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

Statistical Analysis Plan

Version 2

Dated: 20/11/2020

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Trial Identifiers

EudraCT Number:	2018-003462-14
IRAS Number:	262209
Other Trial Identifiers:	N/A

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This document contains up to date statistical analysis plans (with version numbers and dates).

A) Quantitative Analysis Plan

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2 A) QUANTITATIVE ANALYSIS PLAN

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3 Description of the trial

3.1 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 of ChAdOx2 HAV and MVA HAV in patients with active Crohn's disease 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

3.2 Principal research objectives to be addressed

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

3.2.1 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.

3.2.2 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 3.2.3.

The following parameters will be used to assess response and safety 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.

3.2.3 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.

3.3 Secondary objective

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.

3.3.1 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), 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 1and 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 an additional 20ml on 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), but samples would be anonymised. Participants will be consented for this.

4 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 of the protocol and summarized in the table below:

Table 1 Summary of dosing regimen

Group	Dose
1 (n=6)	ChAdOx2 HAV, 2.5x1010 vp
2 (n=6)	ChAdOx2 HAV, 5x1010 vp
3 (n=3)	MVA HAV, $5x10^7$ pfu
4 (n=3)	MVA HAV, $2x10^8$ pfu
5 (n=10)	Prime boost Regime (doses depended on the results from Groups 1-4)

4.1 Participants

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

4.2 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.
4. Active Crohn's inflammation in at least one segment of ileum or colon on a colonoscopy or flexible sigmoidoscopy.
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.

4.3 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 in the last 12 months.
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 swab 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.

4.4 First participants

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 (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 from that participant will be examined and no other 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 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 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 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 a further 48 hours. After 3 subjects 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.

4.5 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.

4.6 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 participants. The profile of adverse events from that participant will be examined and no other participants 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.

4.7 Sequence of Enrolment and Vaccination of Participants (groups 3-5)

The first participant in group 3 will receive 5 x10^7 pfu of MVA HAV. This participant will be vaccinated ahead of any other participants and the profile of adverse events will be examined. No other participants 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 will be participant. 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 volunteer 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 volunteer. 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 subjects in group 4 have been vaccinated and followed up for 7 days, an interim safety review will be performed.

The CI and the Safety Review Committee will be asked to provide the decision on whether to proceed with vaccinations in group 5 where 10 participants will be vaccinated with 5 x 10^{10}vp ChAdOx2 HAV followed by 2 x 10^8 pfu MVA HAV (8 weeks apart).

4.8 Assessment of severity

The severity of clinical and laboratory adverse events will be assessed according to the scales in Table 2-4.

Table 2 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 3 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 4 Severity grading criteria for local and systemic AEs.

GRADE 0	None: Symptom not experienced
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.

4.9 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

4.10 Definition of start and end of trial

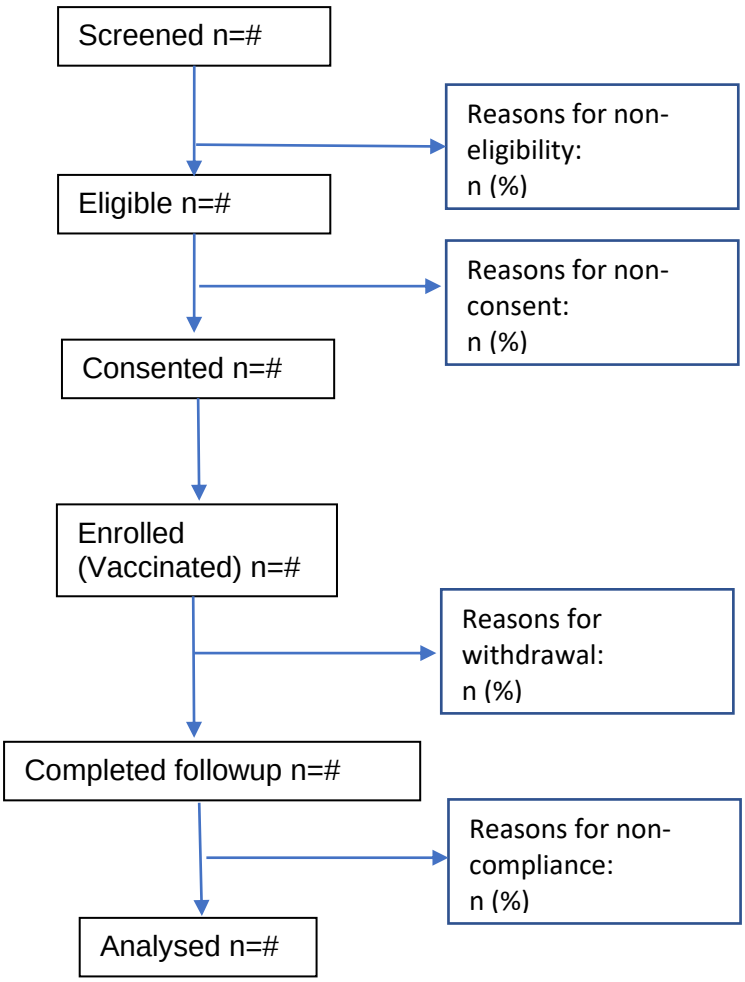
The start of the trial is defined as the date of consent of the first participant. The end of the trial is the date of the last visit of the last participant.

5 Data analysis plan – Data description

5.1 Recruitment and patient progression

A CONSORT flow chart will be constructed – see Figure 1. This will include the number of eligible patients, number of patients agreeing to enter the trial, number of patients refusing, the number continuing through the trial, the number withdrawing, the number lost to follow-up and the numbers excluded/analysed.

Figure 1 Template CONSORT diagram for HAV002 trial



5.2 Patient status

Status for all enrolled participants will be given according to the sample table below:

Table 5 Patient status

Participant	Date screened	Date consented	Date of enrolment/ Vaccination	Group number (* denotes the first patient for that group)	Completed trial (Y/N)	Withdrawal date	Reason for withdrawal
Patient 1							
Patient 28							

5.3 Baseline description of groups

Baseline descriptions of all enrolled participants (e.g. age, gender, ethnicity) will be given overall and by group, using means and standard deviation or numbers and proportions as appropriate.

5.4 Protocol deviations

Details of protocol deviations, such as visits outside of allowable window, missing procedures or procedures taken outside of visit will be given overall by group.

5.5 Loss to follow-up and other missing data

The proportions of participants missing each variable will be summarised at each time point.

The baseline characteristics of those missing follow up will be compared to those with complete follow up.

The reasons for withdrawal from the trial will be summarised.

5.6 Adverse event reporting

Local and systemic Adverse events (AE), adverse reactions (AR), serious adverse events (SAE) and serious adverse reactions (SAR) will be summarised overall by group.

6 Data analysis plan – analysis of endpoints

6.1 Analysis of primary endpoint

Determining the Maximum Tolerated Dose (MTD) for ChAdOx2 HAV will depend on the response of patients in Group 1 and 2. As stated in the Group Holding rules, if 2 or more participants in Group 2 experience Grade 3 SAR lasting more than 48 hours, then the previously tolerated dose will be recorded as the MTD. If 2 or more participants in Group 1 experience Grade 3 SAR lasting more than 48 hours, then the trial will be put on hold pending an internal safety review.

If less than 2 participants in Group 2 experience the outcome stated above, then participants in Group 3 and 4 will be subject to the same criteria for the two doses of MVA HAV. If 2 or more participants in Group 4 experience Grade 3 SAR lasting more than 48 hours, then the previously tolerated dose will be recorded as the MTD. If 2 or more participants in Group 3 experience Grade 3 SAR lasting more than 48 hours, then the trial will be put on hold pending an internal safety review.

Once the MTDs for ChAdOx2 HAV and MVA HAV is determined, then participants in Group 5 will be subject to these doses. If 2 or more participants in Group 5 experience Grade 3 SAR lasting more than 48 hours, then the trial will be put on hold pending an internal safety review.

The proportion of all individuals per group who experience Grade 3 SAR lasting more than 48 hours at the end of follow-up will be estimated with a 95% exact confidence interval. The number with expected and unexpected AEs and all SAEs will be presented alongside, according to the sample table below:

Table 6 Summary of adverse events by Group

	Total enrolled	Number experiencing Grade 3 SAR lasting more than 48 hours	Proportion experiencing Grade 3 SAR lasting more than 48 hours (95% CI)	Number of expected local reactogenicity signs and symptoms for 7 days following the	Number of expected systemic reactogenicity signs and symptoms for 7 days following the	Number of unexpected adverse events for 28 days following the vaccination	Number of serious adverse events during the whole study duration

				vaccination	vaccination		
Group 1							
Group 5							

The median and range of values of safety laboratory measures will be plotted by group at baseline (vaccination visit) and 7 days post vaccination.

6.2 Analysis of secondary endpoints

1. Immunogenicity: The median and range of values of ELISPOT response will be plotted by group at baseline and follow-up.
2. Immune and clinical responses to Vaccination: medians and IQR or numbers and proportions (as appropriate), will be presented at baseline and follow up for the following outcomes:
   - Crohn's disease activity
   - Quantity of MAP in blood

6.3 Statistical considerations

Time points

The primary outcome is measured across all follow-up time period (2 or more participants experiencing Grade 3 severe adverse reactions (SAR) lasting more than 48 hrs). Secondary outcomes are presented using descriptive statistics for baseline and follow-up timepoints. Data will be presented using descriptive statistics and no repeated measures analysis will be conducted.

Stratification and clustering

There will be no stratification or clustering

Missing baseline data

Individuals must have all baseline measurements as part of screening and as standard of care. This data will not be imputed for the primary analysis.

Missing outcome data

Individuals will be monitored for the entire followup duration and thus there will be no missing data for the primary outcome. If data is missing for the secondary or exploratory outcomes analysis will be carried out on a complete case basis with no imputation.

Method for handling multiple comparisons

Bonferroni adjustment for multiple comparisons will be used.

Method for handling non-compliance (per protocol/CACE analyses)

Since this is a single arm trial CACE analysis is not possible

Model assumption checks

Only descriptive statistics will be presented for all outcomes and thus no model assumption checks are needed. As the sample size is small, medians and IQRs will be presented instead of means and standard deviations.

Sensitivity analyses

No sensitivity analyses are planned

Planned subgroup analyses

No subgroup analyses are planned

Interim analysis

Interim safety reviews are planned after vaccinating the first participants in all groups, and prior to moving up a dose. The safety reviews are summarized in the table below, and described in more detail in section 4.4 to 4.7

Safety reviews

Table 7 Planned interim safety reviews

Interim safety review	**When**
1	After vaccination of the first participant in Group 1
2	After vaccination of the participant 2 and 3 in Group 1, and prior to vaccinating participants 4-6 in Group 1 and participant 1 in Group 2
3	After vaccination of the first participant in Group 2
4	After vaccination of the first participant in Group 3
5	After vaccination of the participant 2 and 3 in Group 3, and prior to vaccinating the first participant in Group 4
6	After vaccination of the participant 2 and 3 in Group 4, and prior to vaccinating the first

	participant in Group 5

7 Software

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. The CTO Data Manager will extract data periodically as needed and provide these in comma separated (.csv) format.

Statistical analysis: R (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.) will be used for data description and the main inferential analysis.

8 B) SCHEDULE OF ASSESSMENTS AND MEASURES

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**	
Exploratory microbiology (ml)		**10**				**10**	**10**	
Urinalysis	**x**							
Urinary β-HCG (women only)	**x**	**x**						
HLA typing (ml)		**4**						
HBsAg, HCV Ab, HIV serology (ml)	**5**							
SARS-CoV-2 swab test	**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 swab test	**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	**x**	**x**											

criteria													
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**				**20**	**30**				**20**	**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 swab test	**x**												
Total blood volume per visit (ml)	**23**	**42**	**8**	**8**	**0**	**28**	**42**	**8**	**8**	**0**	**28**	**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.*

Amendments to version 1

LIST HERE ANY AMENDMENTS TO THE SAP THAT WERE MADE AFTER THE SAP WAS SIGNED OFF BY THE TSC

Version Name, number, date	Changes from previous version
SAP, V1, 22/09/2020	-
SAP, V2, 20/11/2020	Changes made to align with the updated protocol (Version 3.0, 15/10/2020)

9 Signatures

Statistical Analysis Plan

Version Number and Date:	V2 dated 20.11.2020
Sponsor:	HAV Vaccines Limited
Chief Investigator:	Professor Jeremy Sanderson

18/01/2021

Dr Abdel Douiri, Statistician — Date (dd-mmm-yyyy)

_____ _____

Professor Jeremy Sanderson, Chief Investigator — Date (dd-mmm-yyyy)