A phase 1b clinical trial to determine the safety, tolerability and immunogenicity of simian adenovirus and poxvirus vectored vaccines against a *Mycobacterium avium* complex subspecies in patients with active Crohn's disease

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Summary

Background Crohn's Disease (CD) is a chronic, debilitating condition hypothesised to be associated with *Mycobacterium avium* ssp *paratuberculosis* (MAP) infection. It is the causative pathogen of the granulomatous inflammatory enteritis in ruminants, Johne's Disease. A developing treatment approach is utilising heterologous prime-boost viral vectored vaccines. We report a Phase 1b dose-escalation trial to determine the safety, tolerability and immunogenicity of candidate recombinant ChAdOx2 and MVA vectored vaccines against MAP in patients with CD.

Methods 28 patients with mild to moderate CD, aged 18–50, were randomly allocated into 5 groups. Group 1 and 2 were vaccinated with ChAdOx2 HAV, Groups 3 and 4 with MVA HAV and Group 5 with both vaccines in a prime-boost regimen. A 112-day follow-up period assessed safety and tolerability by recording adverse events (AEs) and serious adverse events (SAEs). Secondary objectives of immunogenicity were assessed by ELISpot (enzyme-linked immunosorbent spot) and clinical response by Crohn's Disease Activity Index (CDAI) and Simple Endoscopic Score for Crohn's Disease (SES-CD).

Findings 28 participants received either a single dose of ChAdOx2 HAV (n = 12), a single dose of MVA HAV (n = 6) or a prime dose of ChAdOx2 HAV (n = 10) followed by an MVA HAV (n = 9) boost. Solicited AEs were 196 in all participants, one AE was graded as severe but resolved within 24 h. The majority of solicited AEs were graded as mild (149/196; 76%, 95% CI 69%–82%) or moderate (45/196; 23%, 95% CI 17%–29%). ELISpot responses increased in Groups 1 and 2 and significantly more after boosting with MVA HAV.

Interpretation Candidate vaccines ChAdOx2 HAV and MVA HAV were safe, well-tolerated and immunogenic in patients with active CD. A heterologous prime-boost schedule induces a T cell-mediated immune response. Further studies are required to determine the efficacy and optimal regime of the vaccines.

Funding HAV Vaccines Limited funded the trial and acted as trial sponsor. The Sponsor was involved in protocol development, trial conduct, including data monitoring and analysis, and the preparation of this manuscript in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 and amendments.

Keywords: Viral vectored vaccines; *Mycobacterium avium* ssp *paratuberculosis*; Crohn's disease; Johne's disease

Introduction

Crohn's Disease (CD) is a chronic, incurable and progressive inflammatory bowel disease characterized by a variety of intestinal and extra-intestinal manifestations that impair an individual's quality of life. Although much has been learned in recent years about the disease

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Research in context

Evidence before this study

Little progress has been made in recent years in identifying the underlying cause of CD and current treatments for the disease are targeted at mitigating its symptoms using immunomodulating, immunosuppressive and anti-inflammatory medications. Based on historic evidence that MAP is associated with the development of CD, two virally vectored vaccines, ChAdOx2 HAV and MVA HAV, were developed to be used in a prime/boost regimen as a therapeutic treatment against MAP infection. A Phase 1a trial in 2019 showed these vaccines to be safe and well tolerated in healthy adults. A T cell immune response was observed following ChAdOx2 HAV prime which was significantly boosted by MVA HAV following ChAdOx2 HAV prime.

Added value of this study

We report here a Phase 1b trial of the vaccines on participants with mild to moderate active CD. We demonstrate that the vaccines are safe and well tolerated with a signal suggesting that the ChAdOx2 HAV prime/MVA HAV boost regimen is as immunogenic in patients with active CD as it was in healthy adults.

Implications of all the available evidence

ChAdOx2 HAV and MVA HAV have been shown to be safe and well tolerated. Participants in the Phase 1a and Phase 1b trials when vaccinated in a prime/boost regimen developed an immune response to certain key MAP antigens. These findings warrant further clinical trials on larger numbers of candidates with longer follow-up periods and different trial designs to assess efficacy further and to investigate the optimal regimen.

mechanisms, there has been little progress in the recognition of disease causation with current treatments aimed at mitigating an exaggerated and inappropriate immune response using immunomodulating, immunosuppressive and anti-inflammatory medications. Current reviews refer to CD as being auto-immune or auto-inflammatory in nature[1,2] but this is still unclear.

For over a century, researchers have explored the association between *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and the development of CD.[3] However, establishing a causal relationship between MAP and CD has been challenging due to the absence of a reliable and validated diagnostic test for MAP in humans, making it difficult to determine whether MAP is a direct contributor to the disease or merely a bystander in its development.

Despite the complex pathogenesis of CD, a therapeutic vaccine against MAP might provide an alternative treatment strategy to current therapies. Adenovirus-based viral vectors have now been extensively evaluated in numerous human studies and use of heterologous prime-boost strategies are known to substantially increase immune responses induced by viral vectored vaccines.[4] The safety and immunogenicity of the recombinant simian adenovirus vector, ChAdOx2, has been previously reported[5] and a second vaccine based on this construct is also progressing through clinical development against rabies.[6]

Two therapeutic vaccines were developed: a simian adenovirus vectored vaccine, ChAdOx2 HAV, and poxvirus Modified Vaccinia Ankara vectored vaccine, MVA HAV, each expressing four MAP antigens.

The transgene in the HAV vaccine construct comprises a string of four antigens: AhpC, Gsd, p12 and mpa. 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 MAP's intrinsic resistance pattern. Similar sequences of all 4 of these MAP genes can be found in important secondary co-pathogens in CD, including *E. coli* and other *M. avium* ssp.[7–10]

In a study in cattle, where MAP is known to cause a near-identical granulomatous inflammatory enteritis termed Johne's Disease, a prime-boost vaccination regime, utilising viral vectored vaccines with the HAV insert, was well-tolerated and demonstrated a significant reduction in shedding of MAP compared with controls. This was associated with ex vivo bacterial killing activity and induction of antigen specific $CD4^+$ and $CD8^+$ T cell responses.[11]

It has previously been reported that a poxvirus vectored vaccine, MVA HAV, as a single dose or as a booster vaccine following a simian adenovirus, ChAdOx2 HAV prime in healthy UK adults in a Phase 1a trial was safe, well-tolerated and immunogenic.[12] This allowed progression to this Phase 1b trial in patients with active CD. Here we report on the safety and immunogenicity of ChAdOx2 HAV and MVA HAV vaccines, as single dose or in prime-boost regimen, in patients with active CD.

Methods

ChAdOx2 HAV and MVA HAV vaccines

The antigen for both vectored vaccines used in the trial consists of a 95 kDa fusion construct from four MAP genes which are present in all MAP strains, named HAV: 1589c (AhpC), MAP 1234 (Gsd), 2444c (p12) and

1235 (mpa). To summarise, ChAdOx2 consists of a replication-deficient simian adenovirus (E1 and E3 genes deleted) derived from the AdC68 strain. MVA is a highly attenuated poxvirus vector which has been extensively used as a vaccine since the 1970s[13] and more recently, in vaccine clinical trials for multiple different diseases.[14] ChAdOx2 HAV and MVA HAV were manufactured to current good manufacturing practice standards by the Clinical Biomanufacturing Facility (University of Oxford, Oxford, UK) and IDT Biologika GmbH (Dessau-Rosslau, Germany), respectively.

Trial design and participants

Enrolment into the trial commenced in September 2021 and was undertaken at the Clinical Research Facility at Guy's Hospital (Guy's & St. Thomas' Hospitals NHS Foundation Trust), London (Fig. 1).

Twenty-eight participants were enrolled according to the eligibility criteria described in Table 1. Participant sex was self-reported by individuals recruited from clinical practice. Sample size was determined based on practical considerations, such as the number of participants needed to adequately assess safety and tolerability. It was also consistent with the previous Phase Ia clinical trial in healthy volunteers, ensuring adequate numbers for safety and immunogenicity assessments.[12]

Accepted participants were randomly assigned to one of five vaccine groups set out in Table 2 and dose escalated.

Initially recruited patients were assigned according to time of recruitment. The only alteration to this process was ensuring that any patient in Group 5 would have disease assessable for flexible sigmoidoscopy. A staggered-enrolment approach was used for the participants in each group and interim safety reviews conducted prior to dose escalation. All participants were excluded from taking any treatments that might affect CD during the follow-up period following vaccination, including immunomodulating, immunosuppressive, anti-inflammatory and antimicrobial medication.

The primary objective was to assess the safety of ChAdOx2 HAV and MVA HAV administered alone and in a prime-boost regimen in adult volunteers with active CD. Safety was assessed by recording solicited adverse events (AEs) for 7 days, unsolicited AEs for 28 days post-vaccine administration and the occurrence of any serious adverse events (SAEs) throughout the trial. The primary outcome measure was to define the Maximum Tolerated Dose (MTD) of ChAdOx2 HAV and MVA HAV in patients with active CD, not receiving immunosuppressive therapy.

The following parameters were assessed for all 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.

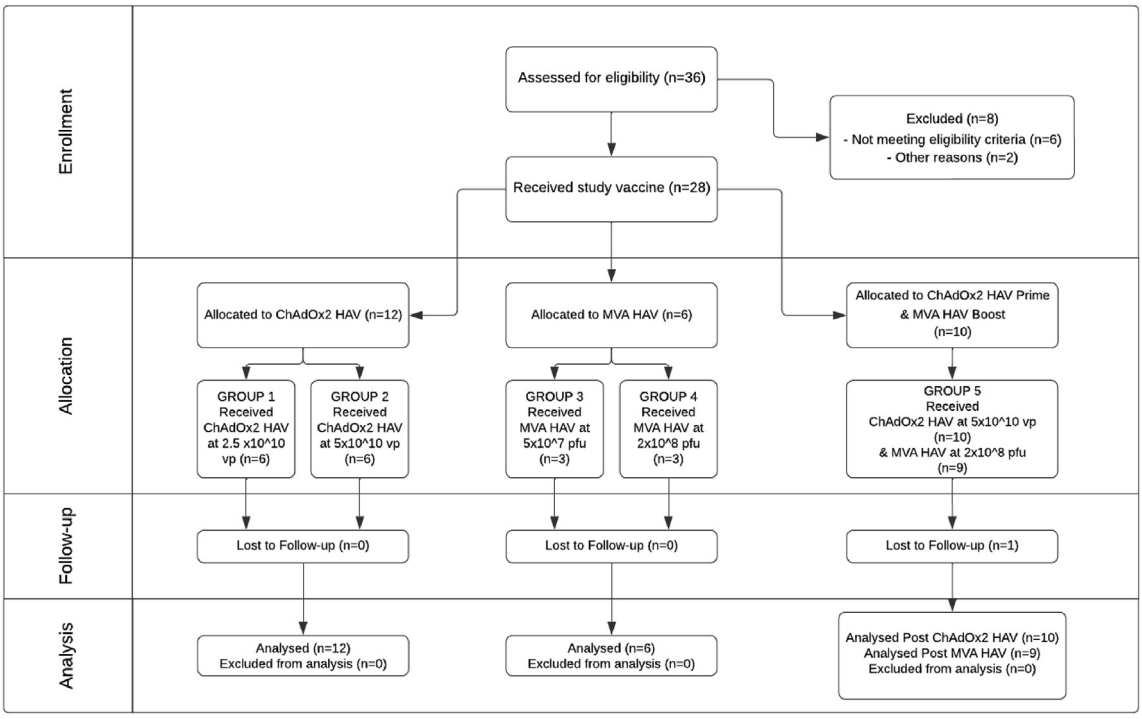

Fig. 1: CONSORT diagram.

Inclusion criteria
1. Age 18–50 years.
2. Confirmed diagnosis of CD 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 >10 mg/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.
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 haematology 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.

Table 1: **Eligibility criteria for enrolment.**

- Occurrence of adverse events for 28 days following the vaccination.
- Change from baseline for safety laboratory measures (Supplementary Figs. S2 and S3)
- Occurrence of serious adverse events during the whole trial duration.

The secondary objective was to assess the immunogenicity in individuals with active CD of ChAdOx2 HAV administered alone and in prime-boost regimen with MVA HAV. To assess cellular immunogenicity, ex vivo interferon-γ (IFNγ) enzyme-linked immunospot (ELISpot) assays were completed. This required freshly isolated peripheral blood mononuclear cells (PBMC) stimulated with pools of peptides spanning the HAV vaccine construct. Methodology and calculation of results has been described previously elsewhere.[12]

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

Table 2: **Summary of group numbers and dosage.**

While this trial was not designed or powered to assess efficacy, the CD Activity Index (CDAI) was assessed for Groups 1, 2 and 5. CDAI is a weighted index comprising eight clinical and laboratory variables that estimate disease activity in CD[15] and is the most used tool in clinical trials that assess efficacy of CD treatments. A clinical response is generally considered to be a decrease of at least 100 points in CDAI score,[16] with an absolute score of less than 150 being often defined as clinical remission.[17]

For Group 5, scoring by endoscopy was included to obtain a Simple Endoscopic Score for Crohn's Disease (SES-CD) at screening and at Day 112 It has been

argued that CDAI is poorly associated with intestinal inflammation[18,19] and current practice in clinical trials is to include SES-CD,[20] as an adjunct to assess disease response and a marker for mucosal healing. Endoscopic response in CD clinical trials is currently widely accepted as a reduction of 50% in baseline SES-CD score,[21,22] with inactive disease or remission being defined as an SES-CD score of 0–2[23,24] or 0–3.[25,26]

Participants in Groups 1 and 2 underwent clinical follow up for 20 weeks following completion of the vaccination regimen. For participants in Groups 3 and 4, who received the MVA only based constructs, a shorter follow-up period of 12 weeks was considered appropriate as MVA vectored vaccines have been used extensively with no significant safety concerns reported to date. Participants in Group 5 were only vaccinated with the prime-boost regimen after data generated from the individual vaccine.

Groups 1–4 had confirmed it was safe to proceed with the proposed higher doses in combination.

Participants in Group 5 underwent clinical follow-up for a further 20 weeks following completion of the boost vaccination regimen. The duration of follow up reflects the desire to obtain sufficient safety data with the use of ChAdOx2 HAV, MVA HAV and a prime-boost regimen with ChAdOx2 HAV/MVA HAV in humans with CD.

Procedures

All vaccines were administered intramuscularly given the favourable safety and immunogenicity profile of this route of administration with viral vector vaccines. Safety and immunogenicity data generated from participants receiving a single dose of ChAdOx2 HAV in Groups 1 and 2 was used to inform the dose to be used in the prime-boost Group 5. The doses of MVA HAV to be used in this trial (5×10^7 and 2×10^8 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.[27–29] 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. Lower doses of MVA enable an acceptable reactogenicity profile without significantly compromising vaccine immunogenicity.

Participants were asked to record any adverse events (AEs) using paper diaries and were regularly reviewed at follow-up visits up to 140 days post-vaccination. Investigators assessed the severity of AEs using the following criteria: (a) Grade 1 mild (short-lived or mild symptoms with no limitation to usual activity); (b) Grade 2 moderate (mild to moderate limitation in usual activity); and (c) Grade 3 severe (considerable limitation in activity, medication or medical attention required). Unsolicited AEs were reviewed for causality by an independent clinician and events were categorised as possibly, probably or definitively related to the vaccines. Laboratory AEs were graded using site-specific toxicity tables which were adapted from the US Food and Drug Administration toxicity grading scale.

A timetable of assessments, assays and procedures for each Group are set out in Table 3.

Statistical analysis

The trial design, including eligibility criteria and definitions of active disease, was reviewed and approved by the GSTT R&D Gastroenterology Group and Clinical Research Facility Review Board. As a Phase I trial, the trial's primary focus was on safety and tolerability, rather than efficacy. The sample size was determined based on considerations such as the number of

Day	Procedure	Groups 1 & 2	Groups 3 & 4	Group 5
Screening	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology	X	X	X
	Urinalysis	X	X	X
	CDAI	X	X	X
	SES-CD			X
0	Vaccination	X	X	X
	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology	X	X	X
	Immunology Assay	X		X
2	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology	X	X	X
7	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology	X	X	X
14	Vital Signs & Physical Exam	X	X	X
28	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology	X	X	X
	Immunology Assay	X		X
56	Vaccination Boost			X
	Vital Signs & Physical Exam	X	X	X
	Biochemistry & Haematology			X
	Immunology Assay	X	X	X
	CDAI	X	X	X
58	Vital Signs & Physical Exam			X
	Biochemistry & Haematology			X
63	Vital Signs & Physical Exam			X
	Biochemistry & Haematology			X
70	Vital Signs & Physical Exam			X
84	Vital Signs & Physical Exam			X
	Biochemistry & Haematology			X
	Immunology Assay			X
112	Vital Signs & Physical Exam			X
	Immunology Assay			X
	CDAI			X
	SES-CD			X

Vital signs included pulse, blood pressure and temperature. Physical examination, other than at screening and vaccination, only took place if considered necessary. Biochemistry included sodium, potassium, urea, creatinine, albumin and liver function tests. Immunology assay included ex vivo ELISPOT responses to interferon gamma. CDAI is Crohn's Disease Activity Index score. SES-CD is Simple Endoscopic Score for Crohn's Disease.

Table 3: **Schedule of procedures and assessments.**

participants needed to adequately assess safety and tolerability. It is also consistent with the previous Phase Ia clinical trial in healthy volunteers, ensuring adequate numbers for safety and immunogenicity assessments. Safety endpoints are described as frequencies with their respective percentages alongside their exact 95% confidence intervals (CI). It should be noted that this method is conservative for small samples leading to wide confidence intervals whose coverage probabilities tend to be too large.

Ethics

The trial was registered with EudraCT (reference number 2018-003462-14) and submitted to the ISRCTN registry on 24th May 2019. The Medicines and Healthcare products Regulatory Agency (MHRA) granted Clinical Trial Authorisation (CTA) as an Acceptance of the Amended Request CTA 51689/0001/001-0001 on 5th September 2019. Ethical approval was received from the London Westminster Research Ethics Committee (reference number 19/LO/1738) on 19th December 2019. The trial was registered with ISRCTN (identifier ISRCTN36126048) on 3rd March 2020. However, trial activation was delayed due to the COVID-19 pandemic. Initial Research and Development (R&D) approval was provided by Guy's and St Thomas' (GSTT) R&D on 25th May 2021, allowing investigators to share participant information sheets with potential participants.

Written informed consent was obtained from all participants before their involvement in the trial, in compliance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. Recruitment commenced following the GSTT R&D 'green light' on 16th August 2021, with the first participant consented and screened during the week of 20th September 2021. The first vaccination was administered during the week of 27th September 2021. This trial adhered to the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trials) Regulations 2004 and its amendments. For further details on the trial approvals and to view the clinical trial protocol, please refer to the ISRCTN registry.

Role of funders

HAV Vaccines Limited funded the trial and acted as trial sponsor. The Sponsor was involved in protocol development, trial conduct, including data monitoring and analysis, and the preparation of this manuscript in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 and amendments.

Results

Trial population

Between 20 Sept 2021 and 23 Aug 2022, twenty-eight adult participants with active CD were screened and received either a single dose of ChAdOx2 HAV (n = 12), a single dose of MVA HAV (n = 6) or a prime dose of ChAdOx2 HAV (n = 10) followed by an MVA HAV (n = 9) boost 8 weeks apart. The baseline characteristics of these five cohorts are summarised in Table 4. One volunteer received a prime dose of ChAdOx2 HAV but withdrew consent before their booster appointment and left the trial.

Primary endpoints: vaccine safety and tolerability

ChAdOx2 HAV and MVA HAV vaccines were safe and well-tolerated in all groups. Total number of solicited AEs was 196 in all 28 (100%) participants. Of all solicited AEs, 149/196 (76.0%, 95% CI 69.6%–81.5%) were graded as mild and 45/196 (23.0%, 95% CI 17.2%–28.8%) were graded as moderate. Three AEs were graded as severe (grade 3) in Group 1 (Table 5).

Most of solicited AEs completely resolved within 7 days (187/196, 95.4%) and the majority 111/196 (56.6%) had their onset within the first 72 h post vaccination (64/196, 32.6% at D0, 27/196, 13.8% at D1 and 20/196, 10.2% at D2). In Group 5, vaccination arm pain was the most common local AE, reported by 6/10 (60%) participants after ChAdOx2 HAV and 8/9 (89%) after MVA HAV and was predominantly mild in severity and in the majority resolved after 48 h (Supplementary Table S1).

A total of 164 local and systemic solicited AEs were reported by 28/28 (100%) participants within 7 days post vaccination. Most of these solicited AEs were mild (129/165; 78.2%, 95% CI 71.3%–83.8%) or moderate (35/165; 21.2%, 95% CI 15.7%–28.1%). One solicited AE was graded as severe and lasted for 1 day (severe migraine attack in a Group 1 participant). Percentages of local and systemic solicited AEs reported during the first 7 days are summarised in Supplementary Fig. S1. A summary of adverse events by group is shown in Supplementary Table S2. Grade 3 adverse events are shown in Supplementary Table S3. There were two SAEs recorded, one a participant of Group 4 and the

	N/Median (IQR: Q1, Q3)					
	Total patients (N = 28)	Group 1 (N = 6)	Group 2 (N = 6)	Group 3 (N = 3)	Group 4 (N = 3)	Group 5 (N = 10)
Sex (Male)	17	3	4	3	1	6
Sex (Female)	11	3	2	0	2	4
Age (Years)	34.5 (30.5, 40.0)	34.5 (29.25, 39.0)	36.0 (34.5, 39.0)	32.0 (31.0, 38.0)	29.0 (29.0, 35.5)	34.0 (31.0, 36.0)

Table 4: **Demographics of trial participants.**

other of Group 5, being a Crohn's flare (thought to be secondary to gastroenteritis) and a flare of a chronic Crohn's-related perianal abscess. These were categorised as unlikely or unrelated to the vaccination (Table 6).

Secondary endpoints: cellular immunogenicity

Prior to vaccination, ELISpot responses to the HAV vaccine insert were low with a median of 53 SFC/10^6 PBMC. One month after vaccination with ChAdOx2 HAV alone (in Groups 1 and 2) the response to vaccination with doses of 2.5×10^{10} vp and 5×10^{10} vp had increased to a median of 87 SFC/10^6 PBMC and 131 SFC/10^6 PBMC respectively (Fig. 2A and B). In Groups 3 and 4 (MVA HAV only), ELISPOT assays were not performed as T cell responses were expected to be low or undetectable. For the nine participants in Group 5 who received the higher ChAdOx2 HAV dose and boosted eight weeks later with the higher dose of MVA HAV, responses twenty-eight days after boosting (D84) increased significantly above that at D28, reaching a median of 1335 SFC/10^6 PBMC. This remained high at a median of 544 SFC/10^6 PBMC two months after boosting (D112) (Fig. 2A and B).

Responses to individual antigens in the HAV vaccine construct were assessed in the 9 participants that were

Group	Adverse Event	Time elapsed since vaccination in days	Time of event in days	Relation
1	Severe Migraine Attack	20	3	Probable
	Flare up of Neck pain	<1	<1	Possibly
	Feverish	<1	1	Probable

Table 5: **Grade 3 Adverse Drug Reactions (AEs that are possibly/probably/definitely related to study drug).**

Group	Adverse event	Time elapsed since vaccination in days	Time of event in days	Severity	Relation
4	Crohn's Flare	61	4	Grade 2	Unlikely
5	Perianal abscess	95	4	Grade 3	No relationship

Table 6: **Serious adverse events.**

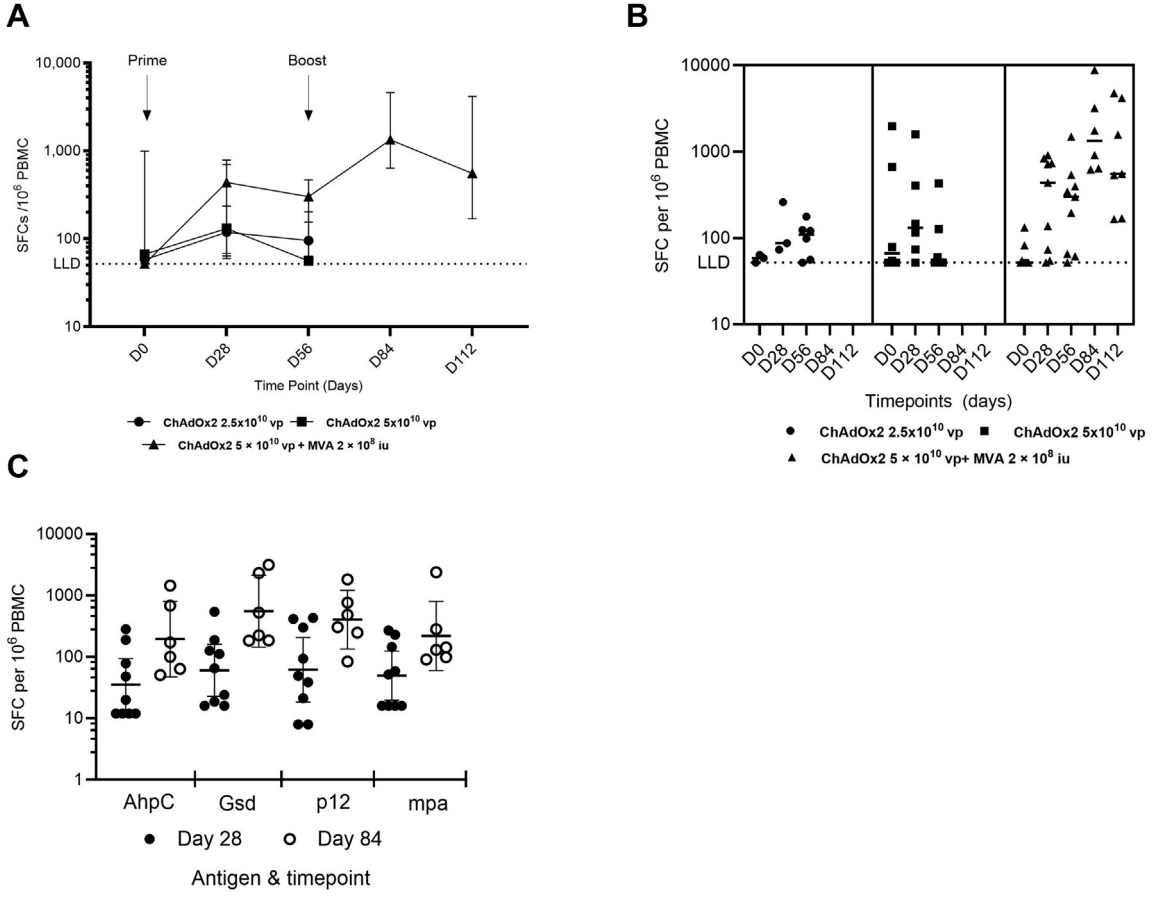

Fig. 2: T cell response to vaccination. A. IFNγ ELISPOT responses to the entire HAV vaccine insert according to vaccine regimen. Lines represent medians with IQR. B. Individual IFNγ ELISPOT responses by time point and vaccine regimen. Lines represent medians. C. Responses to individual antigens at post-prime (day 28) and post-boost (day 84) time points. Lines represent medians with IQR.

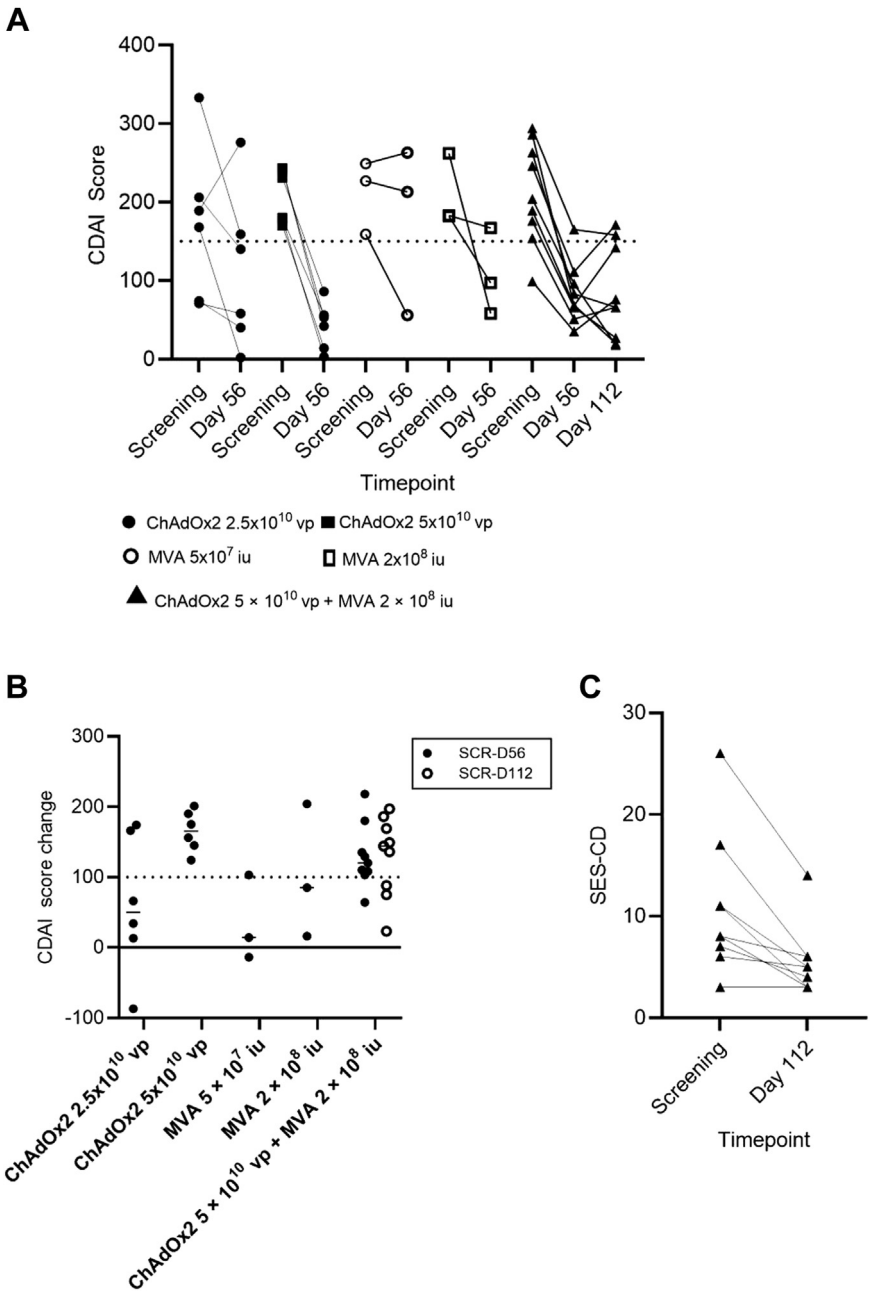

Fig. 3: Changes in clinical markers of CD severity. Diagram A: CDAI at baseline and after vaccination in each vaccine group. A score below the dotted line represents inactive disease. Diagram B: Change in CDAI score after either priming alone or prime-boost vaccination. A change above the dotted line represents clinical response, however this trial was not designed or powered to assess efficacy. Lines represent medians. Diagram C: SES-CD score at screening and post prime-boost vaccination.

primed and boosted. Responses to all four antigens increased significantly after boosting with MVA HAV (Fig. 2C).

Secondary endpoints

CDAI scores for Groups 1–5 are set out in Supplementary Table S4. A marked decrease in CDAI was observed in all vaccine groups and this was significant in Groups 2 and 5. For all six Group 2 participants who received a single higher dose of ChAdOx2 HAV, a significant CDAI response was evident with a median score of 205.5 having decreased to 47.5 two months after vaccination (Fig. 3B). The individual scores of all six participants decreased by more than 100 points and

Participant number	CDAI at D0	CDAI at D112	SES-CD at D0	SES-CD at D112	SES-CD reduction at D112 (%)
13	204	18	3	3	0.0
19	99	76	17	6	64.7
22	246	171	26	14	46.2
23	154	66	8	6	25.0
24	263	66	6	5	16.7
28	286	142	11	5	54.5
29	189	20	8	3	62.5
30	176	27	7	4	42.9
31	294	158	11	3	72.7

Table 7: CDAI and SES-CD scores for participants in group 5 (Prime-Boost) at baseline (D0) and Day 112 (D112).

were below 150 after two months, compared with their level of CD activity at screening (Fig. 3B and A, respectively). This response contrasts with the scores of Group 1 participants, who received half the Group 2 dose, where only two participants' scores decreased by more than 100 points (Fig. 3B), suggesting a possible dose effect. One participant in Group 5 showed minimal CD activity at screening based on CDAI score. For the remaining eight Group 5 participants who, based on their CDAI scores, exhibited mild to moderate active CD at screening, a signal of clinical improvement was observed that was similar to that seen in Group 2. Their median CDAI score declined from 225 to 76 after two months and remained low at 66 two months after booster vaccination with MVA HAV (Fig. 3B). The individual CDAI scores of all these eight participants decreased by more than 100 points with six of the eight remaining in that state two months after boosting (Fig. 3B). Seven of the eight had a score of below 150 two months following priming vaccination with six of the eight remaining below 150 two months after boosting (Fig. 3A).

As regards SES-CD, of the eight Group 5 participants exhibiting mild to moderate CD activity at screening (SES-CD>3), four showed a decrease of >50% and a further two marginally less than 50% (see Table 7 and Fig. 3C) with an overall decreased in median score from 9.5 to 5.

Discussion

This Phase 1b Clinical Trial demonstrated that the candidate MAP vaccines, ChAdOx2 HAV and MVA HAV are safe, well-tolerated and immunogenic in patients with active CD, when given on their own or as part of a heterologous prime-boost regimen. Most AEs were mild or moderate in severity, and all were self-limiting. The profile of adverse events reported here is similar to that for other simian adenovirus and MVA vectored vaccines expressing different antigens.[27]

Modest T cell responses were observed following ChAdOx2 HAV single dose vaccinations. However, T cell responses were significantly boosted by MVA HAV following ChAdOx2 HAV prime, which persisted above baseline levels for at least two months post boost. This is consistent with results seen in previous reports.[5,12] As such, a heterologous prime-boost approach is preferred over a prime only strategy with ChAdOx2 HAV and MVA HAV vaccines in patients with active CD. This is in keeping with T cell, rather than antibody, responses being considered responsible for protection against intracellular agents, such as those in the *M. avium* complex.

The immunogenicity results show that ChAdOx2-HAV prime with MVA-HAV boost regimen is equally immunogenic in both healthy adults and patients with active CD. Within the HAV insert, none of the individual antigens drive the immune response more than the others and boosting does not affect this (Fig. 2C).

This trial was not designed or powered to assess efficacy, however, a signal of clinical improvement in disease markers was observed. CDAI score decreases in all participants who received a heterologous prime-boost regimen. At a mucosal level, over the 4 months, all eight Group 5 participants with endoscopic evidence of active CD at screening (SES-CD >3) showed a decrease in the SES-CD score. Four participants showed a defined clinical response of >50% and a further two between 40% and 50%. It should be noted that CDAI and SES-CD scores do not always correlate and patients may report symptoms that differ from the level of active mucosal inflammation at endoscopy. All Group 5 participants, however, showed both mucosal response and CDAI improvement, though the signal identified does suggests that larger numbers are required to assess this relationship and response more accurately.

Limitations in the trial include the relatively short follow-up period, small sample size and open-labelled, non-randomised, uncontrolled trial design. It should be noted that the method used for calculating confidence intervals is conservative for small samples. Extrapolation of the trial findings to clinical and mucosal responses are limited, as this trial of CD volunteers was designed to assess safety and tolerability. A further limitation of this trial is the lack of available and validated diagnostic tests for MAP, especially assays that can offer semi-quantitative analysis. Such assays could measure levels of MAP directly and, thus, the effect of regimes and dosing. Nonetheless, the patients in this

trial have shown that they are able to develop an immune response to certain key MAP antigens.

In conclusion, ChAdOx2 HAV and MVA HAV were safe and well tolerated in patients with active CD when given alone or as a prime-boost strategy. T cell responses significantly improved and were sustained for at least 2 months post-boost, when given as part of a heterologous prime-boost regimen. Finally, a signal of clinical improvement was observed in all Group 5 participants as measured by CDAI and in half the participants as measured by SES-CD. These findings warrant further clinical trials to assess efficacy and to determine the optimal regime and number of doses of the vaccines.

Contributors

JS, MS, SCG, AVSH and KJE initiated the project and completed the trial design; KJE, JA, RM, KR, SPM and LS completed the immunological testing and analysis. JS, IL, NH, LW and EGray were involved in trial procedures, data collection or quality control. LW, EGray and PIC oversaw trial management; AL and FEO oversaw IMP management; AD, EG and RM completed the statistical analysis. JS, PIC, MS and KJE reviewed all results and wrote the manuscript. JS, JA, LW, EGray, PIC, AD, EG, RM, and KJE accessed and verified the underlying data. All authors read and approved the final version of the manuscript.

Data sharing statement

In compliance with data sharing policies, we confirm the availability of the collected data for this trial. The deidentified participant data can be made available on request. Related documents such as the trial protocol and statistical analysis plan are available as part of the ISRCTN record: https://www.isrctn.com/ISRCTN36126048.

Declaration of interests

This trial was funded by HAV Vaccines Limited. MS is a director and shareholder in HAV Vaccines Limited. HAV Vaccines Limited holds patents relating to the HAV insert of the vaccines ChAdOx2 HAV and MVA HAV which were granted between 2010 and 2014. PIC and FEO are independent consultants to HAV Vaccines Limited. AVSH and SCG are co-founders of Vaccitech Limited, now Barinthus Biotherapeutics, which is developing adenoviral vectored vaccines and inventors on intellectual property filings and patents related to ChAdOx and MVA vectors. SCG and AVSH are inventors on patents related to viral vectored vaccine immunisation regimens. KJE is also an inventor on intellectual property filings and patents related to the ChAdOx2 vaccines. SCG is now at the Pandemic Sciences Institute, IMS-Tetsuya Nakamura Building, Old Road Campus, Roosevelt Dr, Headington, Oxford, OX3 7TY. KJE was an employee of the University of Oxford at the time of the work and is now an employee of the GSK Vaccines Institute for Global Health (Global Health Vaccines R&D), GSK, Siena, Italy. KJE holds restricted shares in the GSK group of companies. The other authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105570.

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