB (Ireland) this was a novel product and study type. In line with
existing legislation the IMB undertook their own assessment of the manufacturing and pre-clinical data rather than gaining opinion from the MHRA. This resulted in the IMB being unable to complete the assessment within the specified timelines and they were likely to reject the application. Instead of this we voluntarily withdrew and resubmitted, thus allowing more time. In parallel developments the European Union has been moving towards harmonisation of national medicines regulatory authorities. The new Regulation directly applies to all individuals in the European Union. It was adopted by the European Parliament (02 April 2014) and by the Council of Ministers (14 April 2014) and signed off on 16 April 2014. It was published in the Official Journal on 27 May 2014[91] and is expected to become effective on 15 June 2016. It increases the harmonisation of clinical trials across EU member states. In particular it allows for CTA in one member state to be acceptable in another member state. This should greatly enhance the approval process in future as investigators and national regulators will be able to get easier access to timely expert reviews.

3.2 Recruitment
3.2.1 Timelines
Recruitment took place between December 2011 and July 2012. Twenty-four healthy malaria-naïve adult volunteers (12 female and 12 male) were enrolled, immunized and followed up (Figure 3.4). The mean age of volunteers was 30 years (range 21 – 46). Full demographic information on volunteers is available in Table 3.1. Vaccinations began in January 2012 and all follow-up visits were completed by November 2012. All volunteers attended all visits as scheduled and completed the study. The rate of enrolment into the specific study groups is shown in Figure 3.5. The gap between completion of enrolment of groups 1A and 1B and commencement of enrolment of groups 2A and 2B was a requirement of the Irish Medicines Board. Enrolment of groups 2A and 2B could only commence following a safety review of the Data Safety Monitoring Board (DSMB).
39 volunteers were screened. Reasons for not meeting the inclusion criteria in 7 excluded volunteers were: psychiatric morbidity (2), history of malignancy (2), one each of: history of headaches, Carbohydrate Deficient Transferrin (CDT) >3% and neutropenia.

Figure 3.4: CONSORT diagram of study progress. 39 volunteers were screened. Reasons for not meeting the inclusion criteria in 7 excluded volunteers were: psychiatric morbidity (2), history of malignancy (2), one each of: history of headaches, Carbohydrate Deficient Transferrin (CDT) >3% and neutropenia.

Figure 3.5 Enrolment of volunteers to various groups over time. Lines terminate when target number enrolled; 1A 4, 1B 8, 2A 4 and 2B 8, volunteers. All volunteers were enrolled after 31 weeks. Week one was the first week of January 2012.
Table 3.1 Demographics of volunteers enrolled by group.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
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<td>2</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>24</td>
<td>30</td>
<td>27</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>18-20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td></td>
<td>10</td>
</tr>
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<td>41-50</td>
<td>2</td>
<td></td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ethnicity</td>
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<td></td>
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<td>4</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>24</td>
</tr>
</tbody>
</table>

3.2.2 Mode of recruitment

As soon as clinical trial authorization was granted by the Irish Medicines Board, recruitment could begin. Initially volunteers who had expressed an interest or been screened in the separate volunteer screening study were contacted by email and invited to enrol in the vaccine trial. Posters were placed in local colleges, shopping centres and other public places with the permission of the owner or managing authority. Permission was sought for the use of email distribution lists and advertisements were placed in the local papers. A dedicated email address and answer phone service were used to collect expressions of interest. All expressions of interest were followed up with an email and or phone call. The phone call involved the use of a brief screening questionnaire to assess volunteer suitability. If volunteers agreed to proceed, the volunteer information sheet and consent forms were forwarded for their consideration. An appointment, at least 72 hours later, was then arranged for a screening visit if the volunteer wished to proceed. Figure 3.6 Mode of Recruitment, below shows the various sources of enquiries, the conversion rate into screening visits, and ultimately enrolment. Newspaper advertisements led to the greatest number of enquiries, followed by email distribution lists, however they had a low rate of conversion to screening (approximately 10%) and enrolment 6%. Posters and word of mouth resulted in far fewer enquiries, but a much greater conversion rate into screening 100% and 41% respectively. Subsequent conversion to enrolment was also high at 100% and 29% respectively.
3.2.3 Volunteer motivation and experience study

A sub-study entitled “Motivations and Experiences of Healthy Volunteers screened for a phase I Malaria Vaccine Study” was performed as mentioned in Methods 2.4 and 2.5. For full questionnaire see appendix 9.1. There was a 66% response rate (N=26) 50% male, 50% female. 100% of respondents were of European descent. 58% were married or in a relationship and 42% were single. 31% of respondents earned between €1000 and €2000 per month and 31% earned between €2000 and €4000 per month. The remaining 38% earned below €1000 per month. 42% were students. 86% were educated to degree level or higher. 85% had never participated in a research study before.

Motivations

Each of the respondents was asked to rate the importance of various factors had on their decision to participate. Figure 3.7 provides details on the qualitative responses on motivation. Respondents gave a weighting of 1 to 5 (1 being unimportant, 5 being very important) as to the influence of each parameter on their decision to participate. There did not appear to be a difference between male and female respondents. 88% of respondents acknowledged a contribution to the progress of medicine and helping others as their highest motivation for participating. Only 23% of respondents identified financial compensation and free medical check-ups as their most significant motivation. 57% of respondents felt the amount of
financial compensation they received was reasonable. 60% of respondents reported that in all likelihood, they would have proceeded with the study, even if there had been no financial compensation. 52% cited the topic (malaria vaccine) as an influence in their decision to participate in the study. 100% of participants felt they had received sufficient information about the study prior to consent. 73% were not concerned about the possibility of a serious complication occurring as a result of their participation in the study. 56% would recommend participation to a friend/family member. 50% would definitely participate in another Phase I study. There were no significant differences in responses between male and female respondents or by age. Figure 3.8 displays the influence of subject matter on decision of volunteers to participate. It would appear that the subject matter was a very significant factor in volunteers decision making. Finally Figure 3.9 shows attitudes to the level of financial compensation for trial participants. Financial payment for participation in a clinical trial is supposed to compensate volunteers for their time and inconvenience. Despite the variety of backgrounds and income levels amongst volunteers over 92% of them responded that the compensation was reasonable or better (Figure 3.9). By asking how volunteers perceived the level of risk of a serious complication we sought to gauge perceptions of the study team and information provided to volunteers. Over 73% had no concern of such a complication (Figure 3.10).
Figure 3.7 Volunteer motivation. N = 26. Volunteers were asked to rank the contribution of each factor from 1 to 5 (1 being unimportant and 5 being very important) that influenced their decision to volunteer for the study. The two altruistic responses, 'progress of medicine' and 'to help others' have the greatest number '4' or '5' responses, whilst the personal gain responses, 'free medical check-up' and 'financial compensation' received the majority of '1' responses, suggesting they were not important to the majority of respondents.
Figure 3.8 Influence of subject matter on decision to participate. This graph displays grouped responses of volunteers to the question; “did the topic under investigation (Malaria Vaccine) have any influence on your decision to participate in the study”. The majority, 76% responded that the subject matter either definitely or probably influenced their decision.

Figure 3.9 Attitudes to the level of financial compensation for trial participants. Response to the question, ‘what did you think of the amount of the financial compensation for participation?’ Over 92% felt that the financial compensation was reasonable or better.
Figure 3.10 Volunteers perception of risk in this study. Response to the question, ‘were you concerned about the possibility of a serious complication occurring as a result of your participation in the study?’

3.2.4 Discussion of volunteer screening and enrolment

Volunteer screening and enrolment proceeded well and all groups completed allocation within 24 weeks. This included a period of suspension of enrolment so that a mandated DSMB review could occur. In terms of attracting enquires newspaper advertisements clearly yielded the most enquires, but with a low conversion rate to screening visits and ultimately enrolment. Posters and word of mouth were at the other end of the spectrum, yielding lower numbers of enquires, but with a much higher conversion rate into screening and enrolment. The large volume of enquires generated by newspaper advertisements led to a significant amount of work for a relatively low return. Targeted email or online distribution, the modern ‘word of mouth’ may access individuals more likely to proceed to screening and enrolment. This should be considered in future trials. The volunteer motivation and experience study, essentially an anonymous survey of those screened, provides insight into the decision process for potential volunteers in this study. There is a general perception that volunteers are generally involved in clinical trials for the money[85], but this was not borne out in this study. 88% acknowledged a contribution to the progress of medicine and helping others as their highest motivation for
participating, and over 92% felt that the financial compensation was reasonable or better. Indeed most respondents ranked financial or access to a free medical check-up low in the ranking of motivating factors. The subject matter seems to have been a large factor. Altruism, not financial gain was the most significant factor. Combining this motivational data with the modes of recruitment data, suggest that targeting informed, altruistic populations via modern ‘word of mouth’ media would be the most effective strategy in future recruitment campaigns for clinical trials such as this.
4. Safety and Immunogenicity

4.1 Safety

No unexpected or serious AEs occurred and no volunteers were withdrawn due to AEs. Table 4.1 provides details of AEs deemed possibly, probably or definitely related to vaccination. ChAd63 CS demonstrated a good safety profile with the majority of AEs being mild in severity (91%) and 80% of all AEs resolved within 48 hours (Figures 4.1 & 4.2). Overall, 14 out of 24 volunteers (58%) experienced one or more local AEs related to ChAd63 CS; all of which were mild. 20 out of 24 volunteers (83%) experienced one or more systemic AE related to ChAd63 CS and a dose response was seen for systemic reactogenicity, with a greater proportion of volunteers receiving $5 \times 10^9$ vp experiencing a systemic AE than volunteers receiving $5 \times 10^9$ vp. The majority of these AEs were mild in severity. MVA CS administered 8 weeks after the ChAd63 CS was more reactogenic, with 14 out of 16 volunteers (87%) experiencing at least one local AEs, mainly pain, erythema and warmth. 15 volunteers (93%) experienced at least one systemic AEs, including feverishness, myalgia, fatigue, malaise and headache in the 24 hours following vaccination, though the majority of these were mild in severity. This AE profile is similar to the flu-like symptoms that have been reported in the past with similar doses of MVA vectored vaccines expressing other antigens[59, 61, 66, 68, 74, 86, 92-95]. The neutrophil count of one volunteer dropped from $1.56 \times 10^9$/L at screening to $1.09 \times 10^9$/L 28 days post vaccination with ChAd63 CS at a dose of $5 \times 10^9$ vp and remained less than $1.56 \times 10^9$/L throughout follow up.
Table 4.1 Local, systemic and laboratory adverse events post immunization. Adverse events deemed possibly, probably or definitely related to vaccination are shown. ‘Other systemic’ following MVA CS included nasal congestion, laryngitis and pharyngitis. The highest intensity adverse event per subject is listed. Other local AEs included paraesthesia. All ‘other’ AEs were considered possibly related to vaccination due to a temporal association. "The two laboratory AEs related to the same volunteer, who experienced neutropenia following both vaccination. “Three severe systemic AEs followed MVA CS, all experienced by one volunteer simultaneously and resolved within 48 hours of vaccination.

<table>
<thead>
<tr>
<th>Sign or Symptom</th>
<th>ChAd63 CS 5x10⁸</th>
<th>ChAd63 CS 5x10⁹</th>
<th>MVA CS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(% of volunteers)</td>
<td>(% of volunteers)</td>
<td>(% of volunteers)</td>
</tr>
<tr>
<td>LOCAL</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Pain</td>
<td>2(17%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythema</td>
<td>4(33%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Warmth</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Swelling</td>
<td>2(17%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Scaling</td>
<td>1(8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2(17%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total local AEs</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SYSTEMIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feverish</td>
<td>1(8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2(17%)</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3(25%)</td>
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<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>2(17%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other systemic AEs</td>
<td>1(8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory</td>
<td>1(8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total systemic AEs</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 4.1 Local and systemic adverse events (AEs) possibly, probably or definitely related to vaccination shown as percentage of volunteers affected. (a) Post ChAd63 CS $5 \times 10^9$ vp; (b) Post ChAd63 CS $5 \times 10^{10}$ vp; (c) Post MVA CS $2 \times 10^8$ pfu.
Figure 4.2 The mean duration and range of duration of local and systemic AEs possibly, probably or definitely related to vaccination. (A) post ChAd63 CS 5x10^9 vp; (B) post ChAd63 CS 5x10^10 vp; (C) post MVA CS.

4.2 Immunogenicity

4.2.1 Cellular immunogenicity

4.2.1.1 ELISPOT

Antigen-specific T cell responses in all volunteers as measured by ex-vivo IFN-γ ELISPOT are shown in Figure 4.3. When comparing the responses to two different doses of ChAd63 CS, no significant difference was seen between group 1 (ChAd63 5 x 10^9 vp) and group 2 (ChAd63 CS 5 x 10^10 vp) at the peak of the response on day 14 (median 423 [range 12.5 – 1590] vs 178 [range 52.5 – 1795] SFC/million PBMCs, p = 0.54 Mann-Whitney test). Thereafter T cell responses gradually contracted to day 56 (Figure 4.3 panels A, C and E). Administration of MVA CS at day 56 significantly boosted responses in all volunteers as measured 7 days later on day 63 (Figure 4.3, panels B, D and E). No significant difference was seen when comparing day 63 ELISPOT responses between groups 1B & 2B (median 1523, [range 380 – 4125] vs 1048 [range 320 – 5450] SFC/million PBMCs in groups 1B and 2B respectively, n=8 v 8, p = 0.5 Mann-Whitney test). ELISPOT responses subsequently contracted but remained above baseline.
when measured at the last time-point, day 140, (median 1B 346.25 [range 17.5 – 1607], median 2B 176 [range 102 – 707] SFC/million PBMCs), Figure 4.3. There was no significant difference between the groups at this time-point (p = 0.16 Mann-Whitney test). Median ELISpot response in groups 1B and 2B were significantly different when all time-points were analysed (p < 0.0001 for both groups, Kruskall-Wallis). Analysis of individual volunteers in group 1B over time showed significantly different responses; day 0 to 14 p = 0.0156; day 14 to 63 p = 0.0391 and day 14 to 63 p = 0.0391 (Wilcoxon matched pairs test). A similar pattern was found analysing group 2B individuals over time; day 0 to 14 p = 0.0156; 14 to 63 p = 0.0547 ; 0 to 63 p =0.0078. No significant difference was seen amongst individuals in group 1A; day 0 to 14 p = 0.25; or group 2A; day 0 to 14 p = 0.37.
Figure 4.3 Summary of PBMC IFN-γ ELISpot responses of volunteers in each group. Summed SFC / million PBMCs. (A) and (B) individual responses for groups 1A and 1B respectively over time. (C) and (D) show individual responses for groups 2A and 2B respectively over time. (E) median ELISpot response by group by time-point, changes were significant over time; for group 1B, day 0 to 14 $p = 0.0063$; day 14 to 63 $p = 0.0148$; day 0 to 63 $p = 0.0002$, Mann-Whitney test; and group 2B, day 0 to 14 $p = 0.01$; day 14 to 63 $p = 0.0074$; day 0 to 63 $p = 0.0002$, Mann-Whitney test. (F), (G) & (H) show individual responses by group at days 14, 63 and 84 or 90 respectively.
4.2.1.2 Intracellular cytokine staining (ICS)

Antigen-specific CD3+ T cell functionality was also assayed by ICS at the days 14, 63 and 84/90 time-points. Following peptide re-stimulation, detectable CSP-specific CD3+ T cells consisted of a mixed CD4+ and CD8+ phenotype, Figure 4.5. It should be noted that the ELISpot and intracellular cytokine staining (ICS) assays vary in methodology (including the use of multiple versus a single peptide pool respectively, differences in peptide concentration, use of co-stimulatory antibodies and the use of fresh versus frozen PBMC). As no difference in ELISpot response was seen between volunteers receiving different doses of ChAd63 CS, data for ICS was combined across groups 1B & 2B and assays were performed where cell numbers allowed. Figure 4.6 presents the relative proportion of multifunctional cells at the peak observed at day 63 with detail on which cytokine is being produced tabulated at the base. The different pie segments represent number rather than type of cytokine being produced.

Across all three time-points analysed CD107a (marker of degranulation) expression was up-regulated by both CD4+ and CD8+ T cells. Figure 4.6. CD4+ cells produced higher levels of TNFα than CD8+ cells at all time-points, but this was not statistically significant (p = 0.58, p = 0.31 and p = 0.48 for days 14, 63 and 84 respectively, Mann-Whitney test). CD4+ cells also produced greater levels of IL-2 compared to CD8+ cells at all time-points, however this did not reach significance (p = 0.77, P = 0.59 and p = 0.32 for days 14, 63 and 84 respectively, Mann-Whitney test) (Figure 4.7). Negligible levels of IFN-γ were produced by either CD4+ or CD8+ cells at days 14 and 63. Levels comparable to IL-2 and TNFα were observed at day 84. Distinct populations of CD4+ and CD8+ T cells expressing 1+, 2+, 3+ or 4+ functional markers / cytokines were evident following a Boolean gate analysis (Figure 4.5, 4.6 and Table 4.2).
Figure 4.5 Gating strategy for analysis of CSP-specific T cell responses. Representative flow cytometry plots are shown for the analysis of CSP-specific T cell responses from volunteers immunized with ChAd63-MVA CSP. (A) Initial gating used (from top left to bottom right) forward scatter area (FSC-A) versus forward scatter height (FSC-H) to remove doublet events and select singlet cells; then following this small lymphocytes were gated using FSC-A versus side scatter area (SSC-A); then live CD14− CD20− CD3+ cells were selected; then CD4 versus CD8 was used to select the total CD4+ CD8− cell population and vice versa for the CD8+ CD4− population. Cytokine (IFN-γ, IL-2 and TNFα) and CD107a gating using bivariate plots is shown for (B) CD4+ cells and (C) CD8+ cells. (B) Representative plots for un-stimulated (UNS), CS peptide stimulated (CS), SEB stimulated samples are shown. IFN-γ (top row), IL-2 (second row), TNFα (third row) and CD107a (bottom row) for the CD8− CD4+ T cell population were analyzed using bivariate plots. Percentages refer to the % of CD8− CD4+ cells that express the specific cytokine or marker. Background responses in UNS control cells were subtracted from the CS response respectively during the analysis. (C) Same analysis as in (B), except for the CD4− CD8+ T cell population.
Figure 4.6 T cell multi-functionality following ChAd63-MVA CS immunization. The multi-functionality of the CD4+ and CD8+ T cell responses was assessed by polychromatic flow cytometry and ICS. Frozen PBMCs from day 63 were re-stimulated with a pool of CSP peptides and cells stained as described in methods above. Gating strategy and representative plots are shown in Figure 4.5. Responses are grouped and colour-coded according to the CD4+ and CD8+ subsets, and the number of functions detected for each T cell population. Individual data points showing the percentage of the parent CD4+ or CD8+ response are shown for each of the functional populations indicated on the X-axis. The pie charts summarize the fractions of CSP specific CD4+ or CD8+ T cells that are positive for a given number of functions (CD107a, IFNγ, IL-2 and TNFα). The row at the base of the Figure labelled 'pie chart' provides a key to the colours of pie segments, the darkest colours representing cells that produced 4 cytokines and the lightest colour producing one.

Table 4.2 Percent of parent population (CD4+ or CD8+) producing 1 or more cytokine by group at day 63 post vaccination. Cytokines measured: CD107a, IFNγ, IL2 and TNFα.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD4+</th>
<th>CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>4+</td>
<td>0.0000</td>
<td>0.0277</td>
<td>1.0346</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0.0119</td>
<td>0.0186</td>
<td>0.2990</td>
</tr>
<tr>
<td>2B</td>
<td>4+</td>
<td>0.0055</td>
<td>0.1566</td>
<td>0.6155</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0.0153</td>
<td>0.0704</td>
<td>0.2080</td>
</tr>
</tbody>
</table>
Figure 4.7 Cytokine production by cell type and time-point assessed by 7 colour flow cytometry. Mean percent and standard error of the mean (SEM) of CD4+ and CD8+ PBMCs producing antigen-specific cytokines at given time-point post vaccination are shown for each cytokine. (A) percent CD4+ and (B) percent CD8+ PBMCs producing CD107a, IFNg, IL2 and TNFa at day 63. (C) percent CD4+ and (D) percent CD8+ PBMCs producing CD107a, IFNg, IL2 and TNFa at day 63. (E) percent CD4+ and (F) percent CD8+ PBMCs producing CD107a, IFNg, IL2 and TNFa at day 84.
4.2.1.3 Breadth of response

T cell responses in all volunteers were detected in multiple peptide pools spanning the entire CSP vaccine insert in the ex-vivo IFN-γ ELISpot assay, (Figure 4.8). Following priming immunization with ChAd63 CS individual responses were seen across all pools with no apparent immune-dominant region in CS detected. One week post boost with MVA CS, responses were again observed across all pools. Pools 1, 2, 5, 6 showed a significant increase in ELISpot response when comparing pre and post boost results, with p values of 0.0229, 0.0021, 0.0227, 0.0026 respectively (Wilcoxon matched pairs signed rank test).

The 6 highest responders at day 63 were assessed by ex-vivo IFN-γ ELISpot for responses to single peptides in an attempt to map the most immunogenic epitopes of the CS insert. Median responses to peptides 1 to 43 (15mer peptides overlapping by 10aa) are shown in Figure 4.9. Peptides 1, 3, 5, 8, 42 and 43 were identified as containing dominant epitopes, although it is likely that there is a single epitope spanning peptides 42 and 43.

![Figure 4.8 Individual ELISpot responses in SFC/10^6 PBMC at day 63 by peptide pool. Bar represents median, whiskers; interquartile range.](image)
4.2.2 Antibody immunogenicity

The kinetics and magnitude of the serum IgG antibody response against CS were assessed over time by ELISA (Figure 4.10). All volunteers had IgG titres below the limit of detection at day zero. CS-specific IgG was induced in all volunteers. Mean responses peaked at day 14 with CS antibody titre of 631 and 713 for groups 1(A&B) and 2(A&B) respectively. Boosting with MVA CS resulted in a significant increase in antibody concentration in group 1B, compared to the unboosted group 1A, as measured at day 84/90 (p = 0.037; Mann-Whitney test). However this was not seen when comparing groups 2A and 2B at the same time-points (p = 0.49 Mann-Whitney test). Mean antibody response was higher in group 2B compared to group 1B at day 140, but this difference was not statistically significant (p = 0.87 Mann-Whitney test). Samples from any volunteer with ELISA titres above the lower limit of detection (LLD) at both day 28 and 84/90 post ChAd63 CS, were also assessed by IFA. No significant boosting effect was observed in either group 1B and 2B when analysing by IFA, p=0.08 (Mann-Whitney test).
4.2.3 HLA typing

Previous studies have identified class I and II CSP epitopes and their HLA restriction, [96-98] so all volunteers were typed for major HLA types; A, B and DR (Table 4.3). The apparent immune-dominance of peptide pools 1 and 6 may be explained by the fact that HLA super types A1 and A2, predominate in our volunteers and that it has previously been shown that epitopes contained within these pools are restricted to these HLA super types.[97-99]
Table 4.3  HLA typing of all volunteers enrolled in study. (*) denotes allele could not be further characterised.

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4.3 Discussion

Safety

No serious adverse events occurred during the course of the trials. The majority of AEs observed were mild in intensity and resolved rapidly. Over 300 healthy volunteers have now received ChAd63 encoding the malaria antigens ME-TRAP, MSP1 and AMA1.[58, 59, 66, 74, 100] The safety profile seen with ChAd63 CS was very similar to that of other ChAd63 vectored vaccines. [66-68, 74] MVA CS at a dose of 2 x 10⁸ pfu was considerably less reactogenic than was observed at higher or same dose of MVA expressing different antigens.[66] [68, 74] 1-2x10⁸ pfu has consistently been shown to be the optimal dose of MVA.[61, 66, 68, 69, 73, 86, 93-95, 100] The majority of volunteers who received MVA CS
experienced a range of symptoms comprising of feverishness, fatigue, headache and myalgia. These were mild in severity in the majority of cases and all resolved within 48 hours. Comparing ChAd63 CS to NMRC-M3V-Ad-PfC, the other viral vectored CS vaccine which has been studied in humans, both result in mainly mild local and systemic AEs. ChAd63 CS at both doses resulted in less local pain than NMRC-M3V-Ad-PfC, with 17%, 58%, 67% and 86% of volunteers experiencing injection site pain following ChAd CS(5x10⁹ vp), ChAd CS(5x10¹⁰ vp) and NMRC-M3V-AdPfC, first immunization and second immunization, respectively.[96] ChAd63 CS, at either dose, resulted in local erythema in a higher percentage of volunteers, 33%(5x10⁹ vp) and 25%(5x10¹⁰ vp) than NMRC-M3V-Ad-PfC, 0%. [96] ChAd63 CS at the higher dose (5x10¹⁰ vp) was also more reactogenic in terms of the systemic AEs of myalgia, headache and fatigue, than NMRC-M3V-Ad-PfC, but the majority were of mild severity and resolved within 48 hours.[96] The safety data collected in this study adds to the already significant body of data supporting the excellent safety profile of this vaccine delivery platform.

Immunogenicity
MVA expressing CS has been studied in humans in the past and IFN-γ ELISpot results have varied from a mean of 79 SFC/10⁶ PBMC when combined with DNA prime[58], to 250 SFC/10⁶ PBMC, when primed with RTS,S, to 1000 SFC/10⁶ PBMC when primed by attenuated fowl-pox virus (FPV).[60] ChAd63–MVA vectored vaccines have consistently yielded high levels of T cells.[66-69, 73, 74, 100] Despite the fact that CS was one of the earliest recognised target antigens in the development of a malaria vaccine, it remains a leading antigen for vaccine development. Others have also recently published data on a human adenovirus vectored vaccine expressing CS (NMRC-M3V-Ad-PfCA), which yielded a peak mean IFN-γ ELISpot CS of 422 SFC/million.[96, 97] ChAd63–MVA CS yielded a peak mean three fold greater at 1523 SFC/million. Protection assessed by controlled human malaria infection was disappointing in the case of NMRC-M3V-Ad-PfCA, with 2/11 volunteers showing delayed onset of parasitaemia with no volunteer protected.[96] Peak antibody responses to CS for both vaccines measured by ELISA titre were modest, with NMRC-M3V-Ad-PfCA yielding median titres of 300 and 692 in separate studies[96, 97] and Ch63-MVA CS yielding a median titre of 631. RTS,S is the malaria vaccine most advanced in clinical development and is currently undergoing phase III trials. [101] It is formed from the fusion of CS to the surface antigen of hepatitis B virus to form virus-like particles. Analysis of the immunological correlates of immunity induced by the RTS,S/AS01 vaccine and adjuvant suggest that very high levels of antibodies to CS correlate with protection in humans.[102, 103]. However, this correlation is relatively weak and there might be a component of T cell mediated protection induced by the vaccine, even though the magnitude of the T cell response measured after vaccination is modest.
(mean of approximately 150 SFU / million PMBCs on ELISpot).[104, 105] The prime-boost strategy of the viral vectored vaccines, ChAd63 and MVA both expressing CS presented here, has produced T cell responses much greater (mean 1,947 SFC/million PMBCs on ELISpot) than RTS,S. The production of CD8+ monofunctional cytokine producing cells which has been shown in this study, has been correlated with protection to controlled human malaria challenge in the past.[73] The breadth of the ELISpot response observed may also be relevant to efficacy. The greater magnitude of T-cell immunogenicity induced by ChAd-MVA heterologous prime-boost immunization correlates with an increase in the number of detectable epitopes recognized[67, 104, 106] so it is likely that increased breadth also correlates with efficacy.

There is a lack of standardisation of reporting antibody response across vaccine studies. Some use ELISA titre [96] and others use μg/mL.[106] CSP specific antibody responses seen with ChAd63 CS followed by MVA CS were modest (mean of 1.9 μg/mL for groups 1B and 2B 7 days post MVA CS). In contrast RTS,S has yielded mean antibody responses of 78 μg/mL in non-immune adult vaccinees.[106] The significant levels of T cells yielded by vaccination with ChAd63 MVA CS and the high antibody levels produced by RTS,S, raises the possibility that combining the platforms to provide potentially complementary immune responses might provide better protection. There may also be benefit in combining ChAd63 MVA CS with other antigens, also delivered by the same viral vectors which have shown to have protective efficacy.[73]

Clearly there are limitations to a phase I study, the small numbers and fact that volunteers were malaria naïve. However both of these were requirements given that it was the first time that these products were administered to humans. There was no placebo group, however, these vectors, expressing a range of antigens, have been administered to over 460 individuals and have shown a consistent reactogenicity profile, so this was thought to be unnecessary.
5. Efficacy

5.1 Controlled Human Malaria Infection study (challenge study)

Following the phase I study on ChAd63 - MVA CS a phase IIa study was undertaken (VAC045). This involved Controlled Human Malaria Infection (CHMI) to assess clinical efficacy of the vaccine. Details of the study design can be found in section 2.13 Materials and Methods – Controlled Human Malaria Infection. In VAC045 a prime boost vaccination schedule of ChAd63 CS followed by MVA CS was compared to ChAd63 ME-TRAP followed by MVA ME-TRAP. ChAd63-MVA expressing ME-TRAP, another pre-erythrocytic malaria vaccine that has been shown sterile protection in 21% of malaria-naïve volunteers in controlled human malaria infection (CHMI)[73]. ME-TRAP is discussed in the introduction section in greater detail. 15 volunteers were vaccinated with ChAd63 / MVA CS, 15 with ChAd63/MVA ME-TRAP and 6 acted as controls.

Figure 5.1 Flow of study design and volunteer recruitment.[107] Twenty volunteers were excluded following screening for the following reasons: psychiatric history (n = 3), no medical screening letter returned (n = 3), multiple medical problems (n = 2), excessive alcohol use (n = 2), syncope (n = 1), connective tissue disease (n = 1), iron deficiency (n = 1), raised alanine aminotransferase level (n = 1), poor venous access (n = 1), gastrointestinal problems under investigation (n = 1), family history of heart disease (n = 1), lost to follow-up (n = 1), unavailable during challenge (n = 1), and history of recreational drug use (n = 1). Furthermore, 7 volunteers withdrew consent after screening but before enrolment. All immunizations were administered intramuscularly with sequential vaccines administered into the deltoid of alternating arms. No enrolled volunteers withdrew from the study and all volunteers completed study visits as scheduled. Abbreviations: ChAd63, simian adenovirus 63; CS, circumsporozoite protein; ME-TRAP, multiple epitope–thrombospondin-related adhesion protein; MVA, modified vaccinia virus Ankara; pfu, plaque-forming units; vp, viral particles.
5.2 Safety and Immunogenicity

Adverse events following vaccination were recorded in the same manner as during the phase I study. As this study was designed as direct comparison between ChAd63 CS followed by MVA CS and ChAd63 ME-TRAP followed by MVA ME-TRAP adverse events related to both vaccines are presented here. The adverse event profile was in line with that expected with ChAd63-MVA vectored vaccines. As has been observed in the phase I study the MVA vectored vaccines resulted in more local and systemic events, that tended to be of greater severity than those following ChAd63 vaccines. The majority continued to be mild and resolved with 24 hours. Details are displayed in Figure 5.2.
Figure 5.2: Adverse Events deemed possibly, probably or definitely related to administration of ChAd63 or MVA vectored vaccines (Groups 1 and 2). Only the highest intensity of each AE per subject is listed. There were no immunization-related serious adverse events. (A) Local AEs after administration of ChAd63 CS (Group 1) and ChAd63 ME-TRAP (Group 2). The “Other” local AE in Group 1 was mild vaccine site paraesthesia. (B) Local AEs after administration of MVA CS (Group 1) and MVA ME-TRAP (Group 2). The “Other” local AEs in Group 1 was mild vaccine site bruising. (C) Systemic AEs after administration of ChAd63 CS (Group 1) and ChAd63 ME-TRAP (Group 2). “Other” systemic AEs in Group 1 were mild insomnia, diarrhoea, abdominal cramps, neck pain, upper back pain, leucopenia (3.3 x 10^9/l), elevated ALT (70 IU/l), and moderate abdominal pain and lymphopenia (0.91 x 10^9/l). “Other” systemic AEs in Group 2 were mild rash, pharyngitis and coryzal symptoms. (D) Systemic AEs after administration of MVA CS (Group 1) and MVA ME-TRAP (Group 2). “Other” systemic AEs in Group 1 were mild light-headedness, disorientation, bruising at injection site, leucopenia (3.18 x 10^9/l) and episode of vasovagal syncope. The “Other” systemic AE in Group 2 was a mild rash.
Figure 5.3 Antigen-specific T-cell responses to vaccination measured by interferon γ enzyme-linked immunosorbent spot assay. Kinetics of T-cell responses after vaccination with ChAd63-MVA encoding circumsporozoite protein (CS; group 1; A). Each line represents an individual volunteer. **P < .01 by the Kruskal–Wallis test with the Dunn multiple comparison test.

T cell Immunogenicity to ChAd63-MVA CS and ME-TRAP T-cell responses followed the expected kinetics after ChAd63 administration [66, 67, 73, 74, 100], with peak responses seen 28 days after ChAd63 receipt (group 1 [CS]: GM, 343 spot-forming cells (SFCs)/million PBMCs [95% CI, 191–617]; group 2 [ME-TRAP]: GM, 553 SFCs/million PBMCs [95% CI, 330–925]). The peak T-cell response after boost was seen at day 63 after receipt of MVA CS for group 1 (GM, 1017 SFCs/million PBMCs [95% CI, 630–1641]) and at 1 day before CHMI after MVA ME-TRAP receipt for group 2 (GM, 2027 SFCs/million PBMCs [95% CI, 1472–2792]). There was no significant difference in T cell responses between day 63 after vaccination and 1 day before CHMI for either group. Responses to both antigens were well maintained, with GMs of 285 SFCs/million PBMCs (95% CI, 156–520) to CS and 659 SFCs/million PBMCs (95% CI, 418–1036) to ME-TRAP 16 weeks after MVA receipt in groups 1 and 2, respectively. ELISpot responses of individual volunteers are shown in Figure 5.3 above. Detailed mapping of T cell responses to CS peptides was not performed because this was described recently in detail with several HLA class I–restricted epitopes [108].

5.3 Efficacy

The infectivity controls (group 3) and 27 of 30 vaccinees were diagnosed with malaria. One volunteer (7%) in group 1 (who received ChAd63-MVA CS) and 2 volunteers (13%) in group 2 (who received ChAd63-MVA ME-TRAP) were steriley protected (Figure 5.4 A). The control
volunteers (group 3) were diagnosed after a median time of 10.3 days, mean time of 10.5 days (range 8.0–14.0, SD 2.2). Three vaccinees (20%) in group 1 and 5 vaccinees (33%) in group 2 demonstrated a delay in time to treatment, relative to controls. There was no significant difference between unvaccinated controls and vaccinees in the protocol-specified endpoint of time to treatment for malaria (Figure 5.4 A). However, when comparing the time to collection of the first sample after CHMI with either >500 parasites/mL (Figure 5.4 B) or >20 parasites/mL (Figure 5.4 C), a significant difference was seen between unvaccinated controls and vaccinees receiving ChAd63-MVA ME-TRAP ($P = .01$ and $P = .005$, respectively).

**Figure 5.4** Efficacy of ChAd63-MVA circumsporozoite protein (CS) and ME-TRAP immunization following *Plasmodium falciparum* 3D7 sporozoite challenge. Kaplan–Meier survival analyses. Log-rank test for significance. A, Kaplan–Meier survival analysis of time to treatment. Median time, 12.0 days for group 1 (CS), 12.5 days for group 2 (ME-TRAP), and 10.3 days for unvaccinated controls. B, Kaplan–Meier survival analysis of time to first sample with >500 parasites/mL detected by quantitative polymerase chain reaction (qPCR). Median time, 10.5 days for group 1 (CS), 12.0 days for group 2 (ME-TRAP), and 7.5 days for unvaccinated controls. C, Kaplan–Meier survival analysis of time to first sample with >20 parasites/mL detected by qPCR. Median time, 7.5 days for
group 1 (CS), 9.0 days for group 2 (ME-TRAP), and 7.0 days for unvaccinated controls. Abbreviations: CHMI, controlled human malaria infection; controls, unvaccinated volunteers undergoing CHMI; ME-TRAP, multiple epitope–thrombospondin related adhesion protein.

5.4 Quantitative PCR Data
Primary analysis comparing the mean parasite density 7.5 days after CHMI (a measure of the liver to blood inoculum) showed a significant reduction when vaccinees receiving ChAd63-MVA ME-TRAP but not ChAd63-MVA CS were compared with unvaccinated control volunteers (P = .01 and P = .08, respectively, by the Mann–Whitney U test; Figure 5.5). The same comparison performed using negative binomial regression gave P values of 0.03 and 0.05, and a similar result was seen when the liver to blood inoculum was estimated 7.5 days after CHMI by using non-parametric regression (P = .01 and P = .05, by the Mann–Whitney U test). Mean total number of parasites 7.5 days after CHMI was a strong predictor of the time to treatment (hazard ratio [HR], 1.003974 [95% CI, 1.002272–1.00568], by Cox proportional hazards regression analysis; P ≤ .0001). This is noteworthy as treatment signifies that a clinical endpoint had been reached. The trial physicians were blinded to the qPCR results and determined endpoints based on clinical assessment and blood film reading. The close correlation between mean number of parasites at 7.5 days after CHMI and subsequent endpoints support the idea that early qPCR kinetics could be used in future CHMI removing the need for more costly, labour intensive and potentially hazardous stages of CHMI studies. In the future CHMI may be able to use early qPCR to reliably predict parasite kinetics and avoid the need for volunteers to reach clinically apparent endpoints.
Figure 5.5 Comparison of mean parasite density, measured by quantitative polymerase chain reaction, 7.5 days after controlled human malaria infection (CHMI) between vaccinees and control volunteers. P values were determined by the Mann–Whitney U test. Abbreviations: ChAd63, simian adenovirus 63; Control, unvaccinated volunteers undergoing CHMI; CS, circumsporozoite protein; group 1, ChAd63-MVA CS recipients; group 2, ChAd63 ME-TRAP recipients; ME-TRAP, multiple epitope–thrombospondin-related adhesion protein; MVA, modified vaccinia virus Ankara.

Sterile protection is the clear goal of vaccine efficacy, but there may be value in analysing vaccines’ ability to reduce parasitaemia or delay onset of patent infection. To this end exploratory analysis of parasite densities by using area under the curve (AUC) were performed and showed that parasite density over the first 3 replication cycles in infected volunteers was a significant predictor of the time to treatment (HR, 1.000015 [95% CI, 1.000008–1.000022], by Cox proportional hazards regression analysis; P < .000; Figure 5.6). Over the first, second, and third blood-stage replication cycles, there was a significant reduction in parasite densities among ChAd63-MVA ME-TRAP vaccinees, as measured by AUC analysis (ie, log [parasite density +1]), compared with unvaccinated controls, when vaccinees who achieved sterile protection were included in the analysis (cycle 1, P = .01; cycle 2, P = .03; and cycle 3 P = .05; by the 2-tailed t test, for all comparisons). Parasite densities in vaccinees receiving ChAd63 CS were significantly less than those in controls over the first blood-stage replication cycle only (P = .05 log [ parasite density + 1], by the 2-tailed t test). AUC analysis showed that, compared with controls, ChAd63-MVA ME-TRAP resulted in a 79% reduction in parasitaemia during cycle 1, whereas ChAd63-MVA CS caused a 69% reduction.
Figure 5.6  Comparison of areas under the curve (AUCs) of parasite densities, measured by quantitative polymerase chain reaction (PCR), between vaccinees and control volunteers. A, Group mean log-transformed PCR data. The AUC of parasite density over the first 3 replication cycles in infected volunteers was a significant predictor of the time to diagnosis (hazard ratio, 1.000015 [95% confidence interval, 1.000008–1.000022], by Cox proportional hazards regression analysis; P < .000). B, AUC analysis of parasite densities, comparing controls to vaccinees at days 6.5–8 (the first cycle after hepatocyte release), days 8.5–10 (the second cycle), and days 10.5–12 (the third cycle) after controlled human malaria infection (CHMI). Means of log [parasite density + 1] were compared for each vaccine group to those of controls, using a 2-tailed t test. Abbreviations: ChAd63, simian adenovirus 63; controls, unvaccinated volunteers undergoing CHMI; CS, circumsporozoite protein; ME-TRAP, multiple epitope–thrombospondin-related adhesion protein; MVA, modified vaccinia virus Ankara; SP, sterile protection.

In group 1 (CS vaccine) but not group 2 (ME-TRAP), IgG antibody responses to CS correlated significantly and negatively with qPCR-determined densities 7.5 days after CHMI (group 1: Spearman r = −0.6 [P = .03]; group 2: Spearman r = −0.3 [P = .34]; Figure 5.7 A and 5.7 C). No significant correlation was seen between IFN-γ ELISpot findings for ME-TRAP or CS and
qPCR findings 7.5 days after CHMI for group 1 or 2 (Figure 5.7 D and 5.7 E), in concordance with previous data in which ELISpot-determined responses did not correlate with vaccine efficacy.[73] Anti-CS IgG antibodies were detected in ME-TRAP vaccinees (group 2) because of the inclusion of 4 copies of the N-acetylneuraminic acid phosphatase (NANP) repeat from the CS antigen in the ME string. No significant correlation with qPCR findings at day 7.5 after CHMI was found (Figure 5.7 B).
Figure 5.7 Associations between immunological outcomes and vaccine efficacy. Correlation between parasite density at day 7.5, measured by quantitative polymerase chain reaction (qPCR), and levels of anti–circumsporozoite protein (CS) immunoglobulin G (IgG) antibody in group 1 (CS; Spearman $r = -0.6; P = .03$; A) and group 2 (ME-TRAP; Spearman $r = -0.3; P = .34$; B). C, Correlation between parasite density at day 7.5, measured by qPCR, and anti-TRAP IgG antibody responses in group 2 (ME-TRAP; Spearman $r = -0.5; P = .05$). D, Correlation between interferon γ (IFN-γ)–secreting T-cell frequency to CS measured by enzyme-linked immunosorbent spot (ELISpot) parasite density at day 7.5 (parasite/mL measured by qPCR) in group 1 (CS; Spearman $r = -0.2; P = .50$. E, Correlation between IFN-γ–secreting T-cell frequency to ME-TRAP measured by ELISpot and parasite density at day 7.5 (parasite/mL measured by qPCR) in group 2 (ME-TRAP; Spearman $r = 0.1; P = .6$). Abbreviations: Black filled points, sterilely protected vaccinees; EU, enzyme-linked immunosorbent assay units; group 1, ChAd63-MVA CS; group 2, ChAd63-MVA ME-TRAP; ME-TRAP, multiple epitope–thrombospondin-related adhesion protein; PBMC, peripheral blood mononuclear cell; SFC, spot-forming cell; unfilled points, vaccinees demonstrating delay to start of antimalarial therapy in comparison to unvaccinated control volunteers.
5.5 Discussion

This was the first CHMI study assessing ChAd63 – MVA expressing CS. The study was designed as head-to-head comparison of the 2 leading pre-erythrocytic antigens, ME-TRAP and CS, delivered in the same vaccine platform. ME-TRAP had greater clinical efficacy, with sterile protection achieved in 13% of vaccinees (2 of 15) and a delayed time to diagnosis in 33% (5 of 15). This efficacy is slightly less than that previously reported in another CHMI study of ChAd63-MVA ME-TRAP [73], despite the induction of similar, very high frequency of antigen-specific T cells (peak median IFN-γ–secreting T cell count, 2027 in this study vs 2436 SFCs/million PBMCs in the previous study). The difference in efficacy between the two studies could be a result of size of the malaria inoculum. This is hinted at with the median time to diagnosis for unvaccinated control volunteers being 1.5 days shorter than in the previous CHMI study.[73] Assuming this to be the case also suggests that the efficacy observed with ChAd63-MVA CS (sterile protection 7% (1 of 15), and a delayed time to diagnosis observed in 20% (3 of 15) are underestimates of what might occur under less stringent CHMI conditions or in naturally occurring infection, where the infectious dose experienced by individuals is considerably less than that used in CHMI studies.[82]

The correlation between anti-CS antibodies and time to treatment suggests that even at low levels this may be contributing to the mechanism of efficacy. This study provided the first evidence that sterile immunity can be generated with viral vectors encoding CS alone, though some sterile efficacy has been reported using combinations of DNA and adenoviral vectors encoding CS and AMA1[109].

Kaplan–Meier analysis of time to diagnosis between vaccinees and unvaccinated controls and numerous analyses of the qPCR data demonstrated significant efficacy for ChAd63-MVA ME-TRAP alone. There was no such statistically significant difference for the ChAd63-MVA CS vaccines using the same analysis. The AUC analysis, comparison of parasitaemia at 7.5 days after CHMI, the evidence of sterile protection, and a delay to diagnosis in certain vaccinees all support the view that ChAd63-MVA CS led to a reduction (by approximately 69%–79%, depending on the analysis) in the number of parasites released from the liver. This study failed to show ChAd63-MVA CS to have efficacy significantly better than that of ME-TRAP. However, given the correlations seen with modest CS antibody levels, there may be a role for the use of this vaccine in combination with other antigens in a multi-stage vaccine.
6. Discussion

The research activities and findings described above have made an impact nationally and internationally. Firstly below is an outline of how the process of conducting this phase I trial in Ireland may have had a positive impact on the clinical trial regulatory process in Ireland for future clinical trials. Following this is a discussion of the influences and societal factors that may have impacted on volunteer recruitment. The results of the studies also add to the already substantial corpus of work about the safety and characteristics of the vaccine response to vaccines vectored with MVA or ChAd63. This is described below. Then follows a summary of the progression of ChAd63-MVA CS and the other candidate vaccines in development, including RTS,S, the current leading malaria vaccine, developed by a partnership between the Walter Reed Army Institute of Research and GlaxoSmithKline (GSK) supported by the Gates foundation. Finally there will be a section on the malaria elimination agenda, the tools needed and the hurdles to overcome.

6.1 Translational research in Ireland

6.1.1 Clinical trials approval

Although both the UK and Ireland had the same legislative 60 day timeline for reviewing this clinical trial, the MHRA (UK) approved this clinical trial within 26 days. The main reason for the difference in review time seems to have be due to the fact that this was the first phase I viral vectored vaccine trial ever to be conducted in Ireland. The MHRA and ethics committees in the UK are very familiar with these phase I vaccine trials and have reviewed numerous applications using viral vectored vaccines over the past 10 years e.g. Malaria, TB, Flu Hep C, HIV and cancer. However this was not always the case, and in 2007 for the first use of simian adenoviral vaccines investigators were required to go through a rigorous lengthy ‘first time in human’ procedure in the UK prior to applying to the MHRA for a clinical trial authorisation (CTA). It is therefore not surprising that the approval of the first viral vectored phase I trial in Ireland took longer to be approved in Ireland than it did in the UK. In addition to the IMB assessors’ review of the application, independent experts’ were appointed by the IMB which ultimately contributed to lengthening the review period. The majority of the expert’s queries related to manufacturing. This meant that a very close and efficient working relationship between vaccine producers, sponsor and investigators was critical in order to answer all queries with clarity, detail and efficiency. Gaining regulatory approval in this jurisdiction was challenging, for both applicant, requiring input from many parties in 4 different European countries and responding to 139 queries, and for the IMB, who had limited prior expertise in
this vectored vaccine area. Ultimately the study was approved showing that this type of novel research can be reviewed successfully and performed in Ireland.

### 6.1.2 Volunteer recruitment in Ireland

There would appear to be a perception that financial reward is the main motivator for people to be involved in clinical trials. However our data found that altruistic reasons, such as contributing to the progress of medicine and helping others were the main motivations for participating in this study. Financial re-imbursement was a motivating factor for only a minority of volunteers. The relationship between these two main factors maybe more complex. A study of 136 volunteers in phase I studies by Almeida et al found that financial reward was the most important motivation and was most valued by subjects with a lower monthly income (p < 0.01) and lower education (p < 0.05)\[85]. The corollary also appeared to be the case, that financial reward was less valued by healthy volunteers with higher income and education. This was also observed in our study, the majority being recruited through university email lists tended to have rather altruistic motivations. Those screened as a result of unselected advertising in newspapers tended to be motivated more by financial reward. Key to completion of any clinical trial is the commitment of the volunteers to attend all follow up and adhere to the protocol. It would seem to me that financial motivation is a less powerful tool for ensuring this than informed altruistic minds. Many of the volunteers recruited to our study in Dublin worked in the pharmaceutical industry or were researchers themselves, both keen to experience and understand the medicines development process from the participants’ point of view. Future studies might well focus advertising on sections of society where motivated and committed volunteers are most likely to be found. The participants’ experiences of being involved in the study were positive across all domains explored. The results of this study can be used to plan recruitment strategies for future phase 1 studies in Ireland and elsewhere and show that there does exist a pool of individuals willing to partake in clinical trials. This trial gained quite an amount of media interest in Ireland, with coverage in the national press, radio and on television. These were opportunities to explain the process of bringing any medical product to market and the function of clinical trials. All too often the only time that the general public hear about clinical trials is when something goes wrong. Cases like TGN1412, a CD28 superagonist antibody, tested by Parexel at London’s Northwick Park Hospital in 2006, that resulted in six volunteers requiring hospital admission, four suffering multiorgan failure and requiring Intensive Care\[110]. And more recently BIA 10-2474, a drug targeting the endocannabinoid system. During the phase I trial in Rennes, Frances in January 2016 five volunteers experienced severe adverse events, including the death of one man\[111]. In addition to these rare events, the history of clinical trials is littered with examples of poor ethical practice and exploitation of vulnerable populations, from impoverished black populations in Alabama enrolled in the
Tuskegee trials on Syphilis, where 399 men did not get access to penicillin to treat their infection, to the intentional infection of mentally disabled children at the Willowbrook State School in Staten Island, New York, with hepatitis B for the purpose of discovering a vaccine. In Ireland, a series of vaccine trials were conducted from 1960s to the 1970s on children in residential homes without parental consent. Whilst there was no evidence of immediate harm to the children, the lack of a proper consent process and the use of a vulnerable population led to public outcry when the story was broken at the end of the 20th century. Processes and ethical standards in clinical trials have changed vastly during the 20th century, but this needs to be explained to a public understandably suspicious. The experience of this phase I vaccine trial in Ireland suggests that more work needs to be done in the area of public engagement in this very important area of clinical trials. The scientific community, in partnership with regulatory agencies, have a responsibility to engage the general public in an open discourse on the role and value of clinical trials. Improvements in medical care can only be made possible by diligent, regulated and safe assessments in clinical trials to generate robust safety and efficacy data. High quality evidence from clinical trials is necessary, not only for licensing of new medicines and devices, but also when evaluating cost-benefit and healthcare planning. The image of clinical trials as experiments using human ‘Guinea pigs’ needs to be challenged and changed into the view that controlled trials in humans are one of the key scientific tools for the improvement of human health.

6.2 Viral vectored vaccines

The value of the exploration of this candidate vaccine goes beyond this product itself. The ChAd63-MVA viral vectored delivery platform remains a potent tool in not only falciparum malaria vaccine development, but is also in use in combating other species of malaria [112] and many other infectious diseases such as HIV[113, 114], TB[115] and Ebola[116]. Thus all data gathered on safety and immunogenicity contributes greatly to many areas of infectious diseases research.

Though field trials have yet to prove the efficacy of viral vectored vaccines, the unique mode of stimulating the immune system, closely mimicking natural infection, and the fact that they can be produced rapidly in large qualities mean they warrant further development. Indeed the ability to rapidly produce large qualities of viral vectored vaccines may be key in combating emerging pathogens such as Ebola, where demand can peak rapidly. Viral vectored vaccines already have a proven record in veterinary medicine, most notably the Raboral V-RG, an oral live vaccinia virus vector expressing the glycoprotein of Evelyn-Rotkitniki-Abelseth rabies virus. Several
countries have used this oral vaccine placed in bait to vaccinate and control rabies in wildlife populations[117].

There are other viral vectored platforms under development. Measles virus (MV) is an exclusively human pathogen. There are well established MV vaccine strains that have proven efficacy and safety profiles and current technology allows for the insertion of more than 5000 nucleotides into the measles virus genome. This makes for a valuable potential vaccine delivery system, particularly the ability to deliver multiple antigens at the same time. The fact that measles replication strictly occurs in the cytoplasm of infected cells without DNA intermediate has important biosafety implications and adds to the attractiveness of MV as a vector[118].

As mentioned earlier, vaccines such as those targeting HPV and Hepatitis B were developed to prevent cancers. More recently vaccines have been developed as adjuvants to cancer therapies. The idea of a therapeutic cancer vaccine originated with the discovery that patients can harbour CD8+ and CD4+ T cells specific for cancer or differentiation antigens[119]. This was first established in metastatic malignant melanoma. Additionally, a relationship between prolonged patient survival and the presence of intra-tumoral CD3+ or CD8+ cytotoxic T cells and an IFN-γ gene signature has been reported.[120, 121] By expressing antigens of the cancer cells and stimulating a T-cell response, these vaccines promote immune recognition and death of tumour cells. In the case of metastatic prostate cancer, a vaccine induces an immune response against prostatic acid phosphatase (PAP), an antigen expressed in more than 95 percent of all prostate cancers[122]. Therapeutic vaccines are also being investigated for the management of cervical cancer[123], head and neck cancers[124], renal cell carcinoma[125] and colorectal cancer[126].

The use of virus specific cytotoxic T-cells in the therapy of resistant infections with CMV, Adenovirus or EBV in patients post allogeneic hematopoietic stem cell transplantation (HSCT) adds yet another dimension to the potential role of T-cell mediated vaccines. Treatment success has been observed in small numbers of patients by utilizing adoptive transfer of virus specific cytotoxic T-cells from donors [127]. The possibility of using a viral vectored vaccine to stimulate specific cytotoxic T-cells in a patient as an active therapy is an exciting prospect.

Finally there may be a role for viral vectored vaccines in the management of HIV infection. Recent work by Soghoian et al identified a specific CD4+ T-cell population that was seen to emerge early during acute HIV infection in individuals who spontaneously control viral replication for a prolonged period of time [128]. The same group has gone on to show cooperativity of HIV-specific cytolytic CD4+ T-cells and CD8+ T-cells in control of HIV.
viraemia [129]. These findings suggest potential targets not only for prevention of HIV infection, but also for prevention of disease progression in individuals already infected. Such specific and potent T-cell responses might be achieved utilising a viral vectored vaccine.

6.3 Malaria control

**ChAd63-MVA CS**

The journey of a new medicinal product from concept to licensure is lengthy and complex. In this work a vaccine candidate based on the leading malaria antigen combined with an exciting delivery platform has been taken from pre-clinical stages to a first in human phase Ia study and on to a phase II human challenge. ChAd63-CS and MVA–CS have been shown to have acceptable safety profiles, and are potently immunogenic, inducing high levels of antigen-specific multifunctional CD4+ and CD8+ T lymphocytes, and significant levels of antibody. The results of the CHMI for ChAd63-MVA CS showed disappointing results with a sterile efficacy of 7%, with ME-TRAP performing better at 13%. The quantitative PCR data allow for a closer interrogation of the effects exerted by a vaccine, particularly when estimating the liver to blood inoculum,[90, 130] but though ChAd63-MVA CS clearly had an effect it was not as marked as that seen with ChAd63-MVA ME-TRAP. Overall these data suggest that ChAd63-MVA CS would not be a candidate as a stand-alone vaccine. Others recently published data on a human adenovirus vectored vaccine also expressing CS (NMRC-M3V-Ad-PfCA) in CHMI with no volunteers achieving sterile protection[96]. Following on from the detailed data presented here, our group trialled a strategy of combining various antigens to enhance efficacy. In VAC052 thirty two healthy adult UK volunteers were infected with *P. falciparum* by mosquito bite: 13 had received both ChAd63-MVA ME-TRAP and CS prime-boost (Group 1), thirteen had received ChAd63-MVA ME-TRAP, CS and AMA1 prime-boost (Group 2) and 6 were unvaccinated (Controls). The number not developing blood stage infection was 3/13 (Group 1), 2/13 (Group 2) and 0/6 (Controls) with corresponding expected delays to patency in other vaccinees (unpublished data). Thus the study did not show an added benefit of ChAd63-MVA CS to ChAd63-MVA ME-TRAP. With these results there does not appear to be an immediate future for ChAd63-MVA CS and at present no further trials of this vaccine candidate are planned.

**Malaria, from control to eradication**

RTS,S/AS01 is currently the leading malaria vaccine candidate, having progressed to the completion of a randomized, double blind placebo controlled trial involving nearly 16,000 infants and young children in seven countries in Sub-Saharan Africa[131]. In 2015 the
European Medicines Agency indicated an acceptable quality and risk-benefit ratio[132], and this was followed by the WHO supporting the four-dose regimen in the 5-to-17-month age group. However, the WHO continued to have concerns over the risk-benefit ratio, noting that “the benefits against malaria-related mortality and all-cause mortality are unknown” and also noting the identified risk of febrile convulsions following vaccination[133]. Thus the WHO has recommended the generation of more evidence from pilot projects to inform the wider role of this vaccine.

Though malaria rates continue to fall around the world, drug resistance is increasing. Resistance to artemisinins, the current most reliable treatment has been documented across South East Asia[134-140]. The mutant K13 allele responsible for falciparum resistance has been tracked and thus far remains isolated in South East Asia[141]. How long this will remain the case remains to be seen. Vector control and bednets have been immensely effective in malaria control but are threatened due to increasing insecticide resistance[142, 143].

The ambition of eradicating malaria was abandoned in 1969 after the conclusion of the WHO's mid-20th century Global Malaria Eradication Programme (GMEP). A strategy of malaria eradication was no longer prioritised and the accepted global approach was one of sustained control [144]. But optimism gradually returned as the Roll Back Malaria Partnership (RBM) in 1998, the Millennium Development Goals in 2000, and the formation of new donor mechanisms including the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) in 2002 and the US President's Malaria Initiative in 2005, saw global malaria morbidity and mortality begin to decrease.

The goal of malaria elimination is now very much alive [145]. Between 2007 and 2013, four countries were certified as malaria-free by WHO (Armenia, Morocco, Turkmenistan, and United Arab Emirates), an additional eight countries moved into the WHO's prevention of reintroduction phase after sustaining at least 3 years of zero local malaria transmission (Argentina, Egypt, Iraq, Georgia, Kyrgyzstan, Oman, Syrian Arab Republic, and Uzbekistan), and five others interrupted local transmission (Azerbaijan, Costa Rica, Paraguay, Sri Lanka, and Turkey)[19]. Secure global funding and political commitment are required to implement the range of measures that will be needed if this goal is to be achieved. Elements of the strategy include vector control, enhanced diagnostics, new drugs to combat resistance, surveillance to inform and a vaccine. A recent modelling study reports that by combining long-lasting insecticide impregnated bednets, indoor residual spraying and three rounds of mass drug administration, malaria transmission would be reduced to less than one case per 1000 people per year in 90% of the population at risk [146]. Over recent decades prophylactic use of anti-
malarials was reserved for travellers and pregnant women in endemic areas [147]. Fears of accelerating drug resistance informed a cautious approach to any widespread Mass Drug Administration (MDA) approach to the control of malaria. Ironically the same issue of drug resistance has prompted the approach of MDA to be tested. Increasing rates of artemisinin derivatives in *Plasmodium falciparum* in South East Asia risked a large scale public health disaster. Guided by routine blood-smear surveillance, high prevalence villages were selected and then intensively sampled with ultra-sensitive qPCR for *P. falciparum* infection. Following MDA with dihydroartemisinin-piperaquine, the sub-microscopic reservoir of *P. falciparum* malaria was eliminated during the six-month follow-up period (prevalence fell from 7 to 0 %)[148]. This was followed by six fold drop in clinical cases in the subsequent rainy season [148]. Many other researchers have reported similar outcomes, though their methodology and programmatic goals vary [149]. It does seem that MDA will play a role in the control of drug resistance and ultimately in the eradication of malaria.

The vaccine envisaged as part of an eradication campaign should be capable of transmission blocking[150]. This is an interesting concept as such a vaccine might not have benefit for the individual who is vaccinated, but by interrupting transmission of effective gametocytes to mosquitos, would prevent onward transmission to other humans. Use of transmission-blocking vaccines (TBVs) targets antigens expressed by the sexual-stages of the parasite (gametocytes) which are infectious to mosquitos, as well as those expressed by the mosquito. The strategic goal of this approach is to reduce and/or block transmission to the vertebrate host [151, 152]. Pfs25 is the current leading transmission blocking malaria vaccine [153], it is being assessed using the same viral vectored platform of ChAd63-MVA used in this study [154], as well as with other platforms.[155, 156] Anti-Pfs25 antibodies have been shown to be functional in the *ex vivo* standard membrane feeding assay (SMFA) and completely block the development of *P. falciparum* in the mosquito [153]. Clinical assessment of this vaccine is ongoing.

The hunt for further antigens that might offer protection continues. Recently PfLSA1 and PfLSAP2 have shown encouraging results in animal models that suggest they may be able to induce sterile protection dependent on the presence of CD8+ T cells[157].

In parallel with the exploration of new antigens is the theory that a combination vaccine, comprising a number of different antigens would provide more effective protection. I was also an investigator on VAC055 in Oxford, where a strategy of combining RTS,S AS01B and ChAd63-MVA ME-TRAP was assessed in a CHMI study. This was the first time that RTS,S, the current leading vaccine candidate was combined with another vaccine. The protection in RTS,S appears to be antibody mediated and that of ME-TRAP T cell related, thus the hope that
a synergistic effect of the combination would be realised. Protective vaccine efficacy was observed in 14/17 (82.4%) subjects who received both vaccines and 12/16 (75%) subjects who received RTS,S alone [158]. 14 protected subjects underwent repeat CHMI 6 months after initial challenge and 7/8 (87.5%) of the combination vaccine group subjects and 5/6 (83.3%) RTS,S subjects remained protected[158]. These encouraging results have led to the planning of further combination vaccines, including new antigens. MultiMalVax, a project funded by the European Union and administered by the European Vaccine Initiative is designed to advance the manufacture and testing of a multi-stage vaccine[159]. A number of steps are planned - to manufacture and test RH5 (a blood-stage antigen) and Pfs25 (a transmission blocking antigen) both expressed and delivered by the ChAd63-MVA viral vector; to manufacture and test R21 (a potentially improved version of the pre-erythrocytic-stage protein particle vaccine RTS,S) and ultimately to test each vaccine in CHMI in combination. The goal is to produce a vaccine that offers individual clinical protection at pre-erythrocytic and erythrocytic stages and has transmission blocking capability.

In summary, the work presented here represents a small but significant step in the development of an effective malaria vaccine. We have produced safety and immunology data that show these vaccines to be safe, well tolerated and immunogenic. Though the human protection shown was not adequate for the vaccines to progress to phase IIb trials, they may well form part of a combination malaria vaccine in the future. Even if these particular vaccines do not proceed to clinical use the knowledge gained may enable the development of more potent vaccines.

Beyond malaria, the horizons for genetically engineered vaccines seem limitless and the work presented here adds to a body of safety and immunogenicity data supporting their use. By our engagement with the media we have fulfilled a critically important role of informing and educating the general population of the value and role of clinical trials. Finally, by conducting the phase I clinical trial in Ireland, expertise has been developed amongst researchers and regulators, which should benefit future research.
7. Publications

Eoghan de Barra¹, Susanne H. Hodgson⁴, Katja J. Ewer⁴, Carly M. Bliss⁴, Kerrie Hennigan¹, Ann Collins¹, Eleanor Berrie³, Alison M. Lawrie⁴, Sarah C. Gilberr², Alfredo Nicosia⁸, Samuel J. McConkey¹, Adrian V. S. Hill²⁴ (2014). "A phase Ia study to assess the safety and immunogenicity of new malaria vaccine candidates ChAd63 CS administered alone and with MVA CS." PLoS One 9(12): e115161.

1 Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland.
2 The Jenner Institute, University of Oxford, Oxford, OX3 7DQ, UK.
3 Clinical Biomanufacturing Facility, University of Oxford, Churchill Hospital, Oxford, United Kingdom
4 Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Churchill Hospital, Oxford, United Kingdom
5 Okairos, Rome

Hodgson, S. H.¹, Katie J. Ewer¹, Carly M. Bliss¹, Nick J. Edwards¹, Thomas Rampling¹, Nicholas A. Anagnostou¹, Eoghan de Barra¹⁶, Tom Havelock¹, Georgina Bowyer¹, Ian D. Poulton¹, Simone de Cassan¹, Rhea Longley¹, Joseph J. Illingworth¹, Alexander D. Douglas¹, Pooja B. Mange¹, Katharine A. Collins¹, Rachel Roberts¹, Stephen Gerry², Eleanor Berrie³, Sarah Moyle³, Stefano Colloca⁷, Riccardo Cortese¹⁰,a, Robert E. Sinden¹⁵,b, Sarah C. Gilbert¹, Philip Bejon¹¹, Alison M. Lawrie¹, Alfredo Nicosia⁷,⁸,⁹, Saul N. Faust⁴ and Adrian V. S. Hill¹ et al. (2015). "Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals." J Infect Dis 211(7): 1076-1086.

Author Affiliations
1. Jenner Institute
2. Centre for Statistics in Medicine
3. Clinical Biomanufacturing Facility, University of Oxford
4. NIHR Wellcome Trust Clinical Research Facility, University of Southampton and University Hospital Southampton NHS Foundation Trust
5. Division of Cell and Molecular Biology, Imperial College London, United Kingdom
6. Royal College of Surgeons in Ireland, Dublin, Ireland
7. Okairos, Rome
8. CEINGE
9. Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Italy
10. Okairos, Basel, Switzerland
11. Centre for Geographical Medical Research (Coast), Kenya Medical Research Institute–Wellcome Trust, Kilifi

Safety and High Level Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP. J Infect Dis 2016, Jun 15.

Tommy Rampling¹, Katie J. Ewer¹, Georgina Bowyer¹, Carly M. Bliss¹, Nick J. Edwards¹, Danny Wright¹, Ruth Payne¹, Navin Venkatraman¹, Eoghan de Barra², Claudia M. Snudden¹, Ian D. Poulton¹, Hans de Graaf², Priya Sukhtankarer³, Rachel Roberts¹, Karen Ivinson³, Rich

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Welzin1, Bebi-Yassin Rajkumar4, Ulrike Wille-Reece4, Cynthia Lee4, Chris Ockenhouse4, Robert E. Sinden6, Stephen Gerry6, Alison M. Lawrie1, Johan Vekemans7, Danielle Morelle7, Marc Lievens7, Ripley W. Ballou1, Graham S. Cooke6, Saul N. Faust3, Sarah Gilbert1, Adrian V.S Hill1.

Affiliations

1 The Jenner Institute, University of Oxford, Oxford, OX3 7DQ, UK.
2 Royal College of Surgeons in Ireland, 123 St. Stephen’s Green, Dublin 2, Ireland.
3 NIHR Wellcome Trust Clinical Research Facility, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK.
4 PATH Malaria Vaccine Initiative, Washington, USA.
5 Department of Life Sciences, Imperial College London, London, UK.
6 Centre for Statistics in Medicine, University of Oxford, Oxford, UK.
7 GSK Vaccines, Rixensart, Belgium.
8 Infectious Diseases Section, Faculty of Medicine, Department of Medicine, Imperial College London, London, UK.


Regulatory approval process for a phase I vaccine trial (VAC038) in Ireland and the UK, a parallel experience. Dr. E. de Barra1, Dr. A. Lawrie3, Dr. S. Sheehy3, Professor A.V.S. Hill2, Professor S. McConkey1. Poster. IMB conference.


EVI rendezvous, oral presentation, University of Heidelberg, Germany - A phase Ia study to assess the safety and immunogenicity of new malaria vaccine candidates ChAd63 CS administered alone and with MVA CS. Eoghan de Barra Invited speaker.

American Society of Tropical Medicine and Hygiene presentations (ASTMH)

Safety, Immunogenicity and Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP Thomas W. Rampling1, Georgina Bowyer1, Daniel Wright1, Nick J. Edwards1, Carly M. Bliss1, Ruth O. Payne1, Navin Venkatraman1, Eoghan de Barra2, Rachel Roberts1, Ian D. Poulton1, Robert E. Sinden3, Karen L. Ivinson4, Bebi S. Yassin-Rajkumar4, Ulrike Wille-Reece4, Cynthia K. Lee4, Johan Vekemans5, W Ripley Ballou5, Marc Lievens5, Alison M. Lawrie1, Katie J. Ewer1, Adrian V. Hill1 The Jenner Institute, University of Oxford, Oxford, United Kingdom, 2 Royal College of Surgeons in Ireland, Dublin, Ireland, 3 Division of Cell and Molecular Biology, Imperial College London, London, United Kingdom, 4 PATH Malaria
A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS. Poster ASTMH 2012


Efficacy of Combination Malaria Vaccine Approaches Using the ChAd63 and MVA Vectors Encoding the Antigens ME-TRAP, CS and AMA1. Poster ASTMH 2013


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9. Appendix

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9.7 Notification of GMO release in national press
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9.9 VAC038 Protocol
9.10 Intracellular Cytokine Staining (ICS) reagents
This is a completely confidential questionnaire. Your identity and answers will not be disclosed.

SOCIO-ECONOMIC DETAILS

1. Are you male or female?
   - [ ] Male
   - [ ] Female

2. What age group do you fit into?
   - [ ] 18-25
   - [ ] 26-35
   - [ ] 36-45
   - [ ] 46+

3. What is your Ethnic Group?
   - [ ] Caucasian
   - [ ] Asian
   - [ ] Black
   - [ ] Mixed
   - [ ] Other

4. What is your Occupation?
   - [ ] Student
   - [ ] Employed
   - [ ] UnEmployed
   - [ ] Homemaker
   - [ ] Retired

5. What is your monthly net Income?
   - [ ] less than €1000
   - [ ] €1000-€2000
   - [ ] €2000-€4000
   - [ ] more than €4000
   - [ ] Do not want to answer

6. Where do you live?
   - [ ] Parents family home
   - [ ] Own home
   - [ ] Rental house/flat
   - [ ] Rental room
   - [ ] Other

7. What is your highest completed level of Education?
   - [ ] Secondary School
   - [ ] Degree
   - [ ] Masters
   - [ ] PhD

8. Are you
   - [ ] Single
   - [ ] In a relationship
   - [ ] Married/living together
   - [ ] Divorced
MOTIVATION TO PARTICIPATE IN A PHASE 1 STUDY

9. Had you ever participated in a Research Study before?
   [ ] YES    [ ] NO

10. If yes, have you ever participated in a Phase 1 study before?
    [ ] YES    [ ] NO

11. What is your understanding of the term ‘Phase 1 Study’? (please give details)

12. Why did you choose to participate in the VAC038 study?
    Please circle the level of importance of each one, with 0 being unimportant and 5 being very important.

    Curiosity  0  1  2  3  4  5
    Progress of Medicine  0  1  2  3  4  5
    Free medical check-up  0  1  2  3  4  5
    Financial compensation  0  1  2  3  4  5
    To help others  0  1  2  3  4  5
    Other Reason  0  1  2  3  4  5

    a. If there is another reason, please specify____________________________

13. How did you hear about the VAC 038 study?
    [ ] Poster    [ ] Email distribution    [ ] Newspaper    [ ] Word of Mouth
    [ ] Other, please
    specify____________________________________________________

14. Before deciding to participate, did you ask someone else’s opinion?
    [ ] YES    [ ] NO
15. If YES, whose opinion did you ask?
   [ ] Family/Partner  [ ] Friend  [ ] GP  [ ] Other

16. How did your family or close friends react to your decision to participate in the VAC038 study?
   [ ] Positively  [ ] Negatively  [ ] No Reaction

17. Did anyone suggest that you should not participate?
   [ ] YES  [ ] NO

18. If yes, what reasons did they give?
   [ ] High Risk  [ ] Guinea Pig role  [ ] Inconvenience
   [ ] Not enough financial compensation  [ ] Other, please give details

19. Did anyone encourage you to participate?
   [ ] YES  [ ] NO

a. If yes, please list the reasons given below

20. What did you think of the amount of the financial compensation for participating?
   [ ] Very Good  [ ] Good  [ ] Reasonable  [ ] Bad

21. Why do you think volunteers are offered financial compensation for participation in a Phase I study?

22. Would you have participated in the VAC038 study if there had been no financial compensation?
   [ ] Definitely  [ ] Probably  [ ] Probably not  [ ] Definitely not

23. Did the topic under investigation (Malaria Vaccine) have any influence on your decision to participate in the VAC038 study?
   [ ] Definitely  [ ] Probably  [ ] Probably not  [ ] Definitely not

24. Would you recommend participation in a phase 1 study to a friend/family member?
   [ ] YES  [ ] NO  [ ] MAYBE
YOUR EXPERIENCE OF PARTICIPATING IN A PHASE I STUDY

25. Was the level of information you were given about the study BEFORE you decided to participate;
   [ ] sufficient   [ ] Not sufficient

26. Were you given the opportunity to ask questions about the study?
   a. Before you decided to participate   [ ] YES   [ ] NO
   b. During the study                   [ ] YES   [ ] NO

27. Were your questions answered to your satisfaction?
   [ ] YES   [ ] NO   Please Comment

28. Were the appointment times for clinic visits convenient for you?
   [ ] All of the time [ ] Most of the time   [ ] Some of the time   [ ] None of time

29. Was the level of physical discomfort you experienced at the clinic visits;
   [ ] more than you expected   [ ] as expected   [ ] less than you expected

30. Were you concerned about the possibility of a serious complication occurring as a result of your participation in the study?
   [ ] Not concerned   [ ] Slightly concerned   [ ] Very concerned

31. How confident were you that you could trust the skills of the clinical staff?
   [ ] Very Confident   [ ] Mostly Confident   [ ] Not Confident

32. Would you agree to participate in a future phase 1 study?
   [ ] Definately   [ ] Probably   [ ] Probably not   [ ] Definitely not

Please give details, regarding your reason

9.2 Severity grading criteria for adverse events

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at injection site</td>
<td>1</td>
<td>Pain that is easily tolerated</td>
</tr>
<tr>
<td>Condition</td>
<td>Score</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Pain that interferes with daily</td>
<td>2</td>
<td>activity</td>
</tr>
<tr>
<td>Pain that prevents daily activity</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Erythema at injection site*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&gt;3 - ≤50 mm</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;50 - ≤100 mm</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;100 mm</td>
<td></td>
</tr>
<tr>
<td>Swelling at injection site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&gt;3 - ≤50 mm</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;50 - ≤100 mm</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;100 mm</td>
<td></td>
</tr>
<tr>
<td>Fever (oral)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37.6°C - 38.0°C</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;38.0°C – 39.0°C</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;39.0°C</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Headache that is easily tolerated</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Headache that interferes with daily activity</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Headache that prevents daily activity</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Nausea that is easily tolerated</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Nausea that interferes with daily activity</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nausea that prevents daily activity</td>
<td></td>
</tr>
<tr>
<td>Malaise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Malaise that is easily tolerated</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Malaise that interferes with daily activity</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Malaise that prevents daily activity</td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Myalgia that is easily tolerated</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Myalgia that interferes with daily activity</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Myalgia that prevents daily activity</td>
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<td>Arthralgia</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>Joint pain that prevents daily activity</td>
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</tr>
<tr>
<td>Urticaria</td>
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</tr>
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<td>1</td>
<td>Requiring no medications</td>
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<td>Laboratory Test</td>
<td>Grade 1</td>
<td>Grade 2</td>
</tr>
<tr>
<td>---------------------------------------</td>
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<td>------------------------------</td>
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<tr>
<td>Hgb (female) – decrease from testing</td>
<td>&gt;1.0 - &lt;1.5</td>
<td>≥1.5 &amp; &lt;2.0</td>
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<tr>
<td>laboratory LLN in gm/dl</td>
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<td></td>
</tr>
<tr>
<td>Hgb (male) – decrease from testing</td>
<td>≥1.5 &amp; &lt;2.0</td>
<td>≥2.0 &amp; &lt;2.5</td>
</tr>
<tr>
<td>laboratory LLN in gm/dl</td>
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<td></td>
</tr>
<tr>
<td>Absolute neutrophil count (ANC,</td>
<td>1000-1499</td>
<td>500-999</td>
</tr>
<tr>
<td>cells/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia (WBC, cells/mm³)</td>
<td>&lt;3500 - ≥2500</td>
<td>&lt;2500 - ≥1500</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>125,000 – 135,000</td>
<td>100,000 – 124,000</td>
</tr>
<tr>
<td>Bilirubin – when accompanied by any</td>
<td>1.1 – 1.25 x ULN</td>
<td>1.26 – 1.5 x ULN</td>
</tr>
<tr>
<td>increase in Liver Function Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>increase by factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>1.25 – 2.5 x ULN</td>
<td>&gt;2.6 – 5.0 x ULN</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.1 – 1.5 x ULN</td>
<td>&gt;1.6 – 3.0 x ULN</td>
</tr>
<tr>
<td>Urine protein</td>
<td>2+ or 0.5-1 gm loss/day</td>
<td>3+ or 1-2 gm loss/day</td>
</tr>
<tr>
<td>Hematuria</td>
<td>2+ confirmed by 5-10 rbc/hpf</td>
<td>3+ confirmed by &gt;10 rbc/hpf</td>
</tr>
</tbody>
</table>
A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS

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1. Purpose
This plan is used to outline specific information relating to the study entitled ‘A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS’ (VAC038), which is not already present in sufficient detail in the study protocol or other existing procedures. Where current standard operating procedures are to be used, these are referenced in the text.

2. Study overview
This is an open label phase Ia clinical trial to assess the safety and immunogenicity of different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime-boost regimen. All volunteers recruited will be healthy adults aged between 18 and 50. Volunteers will be allocated to the groups by the investigators. Safety data will be collected for each of the regimens (Table 4). The immune responses generated by each of these regimens will be assessed.

Objectives

Primary Objective
- To assess the safety in healthy volunteers of two different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime boost regimen.

Secondary Objective
- To assess the immunogenicity in healthy volunteers of two different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime boost regimen.

Table 1. Study Groups

<table>
<thead>
<tr>
<th>Group Number</th>
<th>No. of volunteers</th>
<th>ChAd63 CS Day 0</th>
<th>MVA CS Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>4</td>
<td>$5 \times 10^7$ vp</td>
<td>-</td>
</tr>
<tr>
<td>1B</td>
<td>8</td>
<td>$5 \times 10^8$ vp</td>
<td>$2 \times 10^9$ pfu</td>
</tr>
<tr>
<td>2A</td>
<td>4</td>
<td>$5 \times 10^{10}$ vp</td>
<td>-</td>
</tr>
</tbody>
</table>
Rationale for Trial Design

Administration Schedules

Heterologous prime boost with ChAd63-MVA is, to our knowledge, one of the most potent T cell inducing subunit vaccine regimens which can importantly also induce antibodies. Previous clinical trials using this regimen expressing ME-TRAP, AMA1 & MSP1, have shown that administering ChAd63 as a prime followed 8 weeks later by MVA as a boost is a very immunogenic schedule (O’Hara et al submitted, Sheehy et al submitted). For this reason, and to provide comparability with previous ChAd63-MVA trials we propose to use a similar administration schedule.

Route & Dose

Our choice of the dose and route of vaccines in this study is based on experience using the same vectors in previous Phase I and Phase II clinical trials in the UK and Africa (see investigator brochures) using both intradermal and intramuscular routes.

We have chosen here the intramuscular route of administration for all vaccines given the proven favourable safety and immunogenicity profile of this route of administration with these vectors and because of future practical considerations regarding administration in the field.

ChAd63 has been safety administered to more than 250 healthy individuals and the vector has been shown repeatedly to be safe at the planned dose of $5 \times 10^{10}$ vp (see investigator brochure).

MVA has been administered to more than 120 healthy UK adults following priming with ChAd63 expressing the same antigen, at various doses. Repeatedly, a dose of $1-2 \times 10^8$ pfu MVA has been found to be a suitable dose to balance immunogenic and reactogenicity (see investigator brochure).

Duration of Study

Groups 1A & 2A
The duration of involvement in the study from enrolment will be approximately 6 months.

Groups 1B & 2B
The duration of involvement in the study from enrolment will be approximately 6 months.

**Definition of the Start and End of the Trial**

The start of the trial is defined as the date of the first vaccination of the first volunteer. The end of the trial is the date of the last visit of the last volunteer.

2.1 **Standard Operating Procedures (SOPs)**

The SOPs used in this study come from 3 different sources:

- **CRC SOPs**
  Clinical Research Centre, Beaumont Hospital. The study site, which maintains a set of SOPs cover the running of clinical trials. Master copies held in hard copy in the CRC. Contact Patricia Burke.

- **CCVTM SOPs**
  Centre for Clinical Vaccinology and Tropical Medicine, Oxford University. The study sponsor. Developed and maintained SOPs specific to this trial design. Held digitally in ipassport document management system. The investigators have online access. Contact Eoghan de Barra.

- **RCSI Vaccine Study SOPs**
  Site and Study specific SOPs, written by the local investigators. Hard copies held in the Site File.

3. **Scope**

This plan applies to ‘A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS’ (VAC038), sponsored by the University of Oxford.

4. **Definitions/abbreviations**

<table>
<thead>
<tr>
<th>Short Form</th>
<th>Long Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChAd63</td>
<td>Chimpanzee adenovirus 63</td>
</tr>
<tr>
<td>AdHu</td>
<td>Human adenovirus</td>
</tr>
<tr>
<td>AdHu5</td>
<td>Human adenovirus serotype 5</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AMA1</td>
<td>Apical membrane antigen 1</td>
</tr>
<tr>
<td>CCVTM</td>
<td>Centre for Clinical Vaccinology and Tropical Medicine</td>
</tr>
<tr>
<td>CBF</td>
<td>Clinical Bio manufacturing Facility</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form or Clinical Research Facility</td>
</tr>
<tr>
<td>CS or CSP</td>
<td>Circumsporozoite protein</td>
</tr>
<tr>
<td>ELISPOT</td>
<td>Enzyme-linked immunospot</td>
</tr>
<tr>
<td>FBC</td>
<td>Full blood count</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GIA</td>
<td>Growth Inhibition Assay</td>
</tr>
<tr>
<td>GMO</td>
<td>Genetically modified organism</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B Surface Antigen</td>
</tr>
<tr>
<td>HCG</td>
<td>Human Chorionic Gonadotrophin</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>IDT</td>
<td>Impfstoffwerk Dessau-Tornau</td>
</tr>
<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
</tr>
<tr>
<td>REC</td>
<td>Independent Research Ethics Committee</td>
</tr>
<tr>
<td>LSM</td>
<td>Local safety monitor</td>
</tr>
<tr>
<td>ME-TRAP</td>
<td>Multiple epitopes and thrombospondin related adhesion protein</td>
</tr>
<tr>
<td>MSP1</td>
<td>Merozoite Surface Protein 1</td>
</tr>
<tr>
<td>MVA</td>
<td>Modified vaccinia virus Ankara</td>
</tr>
<tr>
<td>pfu</td>
<td>Plaque forming unit</td>
</tr>
<tr>
<td>PMR</td>
<td>Parasite Multiplication Rate</td>
</tr>
<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>vp</td>
<td>viral particle</td>
</tr>
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</table>
5. Responsibilities

Lead clinical research fellow writes the clinical study plan and ensures that all study staff are aware of its content.

Project/QA manager supports the development and use of the plan.

Principal Investigator reviews and approves the plan.
6. Key timelines and numbers
Site Initiation: 13th December 2011

Proposed commencement of screening 15th December 2011

Proposed first vaccination: 20th December 2011

Following Approval of the Irish Medicine Board (IMB), Beaumont Ethics Committee and the Environmental Protection Agency (EPA) Site Initiation will take place. Thereafter the current Volunteer Information Sheets can be sent to volunteers from the RCSI vaccine research groups database of potential volunteers. These are individuals who have previously participated in the Pre Screening Study carried out at the CRC and consented to being contacted with respect to future studies. The volunteers will then be invited to attend for a VAC038 screening visit.

Using Day 0, the first vaccination day, as a critical time point, the other important timelines in the study can be mapped out as before Day 0 (-) or post (+) Day 0.

6.1 Consent – prior to any study activity. Day -90 to -1

Written informed consent must take place prior to any enrolment. At least 24 hours must have elapsed between volunteers receiving the VIS and consent being taken.

Separate consent for purposes of genetic testing must be obtained. Not consenting to this aspect of the study will not effect volunteers enrolment in the rest of the study.

Documents:  
VAC038 consent form v3.0 Eire 11th Sept 2011
VAC038 consent form DNA v1.0 Eire 11th Sept 2011
CRC SOP 11 Consenting Procedure

6.2 Screening. Day -90 to -1

Volunteers may be screened up to -90 days before vaccination. They must be provided with the Volunteer Information Sheet at least 24 hours prior to screening, and must compete the consent form prior to screening taking place.

See section 11, sample collection for details of samples needed.

Documents:  
VAC038 CRF-1 (Screening) v2.0 21 Nov 2011  
CRC SOP 12 Collecting, handling, storing and transport of biological specimens.
6.3 Vaccination Day 0

Day 0: Results of screening investigations must be reviewed. A review of the eligibility of the volunteer must be performed prior to every vaccination. In the case of female volunteers a urine pregnancy test must be performed. 72 hours must elapse between vaccination of the first volunteer and vaccination of volunteers number 2 and 3, for each vaccine. A further 72 hours must elapse following vaccination of volunteers 2 and 3 prior to any further vaccinations.

Documents: CRF-2 Group 1A v2.0 (or 1B, 2A, 2B)
RCSI SOP Vaccination storage and accountability 2.0
RCSI SOP Vaccination 2.0
CCVTM SOP MC012 Vaccine dilution
RCSI Vaccine study SOP 7 - Autoclave operation, deactivation of GM waste
VAC038 Diary Card 7 day Eire version 1.0 27th May 2011

6.2.1 Observation: In the case of the first 3 subjects to receive each dosing (ChAd63 CS and MVA CS respectively) an observation time of 12 hours must be applied. During this time, the subject should remain in the CRC building and have Blood pressure, temperature and pulse recorded at 60 minutes, 3 hours, 6hours and 12hours post vaccination. For all other volunteers an observation time of 30 minutes will be applied. Symptoms related to the injection site should also be recorded as per the table on page 4 of the CRF.

6.4 Day 1

All volunteers must be reviewed 24 hours post vaccination. Follow the CRF for details of recordings to be made and blood samples to be collected.

6.5 Subsequent visits

See table 2 below for details of events at further visits for volunteers in groups 1A and 2A. See table 3 below for details of events at further visits for volunteers in groups 1B and 2B.

Note that day 56 involves vaccination with MVA – CS for groups 1B and 2B. Thus refer to documents relating to vaccination day 0.

Both groups complete the study with a final review performed on day 180 (+/- 14 days). This can be done by telephone.
As stated above in 1.1 Study groups, groups will be as follows;

**Subgroup A (1A)** 4 volunteers – 1 dose ChAd63 CS 5x10^9 vp

**Subgroup B (1B)** 8 volunteers – 1 dose ChAd63 CS 5x10^9 vp

and 1 dose MVA CS 2x10^8 pfu 8 weeks later.

**Subgroup A (2A)** 4 volunteers – 1 dose ChAd63 CS 5x10^10 vp

**Subgroup B (2B)** 8 volunteers – 1 dose ChAd63 CS 5x10^10 vp

and 1 dose MVA CS 2x10^8 pfu 8 weeks later.
6.3 Dose Escalation

In order to complete the study in the shortest time frame possible, it is advisable to enrol volunteers firstly into group 1B.

Enrollment into groups 2A and 2B can only happen following safety review of the data relating to group 1A and 1B by the DSMB. All 12 low dose vaccination must be administered, the volunteers day 14 reviews completed and the data generated forwarded to the DSMB. Documentary evidence of a favourable DSMB review must be filed prior to enrolment of volunteers to groups 2A and 2B.

7. Key Contacts and communication

7.1 Contacts and Locations

<table>
<thead>
<tr>
<th>Role and location</th>
<th>Name</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle Investigator</td>
<td>Prof. Samuel McConkey</td>
<td>+353 (0) 87 9063375</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:smconkey@rcsi.ie">smconkey@rcsi.ie</a></td>
</tr>
<tr>
<td>Study Coordinator</td>
<td>Alison Lawrie</td>
<td>+44 1865 857382</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:Alison.lawrie@ndm.ox.ac.uk">Alison.lawrie@ndm.ox.ac.uk</a></td>
</tr>
<tr>
<td>Lead nurse</td>
<td>Ann Collins</td>
<td>Tel: +353 (1) 809 3787</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:annmcollins@rcsi.ie">annmcollins@rcsi.ie</a></td>
</tr>
<tr>
<td>Consultant infectious diseases physician</td>
<td>Prof. Samuel McConkey</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="mailto:smconkey@rcsi.ie">smconkey@rcsi.ie</a></td>
</tr>
<tr>
<td><strong>On-call study physician</strong></td>
<td>Call dedicated Mobile phone</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical Research Fellow</strong></td>
<td>Dr. Eoghan de Barra +353 86 8386538 <a href="mailto:edebarra@rcsi.ie">edebarra@rcsi.ie</a></td>
<td></td>
</tr>
<tr>
<td><strong>Lab scientist</strong></td>
<td>Kerrie Hennigan <a href="mailto:Kerrie.hennigan@rcsi.ie">Kerrie.hennigan@rcsi.ie</a></td>
<td></td>
</tr>
<tr>
<td><strong>Biochemistry laboratory</strong></td>
<td>Biomnis Ireland, Three Rock Road, Sandyford Business Estate, Dublin 18, Ireland. Client Services Tel: 1800 252 966 Email: <a href="mailto:client.services@biomnis.ie">client.services@biomnis.ie</a> Logistics Tel: 1800 252 967 Email: <a href="mailto:logistics@biomnis.ie">logistics@biomnis.ie</a> Patrick Clarke Tel: +353 1 295 8545 Fax: +353 1 295 8550 <a href="mailto:patrick.clarke@biomnis.ie">patrick.clarke@biomnis.ie</a></td>
<td></td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
<td>Dr Mary Keogan, Consultant Immunologist Immunology Department, Beaumont Hospital, Dublin 9 Phone: +353 (01) 809 2635 Fax: +353 (01) 809 3933</td>
<td></td>
</tr>
<tr>
<td><strong>Internal Monitor</strong></td>
<td>Mary Dowling <a href="mailto:mcdowkey@hotmail.com">mcdowkey@hotmail.com</a></td>
<td></td>
</tr>
<tr>
<td><strong>External Monitor</strong></td>
<td>Ceri McKenna Appledown Clinical Research Ltd. Ceri McKenna Appledown Clinical Research Ltd. 67, Gregories Road, Beaconsfield, Bucks. HP9 1HL.</td>
<td></td>
</tr>
</tbody>
</table>
| **Tel/Fax:** +44 1494 677447  
| **ceri.mckenna@appledown.net** |
| **Beaumont Hospital** | **Main switch** | **Tel:** +353 1 809 3000  
| **Fax:** +353 1 837 6982 |
| **Infection control – Beaumont Hospital** | **Contact via hospital switch** | **Tel:** +353 1 809 3000 |
| **DSMB** | **Dr. Brian Angus** | Reader in Infectious Diseases,  
|  |  | Director of the Wellcome Trust Centre for Clinical Tropical Medicine, Oxford.  
|  |  | Consultant physician in Infectious Diseases & Acute medicine.  
|  |  | Oxford Radcliff hospitals’ Trust  
|  |  | [Brian.angus@ndm.ox.ac.uk](mailto:Brian.angus@ndm.ox.ac.uk)  
|  |  | Nuffield Dept of Medicine, Level 7, Room 7503, John Radcliff hospital, Oxford, OX39DU. UK  
|  |  | Tel: +44 (0)1865-220289  
|  |  | Fax: +44(0)1865221354  
|  | **Dr Chris Conlon** | Reader in Infectious Diseases and Tropical Medicine, University of Oxford  
|  |  | Consultant Physician in Infectious Diseases & Acute Medicine, Oxford Radcliffe Hospitals’ Trust  
|  |  | Email: chris.conlon@ndm.ox.ac.uk  
|  |  | Address: Nuffield Department of Medicine, Level 7, Room 7503, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom |
Dr Patrick Mallon
Consultant Physician in Infectious Diseases, Mater Misericordiae University Hospital.
Email Address: Paddy.Mallon@ucd.ie
Postal Address: Mater Misericordiae University Hospital, Eccles Street, Dublin 7.
Tel: +353-1-803 2930

Ethics committee
Beaumont Hospital Ethics Committee
Gillian Vale, administrator
Tel: +353 1 8092680
gillianvale@beaumont.ie

<table>
<thead>
<tr>
<th>Location</th>
<th>Use</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCSI, Clinical Research Centre, Smurfit building, Beaumont Hospital, Dublin 9.</td>
<td>Storage IMP, Screening Visits, Administration of vaccines, Follow-up visits</td>
<td>Patrick Connolly, Building Manager – 01 8093733/8093700</td>
</tr>
<tr>
<td>Research Laboratory, Ground floor, RCSI Clinical Research Centre, Beaumont, Dublin 9.</td>
<td>Performing of Elispots and sample storage.</td>
<td>Irene Oglesby, Lab manager - <a href="mailto:ioglesby@rcsi.ie">ioglesby@rcsi.ie</a></td>
</tr>
<tr>
<td>The Jenner Institute Laboratories, ORCRB</td>
<td>Storage of Samples, Performing</td>
<td></td>
</tr>
</tbody>
</table>
7.2 Site communication

Regular teleconferences involving all local site staff with Oxford staff are to be held at least monthly. Teleconferences involving local site staff, Oxford and EVI are to be held every 2 months.

8. Clinical Facilities

8.1 Overview

The Clinical Research Centre (CRC) Beaumont Hospital, is the study site. This building was purpose built for the conduct of clinical trials. On the ground floor there is a reception and a clinical area, with rooms for volunteer review. There are separate secure rooms for storage of medicinal products, including a minus 80 degree Celsius freezer, and processing of biological specimens. The study research nurse has a work place next to these areas. Further back into the building there is a research laboratory, where the investigational immunology work will be performed.

Documents: RCSI Vaccine study SOP 1- Access to the CRC

8.2 Vaccine storage and checks

All persons handling the vaccine must be familiar with the relevant SOPs, covering receipt, storage, vaccination, unintended release / spillage, and reporting mechanisms.

Documents: RCSI SOP Vaccination 2.0

RCSI SOP Vaccination storage and accountability 2.0

RCSI Vaccine study SOP 2 - Training of staff with responsibilities relating to the handling of GMO

RCSI Vaccine study SOP 3 - Emergency response in the event of unintentional GMO release
8.3. Supplies/consumables (including responsibilities)

- **Lead Clinical Research Fellow**: will be responsible for requesting all laboratory supplies and liaising with the Jenner institute for transfer of peptide pools. The Lead Clinical Research Fellow will liaise with the study nurses to prepare volunteer ‘study packs’.

- **Lead nurse**: will be responsible for appropriate numbers of vacutainers for blood sampling (including PAX gene tubes), thermometers, pregnancy tests, cards containing study team contact details a digital camera, food for volunteers, sharps bins and medications for the managements of anaphylaxis are available for the vaccination period. They will also ensure that wall clocks in clinic rooms at the CRC are synched with each other. They will also ensure consumables for the dilution and administration of vaccines are in stock. (see SOP Vaccination, and SOP Vaccine dilutions.)

- **Laboratory assistant**: will be responsible ensuring stock levels of laboratory consumables are sufficient for trial activity. They will also monitor and regulate the liquid nitrogen storage of samples.

9. Data collection and storage

Source data and participant identifiable data

Source data (which includes diary cards and documentation relating to vaccine preparations for each volunteer) will be filed in volunteers’ CRF. Volunteer identifiable data will be stored in volunteers’ CRFs until the end of the study at which time it will be stored separately.

All data including those regarding clinic visits, administration of vaccine and AEs will be documented and stored in paper format in volunteers’ CRFs.

All data, both paper and electronic will be stored securely and only accessed by appropriate personnel. All data entry will be checked for accuracy by the monitor +/- a member of the study team.

Data will be analysed according to the statistics section of the protocol in consultation with the study statistician.
10. **AE/SAE reporting**

AEs will be classified according to criteria outlined in the study protocol. Any SAEs will be managed according to SOP VC004: Safety Reporting.

11. **Sample collection**

Samples will be processed according to SOP VL004: Clinical Trial Sample Handling. All samples will be labelled with volunteer number, which includes study number and time point indicated by “D”. In addition, samples for blood smears and PCR and microscopy slides for blood smears will be labelled with;

1. The time of sampling
2. The visit number

11.1. **Study samples flowchart and collection tubes**

**PAXgene tube**: please fill the green tube first, then the PAX gene tube. The proper performance of the tube is sensitive to the ratio between the amount of blood and the amount of the clear liquid already in the tube. If the PAXgene tube is the first tube inserted in to the blood collection set, then the dead space in the blood collection set will mean there is less than 2.5ml of blood pulled in to the tube. Following blood collection, invert the tube 8-10 times. Store tube upright for a minimum of 2 hours and max of 72 hours before transferring to -20 Freezer. Ensure Paxgene tubes are not frozen in Styrofoam racks. Store at -20 for 24 hours before transferring to -80.

**Centrifuge**: Where ‘centrifuge’ is present in the processing column the following steps must be performed;

1. Invert the tube at least 5 times
2. Leave bottle stand for 30 minutes
3. Centrifuge bottle @ 3000 RPM for 15 minutes within 2 hours of collection

Note some samples should be refrigerated until collection

**Screening**

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Processing</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBC</td>
<td>5ml EDTA</td>
<td>None</td>
</tr>
<tr>
<td>Test</td>
<td>Bottle</td>
<td>Processing</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>5ml Serum</td>
<td>Centrifuge</td>
</tr>
<tr>
<td>Carbohydrate Deficient Transferrin</td>
<td>5ml Serum +4ºC</td>
<td>Centrifuge</td>
</tr>
<tr>
<td>HIV, HBV &amp; HCV</td>
<td>2ml Serum</td>
<td>None</td>
</tr>
<tr>
<td>Urine toxicology</td>
<td>Universal container</td>
<td>None</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>Universal container</td>
<td>In house</td>
</tr>
<tr>
<td>Urine Pregnancy</td>
<td>Universal container</td>
<td>In house</td>
</tr>
</tbody>
</table>

**Day 0 – Pre Vaccination**

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Processing</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ex Imm 22.5ml (Red Top-plain)</strong></td>
<td>BD Red top (10ml)</td>
<td>None</td>
</tr>
<tr>
<td><strong>Ex Imm 45ml (Green Top-LH)</strong></td>
<td>BD Green top (10ml)</td>
<td>None</td>
</tr>
<tr>
<td>HLA</td>
<td>Citrate (4mls)</td>
<td>None</td>
</tr>
<tr>
<td><strong>Ex Imm (Pax Gene)</strong></td>
<td>Blood RNA tube</td>
<td>Freeze -20ºC for 24h then -80ºC</td>
</tr>
</tbody>
</table>

**Day 1**

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Processing</th>
<th>Destination</th>
</tr>
</thead>
</table>
Day 14 – 140

Bloods as per Protocol, below. Also listed in CRF for each visit.

Day 180

No bloods. Clinical review, may be done via telephone.

### 11.2. Prioritisation of samples

1. Safety bloods
2. Plasma (Green Tops for Elispot)
3. Pax Gene
4. Serum (Red Top)

### 11.3. Safety bloods (other labs)

Details of nature, timing and destination of safety bloods have been included in the sample collection section 11.1 above.

**Table 2: Schedule of clinical reviews for Groups 1A & 2A**

<table>
<thead>
<tr>
<th>Review No.</th>
<th>S</th>
<th>ChAd63 CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline (days)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Window (days)</td>
<td>-90</td>
<td>**</td>
</tr>
</tbody>
</table>

Inclusion / Exclusion criteria

- Informed consent: X
- Medical History: X (x) (x) (x) (x) (x) (x)
- Physical Examination: X (x) (x) (x) (x) (x) (x)
- Urinalysis: X

CRC lab: Oxford batched at end of study

Ex Imm 10ml
(Green Top - LH)

BD green top

none

Freeze -20°C for 24h then -80°C
β-HCG urine test | X | X | X
Review contraindications | X | X | X
Vaccination | X
Vital signs | X | X | X | (x) | (x) | (x) | (x)
Local & systemic AEs assessed | X | X | X | X | X | X
Diary cards provided | X
Diary cards collected | X
HLA typing (mL) | 4
HBV, HCV, HIV (mL) | 5
Haematology (mL) | 2 | 2 | 2 | 2 | 2
Biochemistry* (mL) | 4 | 4 | 4 | 4 | 4
Carbohydrate Deficient Transferrin | 5

Exploratory immunology | 70 | 13 | 70 | 70 | 70 | 70
Blood volume per visit (mL) | 16 | 74 | 13 | 76 | 76 | 76 | 76
Cumulative blood volume (mL) | 16 | 90 | 103 | 179 | 255 | 331 | 407

S = screening visit, V = vaccination visit, (x) = If necessary (Windows refer to time since last visit)
* Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests. **Window: -12 hours/ +48 hours. † can be via telephone

Table 3: Schedule of clinical reviews for Groups 1B & 2B

<table>
<thead>
<tr>
<th>Review No.</th>
<th>S</th>
<th>ChAd63</th>
<th>CS</th>
<th>MVA</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline (days)</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>Window (days)</td>
<td>-90</td>
<td>**</td>
<td>±2</td>
<td>±7</td>
<td>±7</td>
</tr>
<tr>
<td>Inclusion / Exclusion criteria</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test / Task</td>
<td>S</td>
<td>V</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>------------------------------------------------------------</td>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>β-HCG urine test</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review contraindications</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local &amp; systemic AEs assessed</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diary cards provided</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diary cards collected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA typing (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV, HCV, HIV (mL)</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology (mL)</td>
<td>2</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Biochemistry* (mL)</td>
<td>4</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Carbohydrate Deficient Transferrin</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploratory immunology</td>
<td></td>
<td></td>
<td>70</td>
<td>13</td>
<td>70</td>
</tr>
<tr>
<td>Blood volume per visit (mL)</td>
<td>16</td>
<td></td>
<td>74</td>
<td>13</td>
<td>76</td>
</tr>
<tr>
<td>Cumulative blood volume (mL)</td>
<td>16</td>
<td></td>
<td>90</td>
<td>103</td>
<td>179</td>
</tr>
</tbody>
</table>

S = screening visit, V = vaccination visit, (x) = If necessary (Windows refer to time since last visit)  
* Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests. **Window: -12 hours/ +48 hours. † can be via telephone.
### 11.4. Samples Storage

<table>
<thead>
<tr>
<th>Purpose Storage</th>
<th>Bottle</th>
<th>Processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex Imm 22.5ml (Red Top-plain)</td>
<td>BD Red top (10ml)</td>
<td>Spin @1800rpm 5 mins store -80°C Separate plasma</td>
</tr>
<tr>
<td>Ex Imm 45ml (Green Top-LH)</td>
<td>BD Green top (10ml)</td>
<td>Isolation of PBMC for Elispot / freezing</td>
</tr>
<tr>
<td>Ex Imm (PaxGene)</td>
<td>Blood RNA tube</td>
<td>Freeze -20°C for 24h then -80°C</td>
</tr>
</tbody>
</table>

-80°C CRC lab

-80°C initially then transfer to Liquid Nitrogen within 24 to 72 hrs. CRC lab

Ship to Oxford batched at end of study
**Laboratory Facilities**

Equipment: Equipment owned by the study group, includes a -20°C freezer and fridge, a microscope, pipettes and a laptop computer. Other equipment listed here is shared in the research lab, fume hoods, -80°C freezer, Liquid Nitrogen storage, Cell culture incubator, centrifuge, water bath, autoclave. Disposal of waste must be done in accordance with local policy, the exception is disposal of GMO waste which is covered in RCSI SOP 7, autoclave operation, deactivation of GM waste.

Main laboratory work space is located in the Respiratory laboratory on the ground floor of the CRC.

Sample processing (see RCSI SOP No. 8, Malaria Vaccine trial sample handling.)

Plate and Sample storage (see RCSI SOP No. 8, Malaria Vaccine trial sample handling.)

**12. Shipment of samples**

**Screening bloods and safety bloods:** Biomnis

Call Biomnis logistics 1800 252967 early on the morning when samples are being taken to ensure they will collect. The latest collection is at 1400 sharp each day. Blood outside this time can be sent using their urgent service, call logistics to arrange.

**HLA typing:** Immunology laboratory Beaumont Hospital

Label with volunteer name and date of birth. Transfer in biohazard bag to Immunology laboratory Beaumont hospital.

All other samples are processed initially in the CRC research lab.

Frozen serum, cells and Paxgene RNA tubes will be shipped to the Jenner Institute Oxford periodically during the study or in one shipment at the end of sample collection. The company ‘World courier’, specialising in logged cold chain shipping will be contracted. Contact is made via Oxford project manager.

**13. DSMB/Monitoring/Auditing**

**14.1 DSMB:** The Local Safety Committee may convene a formal meeting at any time or at the request of the Investigators or Sponsor. The Chair of the LSC will be contacted for advice and independent review in the following situations:

- Following any SAE deemed to be possibly, probably or definitely related to the study vaccine
- In any other situation where the Investigator feels independent advice or review is important or helpful

The LSC has the power to halt or suspend the trial and to make specific recommendations to the Investigators regarding the safety of an individual volunteer or of all the trial volunteers. The LSC or its Chair may also give other advice in response to specific requests from the Investigators. In addition the DSMB must review and give favourable opinion on the day 14 data on all volunteers.
vaccinated with the ChAd63CS vaccine at a dose of \(5 \times 10^9\) vp, before any volunteers can receive the ChAd63CS vaccine at a dose of \(5 \times 10^{10}\) vp.

### 14.2 Monitoring

Local internal monitoring will be carried out throughout the study and in particular in advance of external monitoring visits and auditing. External monitoring will be at site initiation, after enrolment of 3 volunteers and thereafter at the discretion of the monitor.

### 14.3 Auditing

The Irish Medicines Board (IMB), requests that the Investigators notify them upon enrolment of the 4\(^{th}\) volunteer. They intend to audit the study at that point. They may also choose to audit the study at other time points. The Environment Protection Agency (EPA), who issued the deliberate release of GMO permission is entitled to audit the study, focusing on handling and disposal of the GMO. The funders, EVI intend to visit the study site in Spring 2012.
VOLUNTEER INFORMATION SHEET: VAC038

A study to assess new malaria vaccines;
ChAd63 CS & MVA CS

A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS

We would like to invite you to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish.

- Part 1 tells you the purpose of the study and what will happen to you if you take part.
- Part 2 tells you more information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. Part 1

1.1. What is the purpose of the study?
Malaria affects around 300 million people each year about a million of who die from the disease. It is a major problem for those who live in affected areas and for travellers. There is a great need for a safe, effective malaria vaccine. Researchers around the world, including members of Professor Hill's group at the University of Oxford, UK have been investigating malaria for over 15 years. Over the last 10 years, we have been conducting clinical studies of new malaria vaccines.

The purpose of this study is to examine the safety and immune response to two new malaria vaccines; ChAd63 CS (main vaccine) and MVA CS (booster vaccine). We will also be checking the safety of different doses of one of the vaccines (ChAd63 CS).

ChAd63 CS and MVA Cs are vaccines that are made from viruses which are inactivated so that they are unable to multiply within the body. The viruses contain genetic
information (DNA) from the malaria parasite. This genetic material is named CS. The aim is to use these vaccines to help the body make an immune response against parts of the malaria parasite. The ChAd63 CS vaccine is a genetically modified version of an adenovirus (a common cold virus). The strain of adenovirus we use for this vaccine usually affects chimpanzees. The MVA CS vaccine is based on the Modified Vaccinia Virus Ankara (MVA), which is a safer form of the vaccine virus previously widely used for smallpox vaccination and it has also been genetically modified.

The purpose of this study is to assess;

1. The safety of the vaccine schedules in healthy volunteers.
2. The response of the human immune system to the vaccinations

We will do this by giving volunteers one or two vaccinations and doing blood tests to assess the response of the immune system to the vaccines. We hope to recruit 24 volunteers to be vaccinated.

1.2. Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason but you may be asked to return to the clinic for follow up for safety reasons.

1.3. What will happen if I decide to take part?
This study involves having one or two vaccinations and then being followed up with blood tests. Volunteers are enrolled in groups depending on which vaccine schedule they will receive.

Length of research
If you decide to take part in this study, you will be involved in the trial for 6 months.

Am I eligible to be involved in the trial?

In order to be involved in the study you must:

- Be a healthy adult aged between 18 and 50 years.
- Be able and willing (in the investigators’ opinion) to comply with all study requirements.
- Allow the investigators to discuss your medical history with your GP.
- Practice continuous effective contraception for the duration of the study (women only).
- Refrain from blood donation during the course of the study and for 6 months after the end of your involvement in the study.

You cannot participate in this study if:

- You have had malaria before.
- You have travelled to a malaria endemic region in the six months preceding your involvement in the study or are intending to travel to a malaria endemic region during the study.
- You have participated in another research study in the 30 days preceding involvement in this study.
- You are planning to participate in another study at the same time as participating in this study.
- You have previously received an investigational vaccine that could impact on the results of this study.
You have had immunoglobulins and/or any blood products in the three months preceding your involvement this trial.
You have problems with your immune system.
You are pregnant, breast feeding or intend to become pregnant during the study.
You have a history of a severe allergic reaction to a vaccination or an allergy to eggs or a history of any serious reaction following vaccination.
You have a history of cancer.
You have a history of migraine headache.
You have a history of a serious psychiatric condition that may affect participation in the study.
You have any other serious chronic illnesses requiring hospital follow-up. Use of regular medications such as antihypertensives would not necessarily result in exclusion.
You drink on average more than 42 units of alcohol a week (a pint of beer is two units, a small glass of wine 1 unit and a shot of spirits one unit).
You have injected drugs at any time in the last 5 years or currently use opiates, amphetamines, benzodiazepines or marijuana.
You have hepatitis B, hepatitis C or HIV infection.
You are in a particularly dependent relationship with the investigator by way of occupation or otherwise, which in the investigators opinion places you in a vulnerable population.

Mild conditions, such as childhood asthma, which are well controlled, would not automatically exclude you from participating. If you are unclear whether you are eligible to be involved in the study you can contact the study team who will be able to advise you.

CONSIDERATIONS BEFORE TAKING PART IN THIS STUDY

Screening Visit: This takes place up to 3 months before the study starts at your local trial site and lasts up to one and a half hours. The purpose of the screening visit is for you to discuss the trial with us and decide if you still wish to enter the study. If you decide to participate, you will be asked to sign a consent form. We then need to check that you are eligible to participate. You will be asked some medical questions and a doctor will examine you, measuring your blood pressure, pulse and temperature. Some blood tests will be taken to check your red and white cells, your liver and your kidney function. These tests need to be normal for you to be enrolled in the study. Your blood will also be tested for infection with hepatitis B, hepatitis C or HIV. These viruses are transmitted by infected blood and can affect the immune response to infection. If you test positive to any of these infections or antibodies, we will inform you of the result and offer referral for medical review and treatment with your permission. A test called Carbohydrate Deficient Transferrin (CDT) will also be performed on your blood. This is a screening test for chronic alcohol abuse. Chronic alcohol use might also impair your bodies immune response.

All participants are asked for urine samples at screening to check for glucose (to exclude diabetes), protein and blood (which can indicate kidney disease). Your urine will also be tested for the presence of opiates, benzodiazepines, amphetamines and marijuana, as use of them would exclude you from the study. For women, a urine pregnancy test will also be performed.

Blood Donation: Under current regulations, volunteers will not be able to donate blood
during the study or for 6 months after the end of the trial.

Private Medical Insurance: We have contacted the 3 private medical insurance providers in Ireland and discussed the possible effects of participation in this study on their policies. If you have private medical insurance, we can discuss the response of your insurer prior to or at your screening visit.

Malaria Prophylaxis: You should note that the vaccines being tested in this trial are experimental. If you travel to a malaria endemic region in the future you should not assume that the vaccines you received in this study have given you any protection against malaria. Make sure you visit your GP before travelling to a malaria endemic region and use prophylactic anti-malarial medications, bed nets and insect repellent during your trip as directed by your GP.

Contraception: The vaccines being tested in this study are at an early stage of development and it is currently unknown as to whether they are safe in pregnancy. For this reason, it is important that all women use adequate contraception for the duration of the trial.

VACCINATIONS

1.4. What are the vaccines that are being tested?
We are testing two vaccines; ChAd63 CS (main vaccine) and MVA CS (booster vaccine). These vaccines will be given into the muscle of your upper arm(s). Once these vaccinations have been given they cannot be undone, so it is important you are clear of the potential risks of the vaccines before you agree to be involved in the study.

1. ChAd63 CS Vaccine
ChAd63 CS is based on a virus that infects chimpanzees called chimpanzee adenovirus 63 (ChAd63). We have genetically modified the virus so that it is impossible for it to grow in humans, and added a gene encoding a protein from the malaria parasite (the “CS” part of the vaccine). We want to try and make the body develop an immune response to this malaria protein. We have never given this particular vaccine to humans before, however the same chimpanzee adenovirus (with different genes from malaria in it) has been given to more than 250 people in the UK and Africa. In these individuals, the vaccine appears to be a safe and well tolerated, however it can cause short-lived side-effects which resolve rapidly;

Expected Side Effects: Volunteers receiving vaccine (ChAd63 CS) in this trial may experience injection site pain. This is most likely to be mild, however there is a chance this could be moderate or severe in intensity. Volunteers may also experience redness, swelling, itching and warmth at the vaccine site, although these symptoms are likely to be mild if present. Generally volunteers report a transient ‘flu like’ illness within 24 hours of vaccination which resolves within 48hrs. This can include headache, muscle aches, joint
aches, feverishness, tiredness, nausea and feeling generally unwell. The majority of general symptoms are likely to be mild but there is a possibility of moderate or severe headache or feeling unwell.

2. MVA CS Vaccine
MVA CS vaccine is based on a genetically modified virus called modified vaccinia virus Ankara (MVA) and contains the gene encoding the same malaria protein as ChAd63 CS (CS). We have never given this particular vaccine to humans before, however the same MVA virus, with different genes from malaria in it, has been given to more than 940 people in the UK and Africa. In those individuals, MVA appears to be safe and well tolerated, however it can cause short-lived side-effects which resolve rapidly;

Expected Side Effects: Volunteers receiving MVA CS in this trial may experience injection site pain. This is most likely to be mild, but there is a chance this could be moderate in intensity. Volunteers may also experience redness, swelling, itching and warmth at the vaccine site, although these symptoms are likely to be mild if present. Generally volunteers report a transient ‘flu like’ illness within 24 hours of vaccination which resolves within 48hrs. This can include headache, muscle ache, joint ache, feverishness, tiredness and feeling generally unwell. The majority of general symptoms are likely to be mild but there is a possibility of moderate headache, tiredness, muscle aches or feeling unwell.

It is important to remember these are vaccines in the early stage of development, and have not been administered to humans before. For this reason there is a chance you could experience a side effect that is more severe than that described above, or that has not been seen before with these viruses. You are encouraged to consider taking over the counter medications such as paracetamol or ibuprofen, at their recommended doses, if you experience symptoms post vaccination as this is likely to reduce the intensity of any symptoms you have.

Severe Reactions
With any vaccination there is a risk of rare serious adverse events, such as an allergic reaction, which may be related to the nervous system or the immune system. Severe allergic reactions to vaccines (anaphylaxis) are also rare but can be fatal. Reactions in the nervous system are also extremely rare following vaccination and can cause an illness called Guillain-Barré syndrome. Guillain-Barré syndrome is an illness in which people can develop severe weakness and can also be fatal. These adverse events have not previously been seen with the components of the vaccines used in this study. If you experience unexpected events, or become in any way concerned you should contact one of the Investigators (who are available 24 hours a day) using the contact details at the end of Part 2. In addition to the risk of these rare serious adverse events, it should be noted that, the long-term effects on the immune system functions of these vaccines are unknown at this point in time.
1.5. How many vaccinations will I receive?
The number of vaccinations you receive will depend on the group you are in. There are 4 different groups. You will be placed in one of the groups by the study team if you decide to take part. The vaccination groups are summarised in the following table:

<table>
<thead>
<tr>
<th>Group Number</th>
<th>No. of volunteers</th>
<th>ChAd63 CS Day 0</th>
<th>MVA CS Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>4</td>
<td>(low dose vaccine)</td>
<td>-</td>
</tr>
<tr>
<td>1B</td>
<td>8</td>
<td>low dose vaccine</td>
<td>Booster vaccine</td>
</tr>
<tr>
<td>2A</td>
<td>4</td>
<td>higher dose vaccine</td>
<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>8</td>
<td>higher dose vaccine</td>
<td>Booster vaccine</td>
</tr>
</tbody>
</table>

Volunteers in group 1 will receive the lower dose of ChAd63 CS, (low dose). Volunteers in group 2 will receive the higher dose of ChAd63 CS (ten fold higher). We will not give the increased dose of ChAd63 CS to volunteers in group 2 until at least two weeks have passed after the starting dose has been given to volunteers in group 1, in order to allow time to check for side effects. The dose of MVA CS, the booster vaccine, is the same for groups 1 and 2.

Volunteers in group A will receive a single dose of ChAd63 CS alone. Volunteers in group B will receive a single dose of ChAd63 CS followed 8 weeks later by a single dose of MVA CS booster vaccine.

Vaccination Days (Day 0 +/- Day 56)
Visits on vaccination days last approximately 1 hour, except for the first 3 volunteers to receive each dose of either vaccine. They will be observed for 12 hours in the clinical centre following vaccination. Before vaccination you will check you are still fit and healthy and go over any questions you might have. Your blood pressure, pulse and temperature will be measured and blood taken to assess your immune response before vaccination. Women will be asked to provide a urine sample for pregnancy testing prior to vaccination. The vaccine will then be administered as a single injection into the
muscle in your upper arm. We will ask you to wait for 30 minutes after each vaccination to make sure there are no immediate problems. Again if you are one of the first 3 individuals to receive a dose of a vaccine, you will be asked to wait for 12 hours after vaccination for observation. You will be assessed again before leaving and given a diary card, thermometer and tape measure to take away. We will ask you to record your symptoms and the size of any redness or swelling every day for 7 days after each vaccination. We will also ask you to record, on the diary card, the timing and dose of any other medications you may have taken following vaccination like paracetamol or ibuprofen. Your diary card will be collected from you at your next visit. We will also give you the telephone number on which you can contact the trial team 24 hours a day in case of any concerns.

**Follow-Up Visits post Vaccination**

These visits last about 10 minutes each. At these visits we review your diary card and any side effects you may have had, measure your blood-pressure, pulse and temperature and take a blood sample to assess your immune response to the vaccination. We may ask to photograph your vaccination site and you can choose whether or not to agree to this when you sign the consent form. You will not be identifiable in these photographs, as only the vaccination site and your unique trial number will be visible. These photographs may be shown to other professional staff, used for educational purposes or included in a scientific publication.

**Number, timing and purpose of visits**

You will receive between one and two vaccinations and attend a maximum of between 7 and 10 visits in total depending on which group you are enrolled in. Visits may include a medical assessment, temperature, pulse and blood pressure readings, examination by a doctor if needed and blood tests. You will also be contacted by telephone 6 months after vaccination as a final assessment.

**OTHER INFORMATION**

**Blood Tests**

We take blood tests as part of the screening visit to help us to assess your general health. Blood tests are also taken at the study visits in order for us to assess your immune response to the vaccine and for safety reasons. If you would like them, we will give you the results of the blood tests. We only send the results to your GP if you wish us to and will not report them to anyone without your permission. The total volume of blood taken during the study varies according to which group you are enrolled in but will not exceed 572 mls (39 tablespoons) over five months.
The following blood tests will be performed;

- Tests for Hepatitis B, Hepatitis C and HIV are done at the screening visit.
- Carbohydrate Deficient Transferrin (CDT), a screening test for chronic alcoholism.
- HLA typing, a test of a component of the body’s immune system will be done at the first vaccination visit.
- Tests of red and white blood cells and tests of liver and kidney function are done at the screening visit and most of the other visits when you come for vaccination or follow up after vaccination in order to check the vaccines are safe.
- Tests of the immune responses to vaccines are done at most of the visits.
- At some of the visits we also test to see what genes are being used to generate your immune response. We will also do DNA sequencing; that is determining parts of the genetic code that may affect how people respond to the vaccine - you can opt out of DNA testing if you wish, without any effect on the rest of the study.
- The volume of blood taken at each visit ranges from 16 to 76 mls.

Urine Tests

- A urine sample will be tested at screening in order to check for glucose (to exclude diabetes), protein and blood (which can indicate kidney disease).
- Urine will also be tested for the presence of drugs; Cocaine, Opiates, Amphetamines and Marijuana.
- All women will have urinary pregnancy testing at screening and before each vaccination.

Abnormal Results

If abnormal results or undiagnosed conditions are found in the course of the study these will be discussed with you and, if you agree, your GP will be informed. For example, a new diagnosis of high blood pressure might be made. Any newly diagnosed conditions will be looked after by your GP.

Expenses and Payments

You will be compensated for:

- Travel expenses: 10 euros per visit (may vary)
- Time required for visit: 10 euros per hour
- Inconvenience of blood tests: 10 euros per blood donation

<table>
<thead>
<tr>
<th>Group No</th>
<th>Time in Trial</th>
<th>No. of Visits</th>
<th>No. of Blood Tests</th>
<th>Maximum Volume of Blood Taken</th>
<th>Compensation Amount (Euros)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>6 months</td>
<td>7</td>
<td>7</td>
<td>407 mls</td>
<td>200 + travel</td>
</tr>
<tr>
<td>1B</td>
<td>6 months</td>
<td>10</td>
<td>10</td>
<td>572 mls</td>
<td>300 + travel</td>
</tr>
<tr>
<td>2A</td>
<td>6 months</td>
<td>7</td>
<td>7</td>
<td>407 mls</td>
<td>200 + travel</td>
</tr>
<tr>
<td>2B</td>
<td>6 months</td>
<td>10</td>
<td>10</td>
<td>572 mls</td>
<td>300 + travel</td>
</tr>
</tbody>
</table>
If you choose to leave the study early or are withdrawn from the study, you will be compensated according to the length of your participation based on these figures. You should note that compensation payments received in this trial may have an impact on your entitlement to benefits.

What do I have to do?

- You must provide a name and 24 hour phone number for someone who lives near to you and who will know where you are for the duration of the study.
- You must attend all the visits that are outlined above.
- You should record in the study diary all the things you notice about injection sites, any other change in your health or the way you feel after each injection.
- Women must use an effective method of contraception for the duration of the study.
- You must not donate blood during the study or for 6 months after the end of the trial.

1.6. What alternatives are present?
At present, there is no malaria vaccine licensed anywhere in the world. There are other malaria vaccines in various stages of development. This study may help develop an effective malaria vaccine.

1.7. What are the possible benefits of taking part?
This study will not benefit you, but the information gained from the study might help to prevent malaria infection and disease in those who live in areas where malaria is common and in travellers.

1.8. What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

What happens when the research study stops?
If you have any queries or concerns once the study is over please do not hesitate to get in touch with us.

1.9. Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.
2. Part 2

2.1.

2.2. What if relevant new information becomes available?
Sometimes during the course of a research project, new information becomes available about the vaccine that is being studied, for example if unexpected side effects occur. If this happens, we will tell you about it and discuss whether you want to or should continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. On receiving new information, we may consider it to be in your best interests to withdraw you from the study.

2.3. What will happen if I don’t want to carry on with the study?
If, at anytime after agreeing to participate you change your mind about being involved with this study, you are free to withdraw without giving a reason. Your compensation would be paid as a proportion of the total compensation according to the length of your participation. If you withdraw after getting a vaccine you would still be invited to attend for all the safety reviews and blood tests and would be compensated for those visits accordingly.

2.4. What if there is a problem?

Complaints:
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you wish to complain formally about any aspect of the way you have been approached or treated during the course of this study you should approach Professor Samuel McConkey or Dr Eoghan de Barra at Department of Tropical Medicine and International Health, Royal College of Surgeons in Ireland, 123 St Stephen’s Green, Dublin 2. You can call the departmental secretary at 01-4022186 or email mlamb@rcsi.ie.

Harm:
The investigators recognize the important contribution that volunteers make to medical research, and will make every effort to ensure your safety and well-being. In the unlikely event of harm during the research study, compensation will be available from the University of Oxford's insurance scheme. In the most unlikely event of harm being suffered, while the University will cooperate with any claim, you may wish to seek independent legal advice to ensure that you are properly represented in pursuing any complaint. At any time during the study you will be entirely free to change your mind about taking part, and to withdraw from the study. This will not affect your subsequent medical care in any way.
2.5. Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be coded with a study number and kept strictly confidential. The information is available to the study team, the safety monitors, the ethical review committee, the sponsors, government regulatory agencies and external monitors who can ask to audit or monitor the study. Any information about you that leaves the hospital or clinic will have your name and address removed so that you cannot be identified from it. Your information is stored on a secure server and any paper notes will be kept in a locked filing cabinet.

Involvement of the General Practitioner/Family doctor (GP)

2.6. If you have a GP, you will be required to sign a form, documenting that you consent for us to contact your GP. This is to inform them that you are interested in being involved in the study and to ensure there are no medical reasons that they are aware of why this would not be safe. The researchers will not enrol you in the trial if they have any concerns about your eligibility or safety. We will write to your GP to let them know whether you are finally enrolled in the study or not so they can update your medical records accordingly. You do not need to have a GP to participate in this study however, we would encourage you to register with one, not just for the purpose of this study, but for your long term healthcare.

Prevention of ‘Over Volunteering’

Volunteers participating in this study must not be concurrently involved in another study.

2.7. What will happen to any samples I give?
The blood samples that you give will be analysed initially in Ireland and later transferred to Oxford, UK for further tests. Tests that may be performed include measurements of antibody levels, white cell activity and the ability of blood to inhibit the growth of malaria parasites in the laboratory. Samples may also be used to assess what genes are expressed by cells following vaccination, but this is optional for volunteers. Samples will not be stored after the study, any leftover samples will be destroyed. The blood tests mentioned in part 1 will be analysed in the Claymon Biomnis laboratory, Dublin and Oxford University research laboratories.

2.8. Will any genetic tests be done?
Yes. Some blood may be used to look at the pattern of your genes that can affect the immune system (including the Human Leukocyte Antigen or HLA genes). The immune response to vaccines is in part genetically controlled, so knowing your pattern of genes that regulate immune responses (HLA type) may help us to understand the responses to vaccination. We will also look at the expression of certain genes which relate specifically to the immune response to the vaccines and may also perform DNA sequencing on your blood, so we can understand how people respond to the vaccines. A separate consent form is required to allow genetic testing. If you would rather not have genetic testing done on your blood you can still participate in the rest of the study.

2.9. What will happen to the results of the research study?
The results of this research study may be published in a scientific medical journal. This may not happen until 1 or 2 years after the study is completed. If you contact the
researchers in the future you can obtain a copy of the results. You will not be identified in any report or publication. In order to facilitate the commercialisation of this malaria vaccine, which is largely targeted at low income countries, patents may be filed using data derived from this trial and some trial investigators will be amongst the inventors named on any such patent filings.

2.10. **Who is organising and funding the research?**
The study is funded by the European Vaccine Initiative based in Heidelberg, the major contributors to whom are the Swedish, Dutch and Irish (ie Irish Aid) International Development departments. Funding was secured by Professors Hill (Oxford) and McConkey (RCSI), from this organisation, it is an academic initiated publicly funded study. Neither your GP, nor the researchers are paid for recruiting you into this study.

2.11. **Who has reviewed the study?**
This study has been reviewed by Oxford Research Ethics Committee (UK) and The Beaumont Hospital Ethics Committee (Eire) and has been given a favourable ethical opinion. The Irish Medicines Board, which regulates the use of medicine in Ireland, have reviewed the study design and granted permission to use these unlicensed vaccines in this clinical study.

Thank you for reading this information sheet. If you are interested in being involved in the study please contact the study team at your local trial site below to arrange a screening appointment.

2.12.

2.13. **Contact Details for Further Information**

VAC038 Trial Research Nurse

Email: tropmed@rcsi.ie

Tel: +353 (0)1 8093787

Principal Investigator: Prof Samuel McConkey

Trial Physician: Dr Eoghan de Barra

Departmental Secretary: 01 4022186

Department of Tropical Medicine and International Health,

Royal College of Surgeons in Ireland,

123 St Stephen's Green, Dublin 2.
Clinical Malaria Vaccine Trial

Would you like to help in the development of vaccines against malaria?

We are developing a vaccine against malaria and want healthy volunteers to take part in our clinical trial for it.

If you are aged 18-50 and in good health you could be eligible to take part. Participants will be compensated for expenses and time set aside to take part.

For more information please contact:

- tropmed@rcsi.ie
- 01 809 3787
- www.rcsi.ie

The study will involve several short visits to an outpatients clinic over 4 months. A response to this advertisement will be recorded but implies no obligation to take part. If you volunteer you can withdraw at any time from the trial. Your General Practitioner will be informed if you enrol in the trial.

VAC038 Poster Edit version 1.0 27th May 2011
The Royal College of Surgeons in Ireland (RCSI) proposes to undertake a clinical trial of a novel malaria vaccine in 2011 and 2012. This would involve administering either one or two injections containing replication-incompetent, genetically-modified viruses as carriers of information coding for a malaria protein to 24 healthy volunteers. The specific viruses we intend to use are Modified Vaccinia virus Ankara-CS and a chimpanzee virus called Adenovirus Ch 63-CS. In the laboratory these viruses cannot replicate in normal human cells. We propose to do this at the RCSI clinical research facility under clinical research protocols. We have submitted documentation relating to this trial and its safety to the Environmental Protection Agency under the legislation relating to Genetically Modified Organisms (Deliberate Release Regulations), S.I. 500 of 2003. We have also submitted documentation to the Beaumont Hospital Research Ethics Committee and the Irish Medicines Board. For more information regarding this proposed trial please contact Professor Samuel McConkey, Head of Department of Tropical Medicine and International Health, Royal College of Surgeons in Ireland, 123 St Stephen’s Green, Dublin 2 (Tel: 01 4022186; email: smcconkey@rcsi.ie).
9.8 Diary card

Malaria Vaccine Trials

Dear volunteer, please complete the diary card each evening for 7 days following vaccination (including day 0 which is the day of vaccination).

DO NOT HESITATE TO CONTACT US IF YOU HAVE ANY PROBLEMS

01 8003797 during office hours (9am-5pm Mon-Fri)

Out of hours call **Insert Mobile phone number**

Alternatively if your query is not urgent you can email

trayner@rCSI.ie
Local arm symptoms:

<table>
<thead>
<tr>
<th>Date after injection</th>
<th>Date</th>
<th>Time (GMT)</th>
<th>Time (UTC)</th>
<th>Scale</th>
<th>Measurement in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pain Scale Score:
0 = No pain
1 = Sensitivity to touch/ can’t sleep on it
2 = Partial restriction of activity e.g. avoided gym
3 = Pain restricting activities e.g. can’t drive car

Indicate if you have experienced any symptoms since the last time you recorded your observations:

<table>
<thead>
<tr>
<th>Date</th>
<th>Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
</tr>
</tbody>
</table>

Medical Problems:

<table>
<thead>
<tr>
<th>Problem</th>
<th>Did you contact a Doctor?</th>
<th>Any Medications Taken?</th>
<th>Start Date</th>
<th>Stop Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Medications:

<table>
<thead>
<tr>
<th>Medication Name</th>
<th>Date</th>
<th>Reason for Taking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS

Chief Investigator: Professor A.V.S Hill

Sponsor: University of Oxford
Modification History

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>27th May 2011</td>
<td>Susanne Sheehy, Sam McConkey, Alison Lawrie, Sarah Gilbert, Adrian Hill.</td>
</tr>
<tr>
<td>2.0</td>
<td>21st July 2011</td>
<td>Eoghan de Barra, Susanne Sheehy, Sam McConkey, Adrian Hill.</td>
</tr>
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<td>3.0</td>
<td>11th Sept 2011</td>
<td>Eoghan de Barra, Alison Lawrie</td>
</tr>
<tr>
<td>4.0</td>
<td>30th March 2012</td>
<td>Eoghan de Barra, Alison Lawrie</td>
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Details of changes to Protocol from Version 1.0

<table>
<thead>
<tr>
<th>Section</th>
<th>Details of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Study Overview</td>
<td>Volunteer choice in group allocation removed. Investigator will allocate groups.</td>
</tr>
<tr>
<td>5.2</td>
<td>Clarification of role of DSMB</td>
</tr>
<tr>
<td>6.2 Informed consent</td>
<td>Requirement for GP communication and entry in TOPS database limited to UK volunteers only.</td>
</tr>
<tr>
<td>6.4 Withdrawal of volunteers</td>
<td>Added - Any volunteer who is withdraw or are withdrawn, post vaccination, will be invited to attend for all scheduled safety bloods and review as per protocol.</td>
</tr>
<tr>
<td>8.2 Secondary Evaluation Criteria</td>
<td>2.13.1. With reference to freezing and storage of samples for future investigations, this has been limited to UK volunteers only.</td>
</tr>
<tr>
<td>9.6 DSMB</td>
<td>2.13.2. Added - The DSMB will review the data before there is a dose escalation of ChAd63 CS from $5 \times 10^9$ to $5 \times 10^{10}$</td>
</tr>
<tr>
<td>10 Statistics</td>
<td>Added - Data analysis will consist primarily of descriptive summaries for treatment groups. For primary and secondary endpoints descriptive summaries and plots over the time course for both individual patient results and groups will be presented. Due the small number of volunteers in this study, all volunteers receiving the same dose of a given vaccine will be pooled for analysis. Where appropriate highly skewed data will be log-transformed and presented as geometric means</td>
</tr>
<tr>
<td>6.3 Inclusion and Exclusion criteria</td>
<td>Added exclusion criteria; in a particularly dependent relationship with the investigator by way of occupation or otherwise, which in the investigators opinion places the volunteer in a vulnerable population. Removal of Oxford as a clinical site</td>
</tr>
</tbody>
</table>

with 95% confidence intervals.

2.13.3.
A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS

Study Code: VAC.038
EudraCT Number: 2011-001875-38

**Chief Investigator**
Professor Adrian V.S. Hill  
Centre for Clinical Vaccinology and Tropical Medicine  
University of Oxford  
Churchill Hospital, Old Road, Headington  
Oxford, OX3 7LJ  
Email: adrian.hill@ndm.ox.ac.uk

**Principal Investigator**
Professor Sam McConkey  
Clinical Research Centre  
Royal College of Surgeons in Ireland (RCSI)  
Beaumont Hospital  
Dublin 9  
Ireland  
Email: smcconkey@rcsi.ie

**Trial Sites**
PI at site: Professor Sam McConkey

**Sponsoring Institution**
University of Oxford  
Clinical Trials and Research Governance  
Manor House, The John Radcliffe Hospital,  
Headington, Oxford, OX3 9DZ  
Tel: 01865 743004  
Fax: 01865 743002  
Email: heather.house@admin.ox.ac.uk

**External Monitor**
Appledown Clinical Research Limited  
Orchard's End,  
Greenland's Lane,  
Prestwood,  
Gr. Missenden,
Bucks.
HP16 9QX
UK
Tel/Fax: +44 (0)1494 867676
Investigator Agreement
“I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.”

<table>
<thead>
<tr>
<th>Chief Investigator</th>
<th>Investigator Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Professor Adrian Hill</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 Independent 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 Adrian Hill.
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### 1. SYNOPSIS

<table>
<thead>
<tr>
<th>Title</th>
<th>A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial Centres</strong></td>
<td>Clinical Research Centre, Royal College of Surgeons in Ireland (RCSI), Beaumont Hospital, Dublin 9, Ireland</td>
</tr>
<tr>
<td><strong>Trial Identifier</strong></td>
<td>VAC 038</td>
</tr>
<tr>
<td><strong>Clinical Phase</strong></td>
<td>Ia</td>
</tr>
<tr>
<td><strong>Design</strong></td>
<td>Open label observational study</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>Healthy adults aged 18 – 50 years</td>
</tr>
<tr>
<td><strong>Sample Size</strong></td>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td></td>
<td>Subgroup A (1A): 4 volunteers; 1 dose of ChAd63 CS $5 \times 10^9$ vp intramuscularly</td>
</tr>
<tr>
<td></td>
<td>Subgroup B (1B): 8 volunteers; 1 dose of ChAd63 CS $5 \times 10^9$ vp intramuscularly and 1 dose MVA CS $2 \times 10^8$ pfu 8 weeks later intramuscularly</td>
</tr>
<tr>
<td></td>
<td><strong>Group 2</strong></td>
</tr>
<tr>
<td></td>
<td>Subgroup A (2A): 4 volunteers; 1 dose of ChAd63 CS $5 \times 10^{10}$ vp intramuscularly</td>
</tr>
<tr>
<td></td>
<td>Subgroup B (2B): 8 volunteers; 1 dose of ChAd63 CS $5 \times 10^{10}$ vp intramuscularly and 1 dose MVA CS $2 \times 10^8$ pfu 8 weeks later intramuscularly</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>24 volunteers</td>
</tr>
<tr>
<td><strong>Follow-up duration</strong></td>
<td>Minimum 6 months (This is an estimate and may vary in accordance with the specified time windows for each attendance)</td>
</tr>
<tr>
<td><strong>Planned Trial Period</strong></td>
<td>12 months</td>
</tr>
<tr>
<td><strong>Primary Objective</strong></td>
<td>To assess the safety of new candidate malaria vaccines ChAd63 CS administered alone and with MVA CS in a prime-boost regime to healthy volunteers.</td>
</tr>
<tr>
<td><strong>Secondary Objective</strong></td>
<td>To assess the humoral and cellular immune responses generated by ChAd63 CS when administered to healthy volunteers alone and with MVA CS.</td>
</tr>
</tbody>
</table>

### INVESTIGATIONAL PRODUCTS

1. ChAd63 CS (Chimpanzee adenovirus 63 expressing...
circumsporozoite protein)

2. MVA CS (Modified vaccinia virus Ankara expressing circumsporozoite protein)

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
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<tbody>
<tr>
<td>Route of Administration</td>
<td>Intramuscular (IM) needle injection into the deltoid region of the arm</td>
</tr>
<tr>
<td>Dose per Administration</td>
<td>- ChAd63 CS: $5 \times 10^9$ vp, $5 \times 10^{10}$ vp</td>
</tr>
<tr>
<td></td>
<td>- MVA CS: $2 \times 10^8$ pfu</td>
</tr>
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</table>
2. **ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ChAd63</td>
<td>Chimpanzee adenovirus 63</td>
</tr>
<tr>
<td>AdHu</td>
<td>Human adenovirus</td>
</tr>
<tr>
<td>AdHu5</td>
<td>Human adenovirus serotype 5</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AMA1</td>
<td>Apical membrane antigen 1</td>
</tr>
<tr>
<td>CCVTM</td>
<td>Centre for Clinical Vaccinology and Tropical Medicine</td>
</tr>
<tr>
<td>CBF</td>
<td>Clinical Bio manufacturing Facility</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form or Clinical Research Facility</td>
</tr>
<tr>
<td>CS or CSP</td>
<td>Circumsporozoite protein</td>
</tr>
<tr>
<td>ELISPOT</td>
<td>Enzyme-linked immunospot</td>
</tr>
<tr>
<td>FBC</td>
<td>Full blood count</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GIA</td>
<td>Growth Inhibition Assay</td>
</tr>
<tr>
<td>GMO</td>
<td>Genetically modified organism</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B Surface Antigen</td>
</tr>
<tr>
<td>HCG</td>
<td>Human Chorionic Gonadotrophin</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>IDT</td>
<td>Impfstoffwerk Dessau-Tornau</td>
</tr>
<tr>
<td>REC</td>
<td>Independent Research Ethics Committee</td>
</tr>
<tr>
<td>LSM</td>
<td>Local safety monitor</td>
</tr>
<tr>
<td>ME-TRAP</td>
<td>Multiple epitopes and thrombospondin related adhesion protein</td>
</tr>
<tr>
<td>MSP1</td>
<td>Merozoite Surface Protein 1</td>
</tr>
<tr>
<td>MVA</td>
<td>Modified vaccinia virus Ankara</td>
</tr>
<tr>
<td>pfu</td>
<td>Plaque forming unit</td>
</tr>
<tr>
<td>PMR</td>
<td>Parasite Multiplication Rate</td>
</tr>
<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>vp</td>
<td>viral particle</td>
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</table>
3. BACKGROUND AND RATIONALE

2.13.4. The need for a new vaccine against malaria

Although recent and encouraging evidence suggests that the epidemiology of *Plasmodium falciparum* malaria is changing across certain parts of Africa, the worldwide burden of disease from malaria remains a major public health problem, with approximately 250 million cases and over 800,000 deaths worldwide in 2008, mostly in Africa. The enormous economic and social consequences of malaria have been well documented.

The development of resistance both in Anopheles mosquitoes to certain insecticides and of malaria parasites to chemotherapeutic agents has contributed to an increasing need for a new, effective intervention for the prevention or treatment of malaria.

To provide a coordinated global approach to fighting malaria, the Roll Back Malaria (RBM) Partnership was launched in 1998 by the World Health Organization (WHO), the United Nations Children’s Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank. A major goal of the RBM Partnership is to support the development of a vaccine against malaria, felt to be a key future strategy for reducing mortality from malaria and moving towards eradication.

Lifecycle of the malaria parasite

The malaria lifecycle is complex with stages in both human and mosquito hosts (Figure 1). The bite of infected female Anopheles mosquitoes transmits malaria sporozoites to the human host where they travel via the bloodstream to the liver and invade hepatocytes (liver stage). Here they mature into merozoites for 6 to 7 days after which the hepatocytes rupture releasing a large number of merozoites into the bloodstream. Merozoites then invade erythrocytes where they multiply and after 2 days cause the erythrocyte to rupture, releasing progeny merozoites that in turn invade new erythrocytes (blood stage). A small percentage of merozoites differentiate into gametocytes, which when ingested by a mosquito, unite with another gametocyte to create a zygote. The zygote matures and releases sporozoites which migrate to the mosquito’s salivary glands and are injected into the human when the mosquito feeds. Infection by sporozoites and the liver-stage of malaria is asymptomatic. It is the blood stage of infection that is associated with symptoms and potentially severe or fatal complications.

![Figure 1 Lifecycle of Malaria](image_url)

The Circumsporozoite Protein as a Vaccine Antigen

The structural and functional properties of CS were defined in the 1980’s by the study of the mechanisms of the protective immunity induced by immunisation of rodents, monkeys and humans.
with sporozoites attenuated by irradiation.\textsuperscript{5} Irradiated sporozoites invade hepatocytes but their further development is arrested. Protection appears to be dependent on the persistence of these arrested forms in the liver. Protection on sporozoite challenge in humans can be achieved following multiple, repeated bites from irradiated mosquitoes infected with \textit{P. falciparum}, and this protection has been shown to be mediated by antibodies generated to CS.\textsuperscript{7}

CS is expressed by sporozoites and liver schizonts and plays a key role in the attachment phase of sporozoite invasion into hepatocytes.\textsuperscript{6} Anti-CS antibodies can target sporozoites, facilitating destruction of sporozoites prior to hepatocyte invasion. However, since sporozoites travel from the skin to liver within minutes, it may be difficult for a vaccine to achieve complete protection against \textit{P. falciparum} sporozoites based solely on antibodies. The liver stage of infection provides a longer window of opportunity for cell mediated immunity to recognise and destroy infected hepatocytes.

In order to induce T cell recognition, liver stage antigens need to be processed and presented on the surface of infected hepatocytes. A significant obstacle is that the liver stages reside inside a parasitophorous vacuole surrounded by a membrane that is only permeable to small molecules. To date, only CS has been found in the cytoplasm of infected hepatocytes, confirming the importance of CS as a target antigen in liver stage immunity.\textsuperscript{8} Importantly, sporozoites also secrete CS while they glide through or traverse the endothelium. Thus, CD4\textsuperscript{+} T cells may recognize processed CS on the plasma membrane of non-parenchymal liver cells, such as Kupffer cells or dendritic cells that express constitutively class II MHC. If T cell recognition is followed by release of cytokines such as interferon-\textgamma in the proximity of the infected hepatocyte, then the liver stage development will be inhibited.

### Progress towards a pre-erythrocytic malaria vaccine

The candidate pre-erythrocytic malaria vaccine RTS,S is the most advanced and efficacious malaria vaccine in development.\textsuperscript{5,8} It is formed from the fusion of CS to the surface antigen of hepatitis B virus to form virus like particles. This construct, administered with proprietary adjuvants is currently in phase III studies in multiple sites in African infants, where it has been shown to be safe, immunogenic and efficacious, inducing approximately 45\% clinical efficacy which persists up to 15 months.\textsuperscript{9} Whilst these clinical results are the most effective for any malaria vaccine product to date, there remains considerable capacity and need to improve on this limited clinical efficacy, either through modifications to the RTS,S vaccine or by developing vaccine strategies that combine numerous antigens or vaccine strategies.

Analysis of the immunological correlates of immunity induced by the RTS,S/AS02 vaccine in both phase IIa sporozoite challenge studies\textsuperscript{10,11} and a more recent trial in Mozambique\textsuperscript{12} provide evidence that very high levels of antibodies to CS correlate with protection in humans. However, this correlation is relatively weak and there may be a component of T cell mediated protection induced by the vaccine, even though the magnitude of the T cell response measured after vaccination is modest (approximately 150 SFU / million PMBCs on ELIspot).\textsuperscript{13}

Increasing data from animal models, fieldwork and inoculation of volunteers with irradiated sporozoites support an important role for T cells, in particular CD8\textsuperscript{+} cells, in mediating pre-erythrocytic immunity, even in the absence of antibodies.\textsuperscript{14} Whilst pre-clinical studies demonstrate a clear correlation between CD8\textsuperscript{+} T cells and protection,\textsuperscript{15-19} clinical vaccine studies have been hampered by the limited ability of existing vaccine strategies, namely adjuvanted protein constructs, to induce high enough numbers of antigen specific CD8\textsuperscript{+} T cells to confer protection.

Adrian Hill’s group at the University of Oxford have been working for over 10 years to develop a pre-erythrocytic \textit{P. falciparum} malaria vaccine using the sporozoite and liver stage antigen ME-TRAP. This antigen contains a fusion protein of multiple epitopes (ME; a string of 20 epitopes, mainly CD8\textsuperscript{+} T cell epitopes from pre-erythrocytic antigens) and the \textit{P. falciparum} pre-erythrocytic antigen; thrombospondin-related adhesion protein (TRAP).\textsuperscript{20}

Multiple vectors for this antigen have been clinically tested including DNA, fowl pox (FP) and modified vaccinia virus Ankara (MVA), however T cell immunogenicity and clinical efficacy has been limited (Table 1). Most recently, heterologous prime boost with Chimpanzee adenovirus 63 (ChAd63) and MVA ME-TRAP has been shown to be the most immunogenic regimen to date, inducing more than 2400 IFN\gamma producing T cells post boost (Figure 3, O’Hara et al submitted). Sporozoite challenge of malaria naïve individuals vaccinated with ChAd63-MVA ME-TRAP demonstrated significant clinical efficacy of this vaccine strategy, with 3/14 individuals demonstrating sterile protection (21\%) and 5/14 demonstrating partial protection (36\%) (Figure 2A, Ewer et al submitted). Of note, on re-challenge 8 months later, all 3 steriley protected volunteers demonstrated evidence of persisting protection, with 1
volunteer demonstrating sterile protection and the other two partial protection. In this study, protection was shown to correlate strongly with mono-functional CD8+ T cells (Figure 2B).

<table>
<thead>
<tr>
<th>Vaccine encoding ME-TRAP</th>
<th>T cell response mean cells/million PBMCs*</th>
<th>Protective Efficacy on Sporozoite Challenge</th>
</tr>
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<tbody>
<tr>
<td>DNA x 3&lt;sup&gt;21&lt;/sup&gt;</td>
<td>48</td>
<td>Nil</td>
</tr>
<tr>
<td>Fowl-pox x 2</td>
<td>50</td>
<td>Nil</td>
</tr>
<tr>
<td>MVA x 3&lt;sup&gt;21&lt;/sup&gt;</td>
<td>41</td>
<td>Nil</td>
</tr>
<tr>
<td>DNA &amp; MVA&lt;sup&gt;21,22&lt;/sup&gt;</td>
<td>430</td>
<td>23%</td>
</tr>
<tr>
<td>Fowl-pox &amp; MVA&lt;sup&gt;23&lt;/sup&gt;</td>
<td>475</td>
<td>25%</td>
</tr>
<tr>
<td>ChAd63-MVA*</td>
<td>2400</td>
<td>58%</td>
</tr>
</tbody>
</table>

Table 1: Clinical trials of ME-TRAP encoding vaccines by University of Oxford, summarizing maximum T cell response as measured by IFNγ producing ELIspot at peak time point post final boost, and clinical efficacy as measured on sporozoite challenge. *Ewer et al Submitted.

Figure 2: Data from 14 healthy malaria naïve adult volunteers vaccinated with $5 \times 10^6$ vp ChAd63 ME-TRAP intramuscularly, followed 8 weeks later by $2 \times 10^8$ pfu MVA ME-TRAP intradermally. Figure 2A: Clinical Efficacy on heterologous sporozoite challenge with 3D7 P. falciparum conducted in 2 phases. The 12 control volunteers (red line) were all diagnosed with malaria. 57% of vaccines (blue line) demonstrated
Given the proven clinical efficacy with a vaccine encoding CS, evidence of the importance of CD8+ responses in liver stage immunity and the ability of ChAd63-MVA to induce exceptionally potent CD8+ T cells in addition to good humoral responses, the next logical step is to develop and test ChAd63-MVA expressing CS. This vaccine regimen could then be combined with ChAd63-MVA expressing ME-TRAP in order to increase clinical efficacy. Alternatively, ChAd63-MVA CS could be combined with the current leading vaccine RTS,S.

To date there have been a number of attempts to combine RTS,S with viral vectors vaccines; Firstly, a phase I/IIa trial of heterologous prime-boost immunization of RTS,S/AS02 and MVA encoding the entire CS gene construct (CSO) was undertaken in Oxford. In this trial MVA vectored CS, known as MVA CSO was only modestly immunogenic and did not appear to enhance the efficacy of the RTS,S vaccine, although statistical power to assess this was limited. Particularly disappointing was the inability of the prime-boost approach to enhance the T cell immunogenicity to levels greater than RTS,S/AS02 alone. A further phase I/IIa trial of MVA and FP vectors expressing CSO demonstrated only modest T cell immunogenicity and no efficacy on sporozoite challenge.

**An improved insert design for CSP**

The poor immunogenicity of the standard full length CSP insert used in previous vectors in clinical trials (CSO), suggest that there may be an important difference in the intrinsic immunogenicity of CSO compared to the ME-TRAP insert. Using information from multiple sources, we have designed a novel CS antigen, to be used in this study, which omits the extreme C-terminus of the protein that encodes the GPI-anchor sequence and the N-terminal third of the protein N-terminal to the central B cell repeat (see IMP-D for more details). Coincidentally, this creates a sequence encoding amino acids very similar to those of the RTS,S protein (the ‘repeat’ region of CSP consists of multiple repeats of NANP and NVDP. RTS,S contains 16 copies of NANP and none of NVDP. CS contains 13 copies of NANP and 3 of NVDP. The ‘T cell epitope’ region is 100 % identical at the amino acid level in the two sequences). We are confident that use of this novel antigen in the vectors ChAd63 & MVA will be more successful that the CSO antigen used in DNA, MVA and FP9 vectors to date.

**Clinical Trials of CSP Vaccines**

Other than RTS,S the only other vaccine candidates targeting the CS protein currently in clinical development are human adenovirus 35 (Ad35) expressing CS from Crucell and human adenovirus 5 (Ad5) from the US Military Malaria Vaccine Program. This Crucell vaccine development programme is sponsored by the US National Institute of Allergy & Infectious Diseases (NIAID) who are currently conducting Phase Ia & Phase Ib studies of Ad35 CSP administered in homologous prime boost regimens in adults. Data on these trials have yet to be published. (ClinicalTrials.gov identifiers: NCT01018459 & NCT00371189). The US Navy has undertaken unpublished clinical studies of Ad5 CS used alone and in a DNA prime-Ad5 prime-boost regimen. Vaccinees administered Ad5 CS alone reportedly failed to show efficacy against sporozoite challenge. However, in a prime boost regime where CS and AMA1 encoding vectors were mixed prior to administration a regime of DNA vector priming and Ad5 boosting led to 4 out of 15 vaccinees showing sterile protection against sporozoite challenge (T Richie personal communication). This result support further assessment of the utility of CS-based vectors particularly in heterologous prime-boost regimes.

**Adenoviruses as Vectors**

Adenoviruses are attractive viral vectors as they possess a genetically stable virion (so that inserts of foreign genes are not deleted), they can infect large numbers of cells and the transferred information remains epichromosomal, thus avoiding any potential for insertional mutagenesis. Replication defective adenovirus can be engineered by deletion of genes from the E1 locus, which is required for viral replication, and these viruses can be propagated easily with good yields in cell lines expressing E1 from AdHu5 such as human embryonic kidney cells 293 (HEK 293). Previous mass vaccination campaigns using orally administered live human adenovirus serotype 4 and 7 in large numbers of US military personnel have shown good safety and efficacy data.

Human adenoviruses have been used as vaccine vectors for a number of conditions, however a limiting factor to widespread use has been the level of anti-vector immunity present in humans where adenovirus is a ubiquitous infection. Estimates suggest that depending on the geographical region between 45–80%
of adults carry AdHu5-neutralising antibodies. Immunisation with AdHu vectors in animal models in the presence of pre-exposure to human adenoviruses attenuates responses to the vaccine probably due to the removal of virus particles by pre-existing antibodies. Phase I trials of a multiclade HIV-1 vaccine delivered by a replication defective AdHu5 had to exclude volunteers with pre-existing antibodies to AdHu5 at titres greater than 1:12. In recent Phase I placebo controlled human trials of a modified AdHu5 HIV vaccine there were no safety concerns amongst vaccinated volunteers with pre-existing high titre anti-AdHu5 antibodies, indeed less reactogenicity was seen amongst those with high-titre antibodies. Using AdHu5 in a prime boost strategy for HIV-1 gag homologous boosting did not improve the peak post prime levels of gag specific lymphocytes, probably due to anti-vector immunity.

The prevalence of immunity to human adenovirus prompted the consideration of simian adenoviruses as vectors. They exhibit hexon structures homologous to that of human adenoviruses. Indeed, the chimpanzee adenovirus ChAd63's hexons are most similar in sequence to the hexons of AdHu4 previously used by the US military in mass vaccination campaigns where over 2 million adults received tablets of serially passaged adenovirus with good safety and efficacy data (Personal Communication Col. John D. Grabenstein). In chimpanzee adenoviruses the E1 locus can be deleted to render viruses replication deficient and allow transcomplementation on an E1 AdHu5 complementing cell line. An additional attractive observation is that the lack of sequence homology between AdHu5 and simian adenoviruses at the E1 flanking sequence prevents homologous recombination and production of replication competent virus.

Simian adenoviruses are not known to cause pathology or illness in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the US. In Equatorial Africa (the natural habitat for chimpanzees), prevalence is higher but still below that to anti AdHu5 immunity. In a recent study in Kenya, 23% of children aged 1-6 years had neutralising antibodies at a titre greater than 1:200 to AdHu5, whilst only 4% had high-titre neutralising antibodies to ChAd63. Immunity to both vectors was age-dependent. Early murine work using chimpanzee adenovirus 68 (AdCh68, also called C9) expressing gag of HIV-1 showed that in comparison to AdHu5 and poxvirus, AdCh68 was as effective at generating a transgene product specific CD8+ T cell response with approximately 20% of all splenic CD8+ being gag specific. In the same study, pre-exposure to AdHu5 abolished any protection offered by immunisation with AdHu5 but only slightly reduced that elicited by AdCh68, suggesting pre-exposure to human adenoviruses should not reduce the potency of the immune response generated to simian vectored vaccines.

There is no available or validated in vitro cell co-culture method to examine co-infection with human and simian adenovirus vectors as the latter are non-replicating. Due to a lack of any sequence homology between the replication-deficient ChAd63 and MVA vectors, complementation of MVA by ChAd63 does not occur. Pre-clinical bioavailability studies have demonstrated no persistence of the ChAd63 vector 24 hours post intramuscular administration. Therefore, residual priming ChAd63 vector is very unlikely to be present at the time of administration of a MVA boost, 8 weeks later.

### Chimpanzee Adenovirus 63 (see also ChAd63 CS Investigator Brochure)

ChAd63 expressing varying antigens has been administered to over 250 individuals including 24 Gambian children (Table 2) and has demonstrated an excellent safety profile, with doses of up to 2 x 10^{11} vp ChAd63 ME-TRAP found to be safe in UK adults. The vector has been shown to be consistently immunogenic, inducing extremely potent T cell responses (Figure 3) and good antibody responses, especially when combined with MVA. Multiple studies have shown 5 x 10^{10} vp ChAd63 to be the optimal dose, associated with a consistently excellent reactogenicity profile and potent T cell immunogenicity (see investigator brochure).

<table>
<thead>
<tr>
<th></th>
<th>ChAd63 ME-TRAP</th>
<th>ChAd63 MSP1</th>
<th>ChAd63 AMA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults in UK</td>
<td>108</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>Adults in Africa</td>
<td>46</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Children in Africa (2-6 years)</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total No. of Individuals Vaccinated</td>
<td>178</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>Preferred Dose in Adults</td>
<td>5 x 10^{10} vp</td>
<td>5 x 10^{10} vp</td>
<td>5 x 10^{10} vp</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Preferred Dose in Children</td>
<td>5 x 10^{10} vp</td>
<td>Not Known</td>
<td>Not Known</td>
</tr>
</tbody>
</table>

Table 2: Numbers of individuals vaccinated to date with ChAd63 vectored vaccines. Total: 257 individuals. ME-TRAP = Multiple epitopes + thrombospondin-related adhesion protein, MSP1 = Merozoite surface antigen 1, AMA1 = Apical Membrane Antigen 1.

Figure 3: T cell immunogenicity as measured by no. of antigen specific T cells measured by interferon γ ELISpot. Group A = ChAd63 priming vaccination only. Group B = ChAd63 prime & MVA boost. Increasing group number is associated with increasing dose of ChAd63. Priming vaccination takes place on Day 0, boost vaccinations on Day 56. **Figure 3A**: ChAd63-MVA ME-TRAP. Dose escalation of ChAd63 ME-TRAP, Dose of MVA ME-TRAP constant; 2 x 10^8 pfu. **Figure 3B**: ChAd63-MVA MSP1. Dose of ChAd63 MSP1; 5 x 10^9 vp & 5 x 10^{10} vp. Dose of MVA MSP1; 5 x 10^8 pfu. **Figure 3C**: ChAd63-MVA AMA1. Dose of ChAd63 AMA1; 5 x 10^9 vp & 5 x 10^{10} vp. Dose of MVA AMA1 variable.
Concerns exist that pre-existing antibodies to ChAd63 could limit widespread use of the vector. However, data from the Phase IIb efficacy study of ChAd63-MVA ME-TRAP showed no correlation between neutralising antibodies to ChAd63 in volunteers prior to vaccination and their subsequent T cell count post MVA boost, suggesting that even if neutralising antibodies exist they may not limit immunogenicity (Figure 4, Ewer et al, submitted). There is no evidence that pre-existing neutralising antibodies to ChAd63 increase reactogenicity (O’Hara et al. submitted).

MVA as a Vector (See also MVA CS Investigator Brochure)

MVA is an attractive candidate orthopox vaccine vector for safety and immunogenicity reasons. The successful worldwide eradication of smallpox using vaccination with vaccinia virus highlighted vaccinia as a candidate carrier. Although millions of humans have been vaccinated with conventional replication-competent vaccinia virus, its small but definite risk to both researchers and future patients led to the development of several attenuated strains of vaccinia during smallpox eradication and more recently. In particular the host-range restricted MVA proved to be extremely attenuated compared to other vaccinia viruses.

MVA was originally derived from the vaccinia strain Ankara by over 500 serial passages in primary chicken embryo fibroblasts (CEF cells). MVA has six major genomic deletions compared to the parental Ankara genome and is severely compromised in its ability to replicate in mammalian cells. No replication has been documented in non-transformed mammalian cells. The viral genome has been proven to be stable through a large series of passages in chicken embryo fibroblasts. MVA also showed no cytopathic effect or plaque formation in cells of human origin. In irradiated mice, MVA did not elicit any morbidity or lethality even when administered at high doses intra-cerebrally, indicating its safety even in immuno-compromised organisms.

Apart from studies in mice, rabbits and elephants, MVA has been shown to be safe in humans. From 1972 until 1980 (the end of compulsory smallpox vaccination) MVA was licensed in Germany and was included in the official immunisation schedule. In a large field study carried out in Germany in the late seventies, over 120,000 previously unvaccinated individuals were vaccinated with MVA (0.2 mL) administered either intra-dermally or subcutaneously. The study population included high-risk groups such as people suffering from allergies, elderly people and alcoholics. Given intradermally, a red nodule of up to 4 mm in diameter was observed at the injection site at day 4 or 5. Only a small proportion showed any systemic side effects such as fever > 38.5°C. MVA proved to be non-contagious and avirulent. Viral replication is blocked late during infection of cells but importantly viral and recombinant protein synthesis is unimpaired even during this abortive infection. Replication-deficient recombinant MVA has been viewed as an exceptionally safe viral vector. When tested in animal model studies, recombinant MVAs have been shown to be avirulent, yet protectively immunogenic as vaccines against viral diseases and cancer. Recent studies in macaques severely immuno-suppressed by SIV infection have further supported the view that MVA should be safe in immuno-compromised humans.

MVA is currently in development as a vector for multiple diseases including HIV, Tuberculosis, Hepatitis C (Barnes et al submitted), influenza and melanoma. MVA vectored vaccines are particularly suited to boosting immune responses to an antigen following a priming vaccination with another viral vector. In Professor Hill’s group, MVA encoding the malaria antigens has been
administered to over 940 individuals, including children and infants in sub Saharan Africa (Table 3). The optimal dose of MVA has been shown consistently to be 1-2 x 10^8 pfu. Clinical studies have shown intramuscular administration to be associated with fewer and short lived local AEs and no reduction in immunogenicity (O'Hara et al submitted).

<table>
<thead>
<tr>
<th></th>
<th>MVA ME-TRAP</th>
<th>MVA CSO</th>
<th>MVA polyprotein</th>
<th>MVA MSP1</th>
<th>MVA AMA1</th>
</tr>
</thead>
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<tr>
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<td>28</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
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<td>0</td>
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<tr>
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<td>26</td>
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<tr>
<td>Total post ChAd63 prime</td>
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<td>32</td>
<td>26</td>
</tr>
<tr>
<td>Preferred Dose in Adults</td>
<td>2 x 10^8 pfu</td>
<td>1-2 x 10^8 pfu</td>
<td>1-2 x 10^8 pfu</td>
<td>2 x 10^8 pfu</td>
<td>1.25 x 10^8 pfu</td>
</tr>
<tr>
<td>Preferred dose in Children</td>
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<td>1-2 x 10^8 pfu</td>
<td>Not known</td>
<td>Not known</td>
<td>Not Known</td>
</tr>
</tbody>
</table>

**Table 3:** Total numbers of individuals vaccinated to date with MVA vectored malaria vaccines developed by the Hill group, University of Oxford. Total: 946 individuals. ME-TRAP = Multiple epitopes + thrombospondin-related adhesion protein, MSP1 = Merozoite surface antigen 1, AMA1 = Apical Membrane Antigen 1.
4. Study overview

This is an open label phase Ia clinical trial to assess the safety and immunogenicity of different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime-boost regimen. All volunteers recruited will be healthy adults aged between 18 and 50. Volunteers will be allocated to the groups by the investigators. Safety data will be collected for each of the regimens (Table 4). The immune responses generated by each of these regimens will be assessed.

4.1 Objectives

Primary Objective

- To assess the safety in healthy volunteers of two different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime boost regimen.

Secondary Objective

- To assess the immunogenicity in healthy volunteers of two different doses of ChAd63 CS administered alone and with MVA CS in a heterologous prime boost regimen.

4.2 Study Groups

<table>
<thead>
<tr>
<th>Group Number</th>
<th>No. of volunteers</th>
<th>ChAd63 CS Day 0</th>
<th>MVA CS Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>4</td>
<td>5 x 10^9 vp</td>
<td>-</td>
</tr>
<tr>
<td>1B</td>
<td>8</td>
<td>5 x 10^9 vp</td>
<td>2 x 10^8 pfu</td>
</tr>
<tr>
<td>2A</td>
<td>4</td>
<td>5 x 10^10 vp</td>
<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>8</td>
<td>5 x 10^10 vp</td>
<td>2 x 10^8 pfu</td>
</tr>
</tbody>
</table>

Table 4: Overview of trial groups. All vaccinations are intramuscular.

4.3 Rationale for Trial Design

Administration Schedules

Heterologous prime boost with ChAd63-MVA is, to our knowledge, one of the most potent T cell inducing subunit vaccine regimens which can importantly also induce antibodies. Previous clinical trials using this regimen expressing ME-TRAP, AMA1 & MSP1, have shown that administering ChAd63 as a prime followed 8 weeks later by MVA as a boost is a very immunogenic schedule (O’Hara et al submitted, Sheehy et al submitted). For this reason, and to provide comparability with previous ChAd63-MVA trials we propose to use a similar administration schedule.

Route & Dose
Our choice of the dose and route of vaccines in this study is based on experience using the same vectors in previous Phase I and Phase II clinical trials in the UK and Africa (see investigator brochures) using both intradermal and intramuscular routes.

We have chosen here the intramuscular route of administration for all vaccines given the proven favourable safety and immunogenicity profile of this route of administration with these vectors and because of future practical considerations regarding administration in the field.

ChAd63 has been safety administered to more than 250 healthy individuals and the vector has been shown repeatedly to be safe at the planned dose of $5 \times 10^{10}$ vp (see investigator brochure).

MVA has been administered to more than 120 healthy UK adults following priming with ChAd63 expressing the same antigen, at various doses.Repeatedly, a dose of $1-2 \times 10^8$ pfu MVA has been found to be a suitable dose to balance immunogenic and reactogenicity (see investigator brochure).

### 4.4 Duration of Study

**Groups 1A & 2A**
The duration of involvement in the study from enrolment will be approximately 6 months.

**Groups 1B & 2B**
The duration of involvement in the study from enrolment will be approximately 6 months.

### 4.5 Definition of the Start and End of the Trial

The start of the trial is defined as the date of the first vaccination of the first volunteer. The end of the trial is the date of the last visit of the last volunteer.

### 4.6 Potential Risks & Benefits for Volunteers

**POTENTIAL RISKS**

**Phlebotomy:** The maximum volume of blood drawn over the study period (572 mls over approximately 5 months) should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur.

**Vaccination:** Potential expected risks from vaccination, which include local and systemic reactions are specific to each IMP and are described below. It is important to note that ChAd63 CS & MVA CS have not previously been administered to humans. Therefore, although the AE profile can be estimated from previous use of these vectors, the reactogenicity may vary from that seen previously with ChAd63 and MVA encoding different antigens. For this reason, vaccinees will be enrolled in a staggered format (Section 5.2) to allow early identification of any concerning reactogenicity before the majority of individuals have been vaccinated.

As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage including serious allergic reactions may occur but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any serious adverse reactions.

#### 1. ChAd63 CS

Although ChAd63 CS has not previously been administered to humans, the safety data available from the more than 250 individuals who have previously received ChAd63 vectored vaccines can be used to
predict the adverse event profile expected following vaccination with ChAd63 CS in this study; Local adverse events such as pain would be expected to occur frequently. Less frequent adverse events are likely to include erythema, swelling, itching and warmth. Local AEs are likely to be mild in nature and should resolve rapidly, although there is the possibility of moderate or severe arm pain in some cases.

Common systemic adverse events post viral vectored vaccines include headache, feverishness, myalgia, arthralgia, fatigue and malaise. Generally volunteers report a transient flu like illness within 24 hours of vaccination with ChAd63 which resolves completely within 48hrs. The majority of systemic AEs are likely to be mild but there is a possibility of moderate or severe headache or malaise. Given existing data for ChAd63 vectored vaccines, it is anticipated that the majority of systemic adverse events post ChAd63 CS will be mild in intensity.

During the manufacturing process of ChAd63 CS, a biocide named Kathon is used. Kathon is added to body washes, conditioners, liquid soaps, shampoos and wipes as a preservative. The maximum dose is 0.1% for ‘rinse off’ products and for ‘leave on’ products it is 0.05%. It has been approved by regulatory authorities throughout the world as a preservative in these products. As a skin sensitisr it is known to cause contact dermatitis. An internal study was set up to look at the levels of Kathon that were removed during the final purification step of buffer exchange. This study utilized high performance liquid chromatography and showed that trace amounts of Kathon may be left on the column after carrying out the rinse and sanitisation steps. However, the study confirmed greater than 99.9975% removal of Kathon to approximately 30 fold less than the limits for ‘leave on’ products containing Kathon. We will exclude anyone from the study with a history of clinically significant contact dermatitis or sensitivity to Kathon.

2. MVA CS

Although MVA CS has not previously been administered to humans, the safety data available from the more than 160 UK adults who have been boosted with MVA expressing malaria antigens following ChAd63 prime, (particularly those for intramuscular administration and lower doses of MVA) can be used to predict the adverse event profile expected post vaccination with MVA CS in this trial. At the planned dose (2 x 10^8 pfu), it is expected that majority of injection site reactions will be of mild severity. Injection site pain would be expected to occur frequently. Less frequent adverse events are likely to include erythema, swelling, itching and warmth. Local AEs are likely to be mild in nature and should resolve rapidly, although there is the possibility of moderate arm pain in some cases.

Common systemic adverse events post MVA vectored vaccines include headache, feverishness, myalgia, arthralgia, fatigue, and malaise. Generally volunteers report a transient flu like illness within 24 hours of vaccination with MVA which resolves completely. Given existing data for MVA vectored vaccines in Oxford, it is anticipated that the majority of systemic adverse events post 2 x 10^8 pfu MVA CS will be mild or moderate in intensity.

**POTENTIAL BENEFITS**

Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective malaria vaccine regimen. The only benefits for participants would be information about their general health status.
5. INVESTIGATIONAL PRODUCTS

ChAd63 CS

ChAd63 CS was manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility (CBF), Churchill Hospital, Oxford. ChAd63 CS is supplied as a liquid in sterile aliquots in 2.0 mL clear glass vials. Further details relating to batch release and manufacturing can be found in the ChAd63 CS IMP. The concentration of ChAd63 CS is 1.4 x 10^{11} VP/ml and therefore a dilution will be performed to achieve the lower dose of 5 x 10^{9} VP. According to SOP MC012.

MVA CS

MVA CS was manufactured under Good Manufacturing Practice conditions by Impfstoffwerk Dessau-Tornau (IDT) Germany. MVA CS is supplied as a liquid formulation in Tris buffer. The virus suspension is supplied as sterile aliquots in 2.0 mL clear glass injection vials. Final batch certification and associated labelling takes place at the Clinical Biomanufacturing Facility (CBF), Churchill Hospital, Oxford. A dose of 2 x 10^{8} will be administered as a volume of 0.34 ml from 2 vials of MVA CS at concentration of 5.9 x 10^{8} pfu/ml.

5.1 Storage of Vaccines

Vials of ChAd63 CS and MVA CS will be stored between –70°C and –90°C. All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with local SOPs and other relevant local forms.

5.2 Administration of Vaccines

The vaccines will all be administered intramuscularly. The vaccinating investigator will wear gloves and eye protection. During administration of the vaccines, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. Vaccination will be performed and the IMP handled according to the relevant local SOPs. On vaccination day, vaccines will be allowed to thaw to room temperature and administered within 1 hour. Depending on dose, one or more vials of vaccine may be used.

Administration of ChAd63 CS

The first volunteer in group 1 (from group 1A or group 1B) will be vaccinated alone with 5 x 10^{9} vp ChAd63 CS. In the absence of any safety concerns in this first volunteer at least 72 hrs post vaccination, two further volunteers in group 1 (from group 1A or group 1B) will be vaccinated with 5 x 10^{9} vp ChAd63 CS. In the absence of any safety concerns in these two vaccines at least 72 hours after vaccination, the remaining volunteers in group 1 will be vaccinated with 5 x 10^{9} vp ChAd63 CS.

There will be a minimum interval of two weeks between the administration of 5 x 10^{9} vp ChAd63 CS to the last volunteer in group 1 and administration of 5 x 10^{10} vp ChAd63 CS to the first volunteer in group 2. The DSMB will the review the safety data from the low dose volunteers prior to dose escalation to the higher dose vaccine.

The first volunteer in group 2 (from group 2A or 2B) will be vaccinated alone with 5 x 10^{10} vp ChAd63 CS. In the absence of any safety concerns in this first volunteer at least 72 hours post vaccination, two further volunteers in group 2 (group 2A or 2B) will be vaccinated with 5 x 10^{10} vp ChAd63 CS. In the absence of any safety concerns in these two vaccinees in group 2 at least 72 hours after vaccination, the remaining volunteers in group 2 will be vaccinated with 5 x 10^{10} vp ChAd63 CS.

Administration of MVA CS
The first volunteer to receive $2 \times 10^8$ pfu MVA CS (group 1B) will be vaccinated alone. In the absence of any safety concerns in this volunteer at least 72 hours after vaccination, two further volunteers in group 1B will be vaccinated with $2 \times 10^8$ pfu MVA CS. In the absence of any safety concerns in these two vaccines at least 72 hours post vaccination, the remaining volunteers in group 1B and group 2B will be vaccinated with $2 \times 10^8$ pfu MVA CS.

5.3 Minimising environmental contamination with Genetically Modified Organisms (GMO)

The study will be performed in accordance with UK Genetically Modified Organisms (Contained Use) Regulations (2000) and Ireland’s GMO (Deliberate Release) Regulations, S.I. No. 500, 2003. GMO authorisation for deliberate release is obtained from the Irish Environmental Protection Agency (EPA) and approval for use in this trial is sought from the Irish Medicines Board (IMB). In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, the inoculation site will be covered with a dressing after immunisation. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site after 30 minutes ($\pm$ 5 minutes) and will be disposed as GMO waste by autoclaving, in accordance with the relevant local SOPs.

5.4 Vaccine Supply

ChAd63 CS will be supplied to the site by the Clinical Biomanufacturing Unit, University of Oxford, where the vaccine is formulated, vialed and labelled for investigational use only.

MVA CS will be supplied to the Clinical Biomanufacturing Unit, University of Oxford, by Impfstoffwerk Dessau-Tornau (IDT) Biologika GmbH, Germany where the vaccine is formulated and vialed. It will be labelled for investigational use only by the CBF, who will then transfer the vaccine to site.

All vaccines will be certified for release by a qualified person (QP) at the CBF, University of Oxford. The vaccines will be shipped on dry ice directly from the CBF to the RCSI and a temperature monitoring logger will be enclosed to ensure cold chain verification.
6. RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

6.1 Volunteers

Volunteers may be recruited by use of an advertisement +/- registration form formally approved by the ethics committee and distributed, posted or presented in the following places:

- In public places with the agreement of the owner / proprietor
- In newspapers or other literature for circulation
- On radio via announcements
- On a website operated by our group or with the agreement of the owner or operator (including on-line recruitment through our web-site)
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation
- On stalls or stands at exhibitions or fairs or via lectures at public meetings or educational events

6.2 Informed Consent

All volunteers will sign and date the informed consent form before any study specific procedures are performed. The information sheet will be made available to the volunteer at least 24 hours prior to the screening visit. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The volunteer may withdraw from the study at any time
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- The study involves research of investigational vaccines. The possibility of increased local and systemic reactions and other potential vaccine associated events will be stressed. Also that long-term effects on the immune system functions are unknown.
- There is no direct benefit from participating
- The volunteer’s GP will be contacted to corroborate their medical history and confirm that the volunteer is eligible to take part in the study. UK volunteers will only be enrolled in the study if written or verbal information regarding the volunteer’s medical history is obtained from the GP.
- UK volunteers will be registered on the TOPS database (The Overvolunteering Prevention System).
- Separate consent for purposes of genetic testing must be obtained. Not consenting to this aspect of the study will not effect volunteers enrolment in the rest of the study.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the CRF. If addition, if they so wish, they will sign and date two copies of the DNA testing consent form. These forms will also be signed and dated by the Investigator. No trial specific examinations or tests may be performed until the volunteer has consented to participate in the study, and has signed the trial specific consent form.
6.3 Inclusion and Exclusion Criteria

Inclusion Criteria
The volunteer must satisfy all the following criteria to be eligible for the study:

- Healthy adults aged 18 to 50 years
- Able and willing (in the Investigator’s opinion) to comply with all study requirements
- Willing to allow the investigators to discuss the volunteer’s medical history with their General Practitioner
- Women only: Must practice continuous effective contraception for the duration of the study.
- Agreement to refrain from blood donation during the course of the study and for 6 months after the end of their involvement in the study.
- Written informed consent.

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

- History of clinical *P. falciparum* malaria
- Travel to a malaria endemic region during the study period or within the preceding six months with a significant risk of malaria exposure.
- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period.
- Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data.
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
- Pregnancy, breast feeding or intention to become pregnant during the study
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine e.g. egg products, Kathon.
- History of clinically significant contact dermatitis.
- Any history of anaphylaxis post vaccination or any serious reaction following vaccination.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of migraine headache.
- History of serious psychiatric condition that may affect participation in the study.
- Any other serious chronic illness requiring hospital specialist supervision. Use of regular medications such as antihypertensives would not necessarily result in exclusion.
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week or Carbohydrate Deficient Transferrin (CDT) >3%.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Suspected or known use of opiates, cocaine, amphetamines, benzodiazepines or marijuana.
- Seropositive for hepatitis B surface antigen (HBsAg).
- Seropositive for hepatitis C virus (antibodies to HCV).
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination.
• Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

in a particularly dependent relationship with the investigator by way of occupation or otherwise, which in the investigators opinion places the volunteer in a vulnerable population.

Re-vaccination exclusion criteria

The following AEs associated with vaccine immunisation constitute absolute contraindications to further administration of an IMP to a volunteer. If any of these events occur during the study, the subject will be withdrawn from the trial and followed up by the clinical team or their GP until resolution or stabilisation of the event;

• Anaphylactic reaction following administration of vaccine
• Any serious reaction following vaccination
• Pregnancy

The following adverse events constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, or withdrawn, at the discretion of the investigator;

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

6.4 Withdrawal of Volunteers

Volunteers may withdraw or be withdrawn for any of the reasons given below. The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged with the volunteer’s permission, until the AE has resolved or stabilised. Any volunteer who is withdrawn, post vaccination, will be invited to attend for all scheduled safety bloods and review as per protocol. Any volunteer who is withdrawn from the study may be replaced, if that is possible within the specified time frame. The Local Safety Monitor (LSM) may recommend withdrawal of volunteers.

6.5 Discontinuation Criteria

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2008) and any other applicable regulations, 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. The Investigator may withdraw the volunteer at any time in the interests of the volunteer’s health and well-being. In addition the volunteer may withdraw/be withdrawn for any of the following reasons:

• Administrative decision by the Investigator
• Ineligibility (either arising during the study or retrospectively, having been overlooked at screening)
• Significant protocol deviation
• Volunteer non-compliance with study requirements
• An AE which requires discontinuation of the vaccination regimen or results in inability to continue to comply with study procedures.

6.6 Pregnancy

Should a volunteer become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome, with the volunteer’s permission. We will not routinely perform venepuncture on such volunteers.
7. TREATMENT OF TRIAL VOLUNTEERS

7.1 Study procedures

Procedures will be performed on the visit time points indicated in the schedule of procedures (Tables 5 & 6). Additional procedures or laboratory tests may be performed, at the discretion of the investigators if clinically indicated e.g. urine microscopy in the event of positive urinalysis, repeat of an abnormal blood test.

Observations

Pulse, blood pressure and temperature will be measured and documented at screening, immediately pre-vaccination, at visits scheduled 1 day post vaccination, and at any other time point if felt necessary by the clinical team (Tables 5 & 6).

Blood Tests

Blood will be drawn as scheduled (Tables 5 & 6) for the following laboratory tests:

- **Haematology;** Full Blood Count
- **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Bilirubin, Alanine transaminase and alkaline phosphatase
- **Carbohydrate Deficient Transferrin;** screening test for chronic alcoholism.
- **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (Counselling will be given prior to testing blood for these blood-borne viruses)
- **Immunology;** Human Leukocyte Antigen (HLA) typing and *ex vivo* Elispot assays for interferon gamma. Other exploratory immunological assays including flow cytometry assays, cytokine analysis, functional antibody assays including IFA, anti-adenovirus antibodies, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity, RNA analysis by either microarray or RNA Seq or other methods and *in vitro* growth inhibition assays amongst others may be performed at the discretion of the investigators.

Urinalysis

- Urine will be tested for the presence of clinically significant proteinuria, glucosuria or haematuria and drug use (Cocaine, Opiates, Benzodiazepines, Amphetamines and Marijuana).

at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (βHCG) at screening and immediately prior to each vaccination.

Vaccinations

Before each vaccination, the on-going eligibility of the volunteer will be reviewed. The vaccine will be administered as described in section 5.2. The injection site will be covered with a sterile dressing and the volunteer will stay in the clinical area for 30 minutes (+/- 5 minutes) post vaccination. In the case of the first 3 subjects to receive each dosing (ChAd63 CS and MVA CS respectively) an observation time of 12 hours post vaccination will be applied. The sterile dressing will be removed and injection site inspected in all groups at 30 minutes (+/- 5 minutes) post vaccination. An oral thermometer, tape measure and a 7 day diary card for solicited AEs will be given to each volunteer along with the emergency 24 hour telephone number to contact the on call study physician if needed. Volunteers will be advised that they may experience pain at the injection site and that use of paracetamol or Non Steroidal Anti inflammatories is permitted if they desire. Volunteers will be asked to record all medications used independently by them in the diary card provided.
7.2 Clinical Reviews

The clinical reviews and procedures will be undertaken by one of the clinical team. The procedures to be included in each visit are documented in the schedule specific to each group (Tables 5 & 6). Each review is assigned a time point and a window period, within which the review will be conducted. The first clinical review following any vaccination will take place at 24 hours (Tables 5 & 6). In respect of the first vaccinee, no further volunteers can be vaccinated until the first review has been satisfactorily completed on the 1st vaccinee for each vaccine. A final review will take place at 6 months post vaccination, this can be in the form of clinic visit or telephone contact.

All potential volunteers will have a screening visit which may take place up to 90 days prior to vaccination. Informed consent will be taken before screening as described above. If consent is obtained, the screening procedures indicated in the schedule of procedures will be undertaken. To avoid unnecessary additional venepuncture, if the appropriate blood test results for screening are available for the same volunteer from a screening visit for another vaccine study, these results may be used for assessing eligibility (provided the results date within the 3 months preceding enrolment in VAC038).

Abnormal clinical findings from the medical history, examination or blood tests at any point in the study will be assessed by the trial clinician. Any abnormal findings deemed untoward medical occurrences will be recorded as AEs. Findings may be reassessed to determine whether the abnormal finding is an isolated occurrence or a persisting abnormality. If an abnormal finding remains clinically significant, the volunteer will be informed and appropriate medical follow-up and care arranged as appropriate, with the permission of the volunteer. Decisions to exclude the volunteer from the enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator, following procedures for adverse events as described in section 9.
### Table 5: Schedule of clinical reviews for Groups 1A & 2A

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<td>Medical History</td>
<td>X</td>
<td>(x)</td>
</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
<td>(x)</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>β-HCG urine test</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

| Review contraindications      | X |
| Vaccination                   | X |
| Vital signs                   | X | X | X | (x) | (x) | (x) |

| Local & systemic AEs assessed | X | X | X | X | X | X | X |
| Diary cards provided         | X |
| Diary cards collected        | X |
| HLA typing (mL)              | 4 |
| HBV, HCV, HIV (mL)           | 5 |
| Haematology (mL)             | 2 | 2 | 2 | 2 | 2 |
| Biochemistry* (mL)           | 4 | 4 | 4 | 4 | 4 |

| Carbohydrate Deficient Transferrin | 5 |
| Exploratory immunology          | 70 | 13 | 70 | 70 | 70 | 70 |
| Blood volume per visit (mL)     | 16 | 74 | 13 | 76 | 76 | 76 |
| Cumulative blood volume (mL)    | 16 | 90 | 103 | 179 | 255 | 331 | 407 |

*S = screening visit, **V = vaccination visit, (x) = If necessary (Windows refer to time since last visit)  
*Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests.  
**Window: -12 hours/ +48 hours. † can be via telephone
<table>
<thead>
<tr>
<th>Review No.</th>
<th>S</th>
<th>ChAd63</th>
<th>CS</th>
<th>MVA</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline (days)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Window (days)</td>
<td>-90</td>
<td><strong>±2</strong></td>
<td>±7</td>
<td>±7</td>
<td><strong>±2</strong></td>
</tr>
<tr>
<td>Inclusion / Exclusion criteria</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Uralysis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-HCG urine test</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review contraindications</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>Local &amp; systemic AEs assessed</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Diary cards provided</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diary cards collected</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>HLA typing (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV, HCV, HIV (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematology (mL)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Biochemistry* (mL)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Carbohydrate Deficient Transferrin</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploratory immunology</td>
<td>70</td>
<td>13</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Blood volume per visit (mL)</td>
<td>16</td>
<td>74</td>
<td>13</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>Cumulative blood volume (mL)</td>
<td>16</td>
<td>90</td>
<td>103</td>
<td>179</td>
<td>255</td>
</tr>
</tbody>
</table>

*S = screening visit, **V = vaccination visit, (x) = If necessary (Windows refer to time since last visit)  
*Biochemistry will include Sodium, Potassium, Urea, Creatinine & Liver Function Tests.  
**Window: -12 hours/ +48 hours.  
† can be via telephone.
Table 7: Total Blood Drawn During Study:

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximum Total Blood Donated During Study</th>
<th>Max Duration of Involvement in Study (approx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A &amp; 2A</td>
<td>407 mls</td>
<td>6 months</td>
</tr>
<tr>
<td>1B &amp; 2B</td>
<td>572 mls</td>
<td>6 months</td>
</tr>
</tbody>
</table>
8. ASSESSMENT OF SCIENTIFIC OBJECTIVES

8.1 PRIMARY EVALUATION CRITERIA

- Safety of the vaccine regimens will be assessed by analysing actively and passively collected data on AEs from diary cards, clinical review of volunteers and laboratory measurements.

8.2 SECONDARY EVALUATION CRITERIA

- Immunological assays will be conducted according to the procedures established in the test laboratories. With the volunteers’ written informed consent, any leftover cells and serum from UK volunteers will be frozen for up to 15 years for future immunological analysis of malaria-specific responses (A Study of Exploratory Immunological Assays to Provide a Laboratory Based Correlate of Protection From Malaria; OXREC Number: 06/Q1606/123)
- The following parameters will be considered evidence of the impact of vaccination in inducing malaria-specific immune responses. Other laboratory investigations including microarray analysis may be performed.

  (A) Interferon gamma CS peptide ELISPOT.
  (B) Flow cytometry to measure T cell responses to CS
  (C) Antibody response. ELISA will be used to assess the levels of anti-CS antibodies.
9. ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

2.13.6. Definitions

2.13.7. Adverse Event (AE)
An AE is any untoward medical occurrence in a volunteer, including a dosing error, which may occur during or after study vaccination and does not necessarily have to have a causal relationship with vaccination. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with study vaccination, whether or not considered related to study vaccination.

2.13.8.

2.13.9. Adverse Drug Reaction (ADR)
An ADR is any untoward or unintended response to a medicinal product. This means that a causal relationship between the study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

2.13.10.

2.13.11. Unexpected Adverse Reaction
An unexpected adverse reaction is where the nature or severity is not consistent with the Investigator’s Brochure.

2.13.12.

2.13.13. Serious Adverse Event (SAE)
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 the 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 hospitalization) that may, based upon appropriate medical judgment, jeopardize 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.

2.13.15. Suspected Unexpected Serious Adverse Reactions (SUSARs)
A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to the investigational product. Reports of any SUSAR will be sent to the REC, regulatory authority and sponsor according to the sponsor’s SOP and national regulatory requirements. Administration of further vaccines within the trial will be suspended until a safety review is convened.

2.13.16.

2.13.17. Foreseeable Adverse Drug Reactions
Expected local reactions to the vaccine will be recorded as AEs. These include injection site pain, erythema, warmth, swelling or itching. Expected systemic reactions to the vaccine will be recorded as in the CRF as AEs. These include myalgia, arthralgia, fatigue, malaise, nausea, fever, feverishness and headache.

2.13.18.

2.13.19. Foreseeable Serious Adverse Events
No IMP related serious adverse events are expected in this study. If an SAE occurs it will be reported as described in section 9.3 below.

9.1 Causality Assessment
For every AE an assessment of the relationship of the event to the administration of the vaccine will be undertaken. An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine action (table 8).
### Table 8: Guidelines for assessing the relationship of vaccine administration to an AE

<table>
<thead>
<tr>
<th>No Relationship</th>
<th>Unlikely</th>
<th>Possible</th>
<th>Probable</th>
<th>Definite</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>No Relationship</strong></th>
<th><strong>Unlikely</strong></th>
<th><strong>Possible</strong></th>
<th><strong>Probable</strong></th>
<th><strong>Definite</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>No temporal relationship to study product <strong>and</strong> Alternate aetiology (clinical state, environmental or other interventions); <strong>and</strong> Does not follow known pattern of response to study product</td>
<td>Unlikely temporal relationship to study product <strong>and</strong> Alternate aetiology likely (clinical state, environmental or other interventions) <strong>and</strong> Does not follow known typical or plausible pattern of response to study product</td>
<td>Reasonable temporal relationship to study product; <strong>or</strong> Event not readily produced by clinical state, environmental or other interventions; <strong>or</strong> Similar pattern of response to that seen with other vaccines</td>
<td>Reasonable temporal relationship to study product; <strong>and</strong> Event not readily produced by clinical state, environment, or other interventions <strong>or</strong> Known pattern of response seen with other vaccines</td>
<td>Reasonable temporal relationship to study product; <strong>and</strong> Event not readily produced by clinical state, environment, or other interventions; <strong>and</strong> Known pattern of response seen with other vaccines</td>
</tr>
</tbody>
</table>

#### 2.13.20.

### 9.2 Reporting Procedures for Adverse Events

All AEs occurring during the study observed by the investigator or reported by the patient will be recorded in the CRF. AEs that result in a patient’s withdrawal from the study or that are present at the end of the study will be followed up (with the volunteer’s permission) until a satisfactory resolution or stabilisation occurs, or until a non-study related causality is assigned.

The severity of clinical and laboratory adverse events will be assessed according to the scales in Tables 9, 10 & 11.

Laboratory values which fall outside the reference range of the local laboratory processing samples will be deemed laboratory abnormalities. These abnormalities will be assessed by the trial clinician. If deemed an untoward medical occurrence, this abnormality will be documented as an AE and ascribed a severity grading (Table 10). Laboratory abnormalities that are not considered AEs will be collated by the investigators and included in the end of study report.
<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at injection site</td>
<td>1</td>
<td>Pain that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Pain that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Pain that prevents daily activity</td>
</tr>
<tr>
<td>Erythema at injection site*</td>
<td>1</td>
<td>&gt;3 - ≤50 mm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;50 - ≤100 mm</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;100 mm</td>
</tr>
<tr>
<td>Swelling at injection site</td>
<td>1</td>
<td>&gt;3 - ≤50 mm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;50 - ≤100 mm</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;100 mm</td>
</tr>
<tr>
<td>Fever (oral)</td>
<td>1</td>
<td>37.6°C - 38.0°C</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;38.0°C – 39.0°C</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;39.0°C</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>Headache that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Headache that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Headache that prevents daily activity</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td>Nausea that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Nausea that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Nausea that prevents daily activity</td>
</tr>
<tr>
<td>Malaise</td>
<td>1</td>
<td>Malaise that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Malaise that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Malaise that prevents daily activity</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1</td>
<td>Myalgia that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Myalgia that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Myalgia that prevents daily activity</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1</td>
<td>Joint pain that is easily tolerated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Joint pain that interferes with daily activity</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Joint pain that prevents daily activity</td>
</tr>
<tr>
<td>Urticaria</td>
<td>1</td>
<td>Requiring no medications</td>
</tr>
<tr>
<td>------------------</td>
<td>---</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Requiring oral or topical treatment or IV medication or steroids for &lt;24 hours</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Requiring IV medication or steroids for &gt;24 hours</td>
</tr>
</tbody>
</table>

*erythema ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event

---

**Table 10:** Severity grading criteria for laboratory abnormalities

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb (female) − decrease from testing laboratory LLN in gm/dl</td>
<td>&gt;1.0 - &lt;1.5</td>
<td>≥1.5 &amp; &lt;2.0</td>
<td>≥2.0</td>
</tr>
<tr>
<td>Hgb (male) − decrease from testing laboratory LLN in gm/dl</td>
<td>≥1.5 &amp; &lt;2.0</td>
<td>≥2.0 &amp; &lt;2.5</td>
<td>≥2.5</td>
</tr>
<tr>
<td>Absolute neutrophil count (ANC, cells/mm³)</td>
<td>1000-1499</td>
<td>500-999</td>
<td>&lt;500</td>
</tr>
<tr>
<td>Leukopenia (WBC, cells/mm³)</td>
<td>&lt;3500 - ≥2500</td>
<td>&lt;2500 - ≥1500</td>
<td>&lt;1500</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>125,000 – 135,000</td>
<td>100,000 – 124,000</td>
<td>20,000-99,000</td>
</tr>
<tr>
<td>Bilirubin – when accompanied by any increase in Liver Function Test increase by factor</td>
<td>1.1 – 1.25 x ULN</td>
<td>1.26 – 1.5 x ULN</td>
<td>1.51 – 1.75 x ULN</td>
</tr>
<tr>
<td>ALT</td>
<td>1.25 – 2.5 x ULN</td>
<td>&gt;2.6 – 5.0 x ULN</td>
<td>&gt;5.0 x ULN</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.1 – 1.5 x ULN</td>
<td>&gt;1.6 – 3.0 x ULN</td>
<td>&gt;3.0 x ULN</td>
</tr>
<tr>
<td>Urine protein</td>
<td>2+ or 0.5-1 gm loss/day</td>
<td>3+ or 1-2 gm loss/day</td>
<td>4+ or &gt;2 gm loss/day</td>
</tr>
<tr>
<td>Hematuria</td>
<td>2+ confirmed by 5-10 rbc/hpf</td>
<td>3+ confirmed by &gt;10 rbc/hpf</td>
<td>gross, with or without clots, OR red blood cell casts</td>
</tr>
</tbody>
</table>

---

**Table 11:** Functional scale for assessing the severity of AEs

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
<td>Awareness of a symptom but the symptom is easily tolerated</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Discomfort enough to cause interference with usual activity</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Incapacitating; unable to perform usual activities; requires absenteeism or bed rest</td>
</tr>
</tbody>
</table>
2.13.21.

9.3 Reporting Procedures for Serious Adverse Events

The event will be documented accurately and national & sponsor notification deadlines and reporting procedures adhered to (see SOP). In addition to the expedited reporting above, the investigator shall submit once a year throughout the study or, on request, a safety report to the sponsor (CTRG), the regulatory authority and REC.

9.4 Reporting Procedures for SUSARs

The Chief Investigator will report all SUSARs to the sponsor (CTRG), The Medicines and Healthcare products Regulatory Agency, The Irish Medicines Board (IMB) and the RECs concerned within required timelines. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The Chief Investigator will also inform all investigators concerned of relevant information about SUSARs.

For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

9.5 Procedures to be followed in the event of abnormal findings

Any abnormal findings deemed untoward medical occurrences will be recorded as AEs. Findings may be reassessed to determine whether the abnormal finding is an isolated occurrence or a persisting abnormality. If an abnormal finding remains clinically significant, the volunteer will be informed and appropriate medical follow-up and care arranged as appropriate, with the permission of the volunteer. Decisions to exclude the volunteer from the enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator, following procedures for adverse events as described in section 9.

9.6 DSMB

A Drug safety monitoring board (DSMB) will provide real-time safety oversight. The DSMB will review SAEs deemed possibly, probably or definitely related to vaccination. The DSMB will be notified within 1 working day of the investigators being aware of their occurrence. The DSMB has the power to terminate the study if deemed necessary following a vaccine-related SAE. The DSMB will review the data before there is a dose escalation of ChAd63 CS from $5 \times 10^9$ to $5 \times 10^{10}$. The DSMB will be contacted for advice and independent review in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to the study vaccine.
- Any other situation where the Investigator feels independent advice or review is important.

9.7 Safety Profile Review

The safety profile will be assessed on an on-going basis by the investigators. An internal safety group will also review safety issues and SAEs as they arise.
10. STATISTICS

This is an observational, un-blinded, non-randomised safety study. The number of vaccinated subjects in each group recruited for the study will be 4-8. This sample size should allow determination of the magnitude of the outcome measures, especially of serious and severe adverse events, rather than aiming to obtain statistical significance. Data analysis will consist primarily of descriptive summaries for treatment groups. For primary and secondary endpoints descriptive summaries and plots over the time course for both individual patient results and groups will be presented. Due the small number of volunteers in this study, all volunteers receiving the same dose of a given vaccine will be pooled for analysis. Where appropriate highly skewed data will be log-transformed and presented as geometric means with 95% confidence intervals.
11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

**Investigator procedures**
Approved site-specific SOPs will be used at all clinical and laboratory sites.

**Monitoring**
Monitoring will be performed according to ICH Good Clinical Practice (GCP) by the external monitor Appledown Clinical Research Ltd. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

**Modification to protocol**
No amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Chief Investigator and will be made a formal part of the protocol following ethical and regulatory approval.

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the subjects' safety, the objectives of the trial and its progress. An administrative change does not require REC or regulatory approval.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and REC approval has already been given, are not initiated without regulatory and REC review and approval except to eliminate apparent immediate hazards to the subject.

**Protocol deviation**
Any deviations from the protocol will be documented in a protocol deviation form and filed in the site trial master file.

**Audit & inspection**
The QA manager will conduct internal audits to check that the trial is being conducted, data recorded, analyzed and accurately reported according to the protocol, sponsor’s SOPs and in compliance with ICH GCP. The audits will also include laboratory activities according to an agreed audit schedule. The internal audits will supplement the external monitoring process and will review processes not covered by the external monitor.

The sponsor and trial sites may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

**Serious Breaches**
The UK Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the regulatory authority within 7 days of the sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to effect to a significant degree –

- (a) the safety or physical or mental integrity of the subjects of the trial; or
- (b) the scientific value of the trial”.

In the event that a serious breach is suspected the sponsor will be informed as soon as possible and in turn will notify the MHRA and the IMB within 7 days. A copy of this notification will also be forwarded to the Ethics committees.

**Trial Progress**
The progress of the trial will be overseen by the Chief Investigator.
12. ETHICS

12.1 Declaration of Helsinki

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

12.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity to Medicine for Human use (clinical trials) Regulations 2004 and its amendments and with the ICH guidelines for GCP (CPMP/ICH/135/95) July 1996. The trial will also comply with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations, 2004 [S.I. 190 of 2004].

12.3 Informed Consent

Written, informed consent will be obtained, as described above.

12.4 Research Ethics Committee (REC)

A copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to the local RECs for written approval. The Investigator will submit and, where necessary, obtain approval from the local RECs for all subsequent substantial amendments to the protocol and informed consent document. The Investigator will notify deviations from the protocol or SAEs occurring at the site to the sponsor and will notify the local RECs of these if necessary in accordance with local procedures.

12.5 Volunteer Confidentiality

All data will be anonymised; volunteer data will be identified by a unique study number in CRF and database. Separate confidential files containing identifiable information will be stored in secured locations. Only the sponsor representative, investigators, the clinical monitor, the local RECs and the regulatory authorities will have access to the records. Photographs taken of vaccination sites (if required, with the volunteer’s written, informed consent) will not include the volunteer’s face and will be identified by the volunteer’s trial specific identification number only. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.
13. **DATA HANDLING AND RECORD KEEPING**

13.1 **Data Handling**

The Chief Investigator will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The investigators will enter the data into the volunteers’ CRFs, which will be in a paper and/or electronic format. This includes safety data, laboratory data and outcome data.

13.2 **Record Keeping**

The investigators will maintain appropriate medical and research records for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Chief Investigator, co-investigators and clinical research nurses will have access to records. The investigators will permit authorized representatives of the sponsor(s), regulatory agencies and the monitors 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.

13.3 **Source Data and Case Report Forms (CRFs)**

All protocol-required information will be collected in CRFs designed by the investigator. All source documents will be filed in the CRF. Source documents are original documents, data, and records from which the volunteer’s CRF data are obtained. For this study these will include, but are not limited to; volunteer consent form, blood results, GP response letters, laboratory records, diaries, and correspondence. In the majority of cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e. there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, urine assessments, blood results, adverse event data and details of vaccinations. All source data and volunteer CRFs will be stored securely.

13.4 **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 unauthorized third party, without prior written approval of the sponsor.
14. FINANCING AND INSURANCE

14.1 Financing

The study will be funded primarily by a grant from the European Vaccine Initiative (EVI) with further support from other research grants from the Wellcome Trust, the National Institute of Health Research and the Medical Research Council, held by Professor Adrian Hill.

14.2 Insurance

*Negligent Harm:* Indemnity and/or compensation for negligent harm arising specifically from an accidental injury for which the University is legally liable as the Research Sponsor will be covered by the University of Oxford.

*Non-Negligent Harm:* Indemnity and/or compensation for harm arising specifically from an accidental injury, and occurring as a consequence of the Research Subjects’ participation in the trial for which the University is the Research Sponsor will be covered by the University of Oxford.

14.3 Compensation

Volunteers will be compensated for their time and for the inconvenience caused by procedures as below.

**UK volunteers**
- Travel expenses: £6* per visit
- Inconvenience of blood tests: £6 per blood donation
- Time required for visit: £15 per hour

**Republic of Ireland volunteers:**
- Travel expenses: €10* per visit
- Inconvenience of blood tests: €10 per blood donation
- Time required for visit: €10 per hour

*Guide value – this may change depending on individual volunteer’s travel arrangements.*
15. References

## Appendix 9.10 Intra-cellular Cytokines Staining reagents.

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