A clinical trial to determine the safety, tolerability and immunogenicity of the candidate *Mycobacterium avium* subspecies *paratuberculosis* (MAP) vaccines ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease

Study Reference: HAV002
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Full study title A clinical trial to determine the safety, tolerability and immunogenicity of the candidate *Mycobacterium avium* subspecies *paratuberculosis* (MAP) vaccine ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease
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***Modification History* Version**	**Date**	**Author(s)**	**Modifications**
2.0	3rd December 2019	Lindsey West, Paul Cross	Inclusion of final safety data from HAV001 clinical trial
3.0	15th October 2020	Paul Cross	Amendments based on independent review by Statistician, clarification of primary outcome in 5.1.1 Amendment to IMP dose due to transcription error Changes and clarification on Blood Sampling (Exploratory Immunology) Positive swab test for SARS-COV-2 (Covid-19) at screening added to exclusion criteria
4.0	18th March 2021	Paul Cross	7.3.3 Exclusion criteria, point 3: changed from 1 year to 28 days based on emerging data (Ramasamy et al, 2020) Negative test for SARS-COV-2 (Covid-19) required in advance of vaccination visits
5.0	16th December 2021	Paul Cross	Clarification of 7.3.1 Inclusion criteria, points 3 and 4, extra exploratory blood sample (group 5)
6.0	24th March 2022	Paul Cross	Existing biopsies for Group 5: some of the tissue sample may also be used for exploratory analysis
6.1	25th May 2022	Paul Cross	Additional 10ml Day 28 and 84 exploratory analysis samples to be taken at the discretion of the investigator (optional).

Contents

1.0 SYNOPSIS

Trial Title	A clinical trial to determine the safety, tolerability and immunogenicity of the candidate *Mycobacterium avium* subspecies *paratuberculosis* (MAP) vaccine ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease
Trial Centres	Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London, SE1 9RT
Trial Identifier	HAV002
Clinical phase	Ib
Design	Open label, dose escalation, heterologous prime-boost study
Population	Patients with active Crohn's disease aged 18 – 50
Planned Sample Size	Participants receiving ChAdOx2 HAV will be recruited and vaccinated 6 per group, with the usual lead in for each group, up to a maximum of 12 Participants receiving MVA HAV only (n=6) will be recruited into 2 groups. A total of 10 participants will be recruited into a prime-boost group with ChAdOx2 HAV-MVA HAV Maximum possible sample size for study (n=28)
Follow-up duration	20 weeks for participants receiving ChAdOx2 HAV only 12 weeks for participants receiving MVA HAV only 20 weeks for participants receiving ChAdOx2 HAV and MVA HAV
Primary Objectives	To assess the safety and tolerability of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease administered alone and in a prime-boost regimen
Secondary Objectives	To assess the immunogenicity and clinical response of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease on no immunosuppressive therapy when administered alone and in a prime-boost regimen
Investigational Product	**ChAdOx2 HAV** - Viral vectored vaccine using a chimpanzee adenovirus as a vector encoding a *Mycobacterium avium* subspecies *paratuberculosis* (MAP) insert designated HAV MVA HAV - Viral vectored vaccine using a modified vaccinia Ankara as a vector encoding a Mycobacterium avium subspecies paratuberculosis (MAP) insert designated HAV

Finished products and doses	1. ChAdOx2 HAV at 2.5 x 10^{10} vp 2. ChAdOx2 HAV at 5 x 10^{10} vp 3. MVA HAV at 5 x 10^{7} pfu 4. MVA HAV at 2 x 10^{8} pfu
Form	Liquid
Route	Intramuscularly (IM) into the deltoid region of the arm

2.0 ABBREVIATIONS

AdHu	Human adenovirus
AE	Adverse event
AR	Adverse reaction
BAC	Bacterial artificial chromosome
CBF	Clinical Bio-Manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CD	Crohn's disease
ChAd63	Chimpanzee adenovirus 63
CI	Confidence Interval
CRF	Case report form or Clinical Research Facility
CTL	Cytotoxic lymphocytes
CTRG	Clinical Trials Research Governance
Da	Dalton
DSUR	Development Safety Update Report
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme linked immunospot assay
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
GMO	Genetically modified organism
GP	General Practitioner
HAV	Designation of the MAP insert
HBV	Hepatitis B virus
HCG	Human Chorionic Gonadotrophin
HIV	Human Immunodeficiency virus
HLA	Human leukocyte antigen
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IDT	Impfstoffwerk Dessau-Tornau Biologika GmbH
IFN-γ	Interferon-gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
ISF	Investigator Site File
JD	Johne's disease
KHP-CTO	King's Health Partners Clinical Trials Office (part of King's College London)
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
MHRA	Medicines and Healthcare products Regulatory Agency

MLN	Mesenteric Lymph Nodes
MVA	Modified Vaccinia virus Ankara
MVA HAV	Modified Vaccinia Ankara viral vector encoding the HAV insert
µg	microgram
nm	nanometer
PFU	Plaque forming unit
PIS	Participant information sheet
QP	Qualified Person
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SRC	Safety Review Committee
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UOXF	University of Oxford
vp	viral particle

3.0 BACKGROUND & RATIONALE

3.1 Introduction

3.1.1 The opportunity for a therapeutic vaccine against Crohn's disease

Crohn's disease (CD) was first described in 1932 in human populations of W. Europe and North America. It has since been reported in developed and developing countries [1] and is increasing in incidence and prevalence especially in children [2] [3]. Crohn's disease is an incurable, chronic and progressive, inflammatory bowel disease often characterized by a variety of intestinal and extra-intestinal manifestations that impair the patient's quality of life. The natural history of the disease is variable, but the majority of patients tend to have a relapsing and remitting course [4, 5]. Most people with CD will require major surgery and lifelong healthcare support, leading to a substantial economic impact to the patient, their families, and the health care system [6]. Despite improvements in care, mortality in CD remains 50% greater than in the general population [7]. Although much has been learned in recent years about the disease mechanisms, there has been little progress in the recognition of disease causation. Expert reviews refer to CD as being auto-immune or auto-inflammatory [8, 9]. Current available treatments are based on immunomodulating, immunosuppressive and anti-inflammatory agents, which often cause severe side effects.

Mycobacterium avium subspecies *paratuberculosis* (MAP) emerged as the cause of a similar chronic inflammatory bowel disease (Johne's disease - JD) in domestic livestock in W. Europe and North America at the beginning of the 20th century. JD and MAP infection in animals have been identified worldwide since then. The US Department of Agriculture predicts that the current MAP infection rate in US dairy herds is 91.1% [10]. The virulent intracellular MAP phenotype can be found in milk as it is resistant to pasteurisation and infant feed processing [11, 12]. The incidence of CD is rising fast in China following a significant increase of its national dairy herd from importation of large numbers of cattle from Uruguay, Australia and New Zealand where subclinical MAP infection is endemic. Recent data from North and Northeast China and the Shandong province (south of Beijing) reveal that the prevalence of positive MAP tests in dairy and beef herds has reached 20% and 57% respectively [13, 14].

MAP is widely found in the environment. Its natural habitat is the soil where it can persist as an intracellular organism within abundant protists [15, 16] and it is highly resistant to most antibiotics. The presence of farm animal reservoirs and large-scale environmental contamination leads to inevitable human population exposures, not only through the food chain, but also through contaminated water supplies and aerosols [17]. It seems that early life exposure during childhood as well as occupational exposure (farmers and veterinarians) to the extracellular MAP forms shed in faeces and used in veterinary vaccines, may confer some natural protection [18, 19, 20].

There is a clear established association between MAP infection and Crohn's disease, although questions on its definite role remain unanswered [21, 22]. Seven of the eight principal genetic loci for susceptibility to Leprosy, a disease caused by another mycobacteria (*Mycobacterium leprae*), are confirmed risk loci for CD, supporting the hypothesis of a causal relationship between mycobacterial infection and Crohn's disease. [23]

Diagnosis of MAP infection in humans is challenging. It cannot be seen through microscopy in inflamed CD tissues and can only rarely be isolated in culture. As opposed to what is seen in Tuberculosis, MAP specifically minimises its own immune recognition. MAP is also highly resistant to the chemical and enzymic lysis process needed to employ PCR diagnostic tools. The American Academy of Microbiology report 2008 [24] concluded that progress with all the existing uncertainties of a role of MAP as a human pathogen is critically dependent on the availability of a simple, accurate and widely applicable clinical diagnostic test for MAP.

Research at King's College London from 2008 has resulted in the development of a novel diagnostic method based on 5 monoclonal antibodies directed to peptide sequences within a protein unique to MAP. This protein is found to be abundantly expressed and accessible on the microbial surface (John Hermon-Taylor, unpublished data). Two or more of these specific antibodies tagged with different fluorophores co-localising not only on the same host cells but on sub-micrometre MAP organisms within the cytoplasm of infected cells, reinforces the accuracy and precision of the technique. The proposed diagnostic tool may be used in blood and tissue using flow cytometry and routine fixed paraffin embedded histopathology blocks. The method has been tested in 52 patients with CD and MAP infection was found in all of them (John Hermon-Taylor, unpublished data).

A modern viral vectored anti-MAP T-cell vaccine has been developed and tested in animal models with positive results (see below). Clinical trials are now needed to assess the ability of this novel vaccine to attenuate or eradicate MAP infection in humans, potentially leading to significant clinical improvement of Crohn's disease [25, 26].

3.1.2 HAV insert for a viral vectored anti-MAP vaccine

The 'HAV' insert in the vaccine vectors comprises a 95kDa fusion construct from 4 MAP genes 1589c (AhpC), MAP 1234 (Gsd), 2444c (p12) and 1235 (mpa) present in all MAP strains. AhpC is a secreted virulence factor in MAP shared by other pathogenic mycobacteria. Gsd is directly involved in metabolic processes responsible for MAP's relatively inert and highly chemical and enzymic resistance characteristics. P12 is the extracellular portion of the IS900 protein released from the microbial cell and involved in pathogenicity. Mpa may have a pore function which may contribute to the MAP's intrinsic resistance pattern [27, 28]. Similar sequences of all 4 of these MAP genes can be found in important secondary co-pathogens in Crohn's disease, including some *E. coli* and other *M. avium sp*. Overlap of the brisk immunological responses to the HAV insert induced by vaccination are predicted to maximise the efficacy of therapeutic vaccination.

The DNA of the selected genes was codon optimised for mammalian cell expression and strung together to form the single HAV vaccine antigen. For patient safety reasons, this was further edited to remove any genetic sequences with homology to a mammalian sequence.

3.1.3 HAV vaccination in mice

Extensive preclinical prime/boost vaccination studies were carried out in mice using combinations of HAV in plasmid, MVA and hAd5 vectors. None of the animals vaccinated with any of the vectors or vector/insert combinations or dose levels in either prophylactic or therapeutic modes suffered any detected adverse effects. HAV vaccination in all vector combinations was immunogenic inducing T-cells reactive against the vaccine insert antigens. Although there were differences in efficacy between individual animals all vaccinations achieved significant or highly significant reductions in MAP loads compared with control animals (21).

3.1.4 HAV vaccination in cattle

A BBSRC-funded trial of HAV vaccination in protection against experimental MAP infection was carried out (2010-2014) by St George's University of London, The Jenner Institute University of Oxford, The Roslin Institute University of Edinburgh, Animal Health and Welfare and the Agri-Food and Biosciences Institute of Northern Ireland (22).

Six Holstein Friesian calves were given the priming dose of 10^9 viral particles (vp) of hAd5 HAV in 1mL sterile PBS by intradermal injection (id) into the skin of the neck. Five control calves were given the same dose of hAd5 expressing Green Fluorescent Protein (GFP). At 6 weeks calves in the test group received the boosting dose of 10^9 plaque forming units (pfu) of MVA HAV in 1mL sterile PBS id. Control calves received the same id dose of MVA GFP. At 12 weeks all animals received $5x10^8$ live virulent MAP strain R0808 given orally in 20mL PBS on 2 consecutive days. They were then followed for 38 weeks. No adverse effects of vaccination or inflammatory disease over the period of the study were seen in any of the animals.

Blood and faecal samples were obtained before and after each prime and boost vaccination and MAP challenge. Observations were continued with monthly blood and faecal sampling for a period of 38 weeks at the end of which the animals were euthanized. Shortly after oral MAP challenge all animals in the control group shed MAP in their faeces and continued throughout the study. In all test animals HAV vaccination prevented detectable faecal shedding of MAP throughout the study.

All six calves in the test group responded with an increased PBMC release of IFN-γ following PPD-J stimulation not seen in the control group. This was accompanied by a rise in the percentage of CD4+IFN-γ+ and CD8+IFN-γ+ secreting cells which was absent from the control group. Specific cellular immune responses to HAV vaccine peptides were seen in all HAV vaccinated but not in control animals two weeks after boosting and were maintained throughout the study.

Immediately prior to the oral administration of MAP, laboratory tests of the ability of PBMC from control calves to kill MAP were the same as those of PBMC from HAV vaccinated calves. Within 1 week of challenge the *in vitro* MAP killing capacity of PBMC from control calves dropped dramatically by 30% but was unchanged in HAV vaccinated calves. At the same time circulating PBMC from 5/6 HAV vaccinated and 4/5 control calves tested PCR positive for MAP.

A significant impairment of the *in vitro* capacity of PBMC from control calves to kill MAP remained and by week 14 the proportion of these animals with MAP positive PBMC in their blood remained at 4/5. At the same time MAP positive PBMC in blood of vaccinated calves had fallen to 2/6. These trends in blood continued so that over the 19-week second half of the study following MAP infection 3/5 control animals became consistently MAP positive and the other 2 intermittently positive.

By contrast PBMC from 4/6 HAV vaccinated calves remained consistently MAP negative with each of the other 2 animals having only 1 of 5 MAP PCR tests positive over the 19-week period. ELISpot responses of PBMCs to stimulation with HAV specific peptide antigens (which were absent from the control group of animals) continued throughout the study in all vaccinated animals.

At autopsy, full thickness tissue samples were taken from 11 sites throughout the length of the small intestine 2 from the duodenum, 6 from the jejunum and 3 from the ileum together with 4 mesenteric lymph nodes (MLN) and tissue from the spleen. MAP loads in tissues at autopsy were measured by specific qPCR. In the 5 control calves all tissue samples were positive for MAP with microbial loads of up to 5 logs per gram of sample. As a group these tissue samples comprised totals of 10 from the duodenum, 30 from the jejunum, 15 from the ileum, 20 from mesenteric lymph nodes (MLN) and 1 sample each for the 5 spleens.

By contrast, in the 6 HAV vaccinated calves 10 of 12 tissue samples from the duodenum, 28 of 36 from the jejunum, 12 of 18 from the ileum, 7 of 24 from MLN and 3 of the 6 spleens tested negative for MAP. In the residual MAP positive samples, HAV vaccination was associated microbial loads at least 2 orders of magnitude less than corresponding samples in the control group. As with other chronic enteric human pathogens such as Tuberculosis, Yersinia, Legionella and others, MAP demonstrates an ability to persist in MLN for which further strategies may be devised.

3.1.5 Adenovirus-vectored vaccines

Adenoviruses are attractive vectors for human vaccination. They possess a stable genome so that inserts of foreign genes are not deleted. They can infect large numbers of cells without any evidence of 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 cells) [29].
Previous mass vaccination campaigns in over 2 million adult US military personnel using orally administered live human adenovirus serotype 4 and 7 have shown good safety and efficacy data [30]. Human adenoviruses are under development as vectors for malaria, HIV and hepatitis C vaccines, amongst others. They have been used extensively in human trials with excellent safety profile mainly as vectors for HIV vaccines.

A limiting factor to widespread use of human adenovirus as vaccine vectors has been the level of anti-vector immunity present in humans where adenovirus is a ubiquitous infection. The prevalence of immunity to human adenoviruses prompted the consideration of simian adenoviruses as vectors. They exhibit hexon structures homologous to human adenoviruses.

Chimpanzee Adenovirus 63 (ChAd63) hexons are most similar in sequence to the hexons of AdHu4, previously used by the US military mass vaccination campaigns [31]. Simian adenoviruses are not known to cause pathological illness in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the US.

3.1.6 ChAdOx2

The generation of the novel vaccine vector, ChAdOx2, was derived from wild type adenovirus C68 and ChAdOx1, a viral vector developed in the Jenner Institute. ChAdOx1 is described by Dicks et al. [32]. The vector was constructed in a bacterial artificial chromosome (BAC) to facilitate genetic manipulation of genomic clones with improved stability and flexibility. Cellular immunogenicity of recombinant E1 E3-deleted ChAdOx1 was comparable to that of other species E derived chimpanzee adenovirus vectors including ChAd63, the first simian adenovirus vector to enter clinical trials in humans. ChAdOx2 was then produced in the same manner, starting from the replication-competent AdC68 (also known as SAdV-25) and Pan 9, but with further modification to the E4 region.

3.1.7 Development of ChAdOx2 vaccine vector

To generate a molecular clone of the AdC68 genome, a BAC gap repair vector was constructed containing PCR-amplified regions of homology to the left and right flanks of the viral genome as described in Chartier et al [33]. An extra homology flank downstream of the adenovirus E1 region was included to enable deletion of E1 and placement of a unique restriction site at the E1 locus, concomitant with genomic insertion into the BAC. The E1 region is essential for viral replication, hence the ability to delete E1 at this stage renders the new vector immediately replication incompetent. Replication incompetent (E1-deleted) clones were successfully identified by PCR screening and transfection into E1 complementing HEK293 cells confirmed the ability of all candidate clones of the new vector to generate infectious virions.

It is known that the proteins encoded by the E4 region of adenoviruses interact with E1 during viral replication, and the imperfect interaction between the gene products of the AdHu5 E1 gene produced by HEK293 cells and simian E4 gene products has been found to result in impaired viral replication in this cell line, and consequently lower virus yields. In ChAdOx1, Ad5 E4Orf4 has been inserted to replace the homologous simian virus coding sequence, resulting in improved viral replication during vaccine production. Since no replication of the virus takes place after immunization, this replacement has no effect on immunogenicity of the viral vector. In the construction of ChAdOx2, the whole of the native AdC68 E4 region was replaced with the equivalent regions from ChAdOx1.

This will be the second clinical trial using ChAdOx2, a novel chimpanzee adenovirus, as a viral vector. There is already some clinical experience with closely related viral vectors expressing different antigens.

3.1.8 MVA Vectored Vaccination

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 vaccine vector.

Although millions of humans have been vaccinated with conventional replication-competent vaccinia virus, it's very small but definite risk to both researchers and future patients led to the development of several attenuated strains of vaccinia during smallpox eradication and continued 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 [34]. 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 [34].

MVA vectored vaccines encoding several different antigens (malaria, influenza, tuberculosis and prostate cancer) has been administered to thousands of participants in the UK and Africa in clinical trials sponsored by the University of Oxford. Analysis of these clinical trials where MVA vectored vaccines have been used, concludes that this vaccine has an excellent safety record. Adverse events related to the vaccine are mainly local findings consistent with inflammation at the injection site, and nonspecific flu-like systemic symptoms and signs. There have been no serious adverse events related to MVA vectored vaccines to date.

3.1.9 MVA as a boosting agent

MVA vectored vaccines are particularly suited to boosting immune responses to an antigen following a priming vaccination with another viral vector [35]. The ChAd-MVA heterologous prime-boost vaccination approach has been shown to be safe, well tolerated and immunogenic [36-43], eliciting antibodies and high level specific T-cell responses [44] and is now recognised as a highly efficient strategy to boost T cell responses where a second dose is needed, reducing the risks of anti-vector immunity development.

MVA is currently in development as a vector for multiple diseases including HIV-1 [45, 46], tuberculosis [47], HCV (Barnes et al. submitted), influenza [48] and melanoma [49].

3.2 Pre-clinical studies using the HAV insert

3.2.1 Toxicity

A Good Laboratory Practice (GLP) study was conducted at Envigo CRS Ltd to test the potential toxicity of ChAdOx2 HAV and MVA HAV when given by intramuscular administration to Balb/c mice with a 14 days interval. One group, comprising ten male and ten female mice received 65 µL of ChAdOx2 HAV (1 $x10^{10}$ vp) administered by intramuscular injection on two occasions with a 14-day interval followed by a 13-day observation period. The control group consisting of six male and six female mice received phosphate buffered saline (PBS) at the same volume-dose as the treated group.

Treatment was well tolerated and did not evoke any significant signs of systemic toxicity. There were no deaths or adverse effects of treatment. Effects of treatment were mainly confined to those attributable to the administration of a vaccine manifest as inflammatory responses at the injection sites, immune responses in the draining lymph nodes and slight increases in white blood cell numbers, plasma albumin and globulin concentrations (females only) and associated changes in total protein concentrations.

Changes at the intramuscular injection site (inflammatory cell infiltrates) were observed in all five treated males and females that were examined histologically. Enlargement of the right lumbar (draining) lymph node was observed macroscopically for the majority of treated animals, correlating histopathologically with increased cellularity and increased germinal centre development in the majority of treated animals and plasmacytosis observed in a few treated animals. Slightly higher circulating white blood cell numbers (males and females), group mean plasma albumin concentration (females only) and higher group mean plasma total protein concentration (females only) were observed.

Changes in the liver (minimal decreased rarefaction) for treated animals were considered to be a secondary effect, most likely caused by slight variations in food intake prior to necropsy and may be associated with the low plasma triglyceride concentration.

There were no clear histopathological changes that correlated with the slightly increased weight of the spleen recorded at necropsy for treated animals, the slightly high plasma phosphorus and potassium concentrations and the slightly high red cell distribution width and reticulocyte count and slightly low platelet count. However, these changes were considered minor and of no toxicological significance at the degree observed.

In conclusion, treatment with the vaccine ChAdOx2 HAV was well tolerated in mice and was not associated with any adverse effects.

MVA HAV was also tested in a GLP preclinical toxicology study conducted at Envigo CRS Ltd. The objective of the study was to investigate the potential toxicity of ChAdOx2 HAV and MVA HAV to Balb/c mice when administered by intramuscular injection on 2 occasions with a 14-day interval followed by a 13-day observational period.

The intramuscular dose of ChAdOx2 HAV given to each mouse in this study was 600-fold that given clinically and the dose of MVA HAV given to each mouse in this study was 1500-fold the clinical dose. Changes related to treatment with ChAdOx2 HAV followed by MVA HAV vaccine were seen in the tissues of the intramuscular injection site and the right lumbar lymph node (draining lymph node). The changes in the muscular injection sites in the right hindlimb were consistent with inoculation of immunogenic substances such as ChAdOx2 HAV and MVA HAV and were therefore anticipated. The study concluded that treatment with the ChAdOx2 HAV vaccine followed by the MVA HAV vaccine was well tolerated and was not associated with any adverse effects.

3.2.2 Immunogenicity

A mouse *ex-vivo* IFN-γ ELISpot was performed to determine the immunopotency of ChAdOX2 HAV produced by the Clinical Biomanufacturing Facility (CBF). The potency assay was performed according to the relevant SOP.

For the ChAdOX2 HAV clinical lot O1L16-01 (IP-16-181A), four female C57BL/6 mice were vaccinated intramuscularly with 1 x 10^7 i.u in 50µl. The mice were 6 weeks of age when delivered and were vaccinated a week later.

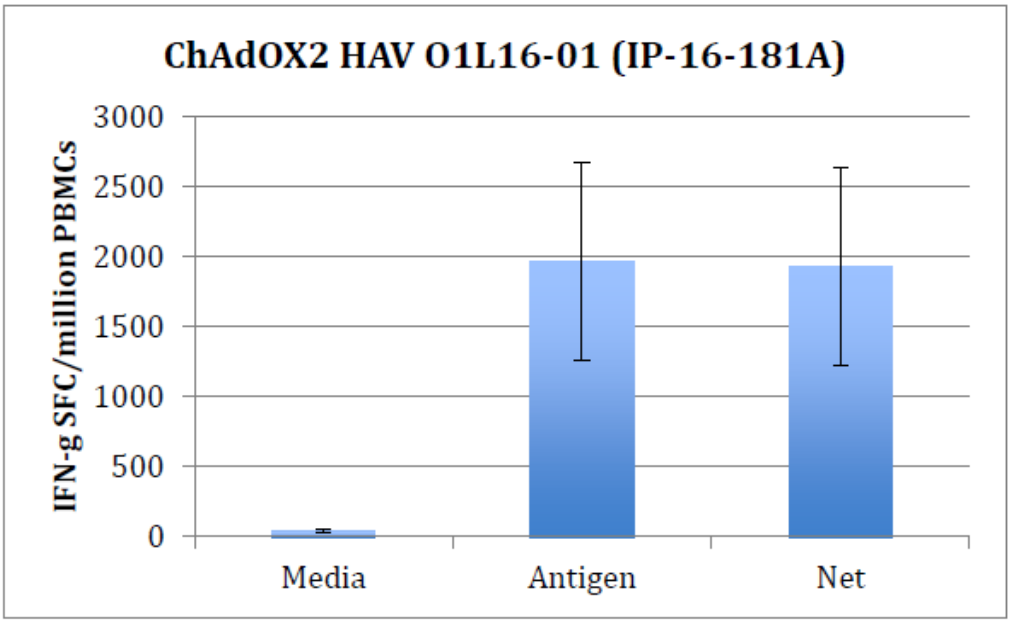

Figure 1. Immunogenicity of 1 x 10^7 i.u. ChAdOx2 HAV, clinical lot O1L16-01 (IP-16-181A). Results show the arithmetic mean of the response per group, error bars represent the standard deviation.

This assay demonstrated that ChAdOx2 HAV - clinical lot O1L16-01 (IP-16-181A), elicits a strong cellular immune response against the HAV antigen in mice, with the mean number of splenocytes in the ELISpot assay greater than 15, after background subtraction, with all mice responding, at a 1 x 10^7 i.u. dose per mouse.

A mouse IFN-γ ELISPOT was also carried out to determine the immunopotency of the IDT manufactured clinical batch of MVA HAV, lot 0020617. Four C57/BL6 mice were vaccinated intra-muscularly with 10^7 pfu of MVA HAV. Thirteen days later the mice were sacrificed and the spleens harvested for in an IFN-γ ELISPOT assay performed.

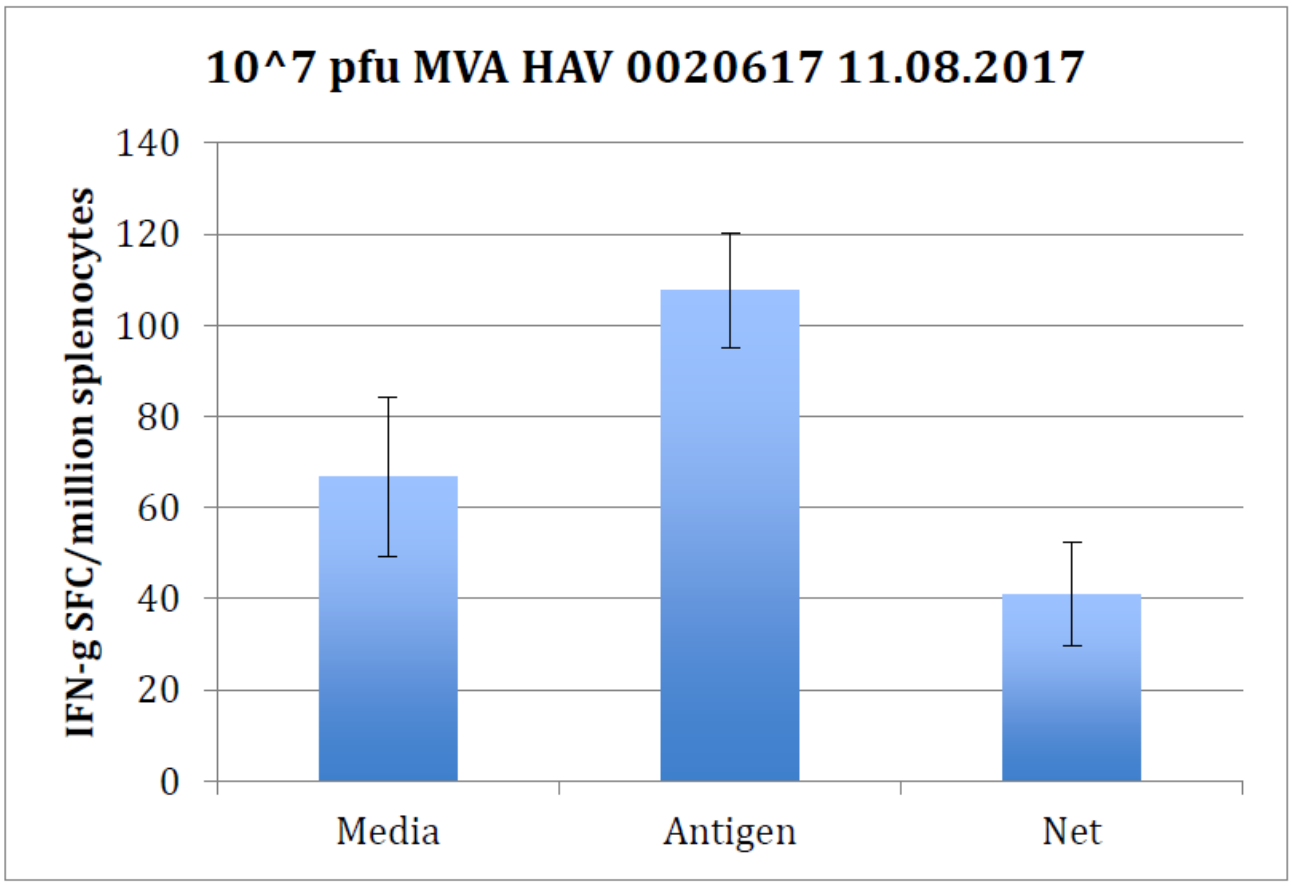

Figure 2. Immunogenicity of 1 x 10^7 pfu MVA HAV, clinical lot 0020617. Results show the arithmetic mean of the response per group, error bars represent the standard deviation.

The potency assay performed demonstrated that IDT manufactured vaccine MVA HAV, lot 0020617 is immunogenic, with the mean number of spot forming cells per million splenocytes in the ELISPOT assay greater than 15, after background subtraction, with not more than 1 non-responding mouse of four tested. The lower limit of detection of the assay for MVA HAV is 15 spots per million splenocytes.

3.3 Previous Clinical Experience

HAV001 was the first in human study employing ChAdOx2 HAV and MVA HAV. During this trial, twelve participants received ChAdOx2 HAV alone, six participants received MVA HAV alone and ten participants received a ChAdOx2 HAV prime followed by a MVA HAV boost, eight weeks apart. The profile of adverse events seen with the prime boost regimen was similar to the ones observed with each vaccine given on its own. No new side effects were identified. However, in addition, ChAdOx1 and MVA vectored vaccines expressing different inserts have previously been tested in clinical studies conducted by Oxford University.

3.3.1 Previous experience with ChAdOx1 and MVA vectored vaccines

ChAdOx1, a closely related chimpanzee adenovirus viral vector, encoding the influenza fusion protein NP+M1 has been safely administered to 161 healthy participants in the UK in two clinical trials conducted by the Jenner Institute. There were no serious adverse events associated with the vaccine and ChAdOx1 was shown to have a good safety profile.

Between May 30 and Aug 8, 2020, 560 participants were enrolled into the COV002 clinical trial: 160 aged 18–55 years (100 assigned to ChAdOx1 nCoV-19, 60 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19: 40 assigned to MenACWY), and 240 aged 70 years and older. In those receiving two standard doses of ChAdOx1 nCoV-19, after the prime vaccination local reactions were reported in 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group, and systemic reactions in 42 (86%) participants in the 18–55 years group, 23 (77%) in the 56–69 years group, and 32 (65%) in the 70 years and older group. As of Oct 26, 2020, 13 serious adverse events occurred during the study period, none of which were considered to be related to either study vaccine [59]

On 30th December 2020, ChAdOx1 nCov-19, Oxford's coronavirus vaccine, received emergency approval from the MHRA and is now being administered as part of the UK's vaccination effort in response to COVID-19.

Replication-deficient recombinant MVA has an excellent safety record. It was administered intradermally to approximately 120,000 people during the smallpox eradication campaign, with an excellent safety record, despite the deliberate vaccination of high-risk groups [50].

The emerging safety profile of recombinant MVA vectored vaccines is supported by data from clinical trials conducted by the University of Oxford. MVA is currently in development as a vector for multiple diseases including HIV-1 [45, 46], tuberculosis [47], HCV [51], influenza [48], melanoma [49], malaria [52] and Ebola [53]. MVA vectored vaccines developed at the University of Oxford has been administered to over 4500 individuals including infants, young children, elderly adults, HIV-infected adults and children and patients with cancer in Europe and Africa without any safety concerns. These studies have demonstrated the safety (side effects and unexpected effects) of these vaccines with the majority of adverse events typically mild to moderate in severity and usually self-limiting.

A summary of 2 influenza trials using a prime-boost strategy with ChAdOx1-MVA vectored vaccines is provided below for reference.

3.3.2 FLU004

Flu004 was a phase I, open-label, non-randomised dose escalation study of ChAdOx1-NP+M1 conducted at the CCVTM, Oxford. A starting dose of 5×10^8 vp, progressing through to 5×10^9, 2.5×10^{10}, and finally 5×10^{10}vp was used. A total of 15 healthy adults (male and female) were vaccinated.

The study demonstrated that ChAdOx1-NP+M1 at a dose of 5×10^{10} vp is safe in healthy participants but had an unacceptable reactogenicity profile with 2 out of 6 participants who received this dose experiencing severe local and systemic reactions (Figures 3 and 4). Within the 3 lowest dose groups (9 participants), ChAdOx1 NP+M1 was well tolerated, with the majority of adverse events (83%) being mild (12% moderate and 5% severe). The local and systemic adverse reactions included fever/febrile symptoms, headache, myalgia, arthralgia, warmth, swelling, malaise, fatigue, pruritus, rhinitis, nausea, vomiting, diarrhoea, abdominal pain, loss of appetite and ocular pain. Laboratory AEs included lymphopaenia and neutropaenia in 3 participants (all in the 5×10^{10} vp group).

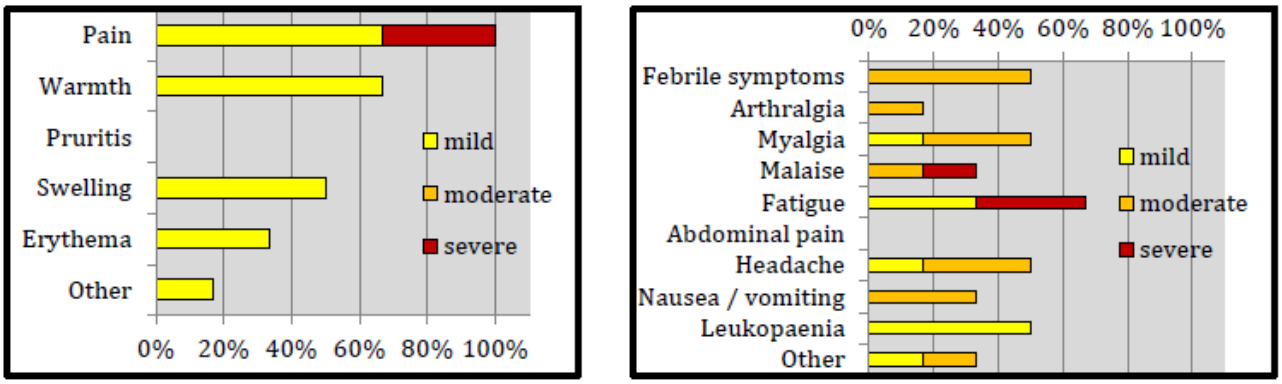

Figure 3. Local and systemic AEs with ChAdOx1 NP+M1 at Dose: 5 x 10^{10} vp

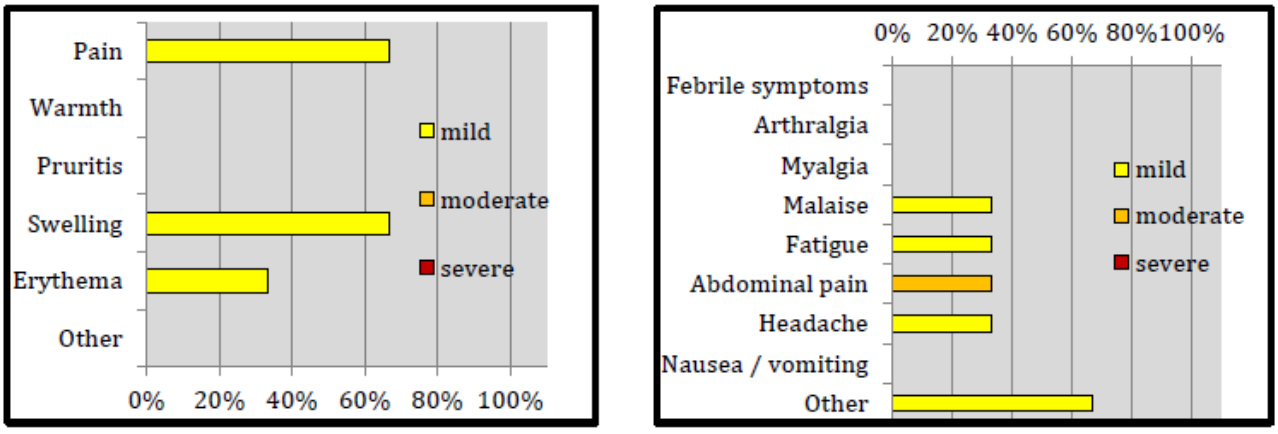

Figure 4. Local and systemic AEs with ChAdOx1 NP+M1 at Dose: 2.5 x 10^{10} vp

Half of the participants (3/6) in the higher dose group were boosted with a vaccination of 1.5 × 10^8 PFUs MVA-NP+M1 7 weeks (n = 2 participants) or 14 weeks (n = 1 volunteer) after ChAdOx1 NP+M1 vaccination. There were no serious adverse events in this study but severe AEs were noted following MVA boost. Two participants complained of severe arm pain and one of these complained of severe systemic AEs (feverishness, myalgia, malaise and fatigue) but these were all self-limited.

The vaccine was immunogenic, inducing ELISPOT responses at all doses, with responses maintained at the day 21 time point (see Figure 5). The dose of 2.5 × 10^{10} vp was chosen for further studies of ChAdOx1 NP+M1. Median ELISpot responses 1 week after MVA-NP+M1 boost following ChAdOx1 NP+M1 were increased approximately threefold and remained at high levels up to 56 days after boost.

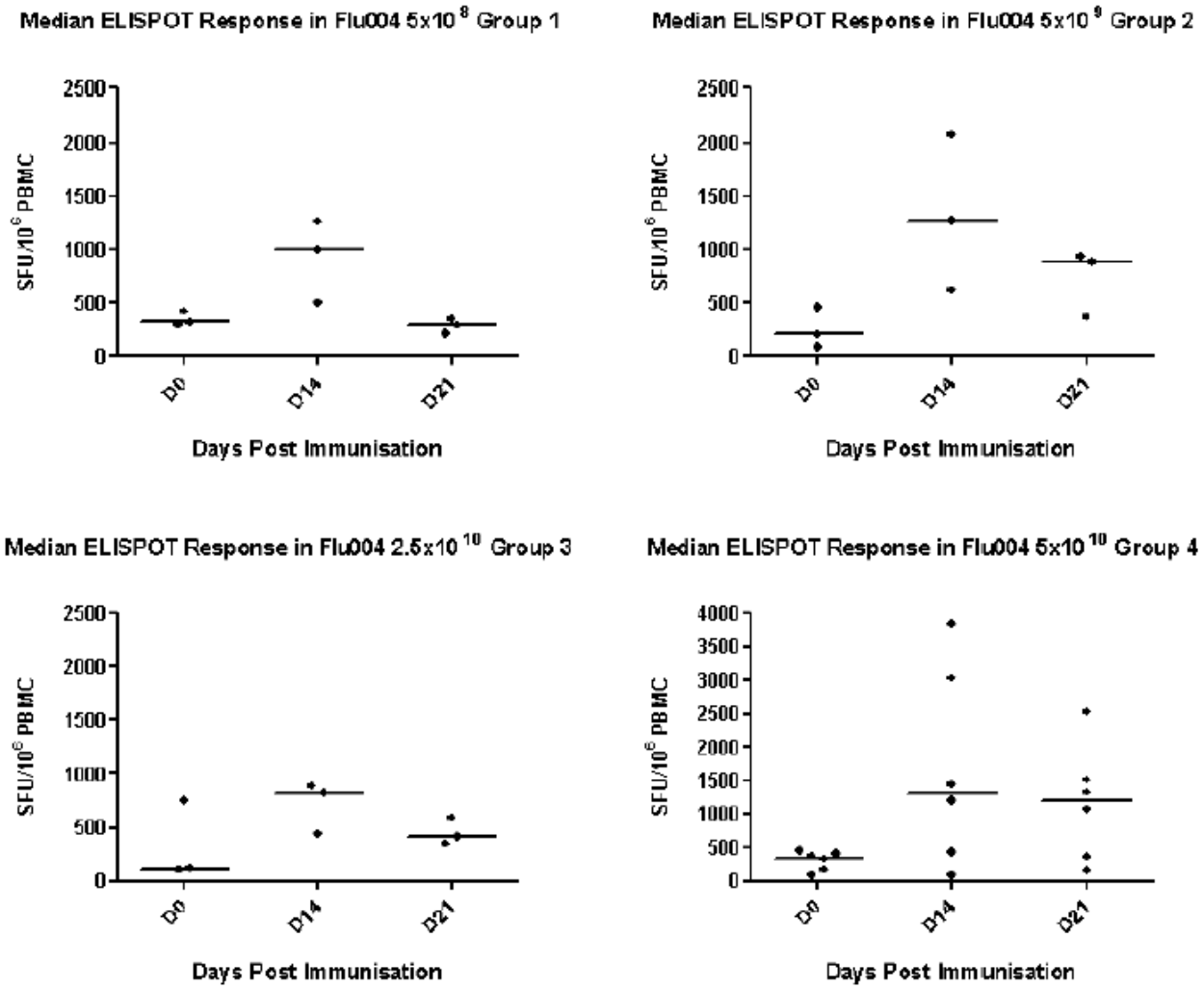

Figure 5. Demonstrates the median ELISPOT responses achieved after vaccination with ChAdOx1 NP+M1 in FLU004 at the different doses. Responses were higher in participants who received higher doses of vaccine, although not statistically significant (small sample sizes). The peak ELISPOT response was seen at day 14, and was highest in Group 4 (5 x 10^{10} vp) at 1325 SFU/106 PBMC. The median ELISPOT response at day 21 in Group 1 (5 x 10^{8} vp ChAdOx1 NP+M1) was 298.3 SFU/106 PBMC, in Group 2 (5 x 10^{9} vp) it was 885 SFU/106 PBMC, in Group 3 (2.5 x 10^{10} vp) it was 411.7 SFU/106 PBMC and in Group 4 it was 1197 SFU/106 PBMC.

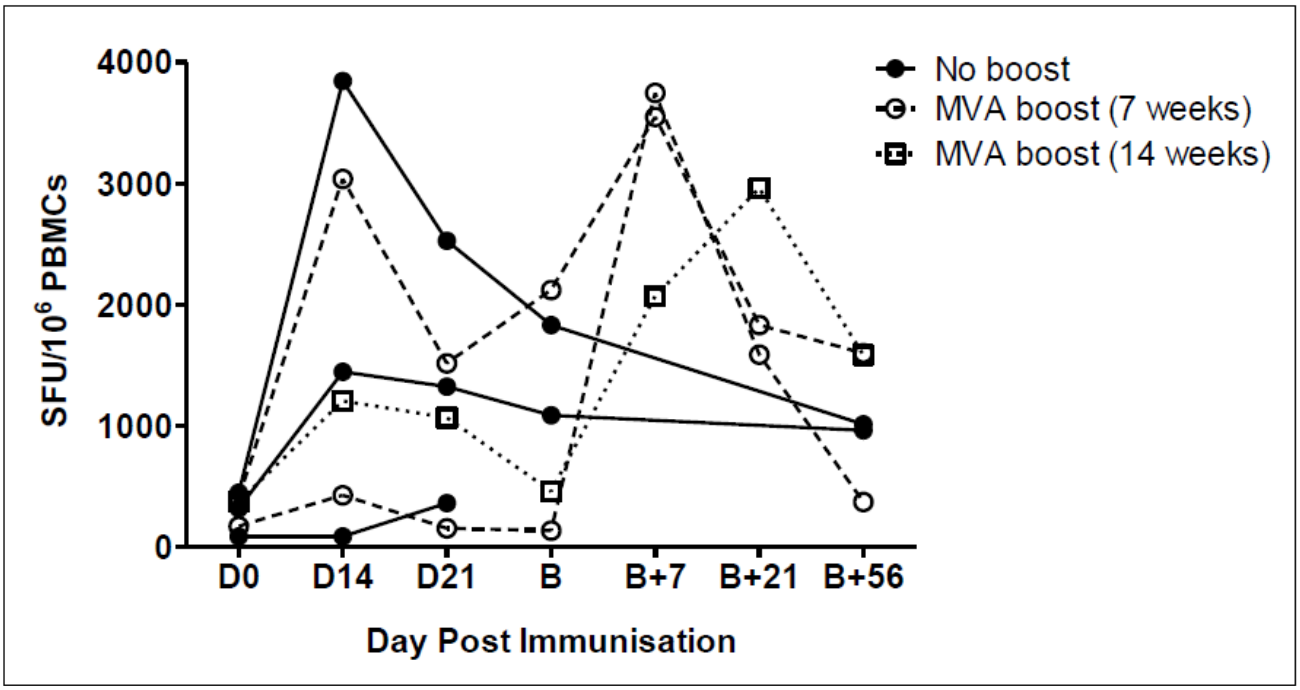

Figure 6. ELISpot responses for 3 participants boosted with MVA NP+M1 following ChAdOx1 NP+M1 prime compared to 2 participants who did not receive a boost in FLU004.

3.3.3 FLU005

Flu005 was a multicentre phase I, randomised observational study to determine the safety and immunogenicity of vaccination regimens employing the candidate influenza vaccines MVA-NP+M1 and ChAdOx1 NP+M1. The trial was conducted at 3 different sites: CCVTM (Oxford), University Hospital Southampton and the University of Surrey (Guildford). Seventy-three healthy adult (male and female) participants have received ChAdOx1 NP+M1 at a concentration of 2.5 x 10^{10} vp.

Administrations of ChAdOx1 NP+M1 and MVA-NP+M1 vaccines were found to be safe and well-tolerated, in agreement with our previous studies [48, 54-57]. The frequency of local and systemic solicited adverse events and majority of adverse events were mild to moderate in nature and lasted for 1-2 days. The most common local adverse event was arm pain at the site of injection and the most common systemic adverse event was mild fatigue and headache. No participant had a documented fever. There were no serious adverse reactions reported in this study. Only mild and transient laboratory adverse events have been reported, except for one volunteer that presented a grade III hyperkalaemia which spontaneously resolved after 1 week.

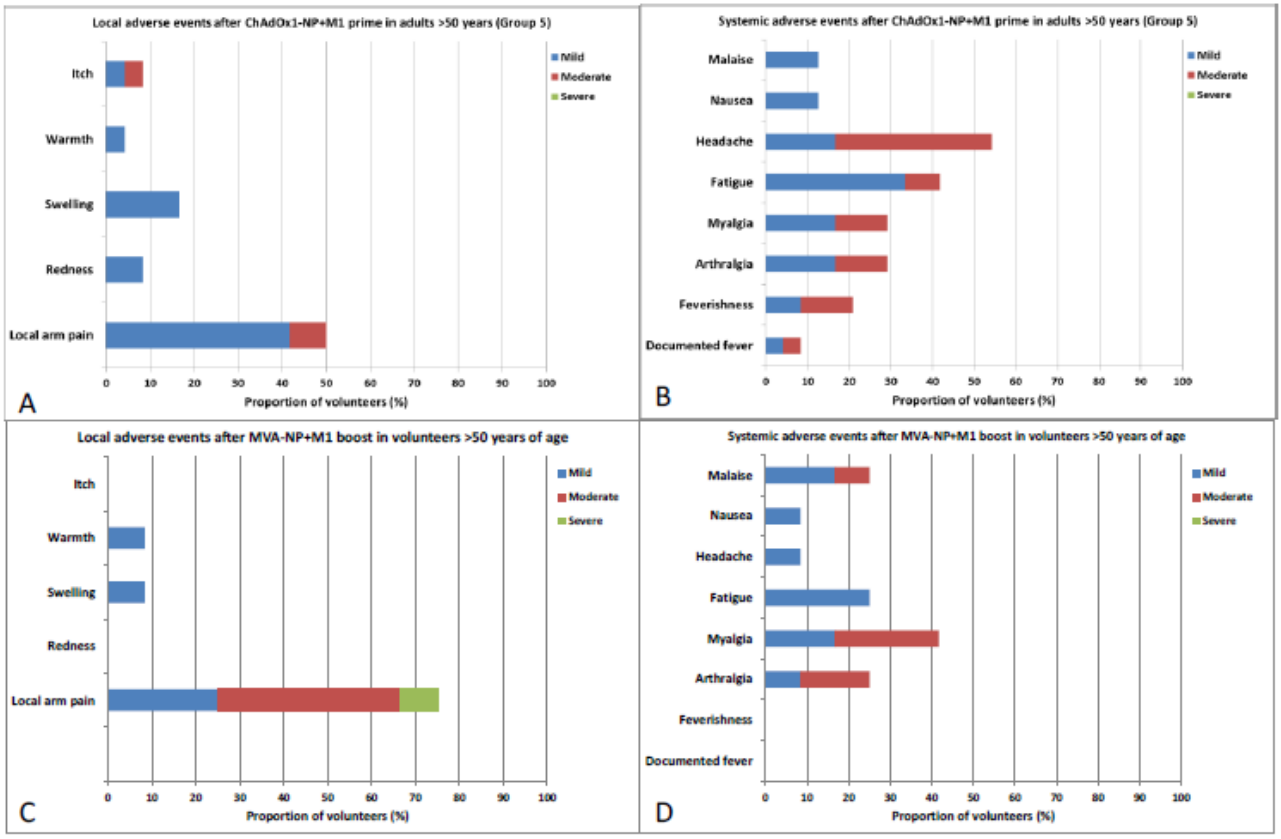

Figure 7. Frequency and severity of systemic and local adverse events after prime vaccination with ChAdOx1 NP+M1 and boost vaccination with MVA NP+M1 in adults >50 years of age (Groups 5, 6). The proportion of participants with mild, moderate and severe solicited local (A) and systemic (B) adverse events after ChAdOx1 NP+M1 vaccination (n=12). The proportion of participants with mild, moderate and severe solicited local (C) and systemic (D) adverse events after MVA NP+M1vaccination vaccination 8 weeks after ChAdOx1 NP+M1 (n=12).

3.4 Rationale

Despite the uncertainties around the role of MAP infection in Crohn's disease, there is a clear and well-established association between MAP infection and CD. Pre-clinical trials in mice and cattle have shown the HAV vaccine is safe and efficacious against MAP infection. The study in cattle was primarily designed to look at a protection role for the vaccine, but it also showed the vaccine reduced bacterial load in an established MAP infection as previously found in mice. The hAd5 HAV prime vaccination with a MVA HAV boost in cattle led to a significant attenuation of MAP microbial load followed by the complete interruption of faecal shedding. To our knowledge, no previous MAP vaccine has been reported to achieve similar results in animal models.

Effects of HAV vaccination on the dynamics of MAP infection have been also shown in blood. All calves receiving an oral MAP challenge developed an established systemic infection. Vaccinated calves were able to significantly reduce or even eradicate the infection at specific tissues. A plausible explanation would be that vaccination has specifically reversed the blinding and dysregulation MAP infection imposes on the immune system.

A novel diagnostic method developed at King's College London revealed that patients with active Crohn's disease tested positive for this chronic enteric pathogen. The hypothesis is that the ChAdOx2 HAV prime vaccine followed by an MVA HAV boost would have similar effects in humans as the ones reported in cattle. There is a potential future application of the vaccine as a therapeutic strategy in humans and the vaccine is expected to have significantly beneficial and long-term disease modifying effects on the clinical course of Crohn's disease.

3.5 Phase Ia Clinical Trial

3.5.1 Phase Ia Design

The phase Ia clinical trial was a first in human open-label dose escalation study to assess the safety and immunogenicity of the candidate Mycobacterium avium subspecies paratuberculosis (MAP) vaccine, ChAdOx2 HAV and MVA HAV boost in healthy participants aged 18-50. Participants were enrolled and doses escalated according to a 3+3 study plan (Dose groups $5x10^9$ vp, 2.5×10^{10} vp and 5×10^{10} vp). Enrolment into the study commenced in March 2017. The study was undertaken at the Centre for Clinical Vaccinology and Tropical Medicine at the Churchill Hospital in Oxford. All vaccines were administered intramuscularly given the favourable safety and immunogenicity profile of this route of administration with viral vector vaccines.

Participants were enrolled in a staggered manner at a lower dose of MVA HAV (5×10^7 pfu). This allowed for interim safety reviews prior to enrolment of participants to receive the full dose of MVA HAV (2×10^8 pfu). Staggered enrolment also applied to the first participants to receive the full dose of MVA HAV.

Ten participants were enrolled in a prime-boost regimen with ChAdOx2 HAV and MVA HAV, following a safety review of the first 3 participants vaccinated with 2×10^8 pfu MVA HAV alone.

Participants were only vaccinated with the prime-boost regimen as the data generated from the individual vaccine groups suggests that was safe to proceed with the proposed doses.
Participants who received the ChAdOx2 based constructs were followed for a longer period of time, considering this was the first use of any ChAdOx2 based constructs. MVA vectored vaccines have been given to thousands of people with no significant safety concerns reported until this date, hence a smaller follow-up period was completed for the MVA only based constructs.

3.5.2 Phase Ia Results

The study demonstrated that ChAdOx2 HAV at a dose of 5×10^{10} vp is safe in healthy participants with an acceptable reactogenicity profile.

Within the 3 dose groups (12 participants), ChAdOx2 HAV was well tolerated, with the majority of adverse events being mild (85%) or moderate (15%) in nature (tables 1 and 2, Figs 6 and 7). The AEs that were considered related to ChAdOx2 HAV included feverishness, headache, myalgia, arthralgia, fatigue, malaise, warmth, pruritus, injection site pain, erythema. Laboratory AEs included mild and transient lymphopaenia in 2 participants (groups 2 and 3) and neutropaenia in 1 volunteer (group 3). One volunteer in group 3 had a moderate and transient thrombocytopenia. The vaccine was immunogenic, inducing significant ELISpot responses at the intermediate dose (see figure 8).

Immunological responses to vaccination with ChAdOx2 HAV in humans were assessed using the interferon-gamma ELISpot assay using freshly-isolated peripheral blood mononuclear cells (PBMC) stimulated with pools of peptides spanning the HAV vaccine construct. Assays were performed prior to vaccination (Day 0) and at one and two months' post vaccination (Day 28 and 56).

Responses to HAV antigens prior to vaccination were low, with a geometric mean response of 109 (95% CI 79-151) spot-forming cells per million PBMC (SFC), which increased to a geomean of 250 SFC (95% CI 107-583) at day 28 taking an average across all dose groups (figure 11). Responses were highest at day 28 in participants immunised with $2.5x10^{10}$ vp and were significantly increased after vaccination only in this dose group. ($p<0.05$, Kruskall-Wallis test with Dunn's multiple comparison test compared with D0 responses). Responses were detected to all HAV antigens at day 28 with geomean responses ranging as follows; AhpC- 56 SFC, Gsd-41SFC, p12-64 SFC, Mpa-52 SFC per million PBMC.

Table 1. Local AEs post ChAdOx2 HAV (all doses combined)

Local Symptoms and Severity	**Number (% of doses)** *Total number of vaccine doses = 12*
Injection site pain	
Mild	6 (50)
Moderate	2 (16.67)
Erythema	
Mild	1 (8.33)
Pruritus	
Mild	1 (8.33)
Warmth	
Mild	4 (33.33)
Swelling	1 (8.33)

Table 2. Systemic AEs post ChAdOx2 HAV (all doses combined)

Systemic Symptoms and Severity	**Number (% of doses)** *Total number of vaccine doses = 12*
Feverishness	
Moderate	1 (8.33)
Pyrexia	0
Arthralgia	
Mild	1 (8.33)
Myalgia	
Mild	3 (25)
Fatigue	
Mild	5 (41.67)
Moderate	1 (8.33)
Malaise	
Mild	2 (16.67)
Moderate	1 (8.33)
Headache	
Mild	6 (50)
Nausea	0

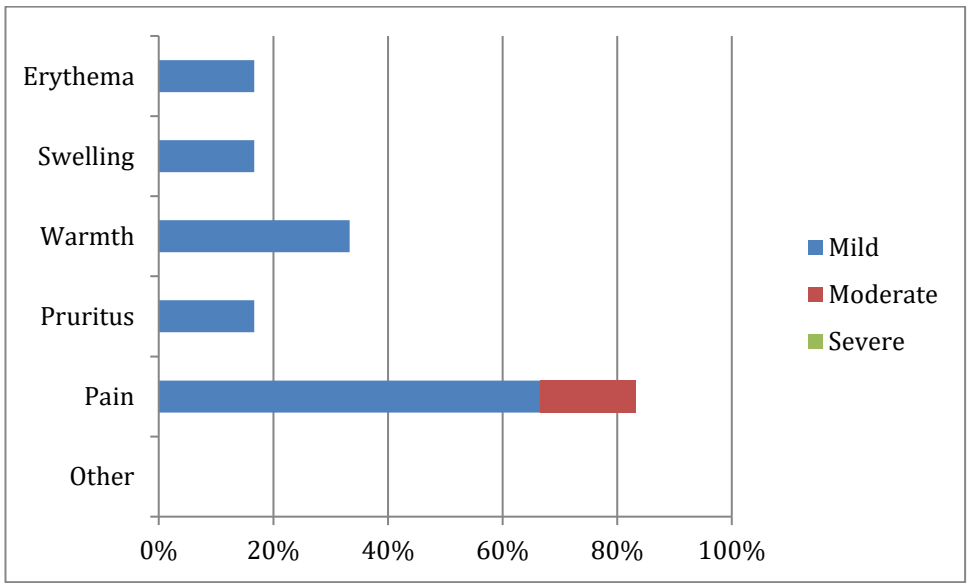

Fig. 8. Proportion of participants reporting local AEs with ChAdOx2 HAV at the 5 x 10^{10} vp dose

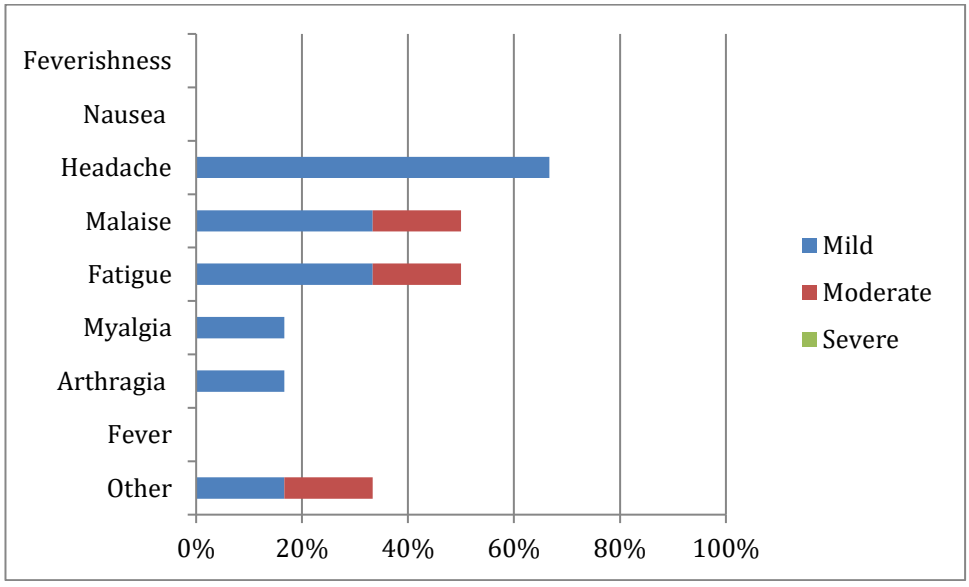

Fig. 9. Proportion of participants reporting systemic AEs with ChAdOx2 HAV at the 5 x 10^{10} vp dose.

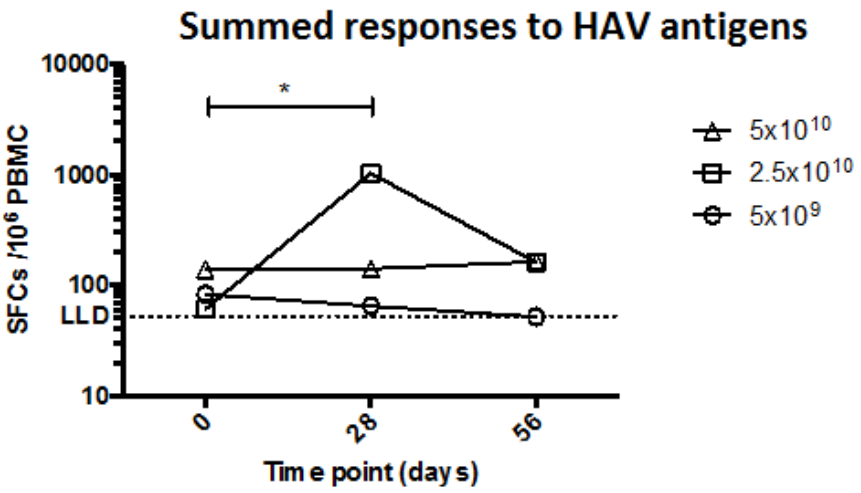

Figure 10. Median summed response to all pools of antigens in the HAV vaccine stratified by dose.* p=0.05 Kruskall-Wallis test, with Dunn's multiple comparison test, comparing the response for each dose group against the average at D0. Lines represent geomeans. B. Responses to individual vaccine antigens at each time point. Lines represent medians

2 further groups have subsequently finished from the Phase 1 incorporating $5x10^7$ pfu (Group 4) and $2x10^8$ pfu (Group 5) of MVA HAV only in healthy adult participants. A summary of the results are described below but no serious adverse events were reported and both groups were signed off by the Local Safety Monitor / Committee Chair and by the Chief Investigator.

Group 4

Solicited AEs after single dose of MVA HAV (5 x 10^7 pfu):

The 7-day data from the e-diaries of all Group 4 participants following their single vaccination has shown no moderate or severe AEs and no SAEs following vaccine administration.

Two participants reported at least one systemic AE after vaccination (feverishness, headache or malaise) but all were mild and short-lived. Local AEs were all mild (pain) and self-limited. All AEs resolved within 24-48hrs.

Unsolicited AEs after single dose of MVA HAV (5 x 10^7 pfu)

Participant HAV-00101023 reported mild (grade 1) 'stiffness in left arm' at D1, where the vaccine was administered. This event resolved within 24h.

Observations:

Observations were taken at each clinic visit from day 0 – 28. All three participants have been reviewed at the day 2 and day 7. One volunteer has been reviewed at day 14 and D28. Observations at all these time-points have been unremarkable.

Laboratory AEs:

Participant HAV-00101032 had a mild (grade 1) lymphopenia (0.91 x109/L) at D2, which resolved by D7.
Participant HAV-00101023 had a mild (grade 1) neutropenia (1.44 x109/L) at D2, which resolved by D7.

For Group 5

Solicited AEs after single dose of MVA HAV (2 x 10^8 pfu):

The 7-day data from the e-diaries of all Group 5 participants following their single vaccination has shown no severe AEs and no SAEs following vaccine administration.

All participants reported systemic AEs after vaccination, but all were mild or moderate and short-lived. Local AEs were all mild and self-limited. All AEs resolved within 24-72hrs.

Unsolicited AEs after single dose of MVA HAV (2 x 108 pfu)

Participant HAV-00101035 reported mild (grade 1) 'sore throat' and 'upset stomach' at D7. This event resolved within 24h.

Participant HAV-00101027 reported mild (grade 1) 'dry skin' at D7. This event resolved within 24h.

All unsolicited AEs reported are unlikely to be attributed to MVA HAV.

Observations:

Observations were taken at each clinic visit from day 0 – 28. All three participants have been reviewed at day 2 and day 7. One volunteer has been reviewed at day 14. Observations at all these time-points have been unremarkable.

Laboratory AEs:

Participant HAV-00101035 had a mild (grade 1) lymphopenia (0.98 x10^9/L) at D2, which resolved by D7.

Participant HAV-00101049 had a mild (grade 1) lymphopenia (0.78 x10^9/L) at D2, which resolved by D7. This participant had a mild (grade 1) neutropenia (1.33 x10^9/L) at D7 but this isn't significantly different than their baseline (1.36 x10^9/L), therefore, this mild neutropenia episode is not related with MVA HAV.

3.5.3 Implications for next study

ChAdOx2 HAV has been demonstrated to be well tolerated and immunogenic in a group of healthy participants. Of the three doses administered in the Phase Ia study, the lowest dose of 5 x 10^9 np is the least immunogenic and can therefore reasonably be omitted from further studies. The intended therapeutic target population for the vaccine is patients with Crohn's disease. Hence, the next study implicated by these results should seek to determine the safety, tolerability and immunogenicity of the ChAdOx2 HAV vaccine in a group of patients with Crohn's disease.

4.0 TRIAL DESIGN

This is a phase Ib open-label dose escalation study to assess the safety, tolerability and immunogenicity of the candidate *Mycobacterium avium* subspecies *paratuberculosis* (MAP) vaccine, ChAdOx2 HAV in patients with active Crohn's disease aged 18-50. Participants will be enrolled and doses will be escalated according to a study plan which will run as outlined in section 8.4.2.2.

4.1 Rationale for selected doses

The design of the study is intended to match the design of the Phase Ia trial as described above but utilising the 2 higher ChAdOx2 HAV vaccine doses of 2.5 x 10^{10} vp and 5 x 10^{10} vp as these doses demonstrated immunogenicity and were both well tolerated. The study will enrol up to 28 participants.

Safety and immunogenicity data generated from participants receiving a single dose of ChAdOx2 HAV in groups 1-2, will be used to inform the dose to be used in the prime-boost group (G5).
The doses of MVA HAV to be used in this study (5x10^7 - 2 x 10^8 pfu) have been chosen in light of reassuring safety and immunogenicity data generated by hundreds of individuals who have safely received MVA vectored vaccines following simian adenovirus vectored vaccines priming (ChAd63 or ChAdOx1) [48, 57, 58].

The optimal dose of MVA has been shown consistently to be 1-2 x 10^8 pfu. Higher doses of MVA (2.5-5 x 10^8 pfu) have been associated with marked reactogenicity, with severe 'flu-like' systemic AEs recorded in a previous study in Oxford. Lower doses of MVA enable an acceptable reactogenicity profile without significantly compromising vaccine immunogenicity.

Clinical studies have shown IM administration to be associated with fewer and short-lived local AEs and no reduction in immunogenicity [48].

4.2 Study groups

	Participants	Dose	Route
Group 1 (n=6)	V1 to V6	ChAdOx2 HAV, 2.5 x10^{10} vp	IM
Group 2 (n=6)	V7 to V12	ChAdOx2 HAV, 5 x10^{10} vp	IM
Group 3 (n=3)	V13 to V15	MVA HAV, 5 x10^7 pfu	IM
Group 4 (n=3)	V16 to V18	MVA HAV, 2 x10^8 pfu	IM
Group 5 (n=10)	V19 to V28	ChAdOx2 HAV, 5 x10^{10} vp followed by MVA HAV at 2 x10^8 pfu (8 weeks apart)	IM

4.3 First participants

The first participant in Group 1 will be vaccinated alone and then reviewed at least 48 hours following vaccination. The chief investigator (CI) and the chair of the Safety Review Committee (SRC) will be asked to provide the decision on whether to proceed after the safety review of the first participant. If there are no safety concerns, another two Group 1 participants will be vaccinated at least one hour apart, and reviewed in a further 48 hours. The CI and the chair of the SRC will be asked to provide the decision on whether to proceed with the vaccination of a further 3 participants in Group 1 and 1 participant in Group 2 following safety review of the previous vaccinated participants. This review will include the results of safety blood tests at day 7 post vaccination.

The same procedure will apply for the first participant in Group 2. He/she will be vaccinated ahead of the other participants. The profile of adverse events and blood laboratory results from that participant will be examined and no other Group 2 participants will be vaccinated until at least 48 hours have elapsed following this participant being vaccinated.

The first participant in group 3 will be vaccinated alone and then reviewed 48 hours following vaccination. The chief investigator (CI) and the chair of the Safety Review Committee will be asked to provide the decision on whether to proceed after the safety review of the first participant. If there are no safety concerns, another two Group 3 participants may be vaccinated at least one hour apart, and reviewed, at least 48 hours later. The CI and the chair of the SRC will be asked to provide the decision on whether to proceed with vaccination at the next highest dose following safety review of the previous vaccinated participants. This review will include the results of safety blood tests at day 7 post vaccination.

The first participant in group 4 will be vaccinated alone and then reviewed at least 48 hours following vaccination. The chief investigator (CI) and the chair of the Safety Review Committee will be asked to provide the decision on whether to proceed after the safety review of the first participant. If there are no safety concerns, another two Group 4 participants may be vaccinated at least one hour apart, and reviewed in at least 48 hours later. After 3 participants in group 4 have been vaccinated and followed up for 7 days, an interim safety review will be performed.

The prime vaccination with ChAdOx2 HAV of group 5 participants will take place on day 0 and the boost vaccination of MVA HAV on day 56, for all participants.

4.4 Duration of study

The total duration of the study will be 20 weeks from the day of enrolment for participants enrolled in groups 1-2. The total duration of the study for participants enrolled in groups 3 and 4 will be 12 weeks from the day of enrolment. The total duration of the study for participants enrolled in group 5 will be 20 weeks from the day of enrolment

4.5 Definition of Start and End of Trial

The start of the trial is defined as the date of consent of the first participant. The end of the trial is the date of the last visit of the last participant.

4.6 Potential Risks for participants

The potential risk to participants is considered as low. The potential risks are those associated with phlebotomy and vaccination.

Phlebotomy:

The maximum volume of blood drawn over the study period (approximately 225mL) should not compromise these otherwise healthy participants. 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 include local effects such as pain, redness, warmth and swelling and systemic effects including a mild self-limiting flu-like illness. As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage may occur, but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason participants will be vaccinated in a clinical area where resuscitation facilities are available.

5.0 OBJECTIVES AND ENDPOINTS

The number of participants has been chosen to generate adequate safety and immunogenicity data to meet these objectives, whilst minimising the number of participants exposed to a new vaccination regimen.

5.1 Primary Objective

To assess the safety and tolerability of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease on no current immunosuppressive therapy when administered alone and in a prime-boost regimen.

5.1.1 Primary Outcome Measures

To define the Maximum Tolerated Dose (MTD) of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease on no current immunosuppressive therapy when administered alone and in a prime-boost regimen. The definition of MTD is defined as per the Group Holding Rules in Section 7.6.1.

The following parameters will also be assessed for all study groups:

- Occurrence of local reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Participants in groups 1-2 will undergo clinical follow up for a further 20 weeks following completion of the vaccination regimen. SAEs will be collected throughout the study.

Participants in groups 3-4 will undergo clinical follow-up for a further 12 weeks following completion of the vaccination regimen. SAEs will be collected throughout the study. Participants in group 5 will undergo clinical follow-up for a further 20 weeks following completion of the vaccination regimen. The duration of follow up reflects the desire to obtain sufficient safety data with the first use of ChAdOx2 HAV, MVA HAV and a prime-boost regimen with ChAdOx2 HAV-MVA HAV in humans with Crohn's Disease. Considering the pre-existing safety data on several other MVA based constructs, participants receiving MVA HAV will be followed by a shorter period.

5.2 Secondary Objectives

To assess the immunogenicity and clinical response of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease on no immunosuppressive therapy when administered alone and in a prime-boost regimen.

5.2.1 Secondary Outcome Measures

Immunogenicity

Measures of immunogenicity may include:

- ELISPOT to enumerate IFN-γ producing T cells

Other exploratory immunology may be carried out in collaboration with other specialist laboratories. This would involve transfer of serum/plasma and/or peripheral blood mononuclear cells (PBMC)/biopsy tissue, but samples would be anonymised. Participants will be consented for this.

Sampling of exploratory immunology responses

20ml of whole blood will be taken at baseline (D0) and days 28 and 56 (groups 1 and 2). For Group 5 10ml of whole blood will be taken at screening, with 30ml taken at baseline (D0) and days 56 and 112 and at least 20ml at days 28 and 84 of the study for use in assays to measure immune responses of various types to the vaccine.

Assessment of clinical response

All participants will undergo an evaluation of Crohn's disease activity at screening and at day 56 for groups 1 – 4. Group 5 will undergo an evaluation of Crohn's disease activity at screening, on day 56 and again on day 112. For Group 5, endoscopic scoring by flexible sigmoidoscopy or colonoscopy will be undertaken by the CD-SES (simple endoscopic score) at screening and at day 112.

Exploratory immunology may be carried out in collaboration with other specialist laboratories. This would involve transfer of serum/plasma and/or peripheral blood mononuclear cells (PBMC)/biopsy tissue, but samples would be anonymised. Participants will be consented for this.

6.0 INVESTIGATIONAL MEDICINAL PRODUCTS

The following vaccination regime, each as single doses, will be given in this study:

1. ChAdOx2 HAV, 2.5 x10^{10} vp
2. ChAdOx2 HAV, 5 x10^{10} vp
3. MVA HAV 5 x10^{7} pfu
4. MVA HAV 2 x10^{8} pfu
5. Prime Boost Regime (doses dependent on the results from Groups 1-4)

6.1 Manufacturing and presentation

6.1.1 Description of ChAdOx2 HAV

ChAdOx2 HAV has been developed and produced at the Jenner Institute, University of Oxford. The ChAdOx2 HAV vaccine consists of the replication-deficient simian adenovirus vector ChAdOx2, containing the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) antigens ahpC/gsd/p12/mpa expressed from the strong CMV IE promoter.

6.1.2 Description of MVA HAV

The vaccine consists of the replication deficient modified vaccinia virus Ankara (MVA) containing the Mycobacterium avium subspecies paratuberculosis (MAP) the AhpC, Gsd, p12 and Mpa genes expressed as a fusion protein.

6.1.3 ChAdOx2 HAV formulation and packaging

ChAdOx2 HAV is manufactured in formulation buffer at a nominal concentration of >1.1 x 10_{11} vp/mL. The drug product is filled into 2mL glass vials with a 13 mm grey bromobutyl rubber freeze- dry stopper (CE Marked, supplied by Adelphi Tubes) and a 13 mm aluminium seal. The nitrogen filled vials are supplied sterile. The containers and closures are tested for compliance with defined specifications. The vials are made from Ph Eur Type 1 glass.

6.1.4 MVA HAV formulation and packaging

MVA HAV is manufactured in formulation buffer at a nominal concentration of 6.9x10^{8} pfu/mL and filled to 0.55 mL with an extractable volume of 0.4 mL.

The drug product is filled into 2mL glass vials with a 13 mm grey bromobutyl rubber freeze-dry stopper (CE Marked, supplied by Adelphi Tubes) and a 13 mm aluminium seal. The nitrogen filled vials are supplied sterile. The containers and closures are tested for compliance with defined specifications. The vials are made from Ph Eur Type 1 glass.

6.2 Supply

ChAdOx2 HAV has been formulated and vialed under Good Manufacturing Practice (GMP) conditions at the CBF, University of Oxford. At the CBF the vaccine will be certified and labelled for the trial by a Qualified Person (QP) before transfer to the clinical site.

MVA HAV has been formulated and vialed under GMP conditions at IDT Biologika. At the CBF the vaccine will be certified and labelled for the trial by a Qualified Person (QP) before transfer to the clinical site.

6.3 Storage

ChAdOx2 HAV and MVA HAV vaccines are stored frozen at a nominal temperature of -80°C.

6.4 Administration of Investigational Medicinal Products

On vaccination day, ChAdOx2 HAV and MVA HAV will be allowed to thaw to room temperature. It will be administered within 1 hour of removal from the freezer. Time of removal from freezer will be recorded. Once thawed, the vaccine will be drawn up into a 1ml syringe and administered intramuscularly into the deltoid of the non-dominant arm (preferentially). All participants will be observed in the Clinical Research Facility for 1 hour (±10 minutes) after vaccination. During administration of the investigational products, Immediate Life Support drugs and resuscitation equipment will be available for the management of anaphylaxis. Vaccination will be performed and the IMP handled according to the relevant SOPs.

6.5 Minimising environment contamination with genetically modified organisms (GMO)

The study will be performed in accordance with UK Genetically Modified Organisms (Contained Use) Regulations (2014). In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, inoculation sites 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 (+15/- 5 minutes) and will be disposed as GMO waste by autoclaving.

7.0 RECRUITMENT AND WITHDRAWAL OF TRIAL PARTICIPANTS

7.1 Participants

Crohn's disease patients will be identified in the Gastroenterology clinics at Guy's and St Thomas' NHS Foundation Hospital.

7.2 Informed consent

All participants will sign and date the informed consent form before any study specific procedures are performed. The information sheet will be made available to the participant at least 24 hours prior to the screening visit. At the screening visit, the participant 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 participant may withdraw from the study at any time
- The participant 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 an investigational vaccine
- There is no direct benefit from participating
- The participant's GP will be contacted to corroborate their medical history
- The participant's blood samples taken as part of the study will be stored indefinitely and anonymised samples may be transferred to UK laboratory for analysis on behalf of the Sponsor.

The aims of the study and all tests to be carried out will be explained. The participant 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 medical records.

7.3 Inclusion and exclusion criteria

This study will be conducted in patients with active Crohn's disease, who meet the following inclusion and exclusion criteria:

7.3.1 Inclusion criteria

1. Age 18 to 50 years.
2. Confirmed diagnosis of Crohn's disease diagnosed according to standard clinical, endoscopic, radiological or histological criteria.
3. Mild to moderately active Crohn's inflammation as defined by one or more of a raised CRP >10mg/L, faecal calprotectin >150 and a CDAI >150 but <320 (Groups 1-5)
4. Active Crohn's inflammation in at least one segment of ileum or colon on a colonoscopy or flexible sigmoidoscopy (Group 5 only)
5. No immunomodulatory treatment (thiopurines, methotrexate, tacrolimus, anti-TNFalpha antibody therapy, anti-alpha4beta7 antibody therapy, anti-p40 antibody therapy) currently or within the last 3 months.
6. Able to comply with all study requirements.
7. For females only, willingness to practice continuous effective contraception (see below) during the study and a negative pregnancy test on the day(s) of screening and vaccination.
8. Agreement to refrain from blood donation during the course of the study.
9. Provide written informed consent.

7.3.2 Exclusion criteria

1. Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the study period.
2. Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data.
3. Prior receipt of an adenoviral vectored vaccine (or any other vaccine) in the last 28 days.
4. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.
5. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections.
6. Any immunosuppressive medication currently or within the preceding 3 months including corticosteroids (except inhaled steroid or topical steroid), thiopurines, methotrexate, tacrolimus and any biological therapy.
7. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine (e.g. Egg allergy)
8. Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
9. Any history of anaphylaxis in relation to vaccination.
10. Unable to provide written informed consent.
11. Pregnancy, lactation or willingness/intention to become pregnant during the study.

12. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
13. History of serious psychiatric condition likely to affect participation in the study.
14. Bleeding disorder (e.g. Factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venipuncture.
15. Any other serious chronic illness requiring hospital specialist supervision.
16. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week.
17. Suspected or known injecting drug abuse in the 5 years preceding enrolment.
18. Seropositive for hepatitis C (antibodies to HCV).
19. Seropositive for hepatitis B surface antigen (HBsAg).
20. Any clinically significant abnormal finding on screening biochemistry or hematology blood tests, urinalysis, or a positive test for SARS-COV-2 (Covid-19) at screening.
21. Any other significant disease, disorder or finding which may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to take part in the study or impair interpretation of the study data.

7.3.3 Effective contraception for female participants

Female participants are required to use an effective form of contraception during the course of the study (i.e. until their final follow up visit). Male participants with female partners of child-bearing potential are not required to use barrier contraception whilst taking part in this study as the risk of excretion of the vaccine is negligible.

Acceptable forms of contraception for female participants include:

- Established use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Total abdominal hysterectomy
- Barrier methods of contraception (condom or occlusive cap with spermicide)
- Male sterilisation, if the vasectomised partner is the sole partner for the participant.
- True abstinence: when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception

7.3.4 Criteria for postponement of vaccination

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 participant may be vaccinated at a later date, or withdrawn at the discretion of the Investigator. The participant must be followed until resolution of the event as with any adverse event:

- 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.
- Participant has a temperature reading of >37.5°C (99.5°F) on the day of vaccination.

7.3.5 Withdrawal of Participants

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a participant 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 Sponsor, Regulatory Authority, Research Ethics Committee and Safety Review Committee may stop the trial at any time. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

Administrative decision by the Investigator.

- Participant becomes Ineligible during the trial in the Investigator's opinion, for any reason.
- Participant non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

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 agreement of the participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned. Any participant who is withdrawn from the study may be replaced, if that is possible within the specified time frame. The chair of the Safety Review Committee may recommend withdrawal of participants. Any participant who fails to attend for two or more follow-up visits during the study will be deemed to have withdrawn from the study.

If a participant withdraws from the study, blood samples collected before their withdrawal from the trial will be used/ stored unless the participant specifically requests otherwise.

In all cases of participant withdrawal, excepting those of complete consent withdrawal, long-term safety data collection, including some procedures such as safety bloods, will continue as appropriate if participants have received one or more vaccine doses.

7.4 Compliance with Dosing Regime

All vaccinations will be administered by the Investigator and recorded in the CRF. The study medication will be at no time in the possession of the participant and compliance will not, therefore, be an issue.

7.5 Pregnancy

Should a participant become pregnant during the trial, she will be followed up as other participants and in addition will be followed until pregnancy outcome. Pregnancy will be reported to the KHP-CTO immediately the Investigator team becomes aware of the Pregnancy, using the SAE report form.

7.6 Safety Holding Rules

'Solicited adverse events' are those listed as foreseeable adverse events in section 8.4.2.1 of the protocol, occurring within the first 7 days after vaccination (day of vaccination and six subsequent days). 'Unsolicited adverse events' are adverse events other than the foreseeable AEs occurring within the first 7 days or any AEs occurring after 7 days post vaccination.

7.6.1 Group Holding Rules

For safety reasons the first participant to receive a new vaccine dose will be vaccinated alone and we will wait 48 hours before vaccinating subsequent participants. Two further participants may be vaccinated 48 hours after the first, and then at least another 48 hours gap will be left before vaccinating the rest of the participants receiving that dose of vaccine.

If the first three 2.5 x 10^{10} vp doses are deemed safe (none of the first three participants experiences a Grade 3 severe adverse reaction lasting more than 48 hours), the first patient in the higher dose of 5 x 10^{10} vp will be vaccinated at the same time as the remaining 3 at the lower dose. Two further participants may be vaccinated at the higher dose, 48 hours after the first, and then at least another 48 hours gap will be left before vaccinating the rest of the participants at the 5 x 10^{10} vp dose.

If 2 or more participants in groups 1-2 experience Grade 3 severe adverse reactions lasting more than 48 hours at a given dose, then no further participants will be vaccinated at that dose or a higher dose and the previously tolerated dose will be recorded as the Maximum Tolerated Dose (MTD).

The group holding rules are as follows (groups 1-5)

- **Solicited local adverse events:**
    - If 2 vaccinations in a group are followed by the same Grade 3 solicited local adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >48 hrs.
- **Solicited systemic adverse events:**
    - If 2 vaccinations in a group are followed by the same Grade 3 solicited systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >48 hrs.
- **Unsolicited adverse events:**
    - If 2 vaccinations in a group are followed by the same Grade 3 unsolicited adverse event beginning within 7 days after vaccination that is considered related to vaccination and persists at Grade 3 for >48 hrs.
- **Laboratory adverse event:**
    - If 2 vaccinations in a group are followed by the same Grade 3 laboratory adverse event beginning within 7 days after vaccination and persists at Grade 3 for >72 hrs.
- **A serious adverse event considered possibly, probably or definitely related to vaccination occurs**
- **Death occurs**
- **A life-threatening reaction occurs**

If a holding rule has been met and following an internal safety review it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The internal safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.

- If appropriate, additional screening or laboratory testing for other participants to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The UK Competent Authority (MHRA) and approving Research Ethics Committee will be notified if the trial is terminated early.

All vaccinated participants will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

7.6.2 Individual Holding Rule – Group 5 only

In addition to the above stated group holding rules, stopping rules for individual participants in groups 5 will apply which are indications to withdraw individuals from further vaccinations - not applicable to participants in groups 1-4 who are scheduled to receive only a single vaccination.
If any of the events listed below occur and are considered possibly, probably or definitely related to vaccination the volunteer will be withdrawn from further vaccination.

- Local reactions: Injection site ulceration, abscess or necrosis
- Systemic solicited adverse events: the volunteer develops a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related to vaccination within 2 days after vaccination (day of vaccination and one subsequent day) and persisting continuously at Grade 3 for > 72hrs
- Laboratory AEs: the volunteer develops a Grade 3 laboratory adverse event considered possibly, probably or definitely related to vaccination within 7 days after vaccination and persisting continuously at Grade 3 for > 72hrs (site-specific laboratory AE reference ranges).
- Unsolicited adverse events:
    - the volunteer has a Grade 3 adverse event considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs.
    - the volunteer has a serious adverse event considered possibly, probably or definitely related to vaccination.
    - the volunteer has an acute allergic reaction or anaphylactic shock following the administration of an investigational product.

As per section 7.3.5, if a volunteer has an acute illness (moderate or severe illness with or without fever) or a fever (temperature greater than 37.5°C) at the scheduled time of administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated participants will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this. In addition to these pre-defined criteria, the study can be put on hold upon advice of the, Chief Investigator, Study Sponsor, regulatory authority, Ethical Committee(s) or Safety Review Committee, for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the participants or the reliability of the data.

8.0 TREATMENT OF TRIAL PARTICIPANTS

This section describes the clinical procedures for evaluating study participants and follow-up after administration of study vaccine.

8.1 Study procedures

All participants in groups 1 and 2 will have the same schedule of clinic attendances and procedures as indicated in the schedules of attendance (Table 4). Participants in groups 3 and 4 will have the same schedule of clinic attendances and procedures as indicated in the schedules of attendance (Table 5). Participants in group 5 will have the schedule of clinic attendances and procedures as indicated in the schedules of attendance (Table 6)

The total volume of blood donated during the study will be approximately 109mL for groups 1 and 2, 49mL for groups 3 and4 and 245mL for group 5. Additional visits or procedures may be performed at the discretion of the investigators, e.g., further medical history and physical examination, urine microscopy in the event of positive urinalysis or additional blood tests if clinically relevant.

8.2 Observations

Heart rate, blood pressure and temperature will be measured at the time-points indicated in the schedule of procedures and may also be measured as part of a physical examination if indicated at other time-points.

8.3 Blood & Urine Tests

Blood will be drawn for the following laboratory tests and processed at the local hospital site clinical laboratory other than in the case of Exploratory Immunology which will be carried out in collaboration with other specialist laboratories:

- **Haematology;** Full Blood Count (White cell count, Red cell count, Haemoglobin, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets, Haematocrit, Mean Cell Volume, Mean Cell Haemoglobin, Erythrocyte Sedimentation Rate).
- **Biochemistry;** Liver Function Tests (Albumin, ALT, ALP, AST, Bilirubin, GGT, Ferritin, CRP), Thyroid Function Tests (FT3, FT4, TSH), Renal Function Tests (e-GFR, Sodium, Potassium, Creatinine, Urea)
- **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses)
- **SARS-COV-2 (Covid-19);** test at screening & on day of vaccination visits
- **Immunology;** Human Leukocyte Antigen (HLA) typing

Exploratory Immunology; Immunogenicity will be assessed by a variety of immunological assays. This may include ex vivo ELISpot assays for interferon gamma and flow cytometry assays, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis, other antibody assays, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies amongst others may be performed at the discretion of the Investigators.

Urinalysis; Urine will be tested for protein, blood and glucose at screening. For female participants only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening, immediately prior to vaccination and as detailed in tables 4, 5 & 6.

Immunological assays will be conducted according to local standard practice. Participants will be informed that there may be leftover samples of their blood/biopsies (after all testing for this study is completed), and that such samples may be stored indefinitely for possible future research, including genotypic testing of genetic polymorphisms potentially relevant to vaccine immunogenicity. Participants will be able to decide if they will permit such future use of any leftover samples. With the participants' informed consent, any leftover cells, urine, biopsies, and serum/plasma will be frozen indefinitely for future analysis of vaccine-related responses. If a participant elects not to permit this, all of that participant's leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

8.4 Study visits

The study visits and procedures will be undertaken by one of the clinical trials team. The procedures to be included in each visit are documented in the schedule of attendances (Tables 4-6). Each visit is assigned a time-point and a window period, within which the visit will be conducted.

8.4.1 Screening visit

All potential participants will have a screening visit, which may take place up to 30 days prior to vaccination. Informed consent will be taken before screening, as described in section 7.2. If consent is obtained, the procedures indicated in the schedule of attendances will be undertaken including a medical history, physical examination and blood tests.

The participant's general practitioner will be contacted with the written permission of the participant after screening to ascertain any significant medical history and as notification that the participant has volunteered for the study. Abnormal clinical findings from the urinalysis or blood tests at screening will be assessed by the lead clinician according to the relevant SOP. Abnormal blood tests following screening will be assessed according to site-specific laboratory reference ranges. Any abnormal test result deemed clinically significant may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the participant will be informed and appropriate medical care arranged with the permission of the participant.

The eligibility of the participant will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

The Principal Investigator or delegate, will confirm eligibility of each participant in the case report form prior to day 0 activities being undertaken. If eligible, a day 0 visit will be scheduled for the participant to receive the vaccine.

Group 5 patients will undergo an index colonoscopy or flexible sigmoidoscopy (chosen according to the previously documented site of Crohn's disease) to assess the activity of their disease at screening. Crohn's disease activity assessments will also be assessed by CDAI score at the screening visit. Each procedure will be undertaken according to standard clinical endoscopy procedures at Guy's & St. Thomas' NHS Trust and will be performed in the Endoscopy Unit at the St. Thomas' Hospital site. Separate clinical written informed consent will be obtained prior to each procedure. Preparation of the bowel will be given according to the local SOP using either an osmotic laxative preparation and dietary schedule prior to colonoscopy or a phosphate enema on the day, immediately prior to flexible sigmoidoscopy. Standard sedation will be used according to patient preference using intravenous fentanyl and midazolam according to local procedure. Where necessary for clinical evaluation, biopsy samples will be sent to the Histopathology laboratory at St. Thomas' Hospital for routine analysis of histology. Some of the tissue sample may also be used for exploratory analysis.

8.4.2 Day 0: Enrolment and vaccination visit

Participants will be considered enrolled in the study at time of consent. Before vaccination, the eligibility of the participant will be reviewed. Pulse, blood pressure and temperature will be observed and if necessary, a medical history and physical examination may be undertaken to determine need to postpone vaccination depending on criteria listed in section 7.3.5. Vaccinations will be administered as described below.

8.4.2.1 Vaccination

All vaccines will be administered intramuscularly to the deltoid of the non-dominant arm (preferentially) according to SOP HAV002-001. The injection site will be covered with a sterile dressing and the participant will stay in the Clinical Research Facility for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (+/- 10 minutes). An oral thermometer, tape measure and diary card (paper or electronic) will be given to each participant, with instructions on use.

Paper diary cards will collect information on the timing and severity of the following solicited AEs:

Table 3. *Solicited AEs as collected on post vaccination diary cards*

Local solicited AEs	Systemic solicited AEs
Pain	Fever
Redness	Feverishness/Chills
Warmth	Joint pains
Itch	Muscle pains
	Fatigue
	Headache
	Malaise
	Nausea

Participants will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

8.4.2.2 Sequence of Enrolment and Vaccination of Participants (groups 1 and 2)

The first participant in Group 1 will be vaccinated alone and then reviewed 48 hours following vaccination. The chief investigator (CI) and the chair of the Safety Review Committee will be asked to provide the decision on whether to proceed after the safety review of the first participant. If there are no safety concerns, another two Group 1 participants will be vaccinated at least one hour apart, and reviewed in a further 48 hours. The CI and the chair of the SRC will be asked to provide the decision on whether to proceed with the vaccination of a further 3 participants in Group 1 and 1 participant in Group 2 following safety review of the previous vaccinated participants. This review will include the results of safety blood tests at day 7 post vaccination.

The same procedure will apply for the first participant in Group 2. He/she will be vaccinated ahead of the other Group 2 participants. The profile of adverse events from that participant will be examined and no other participants in Group 2 will be vaccinated until at least 48 hours have elapsed following this participant being vaccinated.

Therefore, the possible numbers of participants to be vaccinated, assuming the adverse event profile allows progression to the high dose is 6 in each group. All participants will be issued with the telephone number of the investigator and encouraged to contact the investigators if there are any problems.

After all participants in Group 2 have been vaccinated and followed up for 7 days, an interim safety review of the vaccine ChAdOx 2 HAV will be performed and the CI and the chair of the SRC will be asked to provide the decision on whether to proceed with the vaccination of Group 5 participants.

8.4.2.3 Sequence of Enrolment and Vaccination of Participants (groups 3 and 4)

The first participant in group 3 will receive 5 x10^7 pfu of MVA HAV. This participant will be vaccinated ahead of any other Group 3 participants and the profile of adverse events will be examined. No other participants in Group 3 will be vaccinated until at least 48 hours has elapsed following the first participant being vaccinated. The CI and the chair of the Safety Review Committee will be asked to provide the decision on whether to proceed after the safety review of the first participant. Provided no serious adverse reactions have occurred then a further two participants in Group 3 will be vaccinated.

The CI and the chair of the SRC will be asked to provide the decision on whether to proceed with vaccination at the next highest dose following safety review of the previous vaccinated participants in Group 3. This review will include the results of safety blood tests at day 7 post vaccination.

The first participant in group 4 (2 x 10^8 pfu of MVA HAV) will be vaccinated alone and then reviewed 48 hours following vaccination. The Chief Investigator (CI) and the Chairperson of the Safety Review Committee (SRC) will be asked to provide the decision on whether to proceed after the safety review of the first Group 4 participant. If there are no safety concerns, another two Group 4 participants may be vaccinated at least one hour apart, and reviewed in a further 48 hours. After 3 participants in group 4 have been vaccinated and followed up for 7 days, an interim safety review of the vaccine MVA HAV will be performed and the CI and the chair of the SRC will be asked to provide the decision on whether to proceed with the vaccination of Group 5 participants.

8.4.2.4 Sequence of Enrolment and Vaccination of Participants in Group 5

Following the interim safety review of the vaccine ChAdOx2 HAV and the CI and the Safety Review Committee having at that time provided the decision on whether to proceed with vaccinations in group 5, 10 participants will be vaccinated with 5 x 10^{10}vp ChAdOx2 HAV. The ChAdOx2 HAV vaccination will be followed 8 weeks later by 2 x 10^8 pfu MVA HAV vaccination provided at that stage the interim review of the MVA HAV vaccine has been performed, as referred to in 8.4.2.3 above and its use in Group 5 participants sanctioned by the CI and the chair of the SRC.

8.4.2.5 Subsequent visits (groups 1-4): day 2,7, 14, 28, 56

Follow-up visits will take place 48 hours after vaccination and on days 7, 14, 28, 56 (groups 1 to 4. Participants will be assessed for local and systemic adverse events, using diary cards (paper), interim history, physical examination and blood tests at these time points as detailed in the schedule of attendances. Blood will also be taken for exploratory immunology on visits 6 and 7 (groups 1 and 2)

If participants experience adverse events (laboratory or clinical), which are determined to require further close observation, the participant may be admitted to a NHS Hospital for observation and further medical management under the care of the Consultant on call. If the duration or outcome of this hospital admission meets the SAE criteria, this will be reported to the KHP-CTO immediately.

8.4.2.6 Subsequent visits (group 5): days 2, 7, 14, 28, 56, 58, 63, 70, 84 and112

Follow-up visits in group 5 will take place 48 hours and 7, 14, 28 and 56 days after prime vaccination with ChAdOx2 HAV.

The initial ChAdOx2 HAV vaccination day (Day 0) will include assessment of vital signs, prior adverse events, medical history and a physical examination. A 42ml blood draw will be taken for biochemistry, HLA typing and exploratory immunology. Women of childbearing potential will undertake a pregnancy test at day 0. After vaccination administration, patients will be observed for 1 hour for any adverse events as per local IMP protocols. Diary cards will be provided at Day 0.

Visits 3-6 will involve assessment of vital signs, ascertainment of adverse events and a blood draw for biochemistry & haematology. Blood will also be taken for exploratory immunology on visit 6. Diary cards will be collected at day 28. Participants in Group 5 will undergo a test for SARS-CoV-2 in advance of the boost vaccination visit.

The boost vaccination visit (Day 56 – visit 7) will involve assessment of vital signs, ascertainment of adverse events, Crohn's disease activity assessments, a medical history review and physical examination.

A 42ml blood draw will be taken for biochemistry, HLA typing and exploratory immunology. Women of childbearing potential will undertake a pregnancy test at day 56. After vaccination administration, patients will be observed for 1 hour for any adverse events as per local IMP protocols. Diary cards will be provided again at this time point.

Visits 8-12 will involve assessment of local and systemic adverse events, interim history, physical examination and blood tests at the time points as detailed in the schedule of attendances. Blood will also be taken for exploratory immunology on visits 11 and 12. Diary cards will be collected at day 84..

<u>Endoscopy</u>

Group 5 patients will undergo an index colonoscopy or flexible sigmoidoscopy (chosen according to the previously documented site of Crohn's disease) at screening to assess the activity of their disease and at day 112 (visit 12). Crohn's disease activity assessments will also be assessed by CDAI score on day 112. Each procedure will be undertaken according to standard endoscopy local SOP at Guy's & St. Thomas' NHS Trust and will be performed in the Endoscopy Unit at the St. Thomas' Hospital site. Separate clinical written informed consent will be obtained prior to each procedure. Preparation of the bowel will be given according to the local SOP using either an osmotic laxative preparation and dietary schedule prior to colonoscopy or a phosphate enema on the day, immediately prior to flexible sigmoidoscopy. Standard sedation will be used according to patient preference using intravenous fentanyl and midazolam according to local SOP. Where necessary for clinical evaluation, biopsy samples will be sent to the Histopathology laboratory at St. Thomas' Hospital for routine analysis of histology. Some of the tissue sample may also be used for exploratory analysis.

8.4.2.7 Follow-up telephone call

A final review will be conducted by telephone approximately 20 weeks after vaccination for participants enrolled in groups 1 and 2.Participants in groups 3 and 4 will have their final review by telephone approximately 12 weeks after vaccination. Participants in group 5 will have their final review by telephone approximately 20 weeks after their first vaccination (12 weeks after their second vaccine). Any SAEs occurring since the previous visit will be assessed.

Table 4. *Schedule of procedures for study for groups 1 and 2*

Attendance No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Visit	**Screening**	**Vaccination Visit**	**Follow Up 1**	**Follow Up 2**	**Follow Up 3**	**Follow Up 4**	**Follow Up 5**	**Follow Up 6**
Timeline (days)**	**<30**	**0**	**Day 2**	**Week 1 (Day 7)**	**Week 2 (Day 14)**	**Week 4 (Day 28)**	**Week 8 (Day 56)**	**Week 20 (Day 140)**
Time window (days)			**± 1**	**± 1**	**± 2**	**± 7**	**± 7**	**± 7**
Informed consent	**x**							
Review contraindications, concomitant medication, inclusion and exclusion criteria	**x**	**x**						
Vaccination		**x**						
Vital signs ^	**x**	**x**	**x**	**x**	**x**	**x**	**x**	
Ascertainment of adverse events ***		**x**	**x**	**x**	**x**	**x**	**x**	**x**
Diary cards provided		**x**						
Diary cards collected						**x**		
Telephone Contact								**x**
Crohn's disease activity assessments	**x**						**x**	
Medical history	**x**							
Physical examination	**x**	**x**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	
Biochemistry, Haematology (ml)	**8**	**8**	**8**	**8**		**8**		
Exploratory immunology (ml)		**20**				**20**	**20**	
Urinalysis	**x**							
Urinary β-HCG (women only)	**x**	**x**						
HLA typing (ml)		**4**						
HBsAg, HCV Ab, HIV serology (ml)	**5**							
SARS-CoV-2 test	**x**	**x**						
Total blood volume per visit (ml)	**13**	**32**	**8**	**8**	**0**	**28**	**20**	**0**

*(X) = if considered necessary ^ = Vital signs includes pulse, blood pressure and temperature; $ = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests. £ = Exploratory immunology includes ex vivo ELISPOT responses to interferon gamma ** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window. *** Redness and swelling will be assessed by the clinical team following vaccination at days 0, 2 and 7.*

Table 5. *Schedule of procedures for study groups 3 and 4*

Attendance No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Visit	**Screening**	**Vaccination Visit**	**Follow Up 1**	**Follow Up 2**	**Follow Up 3**	**Follow Up 4**	**Follow Up 5**	**Follow Up 6**
Timeline (days)**	**<30**	**0**	**Day 2**	**Week 1 (Day 7)**	**Week 2 (Day 14)**	**Week 4 (Day 28)**	**Week 8 (Day 56)**	**Week 12 (Day 84)**
Time window (days)			**± 1**	**± 1**	**± 2**	**± 7**	**± 7**	**± 7**
Informed consent	**x**							
Review contraindications, concomitant medication, inclusion and exclusion criteria	**x**	**x**						
Vaccination		**x**						
Vital signs ^	**x**	**x**	**x**	**x**	**x**	**x**	**x**	
Ascertainment of adverse events ***		**x**	**x**	**x**	**x**	**x**	**x**	**x**
Diary cards provided		**x**						
Diary cards collected						**x**		
Telephone Contact								**x**
Crohn's disease activity assessments	**x**						**x**	
Medical history	**x**							
Physical examination	**x**	**x**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	
Biochemistry, Haematology (ml)	**8**	**8**	**8**	**8**		**8**		
Exploratory immunology (ml)								
Urinalysis	**x**							
Urinary β-HCG (women only)	**x**	**x**						
HLA typing (ml)		**4**						
HbsAg, HCV Ab, HIV serology (ml)	**5**							
SARS-CoV-2 test	**x**	**x**						
Total blood volume per visit (ml)	**13**	**12**	**8**	**8**	**0**	**8**	**0**	**0**

*(X) = if considered necessary ^ = Vital signs includes pulse, blood pressure and temperature; $ = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests. £ = Exploratory immunology includes ex vivo ELISPOT responses to interferon gamma. ** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window *** Redness and swelling will be assessed by the clinical team following vaccination at days 0, 2 and 7.*

Table 6. *Schedule of procedures for study Group 5*

Attendance No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Visit	**Screening**	**Vaccination Visit**	**Follow Up 1**	**Follow Up 2**	**Follow Up 3**	**Follow Up 4**	**Boost Visit**	**Follow Up 6**	**Follow Up 7**	**Follow Up 8**	**Follow Up 9**	**Follow Up 10**	**Follow Up 11**
Timeline (days)**	**<30**	**0**	**2**	**7**	**14**	**28**	**56**	**58**	**63**	**70**	**84**	**112**	**140**
Time window (days)			**± 1**	**± 1**	**± 2**	**± 7**	**± 7**	**± 1**	**± 1**	**± 2**	**± 7**	**± 7**	**± 7**
Informed consent	**x**												
Review contraindications, concomitant medication, inclusion and exclusion criteria	**x**	**x**											
Vaccination		**x**					**x**						
Vital signs ^	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	
Ascertainment of adverse events ***		**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Diary cards provided		**x**					**x**						
Diary cards collected						**x**					**x**		
Telephone Contact													**x**
Endoscopy	**x**											**x**	
Crohn's disease activity assessments	**x**						**x**					**x**	
Medical history	**x**												
Physical examination	**x**	**x**	**(x)**	**(x)**	**(x)**	**(x)**	**x**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	
Biochemistry, Haematology (ml) $	**8**	**8**	**8**	**8**		**8**	**8**	**8**	**8**		**8**		
Exploratory immunology (ml)	**10**	**30**				**30******	**30**				**30******	**30**	
Urinalysis	**x**												
Urinary β-HCG (women only)	**x**	**x**					**x**						
HLA typing (ml)		**4**					**4**						
HBsAg, HCV Ab, HIV serology (ml)	**5**												
SARS-CoV-2 test	**x**	**x**					**x**						
Total blood volume per visit (ml)	**23**	**42**	**8**	**8**	**0**	**38**	**42**	**8**	**8**	**0**	**38**	**30**	

*(X) = if considered necessary ^ = Vital signs includes pulse, blood pressure and temperature; $ = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests. £ = Exploratory immunology includes ex vivo ELISPOT responses to interferon gamma ** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window *** Redness and swelling will be assessed by the clinical team following vaccination at days 0, 2 and 7. ****at least 20m to be taken, an additional 10ml may be taken at the discretion of the investigator*

9.0 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study. Participants will be encouraged to contact the investigator team to report any severe adverse events between visits.

9.1 Interim Safety Review

Interim safety reviews with the Chair of the Safety Review Committee are planned after the first participant in groups 1-4. Safety reviews with the SRC are planned prior to dose escalation of each vaccine administration. The Chair of the SRC will be consulted to provide a review of safety data and adverse events in the first participant of groups 1-4 before proceeding to the next participant in group 1-4. Interim safety data may also be made available to manufacturers (in coded format) as specified in the contract with the manufacturer(s). Safety reviews will include an assessment of the profile and severity of adverse events reported.

9.2 Definitions

9.2.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a participant, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

9.2.2 Adverse Reaction (AR)

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

9.2.3 Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., IB for an unapproved IMP).

9.2.4 Serious Adverse Event (SAE)

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

- Death
- Life-threatening event (i.e., the participant 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 severe 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 hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the participant 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 hospitalisation.
- Congenital anomaly or birth defect.

9.2.5 Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting Investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

9.2.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product, as set out in the Investigator Brochure.

9.2.7 Important Medical Events (IME) & Pregnancy

Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.

9.3 Foreseeable Adverse Reactions

The foreseeable ARs following vaccination with ChAdOx2 HAV and MVA HAV include; local injection site pain, local erythema, local warmth, local swelling, local pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea.

9.4 Expected Serious Adverse Events

No serious adverse events are expected in this study.

9.5 Causality Assessment

For every AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI-delegated clinician. An intervention-related AE refers to an AE for which there is a 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 therapy (Table 7).

Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to vaccination will be considered and investigated. Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator.

Table 7. *Guidelines for assessing the relationship of vaccine administration to an AE.*

0	**No Relationship**	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
1	**Unlikely**	Unlikely temporal relationship to study product ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study product.
2	**Possible**	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	**Probable**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
4	**Definite**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

9.6 Reporting Procedures for All Adverse Events

All local and systemic AEs occurring in the 28 days following each vaccination observed by the Investigator or reported by the participant, whether or not attributed to study medication, will be recorded.

All AEs that result in a participant's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the participant consents to this). Serious adverse events (SAEs) will be collected throughout the entire trial period.

9.6.1 Reporting Procedures for Serious Adverse Events

HAV Vaccines Ltd have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP-CTO). All SAEs, SARs and SUSARs, will be reported immediately by the Investigator site (and certainly no later than 24hrs) to the KHP-CTO. IME's & Pregnancies will also be reported immediately using the same SAE form.

9.6.2 Reporting Procedures for SUSARS

The KHP-CTO will report SUSARs to the UK regulatory authority (MHRA), The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

9.6.3 Development Safety Update Report

A Development Safety Update Report (DSUR) will be submitted by the KHP-CTO on behalf of the Sponsor, to the competent authority and ethical committee on the anniversary of the first approval date from the regulatory authority for each IMP - the Development International Birthdate (DIBD).

9.7 Assessment of severity

The severity of clinical and laboratory adverse events will be assessed according to the scales in Table 8-10.

Table 8. *Severity grading criteria for local adverse events for intramuscular injections.*

Adverse Event	Grade	Intensity
Erythema at injection site*	1	>3 -≤50 mm
	2	>50 -≤100 mm
	3	>100 mm
Swelling at injection site	1	>3 -≤20 mm
	2	>20 -≤50 mm
	3	>50 mm
Ulceration/necrosis of skin at injection site	1	-
	2	-
	3	Any

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

Table 9. Severity grading criteria for physical observations

	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Fever (oral)	37.6°C -38.0°C	38.1°C –39.0°C	>39.0°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130
Bradycardia (bpm)**	50 – 54	40 – 49	<40
Systolic hypertension (mmHg)	141 - 159	160 – 179	≥180
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80
Diastolic hypertension (mmHg)	91 - 99	100 – 109	≥110

*Taken after ≥10 minutes at rest
**Use clinical judgement when characterising bradycardia among some healthy participant populations, for example, conditioned athletes.
***Only if symptomatic (e.g. dizzy/ light-headed)

Table 10. *Severity grading criteria for local and systemic AEs.*

GRADE 0	None: Symptom not experienced (Grade 0 will not be reported on the eCRF, nor sent to the SRC for review)
GRADE 1	Mild: Short-lived or mild symptoms; medication may be required. No limitation to usual activity
GRADE 2	Moderate: Mild to moderate limitation in usual activity. Medication may be required.
GRADE 3	Severe: Considerable limitation in activity. Medication or medical attention required.

9.8 Procedures to be followed in the event of abnormal findings

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated tests. In general, participants will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the site-specific laboratory adverse event tables (stored in TMF or ISF). Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed and appropriate medical care arranged as appropriate and with the permission of the participant. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

Worsening of Crohn's Disease or the occurrence of a flare

In the event that a participant experiences a flare during the trial, they may need to be treated with steroid therapy and 5-ASA compounds, which is normal standard practice. Therapy of Biologics or immunosuppressants would generally be avoided. Low dose immunomodulators, such as thiopurines/ (azathioprune/ 6-mercaptopurine) can be considered post vaccination.

9.9 Safety Review Committee

An independent Safety Review Committee (SRC) will be convened to review emerging safety data prior to dose escalations for each cohort. A Charter for the SRC will be finalised prior to the start of the first SRC meeting, the Charter will describe the constitution and operating procedures for the SRC. The chair of the SRC may be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

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

9.9.1 Safety Profile Review

The safety profile will be assessed on an on-going basis by the Chief Investigator and Safety Review Committee. The SRC will perform independent safety reviews prior to dose escalations. The Chief investigator and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

10.0 STATISTICS

10.1 Sample Size Selection

This is a descriptive safety study, where participants will be vaccinated with ChAdOx2 HAV and/or MVA HAV. A maximum of 28 participants will be vaccinated in total. Safety data will be presented according to frequency, severity and duration of adverse events. This sample size should allow an initial estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups. It should be noted that this trial is under-powered to detect efficacy, that the efficacy (immunogenicity) outcomes are exploratory so as to evaluate the activity of the agents, and to inform a future RCT.

The analysis for immunogenicity will be to explore the difference in magnitude of MAP specific T-cell responses between the groups. We will assess vaccine immunogenicity by comparing the change in these immunological parameters from baseline to different time points.

The Trial Statistician will write a Statistical Analysis Plan (SAP). HAV Vaccines Limited are responsible for the analysis and production of the Final Study Report.

11.0 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

11.1 Investigator procedures

Approved site-specific standard operating procedures (SOPs) will be used at all clinical and laboratory sites.

11.2 Monitoring

On site monitoring to ensure compliance to GCP and this trial protocol will be performed by the KHP-CTO on behalf of HAV Vaccines Limited. The Investigator site will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor representatives and inspection by local and regulatory authorities.

11.3 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. All amendments will be assessed for substantiality by the KHP-CTO and will be made a formal part of the protocol following ethical and regulatory approval.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and ethical committee(s) approval has already been given, are not initiated without regulatory and ethical committee(s)' review and approval except to eliminate apparent immediate hazards to the participant.

11.4 Protocol deviation

Any deviations from the protocol will be regularly reviewed by the trial team and Sponsor, and reported to the SRC. If there are repeating deviations, then the protocol may need to be re-evaluated. All protocol deviations will be documented in a protocol deviation form and filed in the trial master file.

11.5 Audit & inspection

The Sponsor, or representative of the sponsor, may conduct a clinical site or trial management audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004. The Sponsor, Investigator and KHP-CTO will assist in any inspections and will formally respond to the MHRA as part of the inspection procedure.

11.6 Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA 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 participants 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 within one working day. Notification of Serious Breaches to the MHRA will be performed by the KHP-CTO.

11.7 Trial Progress

The progress of the trial will be overseen by the Chief Investigator.

11.8 Publication Policy

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study.

12.0 ETHICS & REGULATORY APPROVALS

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to an NHS Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation

The Sponsor will submit a final report at conclusion of the trial to the REC and the MHRA within the timelines defined in the Regulations

12.2 Informed Consent

Written, informed consent will be obtained, as described in section 7.2

12.4 Participant Confidentiality

All data will be anonymised: participant data will be identified by a unique study number in the CRF and database. A separate confidential file containing identifiable information will be stored in a secured location in accordance with the General Data Protection Regulation 2018 (GDPR). Photographs taken of vaccination sites (if required, with the participant's written, informed consent) will not include the participant's face and will be identified by the date, trial code and participant's unique identifier. Once developed, photographs will be stored as confidential records, as above.

13.0 DATA HANDLING AND RECORD KEEPING

13.1 Data Handling

The Chief Investigator will be responsible for all data arising during the conduct of the trial at the Investigator Site. The data will be entered into the eCRF system from source data (as below).

13.2 Record Keeping

The Investigators will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP R2 and regulatory and institutional requirements for the protection of confidentiality of participants. The Investigator will permit authorised representatives of the Sponsor(s), as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

13.3 Source Data and Case Report Forms (CRFs)

All protocol-required information will be collected in CRFs reviewed and approved by the Chief Investigator. Source documents are original documents, data, and records from which the participant's CRF data are obtained. Source documents will be defined and their location confirmed, in the Source Document Location list which will be agreed at the Trial Initiation Visit. All essential documents as defined in the UK Clinical Trial Regulations will be stored in the Trial Master File (maintained by the KHP-CTO on behalf of the Sponsor) and/or the Investigator Site File (maintained by the Principal Investigator team).

13.4 Data Protection

The Chief Investigator will act as custodian for the trial data and all personal data will be stored in compliance with the General Data Protection Regulation (GDPR) 2018. The following will be strictly adhered to:

Patient data will be anonymised.

- All anonymised data will be stored on a password protected computer.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the GDPR and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and GDPR.

14.0 DATA MANAGEMENT

The KHP-CTO will provide a fully validated and computer system compliant database for the trial data. Data management including: raising data queries, data extractions for analysis (interim and final) and database locks will be conducted by the KHP-CTO on behalf of the sponsor.

15.0 FINANCING AND INSURANCE

15.1 Financing

The study is funded and sponsored by HAV Vaccines Ltd.

15.2 Insurance

HAV Vaccines Ltd have purchased a clinical trial (no-fault) insurance policy for the conduct of this trial.

15.3 Participant Re-Imbursement for Travel Expenses.

Payment of up to £40 per Clinical Trial Subject, per visit will be reimbursed to cover travel expenses. Written approval from the Sponsor will be required for any travel expenses exceeding the agreed amount. Invoices will be raised based on valid travel receipts'. Payment of up to £10 per Clinical Trial Subject, per visit, will be reimbursed for refreshments. Invoices will be raised based on valid travel receipts.

16.0 SIGNATURES

Digitally signed by Jeremy Sanderson
DN: cn=Jeremy Sanderson, o, ou, email=Jeremy.Sanderson@gstt.nhs.uk, c=GB
Date: 2022.05.27 16:06:09 +01'00'

Chief Investigator — Date

Digitally signed by Michael
DN: cn=Michael, o, ou, email=stalli@hav-vaccines.com, c=GB
Date: 2022.05.25 19:59:51 +01'00'

25 May 2022

Sponsor — Date

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