PROTOCOL TRACKING SHEET

VERSION NUMBER	VERSION DATE	REASON FOR CHANGE (high-level)
V5	March 9, 2022	Date of DCP Final Approval to open study: August 9, 2022
V5.1	June 26, 2023	Added a study continuation phase (Cohort 2). Disapproved by DCP.
V5.2	August 18, 2023	Added study continuation phase and incorporated all requested changes from DCP Consensus Review of Disapproved V5.1
V5.3	October 13, 2023	Addressed requested revisions post-CIRB review of V5.2
V5.4	January 9, 2024	Corrected the dose regimens to the nominal concentration of the vaccine in section 7.1.
V5.5	September 10, 2024	We will randomize 28 participants to Cohort 2 to reach 24 evaluable participants in Cohort 2.
V5.6	February 19, 2025	Replacing “gender” with “sex” in response to EO and investigator at University of Puerto Rico.

COVER PAGE
DCP Protocol #: MDA21-06-01

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PROTOCOL TITLE

A Phase Ib/II Clinical Trial of Nous-209 for Recurrent Neoantigen Immunogenicity and Cancer Immune Interception in Lynch Syndrome

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IND# [redacted]

Agent(s)/Supplier: Nous-209 vaccine composed of GAd20-209-FSP and MVA-209-FSP / NCI DCP

Protocol Version Date: 02/19/2025

Protocol Revision or Amendment # Version 5.6

SCHEMA
A Phase Ib/II Clinical Trial of Nous-209 for Recurrent Neoantigen Immunogenicity and Cancer Immune Interception in Lynch Syndrome

Schema for Cohort 1

Individuals aged 18 years or older with Lynch Syndrome, as defined by:
(a) germline mutation-positive Lynch Syndrome: carrier or obligate carrier (by pedigree) of a pathogenic germline mutation or likely pathogenic mutation in MMR genes (*MLH1*, *MSH2/EPCAM*, *MSH6*, or *PMS2*); or **(b) germline mutation-negative Lynch Syndrome ("Lynch-like Syndrome"):** personal history of a non-sporadic MMR-deficient premalignancy or malignancy associated with loss of MMR gene expression in the absence of somatic *MLH1* hypermethylation and/or *BRAF* mutation, and germline MMR genetic testing showing either a variant of unknown significance, or mutation negative result, or had declined germline MMR genetic testing.

↓

Participants have intact rectosigmoid; no active/recurrent malignancy; no cancer-directed treatment x 6 months; no other medical contraindications

↓

Consent / Registration
(up to 60 potentially eligible participants will be consented and screened)

↓

Eligibility Confirmation
Laboratory tests within acceptable limits and normal physical exam
(up to 45 confirmed eligible participants)

↓

Pre-Treatment Biomarker Acquisition
(1) Tissue (collected via standard-of-care lower GI endoscopy [flexible sigmoidoscopy or colonoscopy]) with biopsies of polyps in the entire colon and grossly normal mucosa in the rectosigmoid
(2) Blood

↓

Intervention
Duration 8 weeks
Intramuscular vaccination with GAd20-209-FSPs (prime) at Week 0 and with MVA-209-FSPs (boost) at Week 8

↓

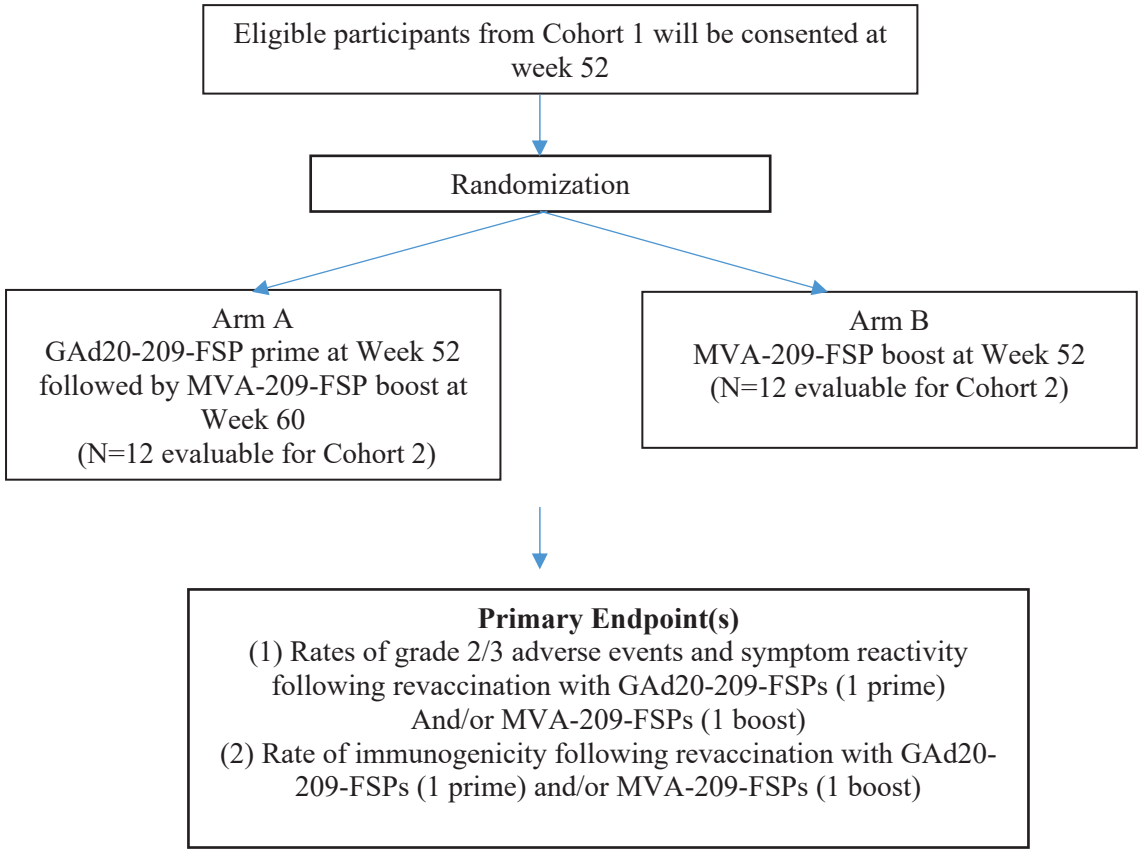
Post-Treatment Biomarker Acquisition
(1) Tissue (collected via standard-of-care lower GI endoscopy [flexible sigmoidoscopy or colonoscopy]) with biopsies of polyps in the entire colon and grossly normal mucosa in the rectosigmoid at Week 52 (12 months) post-vaccination
(2) Blood at Weeks 3, 8, 9, 24, 52

↓

Primary Endpoint(s)
(1) Rates of grade 2/3 adverse events and symptom reactivity following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost)
(2) Rate of immunogenicity following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost)
Secondary Endpoint(s)
(1) Changes in T cell immune profile and T cell receptor (TCR) repertoire in peripheral blood at Weeks 3, 9, 24, and 52 relative to baseline
(2) Changes in TCR repertoire within histologically normal colorectal mucosa at Week 52 (Month 12) relative to baseline
(3) Changes in the gene expression profile and TCR repertoire of tumor infiltrating lymphocytes (TIL) within colorectal adenomas at Week 52 and relative to baseline
(4) T cell cytotoxicity against matched colorectal adenoma organoids derived from participants at Week 52 relative to baseline
(5) Percentage change in the number of colorectal adenomas, advanced neoplasia, and/or carcinomas at Week 52 relative to baseline
(6) Rate of LS-related carcinomas at Week 52 relative to baseline
(7) Change in cfDNA detectability and mutation profile in the peripheral blood of participants with LS at Weeks 3, 9, 24, and 52 relative to baseline
(8) To correlate tobacco and alcohol consumption with the immune response to Nous-209 in trial participants.
(9) To assess the MMR and/or MSI status of polyps (and adjacent normal mucosa as control) detected in the baseline and end-of-the-study colonoscopy using different technologies such as immunohistochemistry, MSI analysis by PCR, or next-generation sequencing

SCHEMA
A Phase Ib/II Clinical Trial of Nous-209 for Recurrent Neoantigen Immunogenicity and Cancer Immune Interception in Lynch Syndrome

Schema for Cohort 2



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1. OBJECTIVES

1.1 Primary Objectives

a) To evaluate the safety and tolerability of GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) vaccination when administered as a single agent (monotherapy) in participants with Lynch Syndrome (LS).

b) To evaluate the neoantigen-specific immunogenicity of GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) vaccination when administered as a single agent (monotherapy) in participants with LS.

c) To evaluate the neoantigen-specific immunogenicity of GAd20-209-FSP prime and MVA-209-FSP boost or MVA-209-FSP boost alone when administered to previously-vaccinated immunogenic participants with LS.

d) To evaluate the safety and tolerability of GAd20-209-FSP prime and MVA-209-FSP boost or MVA-209-FSP boost alone when administered to previously vaccinated immunogenic participants with LS.

1.2 Secondary Objectives

a) To assess the effect of Nous-209 vaccination on T cell immune profile and T cell receptor (TCR) repertoire in the peripheral blood of participants with LS.

b) To assess the effect of Nous-209 vaccination on TCR repertoire within histologically normal colorectal mucosa of participants with LS.

c) To evaluate the effect of Nous-209 vaccination on tumor infiltrating lymphocyte (TIL) immune profile and TCR repertoire within colorectal adenomas in participants with LS.

d) To assess the cytotoxicity of matched T cells on participant-derived colorectal adenoma organoids following Nous-209 vaccination in participants with LS.

e) To evaluate the effect of Nous-209 vaccination on the burden of colorectal adenomas/advanced neoplasia/carcinoma in participants with LS.

f) To assess the effect of Nous-209 vaccination on the burden of LS-related carcinomas in participants with LS.

g) To evaluate the effect of Nous-209 vaccination on cell free DNA (cfDNA) mutation profiles and cfDNA burden in participants with LS.

h) To correlate tobacco and alcohol consumption with the immune response to Nous-209 in trial participants.

i) To assess the MMR and/or MSI status of polyps (and adjacent normal mucosa as control) detected in the baseline and end-of-the-study colonoscopy using different technologies such as immunohistochemistry, MSI analysis by PCR, or next-generation sequencing.

2. BACKGROUND

2.1 Lynch Syndrome

Lynch Syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome, affecting more than one million Americans (1). It is caused by deleterious germline mutations in one of four DNA mismatch repair (MMR) genes, with *MLH1* and *MSH2* mutations causing at least 50% of cases (2) conventionally detected using Clinical Laboratory Improvement Amendments (CLIA)-approved germline genetic testing. However, there is still a fraction of patients suspected to have Lynch Syndrome that do not have any detectable mutations in their germline testing despite of being diagnosed with MMR-deficient tumors with absence of somatic hypermethylation of the *MLH1,* V600E *BRAF* mutations, or double somatic events in one of the MMR genes (double somatic MMR mutations). This subpopulation of patients has been named in the literature as Lynch-like Syndrome and probably have large genomic re-arrangements that are not detectable with conventional genomic tests. In general, the lifetime CRC risk for LS mutation carriers is as high as 70%, depending on the series and the MMR gene that is responsible for LS (3-5). In individuals with LS, normal colorectal mucosa cells acquire somatic "second hit" mutations, thus becoming DNA mismatch repair deficient (dMMR). The MMR system repairs small insertion/deletion (indels) loops occurring in repetitive sequences known as microsatellites, which are widespread throughout the genome. Defects in MMR generate single-base mismatches and indels loops that result in 'microsatellite instability' (MSI) and associated frameshifts, whose open reading frames encode nonsense peptides. LS dMMR CRCs carry exceptionally high numbers of somatic 1-4 bp indels frameshifts and missense mutations (1). Elevated dMMR mutation rates cause some mutations to occur recurrently in tumors from different patients (shared mutations, shared frameshift peptides that become neoantigens). The greatly elevated mutation rates make dMMR CRC molecularly distinct from sporadic MMR-proficient (pMMR) CRC (6, 7). In summary, LS is a well-defined patient population with up to 70% lifetime CRC risk, and these hypermutant tumors are likely to benefit from immune-interception strategies.

Abnormal tumor proteins are processed into short peptides (neoantigens) and presented on the cell surface complexed with major histocompatibility complex (MHC) class I. These frameshift peptides (FSP) can bind to T-cell receptors (TCRs) on cytotoxic CD8+ T-cells, thereby promoting interferon γ (IFNγ) secretion and cancer cell killing (8-10). Neoantigens are also presented in the context of MHC II at the cell surface of dendritic cells (DCs) where they activate CD4+ T-helper cells, which stimulate CD8+ T-cells, natural killer (NK) cells, CD68+ macrophages, and other immune cells to kill neoantigen-expressing cancer cells (11). In contrast, CD4+ CD25+ Foxp3+ regulatory T-cells (Tregs) inhibit CD8+ and CD4+ anti-neoantigen immunity(11-13). Thus, activation of CD8+ and CD4+ T-cells recognizing neoantigens are important for adaptive immunity against tumors, and Tregs suppress the adaptive immune response. LS dMMR CRCs have extremely high numbers of neoantigens (including recurrent shared neoantigens that are repeatedly observed in different patient tumors) (14, 15).

Programmed cell death protein 1 (PD-1) is a receptor expressed on activated immune cells and following binding to its ligand PD-L1 (CD274), promotes activated CD8+ and CD4+ T-cell apoptosis, T-cell exhaustion, and suppression of auto-immunity (10, 16).

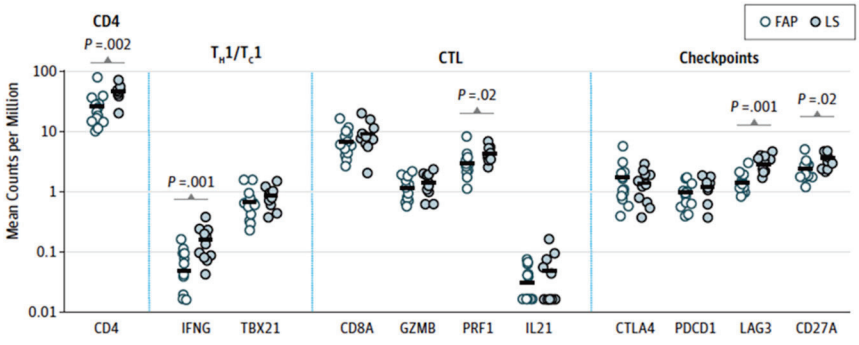


Fig 1. Expression levels of adaptive immunity genes in LS vs hereditary MMR proficient (pMMR) colon adenomas from FAP patients. Data are from RNAseq transcriptomes of 11 LS and 16 pMMR FAP colon adenomas. All *P-values* are adjusted for multiple comparisons. RPKM (Reads/Kb transcript/Million mapped reads; CD274 is PD-L1).

Tumor cells and tumor-associated macrophages commonly express PD-L1, which induces tumor immune tolerance. Anti-PD-1/PD-L1 antibodies reverse T-cell exhaustion and reactivate anti-tumor immunity. Because of their high mutation rates, dMMR tumors are among the most responsive to PD-1/PD-L1 immune checkpoint inhibition (9, 17). However, PD-1/PD-L1 inhibitors also have significant rates of severe adverse events. These include autoimmunity-related lung, hepatic, skin, neuro, colon, and endocrine immune-related adverse events, lymphoma, and other toxicities, some of which may be fatal (18). Thus, while the risk: benefit ratio of PD-1/PD-L1 blockade is acceptable for poor prognosis patients with metastatic tumors, it is unacceptable in the setting of healthy pre-symptomatic LS patients requiring cancer prevention, where the tolerance for side effects is low. PD-1/PD-L1 inhibitors do not, furthermore, have a clear dose response, which makes giving lower doses of these drugs for cancer prevention problematic (18). Therefore, new approaches with lower levels of toxicity are required for LS immune-interception such as preventive pre-cancer and tumor vaccines.

2.2 Nous-209 Prime and Boost Vaccine

Genetic viral vaccines co-opt antigen processing and MHC presentation to activate T-cells and comprise another approach to neoantigen vaccination. Replication deficient adenoviral vectors expressing recombinant antigens may act as T-cell vaccines to induce potent CD8+ and CD4+ T-cell responses and protective immunity against multiple pathogens in pre-clinical and clinical studies (19-27). As reported in the *New England Journal of Medicine*, *Nature Medicine*, and *Science Translational Medicine*, adenoviral T-cell vaccination induces robust CD8+ and CD4+ T-cell responses and protective immunity against Ebola, Malaria, and other pathogens in both patients and mice (20, 25, 26, 28).

Moreover, preclinical data recently published showed that immunization with Nouscom Great Ape Adenovirus (GAd) viral vector encoding neoantigens selected from murine tumor cell line was capable of inducing potent T-cell response (29). Prophylactic vaccination with GAd efficiently controls tumor growth in 100% of treated mice. In the setting of large established tumors, GAd vaccine can eradicate large tumors when combined with anti-PD1 or anti-PD-L1 and enhance the efficacy of such checkpoint inhibitors (29). Vaccination with viral vectors can induce host anti-vector immunity, thus limiting the efficacy of subsequent booster injections. The Nouscom approach employs sequential injections with immunologically unrelated viral vectors to circumvent this problem: (i) a priming infection with a recombinant Gad viral vector encoding a large number of predicted immunogenic neoantigens; (ii) a booster with a genetically unrelated virus, Modified Vaccinia Ankara virus (MVA), that encodes the same neoantigens, but with the neoantigens in a different sequential order so as to minimize potential immune response against junctional epitopes between neoantigens (20, 25, 26, 28) (**Fig 2A**).

Thus, this sequential vaccination strategy using two immunologically distinct viruses carrying the same antigens maximizes the predicted immune response, while minimizing host anti-viral vector immunity. Nouscom computationally predicted 209 recurrent FSP neoantigens in advanced dMMR malignancies from the TCGA data using colorectal, endometrial and gastric tumors according to the pipeline described in **Fig 2B** (30). Then, four GAd (referred as GAd-209-FSP) and 4 MVA (MVA-209-FSP) recombinant viral vector pairs were constructed, each encoding 45-59 predicted immunogenic recurrent dMMR neoantigens for a total of 209 predicted FSPs (**named Nous-209 vaccine**). Details on the list of FSP included in the vaccine are included in Nous-209 Investigator Brochure.

The selection of FSPs included in the Nous-209 vaccine was validated by using an independent cohort of samples of MSI CRC patients. The presence of vaccine-encoded mutations was confirmed in this cohort at the same frequencies observed in the TCGA dataset. Importantly, none of the FS mutations were present in the normal adjacent mucosa of patients with MSI, highlighting the anticipated safety profile of the vaccine (**Fig 2C**) (30).

Next, as proof of principle, the immunogenicity of Nous-209 was evaluated in CB6F1 mice using heterologous prime/boost regimen according to the scheme depicted in **Fig 2D**. Mice were primed at week 0 with GAd-209-FSP. Two weeks later, a group of animals was sacrificed to evaluate GAd-primed immune responses, whereas a second group was boosted with a mix of the MVA-209-FSP. FSP-specific T-cell responses post prime (week 2) and post MVA boost (week 3) were measured by IFN-gamma ELISpot on splenocytes re-stimulated with peptides covering the entire neoantigenic sequence encoded by Nous-209 (**Fig 2B, 2C**). Priming with GAd-209-FSP elicited a strong T-cell–mediated immunity. Responses were efficiently boosted by MVA-209-FSP, with approximately overall 10,000 SFCs/10^6 splenocytes (**Fig 2D**). In summary, Great Apes Adenovirus (GAd) and Modified Vaccinia Ankara Virus (MVA) vector vaccination of dMMR neoantigens can induce potent T cell immune responses in mice.

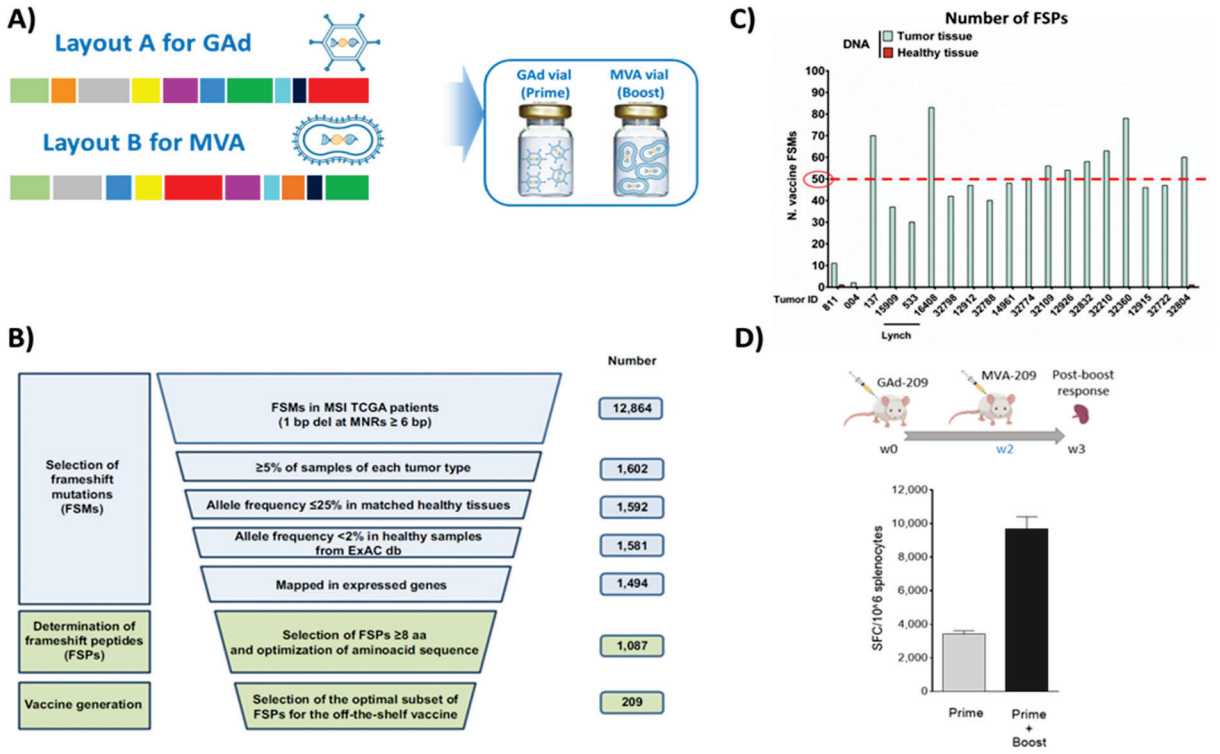


Fig 2. Recurrent LS patient neoantigen viral vaccine immunogenicity. **A.** Schematic of Nous-209 vaccine. NousCom dMMR vaccine is composed of 4 GAd and 4 MVA vector pairs, each coding for ~50 FSP neo-antigens for a total of 209. Each neo-antigen is indicated by a different colored box. Paired GAd and MVA inserts encode the same set of neoantigens in different orders to minimize potential boosting of junctional epitopes between neoantigens. The four vector pairs are pooled together**. B** Scheme to identify the 209 FSPs included in Nous-209 vaccine from TCGA database. **C**. Validation of FSPs selection in MSI patients. Number of FSMs in Nous-209 that were detected on Exomeseq of tumor and healthy tissue from an independent cohort of CRC MSI patients. **D**. Immunogenicity of Nous-209 vaccine in mice. Schematic of immunization schedules and animal groups used. Nous-209 immunogenicity was measured on splenocytes of vaccinated mice by IFNγ ELISpot at 2 weeks post prime with the four GAd vectors (GAd-209-FSP) and one week post boost with the four MVA vectors (MVA-209-FSP). T-cell responses after prime and prime/boost are expressed as the sum of IFNγ SFCs/10^6 splenocytes to the 16 pools of synthetic peptides covering the entire sequence of 209 FSPs. MNR, mono-nucleotide repeat. GAd, Great Apes adenovirus. MVA, modified vaccinia Ankara virus.

Evidence from first-in-human clinical study of Nous-209

The first-in-human (FIH) phase I multicenter, open-label study of Nous-209 (NCT04041310) was designed to evaluate its safety, tolerability, immunogenicity, and efficacy in combination with pembrolizumab – a programmed cell death receptor-1 (PD-1)-inhibitor – for patients with unresectable or metastatic dMMR CRC, gastric cancers, and gastro-esophageal junction (G-E junction) cancers. Nous-209 was administered intramuscularly as a prime (GAd-209-FSP) dose followed by three booster (MVA-209-FSP) doses each given 3 weeks apart (**Fig 3**). Pembrolizumab was administered concomitantly at standard doses according to FDA approved label and schedule.

This study enrolled participants in two cohorts: a "low-dose" escalation cohort and a "high-dose" expansion cohort, with the goal of selecting the recommended phase 2 dose (R2PD) of Nous-209. The target "high-dose" was pre-specified as follows:

- **GAd20-209-FSP polyvalent vaccine:** 1.88 x10^{11} viral particles (vp), comprising a mixture of equal amounts of GAd20-209-FSP-A1; GAd20-209- FSP-A2; GAd20-209-FSP-A3 and GAd20-209-FSP-A4.

- **MVA-209-FSPs polyvalent vaccine:** $1.65x10^8$ infectious units (ifu), comprising a mixture of equal amounts of MVA-209-FSP-B1; MVA-209-FSP-B2; MVA-209-FSP-B3 and MVA-209-FSP-B4.

Accordingly, "low-dose" was defined as $1/10^{th}$ of the "high-dose" (i.e., one log difference in vaccine concentration). The target "high dose" was pre-selected based on safety and immunogenicity data obtained in previous human clinical trials with similar vectors at dosages equivalent to those planned for the FIH study of Nous-209 (as summarized in the Investigator's Brochure).

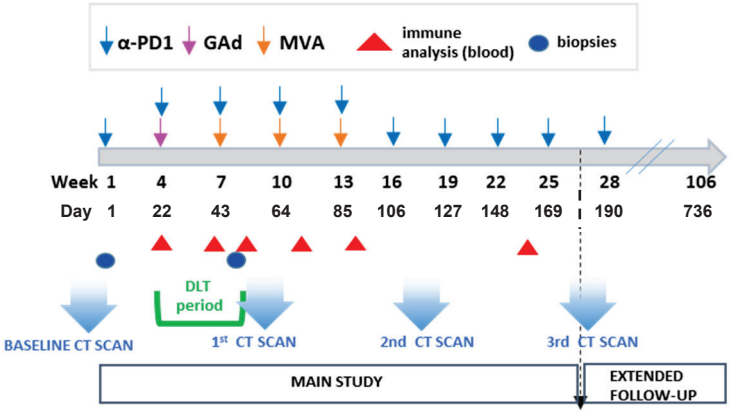


Fig 3. Overall schematic of vaccine administration and safety/efficacy assessments in the FIH Nous-209 study (NCT04041310). GAd, Great Apes adenovirus. MVA, modified vaccinia Ankara virus. a-PD1, anti-programmed cell death receptor-1. DLT, dose-limiting toxicity

Preliminary safety, immunogenicity, and efficacy results of the FIH study have been reported (31, 32). Most recently, data from 12 evaluable participants (3 in the dose escalation cohort, and 9 in the dose expansion cohort) were analyzed as of the reported cut-off date (32). Two participants with LS were among these 12 evaluable participants. With a median follow-up of 20.5 months in dose escalation cohort and 9.4 months in the dose expansion cohort, no dose limiting toxicities, treatment-related deaths, treatment-related adverse events leading to discontinuation of treatment, or serious adverse events were observed. All adverse events related to either GAd20-209-FSP or MVA-209-FSP were grade 1 or 2 (see Section 8.2 for further details).

Immunogenicity was assessed by *ex vivo* IFN-γ ELISpot on peripheral blood mononuclear cells (PBMCs) stimulated with 16 pools of overlapping peptides covering the entire vaccine sequence (**Fig 4**). Across 10 participants with evaluable pre- and post-vaccination PBMC samples, immune response was induced in 67% of participants treated with low-dose FSP-209, and 100% of participants treated with high-dose FSP-209. Vaccine-induced immune responses were potent, with a mean of ~1,500 IFN-γ SFCs/million PBMCs at the peak of the immune response. Based on these data, the target high-dose was selected as the RP2D.

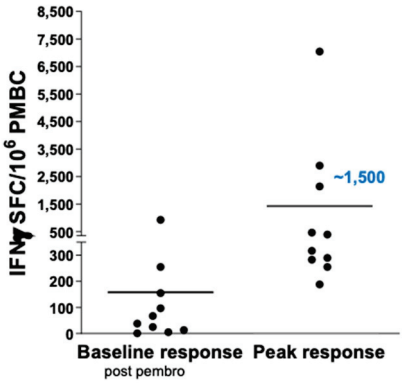


Fig 4. Evaluation of vaccine-induced immunogenicity in the FIH Nous-209 study (NCT04041310). Peak immunogenicity was assessed *by ex vivo* IFN-γ ELISpot on pre- and post-treatment PBMC from 10 evaluable participants using 16 pools of overlapping peptides covering the entire Nous-209 FSP sequence. The number of spot-forming cells (SFCs) per million PBMCs corresponding to the sum of the responses to the single pools are shown. Dot plot represents peak responses for each individual subject (compared with baseline pre-vaccination responses (post pembrolizumab). Lines represent the mean of immune response.

The FIH study also provided early insights into the temporal dynamics of Nous-209-induced immunity. Briefly, in the context of participants with metastatic dMMR/MSI-H cancer, we observed an expected contraction of T cell immune response beginning at 6 months post-vaccination (**Fig 5**). With the expectation of further decline over time, this finding supports the need for future studies to explore optimal Nous-209 revaccination schedules in specific disease contexts.

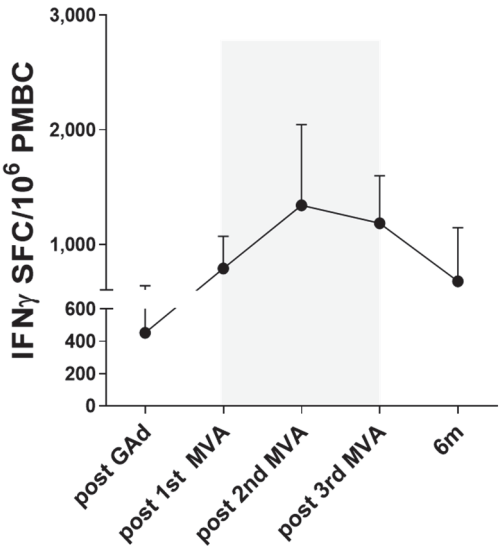


Fig 5. Serial evaluation of Nous-209-induced immunogenicity in the FIH Nous-209 study (NCT04041310). Lines represent the mean *ex vivo* IFN-γ ELISpot response to 16 peptide pools covering the entire Nous-209 sequence.

2.3 Rationale

Vaccine-based immune interception of LS premalignancies

Vaccination represents an important strategy to enhance T-cell immunity against infections and selected tumors (33, 34). Neoantigens are the preferred target antigen for prophylactic cancer vaccines. Specifically, neoantigens derived from indels within coding genes give rise to FSP comprised of novel sequences that are not represented in the human normal proteome and which, therefore, represent the safest and most immunogenic class of neoantigens.

In this context, the Vilar-Sanchez laboratory performed whole transcriptomic analysis of 11 adenomas from patients with LS and 16 MMR-proficient adenomas from patients with familial adenomatous polyposis (FAP). This work revealed that LS adenomas have higher transcript levels of CD4 (consistent with more infiltrating CD4+ T-cells), IFNγ (more activated CD4+/CD8+), PD-L1 (CD274) and LAG3 than sporadic MMR-proficient colon adenomas (**Fig 1**) (35). This finding is consistent with elevated T-cell immune surveillance of LS adenoma neoantigens.

Importantly, the immunogenicity of FSP was demonstrated in T stimulation experiments using blood mononuclear cells isolated both from healthy donors and MSI-H cancer patients (36). Given the observations that LS malignancies contain greatly elevated numbers of neoantigens and that pre-malignant lesions have a less immunosuppressive microenvironment than advanced cancers, we propose that vaccination against LS-associated neoantigens represents a compelling approach for enhancing immune surveillance for LS immunoprevention. Furthermore, based on pre-clinical and clinical evidence outlined above, we hypothesize that vaccination with Nous-209 will induce immunogenicity in T-cells against recurrent MMR-deficient neoantigens contained in the vaccine.

Therefore, we have developed this phase Ib/II study with the primary goal of determining the safety and immunogenicity of Nous-209 vaccination as an "off-the-shelf" immune-interception strategy in LS participants.

The dose of Nous-209 to be evaluated in our trial is the RP2D (i.e., high dose) from the aforementioned FIH phase 1 study (NCT04041310). Our intent is to balance the safety and immunogenicity of GAd-209-FSP MVA-209-FSP, which is supported by results of the initial safety and efficacy analysis summarized in Section 2.2 above. Although no vaccine-related SAEs have been reported with Nous-209 in the FIH clinical trial (NCT04041310) (31, 32), the AE profile could be different in patients with LS without active cancer, who are likely to be more immunocompetent than patients with advanced metastatic cancers. Furthermore, despite the immunosuppressive environment commonly associated with metastatic cancers, Nous-209 is highly immunogenic as defined by the development of T cell responses to at least one FSP pool (31, 32). However, in the healthy LS population, the native antigenic load might be different, thus affecting immunogenicity and efficacy. Therefore, the clinical efficacy of Nous-209 in a healthy LS carrier population warrants separate investigation.

Importantly, our study also aims to evaluate optimal approaches for maintaining long-term immunogenicity in healthy LS participants following initial vaccination with the Nous-209 GAd/MVA series. Specifically, we have noted that revaccination at regular intervals is a common approach to promote long-lasting recall of immune response and protection in the context of infectious diseases. As a pertinent example, Capone et al. (37) have evaluated the safety/tolerability and efficacy of revaccination using Ad and MVA-based vectors against Hepatitis C Virus (HCV) among healthy individuals. The Ad and MVA vectors used in their study were closely related to Nous-209 and participants were revaccinated with additional doses of Ad (prime, n = 9) and/or MVA (boost, n = 14) at varying time intervals (range 8 to 92 weeks). Revaccination with either Ad and MVA was well tolerated regardless of the number of administrations, the order in which they were administered, or the time interval between administration (**Fig 6**). The AE profiles of participants who received a second cycle of Ad (prime) and/or MVA (boost) vaccination were similar to those observed following initial vaccination. In addition, no serious AEs were observed. Among evaluable participants, revaccination with Ad and/or MVA resulted in expansion of vaccine-induced, HCV-specific T cells (**Fig 7**).

While we acknowledge that these data are limited, they highlight the need for further evaluation of Nous-209 revaccination with GAd/MVA (i.e., prime/boost) versus MVA (i.e., boost) only in the context of healthy LS participants, where the goal is to further expand antigen-specific memory T cell response through revaccination of previously-vaccinated participants. As a co-primary endpoint of our study, we have evaluated the rate of adverse events among participants who thus far have received initial vaccination with GAd-209-FSP (prime) and MVA-209-FSP (boost) as of October 3rd, 2023. A summary of the number of participants with their maximum grade event is shown in the table below.

	Maximum Grade	**# Participants**
GAd-209-FSP (prime)	3 2 1 **TOTAL # Participants with AE**	1 5 6 **12**
MVA-209-FSP (boost)	2 1 **TOTAL # Participants with AE**	2 5 **7**

Details on the specific Adverse Events experienced by participants after first dose (so called 'prime') and second dose ('boost') are provided in the following Tables. Overall, the rates of AEs has been low with no unexpected adverse events. A total of 112 adverse events have been observed in 12 participants since vaccination.

Summary of Adverse Events by Grade/Relationship

Time	Grade	Relationship	# Events
Post-Prime	3	Definite	1
	3	Unlikely	2
		GRADE 3 TOTALS	**3**
	2	Definite	10
	2	Probable	5
	2	Possible	2
	2	Unlikely	4
	2	Unrelated	2
		GRADE 2 TOTALS	**23**
	1	Definite	37
	1	Probable	17
	1	Possible	3
	1	Unlikely	7
	1	Unrelated	1
		GRADE 1 TOTALS	**65**
Post-Boost	2	Definite	4
	2	Possible	1
		GRADE 2 TOTALS	**5**
	1	Definite	6
	1	Probable	6
	1	Possible	2
	1	Unrelated	2
		GRADE 1 TOTALS	**16**

The following four participants had adverse events that were considered definitely related to vaccination.

Participants Experiencing Adverse Events Definitely Related to Vaccination

PID	Registration Date	Date_Prime	Date_Booster	CTCAE_Term	Onset_Date	Grade	time
1	11/10/2022	11/15/2022	01/13/2023	Injection site reaction	01/18/2023	2	Post Boost
				Flu like symptoms	11/16/2022	2	Post Prime
				Headache	11/16/2022	2	Post Prime
				Fatigue	11/16/2022	2	Post Prime
				Fever	11/16/2022	2	Post Prime

PID	Registration Date	Date_Prime	Date_Booster	CTCAE_Term	Onset_Date	Grade	time
				Chills	11/16/2022	3	Post Prime
2	12/30/2022	01/19/2023	03/16/2023	Myalgia	01/20/2023	2	Post Prime
				Injection site reaction	01/20/2023	2	Post Prime
				Injection site reaction	01/21/2023	2	Post Prime
				Fatigue	01/20/2023	2	Post Prime
				Musculoskeletal and connective tissue disorder - Other, specify	01/20/2023	2	Post Prime
3	01/18/2023	01/18/2023	03/10/2023	Urticaria	03/10/2023	2	Post Boost
				Nausea	03/10/2023	2	Post Boost
				Pruritus	03/10/2023	2	Post Boost
4	03/04/2023	03/17/2023	05/19/2023	Headache	03/18/2023	2	Post Prime

The following two tables below show maximum toxicity reported. Only those toxicities experienced while on the trial are presented. Should a participant experience the same adverse event but at different grades (say headache at grade 2, 3 and 4), the participant is counted only at the highest (maximum) grade experienced (grade 4 in this case) for that event.

Summary of Adverse Events – Prime Vaccine

Adverse Event	Grade of Toxicity			Total
	1 (Mild)	2 (Moderate)	3 (Severe)	
Anorexia	0	1	0	1
Arthralgia	1	0	0	1
Back pain	0	1	0	1
Bloating	0	1	0	1
Blurred vision	1	0	0	1
Bruising	1	0	0	1
Chills	4	0	1	5
Diarrhea	1	0	0	1
Dizziness	0	1	0	1
Dyspnea	1	0	0	1
Fatigue	7	3	0	10
Fever	6	2	0	8
Flu like symptoms	0	1	0	1
Gastrointestinal disorders - Other, specify	1	0	0	1
Headache	6	2	1	9
Injection site reaction	6	1	0	7
Muscle cramp	0	1	0	1
Musculoskeletal and connective tissue disorder - Other, specify	0	1	0	1
Myalgia	5	2	1	8
Nausea	4	0	0	4
Pain	1	0	0	1
Pain of skin	1	0	0	1
Palpitations	1	0	0	1
Respiratory, thoracic and mediastinal disorders - Other, specify	1	0	0	1
Sinusitis	0	1	0	1

Summary of Adverse Events – Boost Vaccine

Adverse Event	Grade of Toxicity		Total
	1 (Mild)	2 (Moderate)	
Arthralgia	1	0	1
Bruising	1	0	1
Fatigue	2	0	2
Headache	1	0	1
Infections and infestations - Other, specify	1	0	1
Injection site reaction	2	1	3
Musculoskeletal and connective tissue disorder - Other, specify	1	0	1
Myalgia	2	0	2
Nausea	0	1	1
Pain in extremity	0	1	1
Pain of skin	1	0	1
Pruritus	0	1	1
Respiratory, thoracic and mediastinal disorders - Other, specify	1	0	1
Urticaria	0	1	1

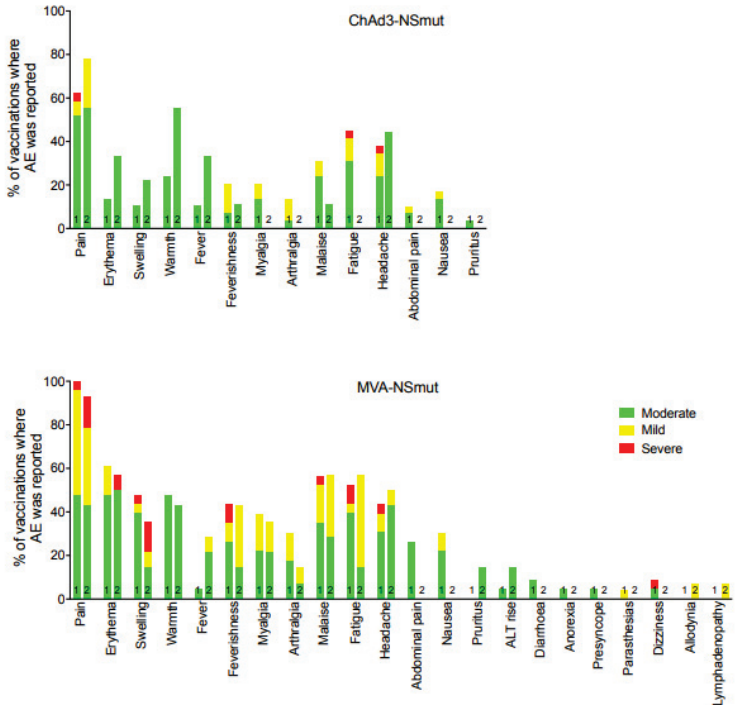


Fig 6. Reboosting with Ad and MVA is safe and well tolerated (Capone et al., Vaccines, 2020). The percentage of volunteers reporting an adverse event following 1st (1) or 2nd (2) vaccination with Ad (top) or MVA (bottom) against HCV. Severity moderate (grade1, green), mild (grade 2, yellow), and severe (grade 3, red)

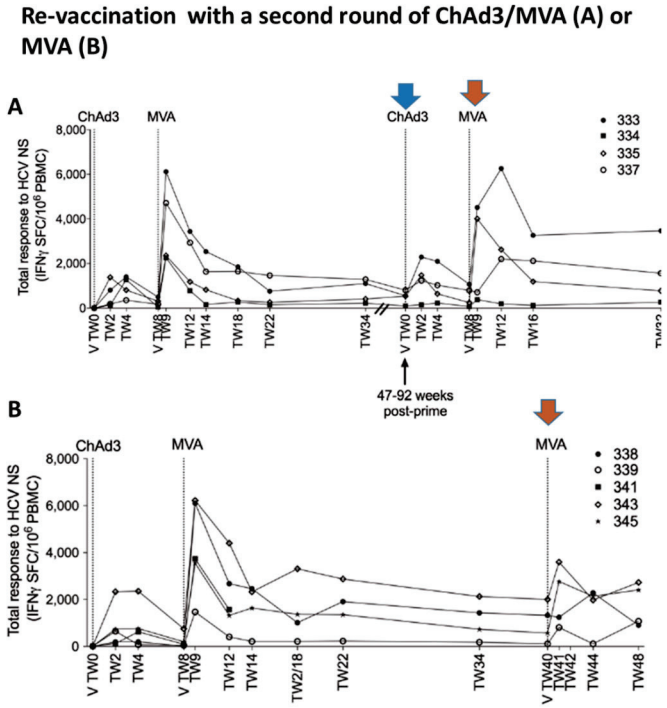


Fig 7. Evaluation of Ad/MVA versus only MVA revaccination against HCV in healthy volunteers (Capone et al., *Vaccines*, 2020). The total ex vivo T cell response to the non-structural (NS) region of HCV encoded within the vaccine is shown over time (IFNγ ELISpot; spot forming cells per 10^6 PBMC).

Our study will aim to have broad eligibility. Namely, we will not exclude participation of LS carriers on the basis of harboring specific MMR germline alterations, and neither will we exclude LS carriers with a prior personal history of premalignancy or malignancy. Nonetheless, it is important to acknowledge that the distinct biological and clinical features associated with right-sided versus left-sided colonic malignancy adds significant complexity to the development of effective immunoprevention strategies. Moreover, in LS carriers with a history of colorectal malignancy, primary treatment is likely to have included surgical resection by a variety of approaches depending on the anatomic location of the tumor (e.g., extended right or left hemicolectomy or partial colectomy, sigmoidectomy, or low anterior resection). To minimize the confounding effects of this biological and clinical heterogeneity, a subset of the secondary objectives in our study are focused on understanding the immunogenicity of Nous-209 vaccination at the level of the rectosigmoid colon (up to 25 cm from the anal verge), which is likely to be present in the majority of LS carriers even after primary treatment.

Our study will be conducted within the context of the NCI CP-CTNet with at least 4 active academic Clinical Cancer Genetics Programs with a history of participation in major national and international clinical trials for hereditary cancer syndromes.

As we explore the efficacy of vaccine-based immunoprevention strategies, it is notable that relatively few data exist regarding the prevalence and incidence rate of premalignant lesions in patients with LS. Based on results of the CAPP-2 study, which examined the prevalence of adenomas and hyperplastic polyps in mutation positive carriers of LS, approximately 10% of and 5% of LS patients presented with adenomatous polyps and hyperplastic polyps in their first colonoscopy in the CAPP-2 study, respectively. In addition, aspirin did not modify the development of adenomas in the CAPP-2 study but provided a reduction of CRC incidence of approximately 50% (IRR 0.56).

Lastly, increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will provide a better understanding the potential relationship

between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, this clinical trial will be including assessment of tobacco and alcohol use at baseline and at completion of the study follow-up period to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

3. SUMMARY OF STUDY PLAN

3.1 Study Design & Objectives

Our study is a phase Ib/II open-label, multicenter prevention study of Nous-209 vaccination as an immune-interception agent in individuals with LS. The study will be performed in two parts:

- Cohort 1 [initial vaccination] includes participants who have not previously received Nous-209 (either as monotherapy or combination). All eligible participants in Cohort 1 will receive one cycle of Nous-209 prime-boost immunization.

- Cohort 2 [revaccination] is comprised of eligible participants from Cohort 1 who received one complete cycle of Nous-209 prime-boost immunization and for whom peak vaccine-induced immune responses have been detected. Participants in Cohort 2 will be randomized to one of two revaccination schedules with Nous-209 prime and/or boost.

Considering both Cohorts 1 and 2, the primary objectives of the study are to evaluate the safety, tolerability, and immunogenicity of GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) vaccination when administered as a single agent (monotherapy) among participants with LS. The secondary objectives are to evaluate the effects of Nous-209 vaccination on a) T cell immune profile and T cell receptor (TCR) repertoire in peripheral blood; b) TCR repertoire within histologically normal colorectal mucosa; c) tumor infiltrating lymphocyte (TIL) immune profile and TCR repertoire within colorectal adenomas; d) cytotoxicity of T cells on matched participant-derived colorectal adenoma organoids; e) the burden of colorectal adenomas/advanced neoplasia/carcinoma; f) burden of LS-related carcinomas; g) cell free DNA (cfDNA) mutation profiles and cfDNA burden in participants with LS; h) to correlate tobacco and alcohol consumption with the immune response to Nous-209 in trial participants; and i) to assess the MMR and/or MSI status of polyps (and adjacent normal mucosa as control) detected in the baseline and end-of-the-study colonoscopy using different technologies such as immunohistochemistry, MSI analysis by PCR, or next-generation sequencing.

Considering Cohort 2, our additional co-primary objective is to ascertain an optimal revaccination schedule for Nous-209 by evaluating the safety, tolerability, and neoantigen-specific immunogenicity of GAd20-209-FSP prime plus MVA-209-FSP boost or MVA-209-FSP boost alone when readministered to previously vaccinated participants with LS.

3.2 Study Population

For Cohort 1 [initial vaccination], our study population is comprised of individuals aged 18 years or older with a diagnosis of LS as determined based on phenotypic and/or genetic criteria. Eligible participants will be those who a) have no active or recurrent invasive malignancy; b) must be at least 6 months from receipt of prior cancer-directed therapy, if any; and c) must have an intact rectosigmoid colon. With an estimated attrition rate of 20%, we will enroll a maximum of 45 participants to the study intervention in order to reach our target of 36 evaluable participants. To account for participants who may be deemed ineligible after consent, we may consent up to 60 individuals to achieve our target of 45 enrolled participants.

For Cohort 2 [revaccination], our study population represents a subset of individuals who were enrolled

into Cohort 1. Specifically, we will be sequentially enrolling eligible participants who a) received a prime/boost vaccination cycle in Cohort 1; and b) for whom peak immunogenicity responses (Week 9 of Cohort 1) are proved by ELISPOT assay. We will enroll 28 participants to Cohort 2 with the goal of reaching 24 evaluable participants.

3.3 Interventions

Cohort 1 [initial vaccination]: At Baseline, all participants will undergo standard-of-care (SOC) lower endoscopy (colonoscopy or flexible sigmoidoscopy) for screening, polyp removal, and research tissue collection. Participants will also have screening at baseline. Upon confirmation of eligibility, enrolled participants will receive Nous-209 vaccination according to the schedule as outlined in **Fig 8**. The initial (priming) vaccination (GAd20-209-FSPs) will be given on day 1 followed by booster (MVA-209-FSPs) at Week 8. Research blood samples will be collected for biomarker assays and AE monitoring on Day 1 (Visit 1), Week 3, Week 8, Week 9, Month 6, and Month 12 (Week 52). All participants who will complete the study in Cohort 1 and who will not continue in Cohort 2 will undergo SOC lower endoscopy (colonoscopy or flexible sigmoidoscopy) with research tissue biopsies collected at Month 12.

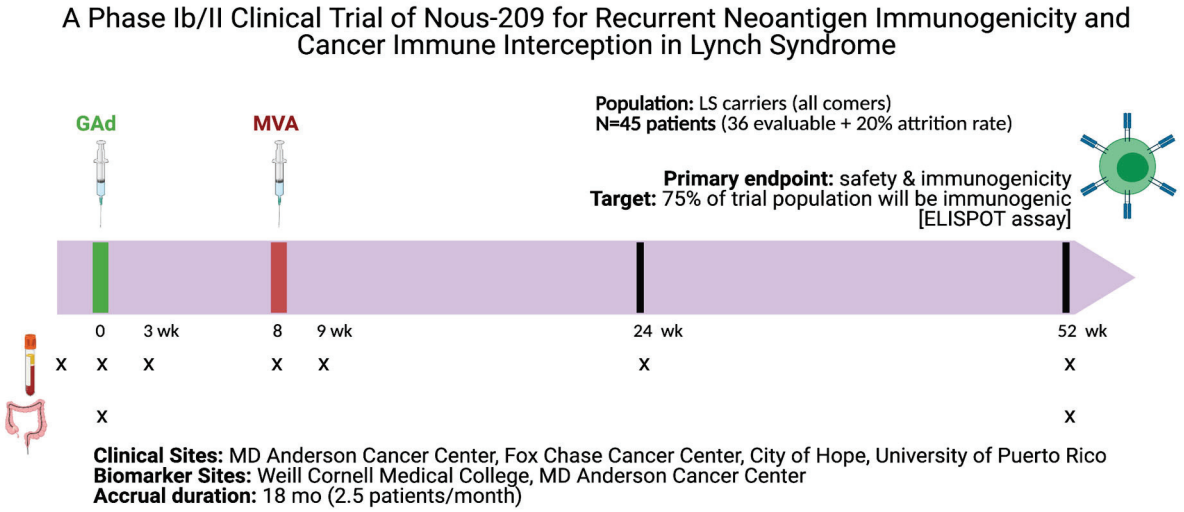


Fig 8. Trial schema with basic procedures and timeline. Accrual duration: 18 mo accrual + 24 mo follow-up. Figure created with BioRender

Cohort 2 [revaccination]: Eligible participants will undergo research blood collection at Month 12 (Week 52) in accordance with study-related procedures for Cohort 1. Participants will then be randomly assigned (in a 1:1 ratio) to one of two revaccination schedules:

- Arm A: GAd20-209-FSP prime and MVA-209-FSP boost
- Arm B: MVA-209-FSP boost only

As outlined in **Fig 9**, participants in Arm A will receive GAd20-209-FSP prime at Week 52, followed by MVA-209-FSP boost at Week 60. Participants in Arm B will receive MVA-209-FSP boost at Week 52. Research blood samples will be collected for biomarker assays at Weeks 52, 60 and 68. All participants in Cohort 2 will undergo SOC lower endoscopy (colonoscopy or flexible sigmoidoscopy) with research tissue biopsies collected at Week 68 instead of Week 52.

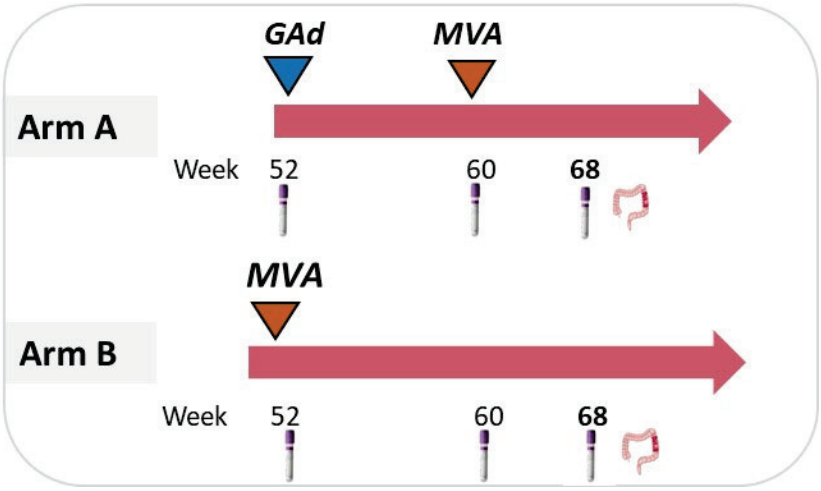


Fig 9. Trial schema for Cohort 2 [revaccination].

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria for Participants in Cohort 1

4.1.1 Participants must have a clinical diagnosis of Lynch Syndrome (LS) as defined by:

a) Mutation-positive LS: documented carriers (or obligate carriers by pedigree) of a germline mutation in MMR genes (*MLH1*, *MSH2/EPCAM*, *MSH6,* or *PMS2)* that is deleterious/pathogenic or suspected to be deleterious/pathogenic (known or predicted to be detrimental/loss of function, respectively). The mutation must have been identified through a CLIA-approved laboratory setting or an equivalent international agency. Final determination of eligibility for any discordant results in pathogenicity of the mutation will be determined by the study investigator. A formal eligibility exception in those instances will not be required as long as approval by the overall study PI has been granted and documented.

b) **Mutation-negative LS (also known as "Lynch-like Syndrome" or "suspected Lynch Syndrome):** individuals with both of the following:

(i) a personal history of a non-sporadic MMR-deficient premalignant lesion (i.e., colorectal polyp) or a non-sporadic MMR-deficient malignant tumor, where "non-sporadic MMR deficiency" is defined by (1) the loss of *MLH1*, *MSH2*, *MSH6*, or *PMS2* expression by immunohistochemistry (IHC), or (2) the detection of MSI by PCR or both, but no evidence of *MLH1* promoter methylation in cases with loss of both MLH1 and PMS2, All testing must have been performed in accordance with local institutional guidelines in a CLIA-approved setting. (Note: central confirmation of MMR expression status, MSI, MLH1 promoter methylation or BRAF mutation is not required.); *and*

(ii) documented results of germline mutation testing performed in a CLIA-approved laboratory environment, demonstrating either a variant of unknown significance in MMR genes or the lack of a clinically significant variant in MMR genes; or, documentation that the individual declined to undergo germline MMR genetic testing.

4.1.2 Participants must have no evidence of active or recurrent invasive cancer for 6 months prior to screening.

4.1.3 Participants must be at least 6 months from any prior cancer-directed treatment (such as surgical resection, chemotherapy, immunotherapy, hormonal therapy, or radiation).

4.1.4 Participants must have endoscopically accessible distal colon and/or rectal mucosa (i.e., participants must have at least part of the descending/sigmoid colon and/or rectum intact).

4.1.5 Participants must consent to standard of care surveillance with colonoscopy with biopsies every 12 months.

4.1.6 Participants must consent to refrain from using aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase (COX) inhibitors for the duration of the trial, except for cardio-preventive aspirin (<100 mg daily). Individuals taking such drugs may not be enrolled unless they are willing to stop the medications (and possibly change to alternative non-excluded medications to treat the same conditions) no less than 1 month prior to enrollment. Participants will discuss with their primary care provider/local provider about the discontinuation of such medication(s) and obtain approval prior to stopping any agent.

4.1.7 Age ≥ 18 years. Because no dosing or adverse event (AE) data are currently available on the use of Nous-209 in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.8 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A)

4.1.9 Participants must have normal organ and marrow function as defined below:

Hemoglobin	≥10 g/dL or Hematocrit ≥ 30 %
Leukocyte count	≥3,500/microliter
Platelet count	≥100,000/microliter
Absolute neutrophil count	≥1,500/microliter
estimated glomerular filtration rate (eGFR) [or creatinine clearance calculated using the Cockcroft-Gault equation]	≥60 mL/min/1.73m2 [mL/min, within institutional limits of normal]
AST (SGOT)/ALT (SGPT)	≤2 times the institutional upper limit of normal (ULN)
Total bilirubin	≤1.5 the ULN; participants with Gilbert's disease may be enrolled with higher Total bilirubin if their Direct bilirubin is ≤ 1.5 times the ULN.

4.1.10 Participants must consent to refrain from receiving any other type of vaccination during the first 10 weeks of the trial.

4.1.11 Participants must consent to refrain from receiving adenoviral-based vaccines for the duration of the trial (including the period from week 9 to week 52).

4.1.12 Willing and able to adhere to the prohibitions and restrictions specified in the final approved protocol.

4.1.13 The effects of Nous-209 on the developing human fetus at the recommended therapeutic dose are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation (12 months) and 6 months after end of study. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.14 Ability to understand and the willingness to sign a written informed consent document (available for both English- and Spanish-speaking individuals).

4.2 Inclusion Criteria for Participants in Cohort 2

4.2.1 Participants must have been confirmed eligible and met all study inclusion criteria for participation in study Cohort 1.

4.2.2 Participants must have completed all baseline procedures and received a complete cycle of GAd20-209 FSP (prime) and MVA-209-FSP (boost) vaccination per protocol for Cohort 1.

4.2.3 Participants must have undergone collection of research blood samples at Baseline (Week 0) and Week 9 for evaluation of the Nous-209-induced immunogenicity endpoint as specified for Cohort 1. Only participants with evaluable and proved immunogenicity response at Week 9 are eligible for participation in Cohort 2.

4.2.4 Participants must consent to refrain from receiving any other type of vaccination during Weeks 52 to 68 of the trial.

4.2.5 Participants must consent to refrain from receiving adenoviral-based vaccines for the duration of the trial (including the period from Week 52 to Week 68).

4.2.6 Participants must consent to refrain from using aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase (COX) inhibitors for the duration of the cohort 2, except for cardio-preventive aspirin (<100 mg daily). Individuals taking such drugs may not be enrolled unless they are willing to stop the medications (and possibly change to alternative non-excluded medications to treat the same conditions) no less than 1 month prior to enrollment. Participants will discuss with their primary care provider/local provider about the discontinuation of such medication(s) and obtain approval prior to stopping any agent.

4.2.7 Female participant must agree to a pregnancy test at week 52.

4.3 Exclusion Criteria

Note: Unless otherwise indicated, exclusion criteria are verified at entry into Cohort 1.

4.3.1 Prior receipt of a recombinant adenoviral or MVA vaccine including COVID-19 Adenovirus vaccines within the previous 6 months.

4.3.2 Histologically-confirmed high-grade dysplasia or cancer on biopsy at screening.

4.3.3 Individuals with active malignancy (excluding non-melanoma skin cancer).

4.3.4 Any serious uncontrolled and/or unstable pre-existing medical disorder (aside from malignancy exception above), psychiatric disorder, or other conditions that could interfere with participant's safety, obtaining informed consent, or compliance to the study procedures.

4.3.5 Active infection (acute and self-limited) or Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), or Hepatitis C virus (HCV) infection. Participants with laboratory evidence of cleared HBV and HCV infection will be permitted.

4.3.6 History of organ allograft or other history of immunodeficiency.

4.3.7 Known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to study drug, excipients, or to egg proteins.

4.3.8 Individuals with a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications (such as infliximab, rituximab, adalimumab, tacrolimus) within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses >10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

4.3.9 Pregnant or breastfeeding or planning to become pregnant within 6 months after the end of study. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with Nous-209, breastfeeding should be discontinued if the mother is treated with Nous-209.

4.3.10 Men attempting or planning to conceive children during the study or within 6 months after the end of the study.

4.3.11 Participants may not be receiving any other Investigational Agents.

4.3.12 Cohort 2 only: participants who experienced grade 3 or higher AE(s) attributed to study drug in Cohort 1, excluding reactogenicity events as defined in Section 13.1.

4.4 Inclusion of Women and Minorities

Our study will include adult (age≥ 18 years) men and women of all races and ethnic groups and who are deemed eligible. Children will not be recruited to the trial. Our recruitment strategies, including minority recruitment, will include identifying participants through The University of Texas MD Anderson Cancer Center Familial High-Risk Gastrointestinal Cancer Clinic, Houston, TX; University of Puerto Rico, San Juan, PR; Fox Chase Cancer Center, Philadelphia, PA; City of Hope National Medical Center, Duarte, CA. Our other sources of recruitment will involve working with the minority community and social network groups available at each accruing site within the US. We will advertise the study on minority and other national websites. Furthermore, our informed consent form will be available in both English and Spanish.

Site staff will be directed to pay special attention to the nature of any disadvantage that potential participants may have, and consider their respective unique needs and vulnerabilities. Consequently, potential participants will receive explanation of the costs of participating in the trial in detail. They will receive explanation the of trial and related procedures in lay language. In addition, when available, family members and caretakers will be in the consent discussion, and will be informed that the protocol allows assent and permits Legally Authorized Personnel.

4.5 Recruitment

This multicenter protocol will be conducted at the following sites: UT MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA; City of Hope National Medical Center, Duarte, CA; and University of Puerto Rico, San Juan, PR. Efforts will be made to enroll participants from diverse ethnic and socio-economic backgrounds. Prior to enrollment on the study, the physician and/or study personnel will discuss the study protocol in detail with the participant, including possible toxicities. The informed consent document will be reviewed by study personnel with the participant. To facilitate accrual, information about the trial will be advertised, for example, on the websites of participating institutions and through social media channels. Recruitment and retention efforts will be evaluated routinely by site coordinators and study staff members. The study recruitment and retention plan will be modified as necessary to promote rapid accrual. Please refer to the study-specific Recruitment and Retention Plan for more details.

4.6 Planned Accrual

Lynch syndrome has no known racial proclivity. Previous studies have reported on only a modest number of African American and Hispanic families with Lynch syndrome. Thus, we will project that the targeted number of participants from ethnic and racial subpopulations in our study will be proportionate to the prevalence distribution of the study cohort in the U.S. population. The planned enrollment estimates are shown below.

DOMESTIC PLANNED ENROLLMENT REPORT

Racial Categories	Not Hispanic or Latino: Female	Not Hispanic or Latino: Male	Hispanic or Latino: Female	Hispanic or Latino: Male	Total
American Indian/Alaska Native	1	0	0	0	1
Asian	0	1	0	0	1
Native Hawaiian or Other Pacific Islander	0	1	0	0	1
Black or African American	2	1	0	0	3
White	16	17	2	2	37
More Than One Race	1	1	0	0	2
Total	20	21	2	2	45

Note: The Planned Enrollment Report table has been revised with the protocol version dated 09/10/2024. The calculations have been adjusted to match the Black or African American enrollment projections to the ethnic and racial subpopulations of the States in which the study is conducted: California (5% Black or African American); Pennsylvania and Texas (12% of Black or African American); and Puerto Rico (17% of Black or African American). Given the historical barriers to Black or African American participant accrual to clinical trials (48), the goal was further adjusted to 3.

5. REGISTRATION PROCEDURES

5.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually which is done via the Registration and Credential Repository (RCR).

To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to Rave or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR (Investigator)	NPIVR (Non-physician Investigator)	AP (Associate Plus)	A (Associate)	AB (Associate Basic)
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to participate in all CP-CTNet clinical trials.

All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be registered in the RCR.

Personnel associated with the five registration types include, but is not limited to, the following:

- **Investigator (IVR)** — MD, DO, or international equivalent.
- **Non-Physician Investigator (NPIVR)** — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD).

- **Associate Plus (AP)** — clinical site staff (e.g., RN or CRA) with data entry access to RAVE. Also includes site administrator, data administrator, and consenting person. Individuals with an auditing role should register as an AP.
- **Associate (A)** — other clinical site staff involved in the conduct of NCI-sponsored trials.
- **Associate Basic (AB)** — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

In addition, the site-protocol Principal Investigator (PI) must meet the following criterion:
- Active registration status
- The IRB number of the CIRB (IRB of record) listed on their Form FDA 1572

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

6. NCI CENTRAL INSTITUTIONAL REVIEW BOARD

The NIH policy on the Use of a Single Institutional Review Board for Multi-Site Research https://grants.nih.gov/grants/guide/notice-files/not-od-16-094.html became effective on January 25, 2018. In compliance with this policy, NCI Central IRB (NCI CIRB) is the sole IRB of record for all accruing sites conducting clinical trials through the CP-CTNet, all CP-CTNet U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB), and utilize the Cancer Prevention and Control CIRB as their IRB of record. International sites should submit Research Ethics Board (REB) approval to the DCP Regulatory contractor following country-specific regulations.

Signatory Institutions must submit a Study Specific Worksheet (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the PIs at the Signatory Institution and the Regulatory Contractor. In order for the SSW approval to be processed, the Signatory Institution must inform which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the CIRB prior to implementation.

7. AGENT ADMINISTRATION

The study agent will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 8.2.

7.1 Dose Regimen and Dose Groups

For Cohort 1 [initial vaccination], our study will be conducted as a single-arm, open-label, non-randomized intervention trial. As such, all eligible study participants in Cohort 1 will be assigned to a single treatment group and receive Nous-209 vaccination as follows:

- Week 0 (Day 1): GAd20-209-FSPs (prime)
- Week 8: MVA-209-FSPs (boost)

For Cohort 2 [revaccination], we will conduct an open-label, randomized intervention. 28 eligible participants in Cohort 2 will be randomly assigned to one of two treatment groups for revaccination (Arm A or B) as follows:

Arm A:
- Week 52: GAd20-209-FSPs (prime)
- Week 60: MVA-209-FSPs (boost)

Arm B:
- Week 52: MVA-209-FSPs (boost)

GAd20-209-FSP polyvalent vaccine comprises 4 replication-incompetent gorilla-derived Great Apes Adenoviruses (GAd20). GAd20-209-FSP polyvalent vaccine is formulated in A195 buffer at the nominal concentration of $2x10^{11}$ viral particles (vp)/mL. The vaccine is vialed in 3 mL Borosilicate Type 1 glass vials. Each vial is used to prepare the vaccine dose for one participant. The vial is wasted after one participant dose preparation and not stored.

MVA-209- FSP polyvalent vaccine comprises 4 attenuated, replication-defective orthopoxvirus, Modified Vaccinia virus Ankara (MVA). MVA-209-FSP polyvalent vaccine is formulated in MVA buffer at the nominal concentration of $2x10^{8}$ infectious units (ifu)/mL. The vaccine is vialed in 3 mL Borosilicate Type 1 glass vials. Each vial is used to prepare the vaccine dose for one participant. The vial is wasted after one participant dose preparation and not stored.

Both vaccines must be administered as a single 1 mL-dose corresponding to the nominal concentration of $2x10^{11}$ vp/mL for GAd20-209-FSP and $2x10^{8}$ ifu/mL for MVA-209-FSP for intramuscular injection.

GAd20-209-FSP and MVA-209-FSP are manufactured under Good Manufacturing Practice (GMP) conditions at Reithera GMP facility (Via di Castel Romano 100, 00128 Rome, Italy).

7.2 Nous-209 Vaccine Administration

On the day of vaccination, GAd20-209-FSP (prime) and MVA-209-FSP (boost) vaccines will be allowed to thaw at room temperature and administered within 1 hour from thawing. Each vaccine will be administered intramuscularly over the deltoid region of the arm at the time points detailed in Section 9. The injection volume is 1 mL.

The medical professional performing the procedure must wear gloves and appropriate eye protection. The 2 vaccines (1 prime and 1 boost) will be administered each time on the contralateral arm compared to the previous administration. In the event that the investigator finds the injection location inappropriate (i.e., due to the loss of muscle mass of the deltoid region), then the vaccination can be performed by injection in the participant's femoral quadriceps region.

During the administration of the vaccines, appropriate medicines and resuscitation equipment must be immediately available for the management of anaphylaxis. In particular epinephrine and equipment for maintaining an airway should be available for immediate use. Following vaccination, participants must remain 1 hour in the unit for observation. The timing of 1 hour is considered adequate for the acute vaccine

reactions management [General Recommendations on Immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP) January 28, 2011].

To minimize dissemination of the recombinant vectored vaccine virus into the environment, the inoculation site must be fully covered with an appropriate dressing immediately following immunization. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site and disposed as GMO waste, in accordance with the current standard US practice.

7.3 Run-in Procedures

A placebo run-in phase will not be used in this study.

7.4 Contraindications

- Participants should not be breast-feeding.
- Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation (12 months) and 6 months after end of study. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

7.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented and will include: 1) start and stop date, dose and route of administration, and indication.

Participants must refrain to receive any other type of vaccination during the first 10 weeks of the trial. In addition, participants must refrain from using aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase (COX) inhibitors for the duration of the trial, except for cardio-preventive aspirin (<100 mg daily) during the first 10 weeks of the trial. Individuals taking such drugs may not be enrolled unless they are willing to stop the medications (and possibly change to alternative non-excluded medications to treat the same conditions) no less than 1 month prior to enrollment. Participants will discuss with their primary care provider/local provider about the discontinuation of such medication(s) and obtain approval prior to stopping any agent.

7.6 Dose Modification

Every effort must be made to administer the GAd20-209-FSP and MVA-209-FSP according to the protocol-mandated schedule outlined in Section 9. Schedule modifications or dose reductions of GAd20-209-FSP and MVA-209-FSP are not permitted in individual participants.

In the event of hypersensitivity reactions following the administration of GAd20-209-FSP and MVA-209-FSP, the participant should be treated according to best medical practice. Acute Hypersensitivity Reactions including anaphylaxis may occur after vaccine injections, therefore appropriate resuscitation equipment should be available at the bedside and a physician readily available during the vaccine administration and

during the first hours after vaccination. All participants should be instructed to immediately report to the Investigator any delayed hypersensitivity reactions, which may manifest as:

- Decreased oxygen saturations (<92%) on room air
- Confusion
- Lethargy
- Hypotension
- Pale or clammy skin
- Cyanosis
- Bronchospasm

In the event of any such clinical findings and suspicion of a severe hypersensitivity reaction, either acute of delayed, participants must be immediately admitted to hospital for observation and monitoring. Participants who experience a severe hypersensitivity reaction following administration of GAd20-209-FSP will not receive MVA-209-FSP.

Exclusive of reactogenicity symptoms (defined in Section 13.1), participants who experience grade 2 adverse events attributed to study drugs (GAd20-209-FSP and/or MVA-209-FSP) persisting > 48 hours or any grade 3 or higher adverse event attributed to study drugs following administration should receive no further doses.

7.7 Adherence/Compliance

7.7.1 Definition of Compliance

Participants who receive at least the GAd20-209-FSP (prime) dose will be included in the evaluation of our safety-related endpoints. In addition, for Cohort 1, we will define compliance as receiving both the GAd20-209-FSP (prime) and MVA-209-FSP (boost) vaccine administrations.

In Cohort 2, participants who receive at least the GAd20-209-FSP (prime) dose in Arm A or MVA-209-FSP (boost) in Arm B will be included in the evaluation of our safety-related endpoints. In addition, for Cohort 2, we will define compliance as receiving either both the GAd20-209-FSP (prime) and MVA-209-FSP (boost) vaccine administrations in Arm A or MVA-209-FSP (boost) vaccine administrations in Arm B.

7.7.2 Monitoring of Compliance

Adherence will be monitored during each study visit. Adherence will be measured by the proportion of missed appointments and drop-out rates. Participants will have a grace period of 7-14 days after the scheduled vaccination time. Adherence rates will be reported descriptively.

For our biomarker-driven endpoints, we perform analyses in two levels: first, by including all participants, both compliant and non-compliant (i.e., intention-to-treat principle); and second, by including only participants who met the definition of compliance.

8. PHARMACEUTICAL INFORMATION

8.1 Nous-209 Vaccine

Nous-209 is a neoantigen vaccine composed of two different products (GAd20-209-FSP and MVA-209-FSP polyvalent vaccines) which are designed to be used in a heterologous prime/boost modality. The GAd20 and MVA viral vectors encode for a set of 209 FSPs identified following an analysis of dMMR/MSI-H CRC, gastric, G-E junction, and endometrial tumor sequences in the TCGA database.

GAd20-209-FSP is a polyvalent vaccine. It is manufactured under Good Manufacturing Practice (GMP) conditions at Reithera GMP facility (Via di Castel Romano 100, 00128 Rome, Italy). GAd20-209-FSP comprises 4 replication-incompetent gorilla-derived Great Apes Adenoviruses (GAd20). The GAd20-209-FSP polyvalent vaccine is formulated in A195 buffer (10 mM Tris, 75 mM NaCl, 1 mM MgCl2, 0.02% PS80, 5% sucrose, 0.1 mM EDTA, 10 mM Histidine, 0.5% ethanol, pH 7.4) at the dose of $2x10^{11}$ vp/mL.

MVA-209-FSP is a polyvalent vaccine. It is manufactured under Good Manufacturing Practice (GMP) conditions at Reithera GMP facility (Via di Castel Romano 100, 00128 Rome, Italy). MVA-209-FSP polyvalent vaccine comprises 4 attenuated, replication-defective orthopoxvirus, Modified Vaccinia virus Ankara (MVA). MVA-209-FSP polyvalent vaccine is formulated in MVA buffer (10 mM Tris, 140 mM NaCl, pH 7.7) at the dosage of $2x10^{8}$ ifu/mL. The vaccine is vialed in 3 mL Borosilicate Type 1 glass vials.

GAd20-209-FSP and MVA-209-FSP are each provided as a glass vial closed by a rubber stopper and sealed with an aluminum tear-off cap. Both vaccine products may be administered as a high-dose (1 mL) intramuscular injection formulated without preservative.

Historically, the agent manufacturer used the actual concentrations of study agents, measured by qPCR, to define the doses of the initial lots produced. In keeping the industry best practices, the agent manufacturer currently uses the nominal concentrations for both GAd-209-FSP and MVA-209-FSP to define the doses after acquiring confidence on the qPCR acceptance range. Changes in the actual concentration within the acceptance range do not cause any difference in the safety and tolerability of the products, and therefore the nominal concertation is to be used to define subsequent lots.

8.2 Reported Adverse Events and Potential Risks

The FIH phase I study of Nous-209 (NCT04041310) is designed to evaluate its safety, tolerability, immunogenicity, and efficacy in combination with pembrolizumab (anti-PD-1) for patients with unresectable or metastatic MMR-deficient CRC, gastric cancers, and gastro-esophageal junction (G-E junction) cancers. Although complete results of the FIH study are not yet reported, preliminary findings support the safety of Nous-209 (31, 32, 38). Participants were enrolled in two consecutive cohorts with one log difference in vaccine dose:

- **Low-dose (cohort A; dose-escalation):** GAd20-209-FSP polyvalent (prime) vaccine at a dose of $1.88\ x10^{10}$ vp and MVA-209-FSPs polyvalent (boost) vaccine at a dose of $1.65x10^{7}$ ifu
- **High-dose (cohort B; dose-expansion):** GAd20-209-FSP polyvalent (prime) vaccine at a dose of $1.88\ x10^{11}$ vp and MVA-209-FSPs polyvalent (boost) vaccine at a dose of $1.65x10^{8}$ ifu

With a safety data cut-off of September 29th, 2021, no treatment-related deaths or treatment-related AEs leading to permanent discontinuation of treatments were observed among 12 evaluable participants (three in the low-dose cohort, and 9 in the high-dose cohort) (32). No DLTs were observed among the three participants in the low-dose cohort.

All AEs related to either GAd20-209-FSP or MVA-209-FSP were grade 1/2 and were comparable between both dose levels. These included injection site pain, fatigue, decreased appetite, and fever. AEs occurring on or after the date of GAd20-209-FSP administration were considered Treatment Emergent AEs (TEAEs). Among the 12 evaluable participants, 5 serious (grade 3/4) TEAEs were observed and included cellulitis, adenocarcinoma of colon, psychotic disorder, pulmonary embolism, and enteritis. None of the serious TEAEs were considered related to GAd-209-FSP or MVA-209-FSP per the Investigator and Sponsor's assessment.

Consistent with the above, several prior clinical studies using the GAd prime and MVA boost vector platform against viral pathogens have demonstrated its favorable safety profile (27, 28, 39-41). In one the largest studies performed to date, NCT01436357 was a phase I/II randomized, double-blind, placebo-controlled trial evaluating the safety and efficacy of prime/boost vaccination in 548 healthy individuals at risk of HCV infection (42). Consistent with prior data, there were no vaccine-related serious adverse events identified in the study.

Altogether, the most common reported vaccine-related adverse events are typically mild (grade 1 or 2) and manifest as *local reaction* and/or *systemic reactions*:

- Local reactions: a local inflammatory reaction that can include redness, swelling, scaling, tenderness, or itching.
- Systemic reactions: flu-like symptoms, such as low-grade fever, chills, and malaise

These reactions typically resolve within 7 days of vaccination.

8.3 Availability

The GAd20-209-FSP (prime) and MVA-209-FSP (boost) vaccines are Investigational Agents supplied to investigators by the Division of Cancer Prevention (DCP), NCI.

The GAd20-209-FSP (prime) and MVA-209-FSP (boost) vaccines are provided to the NCI under a Clinical Trials Agreement (CTA) between Nouscom (Agent Manufacturer) and the DCP, NCI (see §14.7).

8.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of CIRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or his/her authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form: https://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/Investigational-Agent-Request.docx (to include complete shipping contact information) and e-mailing the form to the DCP agent repository contractor:

John Cookinham

MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
E-mail: NCI.DCP@mriglobal.org

8.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility may be delegated to the study staff, institutional pharmacist or their designees. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant. The Investigator at each accruing site will be responsible for study agent accountability for participants.

8.6 Packaging and Labeling

GAd20-209-FSP and MVA-209-FSP will be packaged by Nouscom, Inc. Labeling and distribution to the sites will be done by NCI, DCP or its authorized designee.

8.7 Storage

The storage conditions are specified on the labels. Clinical supplies must be stored in a secure, limited-access temperature controlled location. Receipt and dispensing of trial medication must be recorded by an authorized person at the study site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Refer to the Pharmacy Manual for storage temperature and the Temperature Excursion policy and procedure(s). The Pharmaceutical partner (if required), CP-CTNet LAO, the DCP agent repository contractor, DCP Medical Monitor and Nurse Consultant should be notified in the event of a Temperature Excursion.

8.8 Registration/Randomization

This trial will use a web-based Registration/Randomization System, developed and maintained by the CP-CTNet Data Management, Auditing, and Coordinating Center (DMACC). The Help Desk includes technical personnel and administrators of the registration programs at the Data Management Center in Amherst, NY, USA. The Help Desk is available round the clock 7 days per week, except for New Year's Eve, Memorial Day, Independence Day, Thanksgiving Day, and Christmas Day.

Frontier Science Randomization Help Desk
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716 834 0900 Extension 7301
Email: UserSupport_CP-CTNet@frontierscience.org

Note: The Registration and Enrollment process is documented in the "Stars User Guide".

Screening and Registration into the DMACC Stars System:
To obtain a Screening ID, log into Stars and verify the participant is eligible for contact. Once confirmed, a Screening ID will be assigned. Once informed consent has been signed, the participant can be registered into the DMACC Stars System. The Stars System will assign a Participant's ID upon completion of the registration process.

Enrollment:
Participants will be assigned a Participant ID once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility information has been entered into the Stars System web application. The Participant ID will be generated by the system and assigned to the participant. The data from Stars will automatically transfer to Medidata Rave clinical data management system, which is the CP-CTNet database of record and subject to NCI and Food and Drug Administration (FDA) audits, within 5 minutes of entry into Stars.

Recruitment Journaling and Clinical Study Data in Medidata Rave:
The Affiliated Organizations will enter both Recruitment Journaling information and study data into the Medidata Rave.

Screening/Registration/Enrollment into site-specific databases:
The Medidata Rave is the database of record for the study. Registration and enrollment should occur per the procedures outlined above. If the site staff need to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the PI signoff form. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into Stars and Medidata Rave. If a participant experiences a serious adverse event during the screening process, an SAE form must be completed.

8.9 Blinding and Unblinding Methods

No blinding will be utilized for this study.

8.10 Agent Destruction/Disposal

DCP-supplied agents: at the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "DCP Returned Agents List".

9. CLINICAL EVALUATIONS AND PROCEDURES

9.1 Schedule of Events

9.1.1 Schedule of Events for Cohort 1

Evaluation/ Procedure	**Baseline Testing / Pre-Study Evaluation**		**Study Intervention**					**Follow-up**			
Evaluation/ Procedure	**Screening Visit / Eligibility / Registration / Enrollment [within ≤ 30 days of Day 1]**	**Week #:**	**0 [Day 1]**	**0 [Day 2-4]**	**3 (+/- 7 days)**	**8 (+/-7 days)**	**9 (+7 days)**	**16 (+/-14 days)**	**24 (+/-14 days)**	**36 (+/-14 days)**	**52 (+/- 14 days)**
		Study Visit #:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9[9]**
Informed Consent	X										
Medical History	X										
Baseline Symptoms	X										
Physical Exam / Vital Signs	X										
Height & Weight	X										
BMI	X										
Liver Function Panel	X[1]										
Hematology and Chemistry	X[2]										
Anti-Hepatitis C Virus, Hepatitis B Virus, and HIV serology	X[3]										
HLA typing	X[4]										
Pregnancy Test	X[5]										
Confirm Eligibility	X										
Registration	X										
Lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies	X[6]										X[6]
Research blood sample			X		X	X	X		X		X
Concomitant Medications	X		X	X	X	X	X	X	X	X	X
GAd20-209-FSP (prime) administration			X								
MVA-209-FSP (boost) administration						X					
Adverse Events/Symptom Assessment[7]			X	X	X	X	X	X	X	X	X
Telephone Contact/Telehealth Visit				X				X		X	
Tobacco and Alcohol Assessment[8]			X								X
COVID-19 Assessment[8]											X
Vaccine Report Card[9]			X	X		X					

Study Schedule Footnotes

1. Liver Function Panel: including serum AST/SGOT, serum ALT/SGPT, and serum total bilirubin.
2. Hematology and Chemistry Laboratory Tests: including CBC (complete blood count) with hemoglobin, leukocyte count, platelet count and differential (including absolute neutrophil count); serum creatinine; and serum electrolytes (sodium, potassium); prothrombin time (PT) / international normalized ration (INR), and partial thromboplastin time (PTT)
3. Viral serology tests including: Hepatitis C Virus (HCV) antibody, Hepatitis B Virus (HBV) surface antigen (HBsAg), and Human Immunodeficiency Virus (HIV)-1/2 antibody. Counseling will be given prior to testing blood for these blood-borne viruses.

4. Human Leukocyte Antigen genotyping. The HLA genotyping test performed as part of "Baseline Testing/Pre-Study Evaluations" does not need to be repeated if the patient does not start the vaccine within 30 days of Day 1. The HLA report may be used at any time because the results will not change.
5. In women of childbearing potential, a urine or serum pregnancy test must be done at Baseline as part of eligibility verification.
    a. If the pregnancy test result is positive at Baseline (any time before enrollment), the participant is a Screen Failure.
    b. If a pregnancy is discovered after Day 1, the vaccine will not be given. Take participant off-study, complete the Off-Study eCRFs, follow the pregnancy to term and complete the Outcome of Pregnancy CRF.
6. If indicated, lower GI endoscopy may be performed as standard-of-care flexible sigmoidoscopy in those participants with total or subtotal colectomy; otherwise, complete colonoscopy is the standard of care procedure:
    a. Lower GI endoscopy may be performed within 60 days of Day 1.
7. The Study staff should pay particular attention to the following potential events: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
8. The participants will be given the option to complete paper form questionnaires or complete the questionnaires electronically, either face to face with the study staff or remotely according to the participant's preference. See Appendix B and Appendix C.
9. Each participant will be asked to record symptom reactivity events daily in the Vaccine Report Card, from day 0 to day 6 post vaccine. See Appendix E.

ATTENTION COORDINATORS!

- **If a participant is completing Cohort 1 and *NOT* moving on to Cohort 2, then the participant's visit at Week 52 is their last visit and coordinators must perform all tests and procedures as outlined in § 9.1.1 Schedule of Events. The lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies will be performed at Week 52 (+/- 14 days) if the participant is not continuing on for Cohort 2.**
- **If the participant *IS* moving on to Cohort 2, then coordinators should start the participant's Week 52 visit with the procedures noted in the § 9.1.2 Schedule of Events. For participants who continue to Cohort 2, the lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies will be performed at Week 68 (+/- 14 days) according to the Schedule of Events outlined in Section 9.1.2.**

9.1.2 Schedule of Events for Cohort 2[1]

Evaluation/ Procedure	Study Intervention		
Week #:	**Screening Visit / week 52 (+/- 14 days)[1]**	**Week 60 (+/-7 days)**	**Week 68 (+/-14 days)**
Study Visit #:	**9**	**10**	**11**
Informed Consent[2]	X		
Physical Exam / Vital Signs	X		
Height & Weight	X		
Pregnancy Test[3]	X		
Confirm Eligibility[4]	X		
Randomization/stratification	X		
Research blood sample	X	X	X
Concomitant Medications	X	X	X
Lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies[5]			X
GAd20-209-FSP (prime) administration	Arm A		
MVA-209-FSP (boost) administration	Arm B	Arm A	
Adverse Events/Symptom Assessment[6]	X	X	X
Telephone Contact/Telehealth Visit/ in-person visit.	X	X	X
Tobacco and Alcohol Assessment[7]	X		
COVID-19 Assessment[7]	X		
Vaccine Report Card[8]	X	X	

Study Schedule Footnotes

1. Only for participants who qualify for cohort 2.
2. Participant must be consented before any study procedure is performed; participants who are screened 14 or more days after signing informed consent will need to be re-consented.
3. In women of childbearing potential, a urine or serum pregnancy test must be done as part of eligibility verification for Cohort 2 study.
    a. If a pregnancy is discovered at the Cohort 2 Screening Visit, the vaccine will not be given. Take participant off-study, complete the Off-Study eCRFs, follow the pregnancy to term and complete the Outcome of Pregnancy CRF.
4. Eligibility must be verified prior to randomization.
5. If indicated, lower GI endoscopy may be performed as standard-of-care flexible sigmoidoscopy in those participants with total or subtotal colectomy; otherwise, complete colonoscopy is the standard of care procedure.
6. The study staff should pay particular attention to the following potential events: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
7. The participants will be given the option to complete paper form questionnaires or complete the questionnaires electronically, either face to face with the study staff or remotely according to the participant's preference. See Appendix B and Appendix C.
8. Each participant will be asked to record symptom reactivity events daily in the Vaccine Report Card, from day 0 to day 6 post vaccine. See Appendix E.

9.2 Baseline Testing/Pre-Study Evaluation

Potentially eligible participants are identified through database/charts/clinic lists prior to Baseline Visit. A letter will be mailed to all potentially eligible participants introducing the study to them. Alternatively, or

in addition to the letter, a phone call from the study staff introducing the study may be made.

Note: Receipt of a recombinant adenoviral or MVA vaccine including COVID-19 Adenovirus vaccines within 6 months of Baseline excludes individuals from study participation. Participants must consent to refrain from receiving any other type of vaccination during the first 10 weeks of the trial (in Cohort 1) and weeks 52-68 of the trial (in Cohort 2). Participants must consent to refrain from receiving adenoviral-based vaccines for the duration of the trial (including the period from week 9 to week 68).

Screening Visit: Baseline Testing / Eligibility / Registration / Enrollment [≤30 days of Day 1]

The following procedures will be conducted at the Screening Visit which must occur within 30 days prior to Study Visit 1. Participants who are screened 30 or more days after signing the informed consent will need to be re-consented and have all screening procedures repeated to determine eligibility with the exception of the lower GI endoscopy, which will have a window of up to 60 days of Day 1; and that HLA typing does not have to be repeated if done outside the window of ≤30 days of Day 1.

- Informed consent must be obtained prior to starting any further study procedures.
- Registration: Once informed consent has been signed, participants will be registered into the DMACC Stars System. The DMACC Stars System will assign a participant's ID upon completion of the registration process. Participants will also be registered into site-specific registry databases as applicable.
- Medical history, to include a review of cancer history and previous medical history, previous surgery, chemotherapy, radiation, and other cancer-directed therapy history, family history and genetic diagnosis (including results of clinically-indicated genetic analysis to confirm the presence or absence of germline MMR gene mutation, if available), demographic information, including age and race.
- Baseline symptom assessment will include: constipation, diarrhea, headache, dizziness, drowsiness, lightheadedness, vertigo, easy bruising/bleeding, gastrointestinal bleeding, hematemesis, hematochezia, melena, abdominal pain/cramping, heart burn, irregular heart rate/palpitations, chest pain, shortness of breath, and edema.
- Use of concomitant medications will be collected, with particular attention to chemo-preventive use of non-steroidal anti-inflammatory drugs (NSAIDs), here defined as taking aspirin (>81 mg daily) or naproxen (>200 mg daily) more than 5 days per month; (2) pre- or probiotic supplement use; (3) antibiotic use; and (4) immunosuppressive drug use including (but not limited to) oral glucocorticoids.
- Participants must consent to refrain from using aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase (COX) inhibitors for the duration of the trial, except for cardio-preventive aspirin (<100 mg daily). Individuals taking such drugs may not be enrolled unless they are willing to stop the medications (and possibly change to alternative non-excluded medications to treat the same conditions) no less than 1 month prior to enrolling. Consultation with the participant's primary care provider will be obtained prior to stopping any agent.
- A physical examination including vital signs (temperature, blood pressure, heart rate, respiratory rate), height, weight, BMI.
- Baseline laboratory evaluations include: complete blood count (including hemoglobin, leukocyte count, platelet count) with differential (including absolute neutrophil count); creatinine, electrolytes (including sodium, potassium), AST/SGOT, ALT/SGPT, bilirubin (total); viral serology (including HBV sAg, HCV antibody, HIV-1/2 antibody); HLA genotyping; coagulation panel (including PT/INR, PTT). Standard of care labs are acceptable for use to confirm eligibility if done within 30 days of Study Visit 1. The HLA genotyping test performed as part of "Baseline Testing/Pre-Study

Evaluations" does not need to be repeated if the patient does not start the vaccine within 30 days of Day 1. The HLA report may be used at any time because the results will not change.

- Standard of care lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) is performed to access the residual descending/sigmoid colon and/or rectum in a participant. (See Section 9.7 for details):
    - Lower GI endoscopy may be performed within 60 days of Day 1.
    - Visual counting of any polypoid lesion observed in the entire colon (colonoscopy) or rectosigmoid area (flexible sigmoidoscopy) should be done and recorded;
        - If any abnormal mucosa/polypoid lesions were identified during endoscopy, standard procedures of clinical care of the local enrolling site should be followed for biopsy, tissue handling, and subsequent pathologic assessment of these lesions.
        - Any abnormal mucosa/polypoid lesions tissue collected in the manner described above that may be residual after clinical care pathologic assessment will be collected and retained for research purposes (Refer to Section 12 for procedure for the collection of residual polyp tissue).
    - For research purposes, participants will undergo biopsy of grossly normal mucosa from the intact and accessible rectosigmoid (Refer to Section 12 for collection details).
- For women of child-bearing potential, a urine or serum pregnancy test must be done at Baseline as part of eligibility verification. Results must be known prior to enrollment and initiation of study intervention. The urine pregnancy test must be done in the clinic.
    - If the pregnancy test result is positive at Baseline (any time before enrollment and initiation of study intervention), the participant is a Screen Failure.
    - If a pregnancy is discovered after Study Visit 1, vaccine will not be given. Take the participant off-study, complete the Off-Study CRFs, follow the pregnancy to term and complete the Outcome of Pregnancy CRF.
- Confirm eligibility: At the completion of the screening period all baseline testing and pre-evaluation procedures, eligibility must be confirmed. Only after eligibility is confirmed and eligibility CRF is entered into the web application, eligible participants will be scheduled for Study Visit 1.
- All data will be captured on the appropriate CRFs.

Notes:

- Previously screened participants may be rescreened for enrollment in the study. Participants who are rescreened 30 days after signing the informed consent will need to be re-consented and have all screening procedures repeated to determined eligibility, with the exception of the lower GI endoscopy, which will have a window of up to 60 days of Day 1; and HLA typing does not have to be repeated if done outside the window of ≤30 days of Day 1.
- Any screen failed participant based on medical history, physical exam or laboratory values will need to have a screen failure case report form completed. Information will be recorded on the appropriate CRF(s) up until the point at which the participant was found ineligible and screen failed.
- For any participant who is deemed as screen failure, tissue samples collected at Baseline are to be saved for future use at the respective affiliated organization and shipped to Dr. Eduardo Vilar-Sanchez with the other specimens per the instructions in Section 12.3.

9.3 Evaluation During Study Intervention for Cohort 1

Study Visit 1 (Start of Intervention): Week 0 (Day 1)

- Study Visit 1 is an in-person visit.
- Concomitant medication review.

- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- GAd20-209-FSP (prime) intramuscular injection (see Section 7 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- Tobacco and Alcohol Use Assessment questionnaires. The participants will be given the option to complete paper form questionnaires or complete the questionnaires electronically, either face to face with the study staff or remotely, according to the participant's preference. See Appendix B. Refer to Appendix B for resources for alcohol and tobacco quitting. These resources can be given to individuals if there is concern about alcohol or tobacco dependence. It is not expected that investigators will refer individuals for assistance or that they will undertake the care of individuals for alcohol or tobacco dependence.
- Each participant will be asked to record symptom reactivity events daily in the Vaccine Report Card, from day 0 to day 6 post vaccine. See Appendix E.
- All data will be captured on the appropriate CRFs, including the participant questionnaires.

Study Visit 2 (Telephone/Telemedicine Contact): Week 0 (Days 2-4)

- Study Visit 2 is a telephone/telemedicine visit.
- Concomitant medication review.
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
    - If any AEs concerning to either the participant or the study staff are reported during the telephone contact, the participant will be scheduled for a clinic visit and evaluation, and the study investigator(s) will be notified in a timely fashion.
    - If no events concerning to either the participant or the study staff are reported that warrant a clinic visit, the participant will be scheduled for a subsequent study visit according to the calendar of events.
    - If any Grade 3-4 adverse events occur, the participants must be referred to the appropriate study physician.
- The study staff will remind the participants to complete the VRC. See Appendix E.
- All data will be captured on the appropriate eCRFs.

Study Visit 3: Week 3

- Study Visit 3 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- All data will be captured on the appropriate eCRFs.

Study Visit 4: Week 8

- Study Visit 4 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- MVA-209-FSP (boost) intramuscular injection (see Section 7 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- The study staff will remind the participants to complete the VRC. See Appendix E.
- All data will be captured on the appropriate eCRFs.

9.4 Evaluation at Completion of Study Intervention for Cohort 1

Study Visit 5: Week 9

- Study Visit 5 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- All data will be captured on the appropriate eCRFs.

9.5 Post-intervention Follow-up Period for Cohort 1

Study Visit 6: Week 16 (Month 4)

- Study Visit 6 is a telephone/telemedicine visit.
- Concomitant medication review.
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- All data will be captured on the appropriate eCRFs.

Study Visit 7: Week 24 (Month 6)

- Study Visit 7 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- All data will be captured on the appropriate eCRFs.

Study Visit 8: Week 36 (Month 9)

- Study Visit 8 is a telephone/telemedicine visit.
- Concomitant medication review.
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- All data will be captured on the appropriate eCRFs.

Note: a participant who, for any reason, received the first GAd20-209-FSP (prime) vaccination, but not the booster (MVA-209-FSPs), will be considered for a post-treatment lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) and other study interventions listed under Study Visit 9 if the participant received GAd20-209-FSP (prime) vaccination not more than 15 months prior to the scheduled Study Visit 9 procedures.

Study Visit 9: Week 52 (Month 12)

- For participants who complete Cohort 1 and do not continue in Cohort 2, all study procedures will complete according to the Schedule of Events outlined in Section 9.1.1. The lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies will be performed at Week 52 (+/- 14 days). For participants who continue to Cohort 2, the lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies will be performed at Week 68 (+/- 14 days) according to the Schedule of Events outlined in Section 9.1.2.
- Study Visit 9 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Standard of care lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) is performed to access the residual descending/sigmoid colon and/or rectum in a participant. (See Section 9.7 for details):
    - Visual counting of any polypoid lesion observed in the entire colon (colonoscopy) or rectosigmoid area (flexible sigmoidoscopy) should be done and recorded;
        - If any abnormal mucosa/polypoid lesions were identified during endoscopy, standard procedures of clinical care of the local enrolling site should be followed for biopsy, tissue handling, and subsequent pathologic assessment of these lesions.
        - Any abnormal mucosa/polypoid lesions tissue collected in the manner described above that may be residual after clinical care pathologic assessment will be collected and retained for research purposes (Refer to Section 12 for procedure for the collection of residual polyp tissue).
    - For research purposes, participants will undergo biopsy of grossly normal mucosa from the intact and accessible rectosigmoid (Refer to Section 12 for collection details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- Tobacco, Alcohol Use, and Covid-19 Assessment questionnaires. The participants will be given the option to complete paper form questionnaires or complete the questionnaires electronically, either face to face with the study staff or remotely, according to the participant's preference. See Appendix B and Appendix C.
- All data will be captured on the appropriate eCRFs.

9.6 Evaluation During Study Intervention for Cohort 2

Study Visit 9: Week 52 (Month 12)

- Potentially eligible participants for Cohort 2 will be those who received a prime/boost vaccination cycle in Cohort 1 and for whom peak immunogenicity responses (Week 9 in Cohort 1) are proved by ELISPOT assay. Once such participants are identified, they will be notified that they are eligible for Cohort 2 of the study. A letter may be sent to them by mail, informing them of their eligibility for Cohort 2, alternatively, or in addition to the letter, a phone call from the Study Staff may be made, informing the participant of their eligibility for Cohort 2.
- Informed consent must be obtained prior to starting any further study procedures.
- Confirm eligibility: Once eligibility based on immunogenicity response has been verified, the pregnancy test has been verified negative and pre-evaluation procedures also confirm eligibility, the eligibility CRF should be entered into the web application and eligible participants will be randomized.
- Randomization/stratification: Once registration is complete, eligibility has been confirmed and eligibility CRF is entered into the web application, participants will be assigned a randomization number pre-generated by the database. Participants will be randomized to receive either Arm A or Arm B.
- Study Visit 9 is an in-person visit.
- Gad20-209-FSP (prime) intramuscular injection for Arm A participants or MVA-209-FSP (boost) intramuscular injection for Arm B participants (see Section 7 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- A physical examination including vital signs (temperature, blood pressure, heart rate, respiratory rate), height, weight, BMI.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Tobacco, Alcohol Use, and Covid-19 Assessment questionnaires. The participants will be given the option to complete paper form questionnaires or complete the questionnaires electronically, either face to face with the study staff or remotely, according to the participant's preference. See Appendix B and Appendix C.
- Each participant will be asked to record symptom reactivity events daily in the Vaccine Report Card, from day 0 to day 6 post vaccine. See Appendix E.
- All data will be captured on the appropriate eCRFs.

Study Visit 10: Week 60 (Month 15)

- Study Visit 10 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- MVA-209-FSP (boost) intramuscular injection for Arm A (see Section 7 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.

- The study staff will remind the participants to complete the VRC. See Appendix D.
- All data will be captured on the appropriate eCRFs.

Study Visit 11: Week 68 (Month 17)

- Study Visit 11 is an in-person visit.
- Concomitant medication review.
- Research blood collection (75 mL) for immunogenicity and cfDNA studies (see Section 12 for details).
- Review adverse events / symptom assessment. The study staff should pay particular attention to: abdominal pain, fever>100.4F, irregular heart rate/palpitations, hypotension, confusion, chest pain, shortness of breath, lightheadedness or dizziness, easy bruising/bleeding, and notify the study investigator(s) immediately if reported.
- Standard of care lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) is performed to access the residual descending/sigmoid colon and/or rectum in a participant. (See Section 9.7 for details):
    - Visual counting of any polypoid lesion observed in the entire colon (colonoscopy) or rectosigmoid area (flexible sigmoidoscopy) should be done and recorded;
        - If any abnormal mucosa/polypoid lesions were identified during endoscopy, standard procedures of clinical care of the local enrolling site should be followed for biopsy, tissue handling, and subsequent pathologic assessment of these lesions.
        - Any abnormal mucosa/polypoid lesions tissue collected in the manner described above that may be residual after clinical care pathologic assessment will be collected and retained for research purposes (Refer to Section 12 for procedure for the collection of residual polyp tissue).
- All data will be captured on the appropriate eCRFs.

9.7 Methods for Clinical Procedures

Lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) with biopsies:

1. Standard of care lower GI endoscopy (flexible sigmoidoscopy or colonoscopy) is performed to access the residual descending/sigmoid colon and/or rectum in a participant.
2. Bowel preparation: all patients will undergo an oral bowel prep. No prep should be taken per rectum (i.e., enema or suppository) due to possible rectal mucosal trauma and possible associated changes in prostaglandin levels. Dietary instructions prior to this procedure are according to the standard of care at the local site/provider, but typically include NPO except for medications after midnight.
3. For patients undergoing colonoscopy, sedation is customary. For patients undergoing flexible sigmoidoscopy, sedation is optional.
4. The flexible endoscopy equipment to be utilized is per the standard of care and/or physician preference at the local site. There is no requirement for magnification, narrow-band imaging, dye spray or any other advanced equipment.
5. Patient is placed in lateral decubitus position. The flexible endoscope is advanced via the anus and/or an end ostomy as applicable and traversed proximally until accessible mucosa of the descending/sigmoid colon and/or rectum is visualized.
6. Any polypoid lesion visualized on the mucosa of study interest in the entire colon should be visually counted and recorded.
7. The actual tissue of abnormalities or polypoid lesions visualized on the mucosa anywhere within the colorectum should be handled according to the standard of care at the local site in terms of endoscopic tattoo, biopsy, handling, and clinical care pathologic assessment. Any such tissue

collected in the manner above that may be residual after clinical care pathologic assessment will be collected and retained for research purposes.

8. For research purposes, a total of up to 10 biopsies of grossly normal mucosa from the rectosigmoid colon (up to 25 cm from the anal verge) will be obtained using jumbo biopsy forceps (Radial Jaw 4, 2.8mm bite size and 3.2mm working channel diameter) by opening and pressing the biopsy forceps perpendicular to the mucosal surface with mild pressure. Each biopsy is taken approximately 1 cm or more from other biopsy sites within the rectosigmoid colon that had no visual appearance of trauma or recent biopsy. Endoscopists are encouraged to maintain as much consistency as possible in the biopsy depth into the mucosa.
9. All samples are processed according to procedures specified in Section 12.

10. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

10.1 Primary Endpoint

The primary endpoints of our study are:

a) Rates of grade 2/3 adverse events and symptom reactivity following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost): Each participant will be asked to record symptom reactivity events daily on a memory aid (Vaccine Report Card, Appendix E). Participants will record temperature and the presence and intensity of post-vaccination reactogenicity events, including pain at injection site, muscle aches, chills, headache, nausea, feeling tired, underarm pain, underarm swelling, warmth at vaccination site, itchiness at vaccination site, change in appetite, joint pain, and any other symptoms they might be experiencing after vaccination. Erythema and induration at the vaccination site will be measured in millimeters. Any symptoms still present 8 days after initiation will continue to be followed by participant memory aid notations until 8 days after symptom resolution. Participants will also be asked to record any medications taken and any emergency room or physician visits (other than routine check-ups). The memory aid will be reviewed with each participant at subsequent clinic visits.

b) Rate of immunogenicity following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) (Cohort 1): Immunogenicity will be defined as reactivity to at least 1 of 16 synthetic FSP pools using an enzyme-linked immune absorbent spot (ELISpot) assay. For each participant, the endpoint will be evaluated at baseline and at Week 9 (following vaccination with MVA-209-FSP). In the case of detection of reactivity pools at baseline, an increase of at least 80% of the pre-existing reactivity will be considered as a positive response to the vaccine. This methodology has been used in the evaluation of immunogenicity in the FIH clinical trial presented in section 2.2 of this protocol.

c) Rate of immunogenicity following revaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) or MVA-209-FSP (1 boost) alone (Cohort 2): Immunogenicity will be defined as reactivity to at least 1 of 16 synthetic FSP pools using an enzyme-linked immune absorbent spot (ELISpot) assay. For each participant in Cohort 2, the endpoint will be evaluated at Week 52 and at 8 Weeks after the last boost with MVA-209-FSP, corresponding to Week 68 and Week 60, for arm A and B, respectively. This methodology has been used in the evaluation of immunogenicity in the FIH clinical trial presented in section 2.2 of this protocol.

d) Rates of grade 2/3 adverse events and symptom reactivity following revaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost) or MVA-209-FSPs (1 boost) alone (Cohort 2):

Each participant will be asked to record symptom reactivity events daily on a memory aid (Vaccine Report Card, Appendix E). Participants will record temperature and the presence and intensity of post-vaccination reactogenicity events, including pain at injection site, muscle aches, chills, headache, nausea, feeling tired, underarm pain, underarm swelling, warmth at vaccination site, itchiness at vaccination site, change in appetite, joint pain, and any other symptoms they might be experiencing after vaccination. Erythema and induration at the vaccination site will be measured in millimeters. Any symptoms still present 8 days after initiation will continue to be followed by participant memory aid notations until 8 days after symptom resolution. Participants will also be asked to record any medications taken and any emergency room or physician visits (other than routine check-ups). The memory aid will be reviewed with each participant at subsequent clinic visits.

10.2 Secondary Endpoints

Our secondary endpoints include the following:

a) Changes in T cell immune profile and T cell receptor (TCR) repertoire in the peripheral blood of participants with LS at Weeks 3, 9, 24 (Month 6), and 52 (Month 12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

b) Changes in TCR repertoire within histologically normal colorectal mucosa of participants with LS at Week 52 (Month12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

c) Changes in the gene expression profile and TCR repertoire of tumor infiltrating lymphocytes (TIL) within colorectal adenomas of participants with LS at Week 52 (Month 12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

d) T cell cytotoxicity against matched colorectal adenoma organoids derived from participants with LS at Week 52 (Month 12) relative to following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

e) Percentage change in the number of colorectal adenomas, advanced neoplasia, and/or carcinomas in participants with LS at Week 52 (Month 12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

f) Rate of LS-related carcinomas at Week (Month 12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

g) Change in cfDNA detectability and mutation profile in the peripheral blood of participants with LS at Weeks 3, 9, 24 (Month 6), and 52 (Month 12) relative to baseline following vaccination with GAd20-209-FSPs (1 prime) and MVA-209-FSPs (1 boost).

h) To correlate tobacco and alcohol consumption with the immune response to Nous-209 in trial participants.

i) To assess the MMR and/or MSI status of polyps (and adjacent normal mucosa as control) detected in the baseline and end-of-the-study colonoscopy using different technologies such as immunohistochemistry, MSI analysis by PCR, or next-generation sequencing.

This protocol will retain residual tissue biopsies of adenomatous polyps and normal mucosa in participants with LS to determine future candidate biomarkers measured by genomic and transcriptomic platforms after the samples have been processed for standard pathologic evaluation.

10.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or development of gastrointestinal or other malignancy. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

10.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy, or physician decision to take the participant off study.

Participants found to be ineligible during the screening phase and after signing the Informed Consent document and assigning the PID number, will be considered "screen failures". Such participants will be taken off study and the appropriate end of study CRFs will be completed for these participants. In cases of early termination, when possible, participants will be asked to return to the clinic site prior to going off study for an early termination visit according to the Schedule of Events. A detailed description can be found in Section 8.8.

10.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

11. CORRELATIVE/SPECIAL STUDIES

To evaluate the effect of Nous-209 vaccination on our biomarker endpoints, this study will collect and process samples of peripheral blood, normal rectosigmoid tissue, and adenomatous (polyp) tissue for all eligible participants. Details regarding the planned methodology are provided in the accompanying Pharmacokinetic and Biomarker Methods Development Report.

11.1 Rationale for Methodology Selection

11.1.1 Rationale for Blood Analyses

We will collect 75 mL of blood from each participant at Visit 1 (Day 1), Week 3, Week 8, Week 9, Week 24 (Month 6), and Week 52 (Month 12) [during Cohort 1] and at Weeks 60 and 68 [during Cohort 2] in order to perform the laboratory correlates outlined below and in the accompanying Biomarker Methods Development Report. The correlates will support our primary and secondary objectives to evaluate both the temporal and spatial dynamics of neoantigen FSP immunogenicity following vaccination with Nous-209.

Quantification of immunogenicity: The primary assay to monitor vaccine-induced immunogenicity is the ex-vivo IFN-γ ELISpot. 60 mL of blood will be collected in green vacutainer at pre-prime at Visit 1 (Day 1), post-prime (Week 3), pre-boost (Week 8), post-boost (Week 9) vaccination and then at Months 6 and 12 visits [during Cohort 1] and at Weeks 60 and 68 [during Cohort 2] will be drawn. Peripheral blood cell monocytes (PBMCs) will be isolated and viably frozen using standard protocols. PBMCs will be thawed and placed in microplate wells pre-coated with anti-human IFN-γ antibody. PBMC will be cultured with antigen peptides for 16-20 hours, resulting in the re-stimulation of the precursor cells and secretion of IFN-γ. The assay will measure the number IFNγ-secreting cells from frozen PBMC obtained before and after each vaccination (prime and boosts) and at all defined time points in the study will be compared. ELISpot activity will be measured using a central ELISpot reader following SOPs. For the purpose of the primary endpoint within Cohort 1, PBMCs collected on Visit 1 (Day 1) and post-boost (Week 9) will be used to establish immunogenicity, which will be defined as an increase in the number of spot-forming cells of at least 80% compared to the baseline in at least one of the 16 peptide pools. Similarly, within Cohort 2, PMBCs collected on Week 52 and at 8 Weeks after the last boost with MVA-209-FSP (corresponding to Week 68 and Week 60, for arm A and B, respectively) will be used to evaluate immunogenicity following prime-boost or boost-alone. Immunogenicity results obtained from ELISPOTs will be put into the context of the specific HLA alleles present in each of the participants to verify antigen presentation.

Characterization of T cell receptor (TCR) repertoire in peripheral blood. For participants in Cohort 1, 5 mL will be collected in EDTA vacutainer at Visit 1 (Day 1), post-prime (Week 3), pre-boost (Week 8), post-boost vaccination (Week 9), months 6 and 12. For participants in Cohort 2, samples will also be collected at Weeks 52, 60, and 68. Blood will be aliquoted into cryovials tubes and stored in -80°C. For the analysis, frozen blood will be thawed and DNA from CD3+ T cells will be analyzed using ImmunoSeq Deep (Adaptive) to assess and quantify the T cell repertoire.

Quantification and characterization of cfDNA in peripheral blood. 10 mL blood will be collected in Streck BCT isolation tubes for plasma. Total cell-free DNA (cfDNA) will be purified using standard isolation protocols for the time points described in the ELISPOT section. Sequencing reads will be aligned using different methodologies such as MS_Mutect (43) and others, and cfDNA MSI fraction will be delineated using MSI-Sensor and other methodologies (44-46).

11.1.2 Rationale for Rectosigmoid Tissue Sampling

At Baseline and Month 12 (or Week 68 for Cohort 2), we will obtain up to a total of 10 jumbo random biopsies of macroscopically normal tissue in the visualized rectosigmoid colon. We will collect an additional 1-2 biopsies from polyps larger than 10 mm in size. Further details regarding the collection of biopsies are provided in Sections 9.2 and 9.6. Our planned usage for the tissue samples is outlined in Table 1 below. After standard processing, all clinical and research biopsies will be read at each site by an experienced gastrointestinal pathologist. Standard reporting criteria for adenomatous and non-adenomatous polyps, advanced adenomas and carcinoma will be used. Microscopic inflammation will be reported according to established criteria.

Table 1. Planned use of tissue biopsies

Biopsy #	Tissue type	Planned use
Biopsy 1 (Formalin-fixed)	Normal	Histological evaluations and tissue markers
Biopsy 2-3 (RNALater)	Normal	mRNA for transcriptomic analysis
Biopsy 4 (Flash frozen)	Normal	For future studies
Biopsy 5-10 (Fresh)*/Flash frozen	Normal	T cell isolation
Biopsy 11-12 (Fresh)*/Flash frozen	Adenoma**	T cell isolation

*The fresh biopsies will be collected in MD Anderson participants only; and only when feasible due to timing of procedure/experimental conditions. The rest of the trial sites will be collecting these samples as flash frozen.
** only to be collected in participants with polyps > 10 mm

11.1.3 Rationale for Rectosigmoid Tissue Analyses

T cell TCR repertoire analysis in T cells of normal mucosa and adenomas. Biopsies of normal colorectal mucosa and adenomas will be taken during pre-vaccination and post-vaccination colonoscopies (baseline, month 12). Lamina propria CD3+ T cells will be isolated using CD3 magnetic bead sorting. Autologous PBMCs will be infected with a non-GAd, non-MVA viral vector carrying as insert the 209 neoantigens or insert-less viral vector control and used as antigen presenting cells. ELISPOT will be performed and also DNA from CD3+ T cells will be isolated and analyzed using ImmunoSeq Deep (Adaptive) to assess and quantify the T cell repertoire.

11.2 Comparable Methods

The co-primary and secondary objectives of our study are to provide novel insights regarding the immunogenicity effect of Nous-209 vaccination within normal and pre-malignant intestinal tissue of individuals with LS. To achieve this aim, we will apply validated high-throughput genomic and transcriptomic profiling methods such as ELISPOT and ImmunoSeq. Although the context of these methods is unique to our study, similar methods have been applied in prior and on-going studies of GAd and MVA prime/boost vaccination, including NCT04041310. We anticipate that our results will be comparable to genomics datasets derived from pre-clinical mouse models of LS, as discussed above in section 2.3.

12. SPECIMEN MANAGEMENT

12.1 Laboratories

12.1.1 Tissue Samples

Normal rectosigmoid mucosa	
Biopsy 1 Histopathology and tissue markers	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center
Biopsy 2, 3: RNALater mRNA for genomic analysis	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center
Biopsy 4: Flash frozen Future studies (biorepository)	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center
Biopsy 5-10: Fresh*/Flash Frozen T cell isolation/TCR sequencing	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center
Colon polyp	
Biopsy 11-12: Fresh*/Flash Frozen T cell isolation/TCR sequencing	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center

*The fresh biopsies will be collected in MD Anderson participants only; and only when feasible due to timing of procedure/experimental conditions. The rest of the trial sites will be collecting those samples as flash frozen.

12.1.2 Blood Samples

Whole blood	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center

PBMCs	Central Laboratory (Nouscom, s.r.l.) Italy
Plasma cfDNA	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center

12.1.3 Abnormal mucosa/polypoid lesions tissue

Abnormal mucosa/polypoid lesions tissue Future studies	Eduardo Vilar-Sanchez, M.D., Ph.D. MD Anderson Cancer Center

12.2 Collection and Handling Procedures

Random, blinded, unique ID numbers will be assigned to specimens at baseline and post-treatment and will be distributed with the PID number. All specimens will be labeled with the unique specimen number (XXXX), date (MM-DD-YY) and product code (N) using a permanent, waterproof marker. All efforts will be made to keep laboratories blinded from distinguishing participant information and visit point but will be kept available in a password protected log at each site to indicate which specimen number belongs to which participant and at what visit. Shipped specimens will be tracked by the shipping company per routine.

12.2.1 Fresh Tissue

Rectosigmoid colon mucosa biopsy techniques have been described in Section 9.2 and 9.6. The types of assays planned for each of the tissue biopsy specimens are listed in Table 1, Section 11.

12.2.1.1 Normal rectosigmoid colon mucosa

Biopsy 1: Histopathology and tissue markers

Collected at Baseline and Study Visit 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). Normal rectosigmoid colon mucosa biopsy 1 will be processed following standard pathological procedures to obtain a formalin-fixed paraffin-embedded block of the biopsy. The block will be sent to the Vilar Lab at MD Anderson to prepare an H&E slide for pathology evaluation and verification of the normal status of the mucosa. The rest of the block will be used to determine the type and number of immune and mesenchymal cells changes after exposure to Nous-209. Paraffin blocks obtained from Biopsy 1 will be used to create a TMA and assessed by different multiplex technologies.

Biopsy 2, 3: RNALater

mRNA for genomic analysis collected at Baseline and Study Visit 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). Normal rectosigmoid colon mucosa biopsies 2 and 3 will be kept in RNALater for mRNA extraction and sequencing studies. Immediately upon removal from participant, the tissue should be rinsed in sterile phosphate buffered saline (PBS). Using tissue collection tool, place each biopsy in (1) prefilled cryovial. Store in a secure 4 °C refrigerator (range of 4-6 °C) for a minimum of 24 hours and up to 7 days. Remove the cryovials from the refrigerator and place them in a secure -80 degree Celsius freezer (range -70 °C to -80 °C).

Biopsy 4: Flash Frozen
Future studies (biorepository) collected at Baseline and Study Visit 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). Normal rectosigmoid colon mucosa will be flash frozen in liquid nitrogen. Immediately upon removal from participant, colonic tissue should be rinsed in sterile PBS. All fecal material should be removed from the sample during this process. The biopsies will be placed in cryovials, then placed in liquid nitrogen or on dry ice. Store at a temperature of -80 °C. Biopsies 4 will be retained for those cases in which any of the previous techniques fail to render adequate results due to lack of sufficient amounts of tissue or for future genomic and transcriptomic studies.

Biopsy 5 – 10: Fresh/Flash Frozen
T cell isolation/TCR sequencing collected at Baseline and Study Visit 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). Fresh biopsies will be collected in MD Anderson participants only; and only when feasible due to timing of procedures/experimental conditions. The rest of the trial sites will be collecting these samples as flash frozen. Normal rectosigmoid colon mucosa biopsies 5 through 10 will be plunged into a 50 mL tube containing ice-cold sterile PBS, immediately upon removal. The tissue will then be minced with a sterile scalpel inside a petri dish containing collagenase dissociation solution (DS). The dissected-tissue solution will be incubated at 37°C for 1.5 hours to activate the enzymatic digestion by collagenase. Then, the digested solution will be serially filtered using 70 μm and 40 μm filters to obtain a single-cell suspension, which will be washed with PBS and resuspended in PBS with 0.04% BSA. CD3+ TILs will be isolated from the single-cell suspension using the REAlease® CD3 (TIL) MicroBead Kit (Milteny Biotec, cat#130-121-152). DNA extraction will be performed from the sorted CD3+ TILs using the QIAamp DNA Micro kit. With the DNA, we will perform capture, amplification, and sequencing of the CDR3 region from the TCRβ variable (V), diversity (D) and joining (J) segments using the ImmunoSEQ TCRB Assay by Adaptive Biotechnologies, Inc (Seattle, WA). (edited)

12.2.1.2 Colon polyp

Biopsy 11, 12: Fresh/Flash Frozen
T cell isolation/TCR sequencing collected at Baseline and Study Visit 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). Fresh biopsies will be collected in MD Anderson participants only; and only when feasible due to timing of procedures/experimental conditions. The rest of the trial sites will be collecting these samples as flash frozen. Colon polyp biopsies 11 and 12 will be plunged into a 50 mL tube containing ice-cold sterile PBS, immediately upon removal. The tissue will then be minced with a sterile scalpel inside a petri dish containing collagenase dissociation solution (DS). The dissected-tissue solution will be incubated at 37°C for 1.5 hours to activate the enzymatic digestion by collagenase. Then, the digested solution will be serially filtered using 70 μm and 40 μm filters to obtain a single-cell suspension, which will be washed with PBS and resuspended in PBS with 0.04% BSA. CD3+ TILs will be isolated from the single-cell suspension using the REAlease® CD3 (TIL) MicroBead Kit (Milteny Biotec, cat#130-121-152). DNA extraction will be performed from the sorted CD3+ TILs using the QIAamp DNA Micro kit. With the DNA, we will perform capture, amplification, and sequencing of the CDR3 region from the TCRβ variable (V), diversity (D) and joining (J) segments using the ImmunoSEQ TCRB Assay by Adaptive Biotechnologies, Inc (Seattle, WA). (edited)

12.2.2 Blood samples

PBMC, plasma, and frozen blood

Collected at Study Visits 1, 3-5, 7, and 9 in Cohort 1 and at Visits 9, 10 and 11 in Cohort 2. Heparinized whole blood will be collected and timely processed to collect PBMCs according to established protocols. For preservation until analysis, PBMCs will be mixed in 1:1 ratio with a specialized media in 10% DMSO, slowly cooled at 1°C/min and stored in the vapor phase of liquid nitrogen in backed up freezers at a temperature of -132°C for preservation until analysis. For plasma, blood will be collected using streck tube. Centrifuge upon collection into cryovial tubes and quickly frozen at -80°C. Whole blood collected in EDTA tube will be aliquoted into cryovial tubes and frozen at -80 °C until shipment.

12.2.3 Formalin-Fixed Paraffin-Embedded Tissue

Collected at Baseline and Study Visits 9 for Cohort 1 participants (who do not proceed to Cohort 2) or at Baseline and Study Visit 11 (for participants who continue on to participate in Cohort 2). If any abnormal mucosa/polypoid lesions were identified during Baseline and/or Study Visit 9 and/or Study Visit 11 [for participants in Cohort 2], standard procedures of clinical care of the local enrolling site should be followed for biopsy, tissue handling, and subsequent pathologic assessment of these lesions. This process involves the preservation of tissues in formalin-fixed paraffin-embedded blocks. Any abnormal mucosa/polypoid lesions tissue that may be residual after clinical care pathologic assessment should be collected and retained for research purposes. Residual tissue at every study site after standard pathology assessment will be shipped to Dr. Vilar-Sanchez's laboratory at MD Anderson Cancer Center per shipping instructions provided below.

12.3 Shipping Instructions

The following table describes packaging, carrier requirements, when specimens may be shipped, and name, address, and telephone number of the person to whom the specimens are being sent.

Table 2. Overview of specimen shipment specifications and destinations

Biomarker/ Procedure	Laboratory	Type of specimen	Packaging requirements	Batching Allowed (Yes/No)	Contact person, address and telephone number to whom specimens are being sent
Normal rectosigmoid colon mucosa biopsies #5-10 Colon polyp biopsies #11 and 12	Eduardo Vilar-Sanchez	Tissue (flash frozen)	Dry ice / Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org

Normal rectosigmoid colon mucosa biopsies #5-10 Colon polyp biopsies #11 and 12 Note: MD Anderson site only.	Eduardo Vilar-Sanchez	Tissue (fresh, refrigerated)	Refrigerated (range of 4-6 °C) / Diagnostic Goods Label	No	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org
Normal rectosigmoid colon mucosa biopsy #4	Eduardo Vilar-Sanchez	Tissue (flash frozen)	Dry ice / Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org
Normal rectosigmoid mucosa biopsies #2 and 3	Eduardo Vilar-Sanchez	Tissue (RNALater)	Dry ice / Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org
Any abnormal mucosa/polypoid lesions tissue that may be residual after clinical care pathologic assessment	Eduardo Vilar-Sanchez	Formalin-Fixed Paraffin-Embedded	Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org

Frozen blood	Eduardo Vilar-Sanchez	Blood	Dry ice / Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org
PBMCs	Claudia Massimi	PBMCs aliquots	Liquid Nitrogen/Diagnostic Goods Label	Yes	Central Lab. Nouscom srl Via di Castel Romano 100 Rome, 00128 IT Claudia Massimi 0039 0699775303
Plasma	Eduardo Vilar-Sanchez	Plasma	Dry ice / Diagnostic Goods Label	Yes	Vilar-Sanchez Lab UT – MD Anderson Cancer Center 6767 Bertner St. BSRB, S7.8414 Houston, TX 77030 United States P: (713) 563-9840 / Ext. 7-2170 F: (713) 834-6397 Email: evilar@mdanderson.org

Shipping frequency:

- Batched specimens: Unless otherwise specified in Table 2 above, specimens will be shipped in batches. Each batch must be sent with overnight delivery. For shipping frequency refer to the study lab manual.

Instructions for shipping to MD Anderson Cancer Center (Dr. Vilar-Sanchez):

- Unless otherwise specified in Section 12.2 and Table 2 above, all samples (either tissue or blood) must be kept frozen (-80 °C or below) after preparation and until shipment is initiated.
- Wrap the frozen samples in bundles or place samples in a storage box. Then place in a plastic freezer bag.
- Shipping containers should be insulated and filled with enough dry ice to prevent specimens from thawing. The shipping labels should be affixed firmly to the outside of each container.
- Plasma must be kept frozen (-80°C or below) after preparation and until shipment is initiated.
- All samples must be shipped in accordance with local biohazard requirements. Please refer to your institutional policy for biohazard labeling and packaging for shipment of hazardous and infectious human samples. Place dry ice around the bundles or the storage box. Include enough dry ice to ensure the dry ice does not evaporate before shipment is delivered.
- Ship via overnight express (i.e., FedEx, UPS). Do not ship on Thursday, Friday or the day prior to a public holiday.

Instructions for shipping to Central laboratory – Nouscom, s.r.l. (Attention to: Claudia Massimi):

- MD Anderson will schedule the shipments for all the sites upon agreement with the Central lab.
- PBMC must be kept frozen (-80°C) for less than a week. For long term storage (more than a week) please store in liquid nitrogen until shipment is initiated.
- Shipping containers should be insulated and filled with enough liquid nitrogen to prevent specimens from thawing. The shipping labels should be affixed firmly to the outside of each container.
- All samples must be shipped in accordance with local biohazard requirements. Please refer to your institutional policy for biohazard labeling and packaging for shipment of hazardous and infectious human samples. If shipping in dry ice, include enough to ensure it does not evaporate before shipment is delivered.
- Ship via World Courier.

Include a copy of the corresponding case report form (CRF) matching each specimen being shipped. Before placing in the shipping container, put the CRFs in a plastic bag for protection. Sample shipment should be made to the address provided in Table 2.

All specimens will be shipped in accordance with the International Air Transport Association (IATA) Dangerous Goods Regulations. The study staff from each site will be responsible for shipping specimens to the appropriate laboratory listed in Table 2.

12.4 Tissue Banking

The NCI reserves the right to require the transfer of biologic specimens and data, or true copies of such data, acquired from research supported under this award to an eligible third party. This transfer can occur in order to preserve the specimens and data and/or to continue the research. Third parties supported under this award must be informed of this right.

13. REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not the untoward occurrence is considered drug related. Thus, an AE can include any unfavorable sign (e.g., an abnormal laboratory finding), symptom, or clinical outcome temporally associated with the use of a test drug, active control, or placebo, regardless of whether the event is thought to be related to the drug. An AE can arise with the use of a drug or biologic (e.g., use for a purpose other than FDA-approved indication or in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. A clinically significant lab value is one that indicates a new disease process, an exacerbation or worsening of an existing condition, or requires further action(s) to be taken**.** Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible. (See the *DCP Baseline and Adverse Event Reporting Guidelines* [https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms] for more detail on reporting abnormal clinical laboratory values.)

A list of AEs that have occurred or might occur can be found in §8.2 Reported Adverse Events and Potential Risks, as well as the Investigator's Brochure or package insert.

13.1 Adverse Events

13.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are collected, must be recorded on the AE CRF whether or not related to study agent.

13.1.2 AE Data Elements

The following data elements are required for AE reporting:

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the participant dropped due to the event
- Outcome of the event

13.1.3 Severity of AEs

13.1.3.1 Identify the AE using CTCAE v5.0.

The CTCAE provides descriptive terminology (MedDRA lowest level term) and a severity grading scale for each AE listed. A copy of the CTCAE can be found at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AE severity will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v5.0. as shown in Table 3 below.

Table 3. CTCAE v5.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

13.1.3.2 Injection site reactions and systemic reactogenicity symptoms

Injection site reactions and systemic reactogenicity symptoms, both subsets of all AEs, may occur up to 7 days after administration of study drugs (GAd20-209-FSP and/or MVA-209-FSP). Such events will also be reported by use of a participant-completed Vaccine Report Card (Appendix E), captured on a separate CRF, and reported to NCI, DCP. The following guidelines will be used to evaluate injection site reactions and reactogenicity symptoms, the onset of which is within 7 days after administration of study drugs (GAd20-209-FSP and/or MVA-209-FSP):

Table 4. Injection Site Reaction and Systemic Reactogenicity Grading

	Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Injection Site Reactions				
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/ Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/ Swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Itching	Mild or localized; topical intervention indicated	Widespread and intermittent; skin changes from scratching; oral intervention indicated; limiting instrumental ADL	Widespread and constant; limiting self care ADL or sleep; systemic corticosteroid or immunosuppressive therapy indicated	
Bruising	Localized or in a dependent area	Generalized		
Systemic Reactogenicity Symptoms				
Fever	100.4°F – 102.2°F	102.3°F – 104.0°F	> 104.0°F	
Chills	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	
Malaise	Uneasiness or lack of well being	Uneasiness or lack of well being limiting instrumental	Uneasiness or lack of well being limiting self-care ADL	

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
		ADL		
Fatigue	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self care ADL	
Muscle aches/myalgia	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	
Headache	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	
Vomiting	Intervention not indicated	Outpatient IV hydration; medical intervention indicated	tube feeding, TPN, or hospitalization indicated	
Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake)	
Joint pain/arthralgia	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	

13.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: unrelated, unlikely, possible, probable, definite. Criteria for these classifications are provided in DCP's *Serious Adverse Event Report Form: Instructions for Completion and Submission* **(https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms).**

13.1.5 Follow-up of AEs

All AEs, including laboratory abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

13.1.6 Collection of AEs

AEs will be collected for each participant for a maximum of 52 weeks (12 months) after the receipt of the GAd prime vaccination.

13.2 Serious Adverse Events

13.2.1 Definition

Regulations at 21 CFR §312.32 define an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
  (According to FDA safety guidelines, an AE is considered life-threatening if in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death. Example: An allergic reaction resulting in angioedema of the larynx, allergic bronchospasm or anaphylaxis is considered life-threatening; however, an allergic reaction resulting only in a localized rash is not life-threatening.)
- In patient hospitalization or prolongation of existing hospitalization
  (NCI, DCP uses admission or stay (including emergency room) equal to or greater than 24 hours as the definition of hospitalization. Exceptions are hospitalization for treatment of a pre-existing condition [unless the condition increased in severity on study], outpatient surgery, planned/elective procedures, and procedures described in the protocol [e.g., pharmacokinetic sampling, surgery] even if the hospital stay is of the described length; however, it does include events resulting from any of these that fulfill other serious outcome criteria, e.g., prolongation of hospitalization or life-threatening.)
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the Participant and may require intervention to prevent one of the other outcomes listed above.

13.2.2 Reporting SAEs to DCP

13.2.2.1 The accruing LAO and all Affiliated Organizations (AOs) will report SAEs on the DCP SAE Report Form as described in DMACC's CP-CTNet SOP 02-01, found at CP-CTNet SOP 02-01 Reporting Serious Adverse Events (cp-ctnet-dmacc.org) and DCP's *Serious Adverse Event Report Form: Instructions for Completion and Submission* found at https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms.

13.2.2.2 Contact the DCP Medical Monitor, Protocol PI, and DCP Regulatory Contractor's Safety Department within 24 hours of knowledge of the event. Contact via email is preferred, but phone contact is acceptable.

NCI DCP Medical Monitor:
Luz María Rodríguez, MD, FACS
National Cancer Institute
Division of Cancer Prevention
Gastrointestinal and Other Cancers Research Group
9609 Medical Center Drive, Room: 5E228
Rockville, MD 20850

Phone: 240-276-7039
Fax: 240-276-7848
E-mail address: rodrigul@mail.nih.gov

The contact information for the DCP Regulatory Contractor's Safety Department is: phone: 650-691-4400 x133; email: safety@ccsainc.com).

Include the following information when contacting both the DCP Medical Monitor and the DCP Regulatory Contractor's Safety Department:

- Participant ID
- Date and time of SAE onset
- Date and time the accruing LAO or AO was notified about the SAE by the study participant or other person(s)
- Name of person reporting the SAE
- Call back phone number and email address
- Accruing LAO or AO at which the subject is enrolled
- DCP protocol number
- Title of protocol
- Suspected drugs (if any)
- Description of the SAE, including attribution to the Investigational Agent

13.2.2.3 The accruing LAO and AOs will email written SAE reports to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and the DCP Regulatory Contractor's Safety Department within 48 hours of learning of the event using the Word SAE Report Form.

13.2.2.4 The DCP Medical Monitor and the DCP Regulatory Contractor will determine which SAEs require submission to FDA or the manufacturer as expedited safety reports.

13.2.2.5 The accruing LAO and AOs will comply with applicable regulatory requirements related to reporting SAEs to the CIRB and local IRB/IEC as applicable. Specifically, if an SAE meets the definition of an unanticipated problem (UP; i.e., requires expedited reporting to FDA or the manufacturer as a safety report [serious, unexpected, and related to a study agent]), then it needs to be reported to the CIRB by the Signatory Institution PI at the accruing LAO or AO where the SAE occurred (see SOP 02-02 *Reporting Protocol Deviations* for more information). In addition to CIRB requirements, UPs must be reported to the accruing LAO's or AO's local IRB per local requirements.

13.2.3 Follow-up of SAE

Accruing LAO or AO staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and DCP Regulatory Contractor's Safety Department as soon as available. SAEs related to the study agent will be followed until resolved.

Furthermore, to minimize the number of study participants exposed to a potentially unacceptable risk, study treatment and enrollment will be paused pending a comprehensive safety evaluation by the DCP Medical Monitor, DSMB, and/or IRB/IEC when any of the following death events occur:

- Any death at least possibly related to the study treatment
- Any death within 30 days of receiving the study treatment unless clearly due to disease progression

14. STUDY MONITORING

14.1 Data Management

This study will report clinical data using Medidata RAVE, a cloud–based clinical trials data management system managed by the DMACC. RAVE will be the database of record for the protocol and subject to NCI and FDA audit. All RAVE users will be trained to use the system and will comply with the instructions in the guidelines provided to the LAO by the DMACC as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for this study.

14.2 Electronic Case Report Forms

The System Variable and Attribute Report (SVAR) template will be used to create the study-specific eCRFs or SVAR workbook. The SVAR template contains NCI Common Data Elements (CDEs) to facilitate data collection and analysis across studies. The SVAR template will be modified to capture the unique data elements of each protocol and prepare a protocol-specific SVAR workbook. NCI CDEs, where available, shall be used for the initial SVAR workbooks and all subsequent workbook modifications. The DCP approved SVAR will be used to create the electronic CRF (e-CRF) screens in the Medidata RAVE application. Site staff will enter data into the e-CRFs in Medidata RAVE. SVAR amendments, if needed, will be submitted to the DCP Protocol Information Office for review and approval prior to deployment in Medidata RAVE. Approved changes will be programmed into the Medidata RAVE database by DMACC. Study questionnaires will be collected electronically using the REDCap system.

14.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

14.4 Data and Safety Monitoring Plan

NIH and NCI policy requires a Data and Safety Monitoring Plan (DSMP) to document the institution's procedures to ensure safety of participants, validity of data, and the appropriate termination of studies for which significant benefits or risks have been uncovered or when it appears that the trials cannot be concluded successfully.

The Data and Safety Monitoring Plan for the iCAN-PREVENT Consortium is on file at the DCP. This study will be monitored yearly by the MDACC Data and Safety Monitoring Board (DSMB), the data and safety monitoring board of record for this study. The DSMB reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB

are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;
(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

To minimize the number of study participants exposed to a potentially unacceptable risk, study treatment and enrollment will be paused pending a comprehensive safety evaluation by the DSMB, DCP Medical Monitor, and/or IRB/IEC when any of the following death events occur:

- Any death at least possibly related to the study treatment
- Any death within 30 days of receiving the study treatment unless clearly due to disease progression

Refer to the Data and Safety Monitoring Plan for the iCAN-PREVENT Consortium on file at the DCP for further details.

14.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

14.6 Record Retention

Clinical records for all participants, including eCRFs, all source documentation (containing evidence of study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as CIRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

14.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent supplied by DCP, NCI used in this protocol, is provided to the NCI under a Collaborative Agreement (CTA) between the Pharmaceutical Company (Nouscom) (hereinafter referred to as Collaborator) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" contained within the terms of award, apply to the use of Agent in this study:

14.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a Participant participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

14.7.2 For a clinical protocol where there is an Investigational Agent used in combination with (an) other Investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-party Data").

14.7.3 NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

14.7.4 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own Investigational Agent.

14.7.5 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Investigational Agent.

14.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

14.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

14.7.8 Any manuscripts reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to DCP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to DCP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

14.8 Genomic Data Sharing Plan

Data produced through this protocol will be shared in a manner consistent with data-sharing under the NIH Genomic Data Sharing Policy (NOT-OD-14-124). Several of the exploratory endpoints will generate genome-wide RNA sequencing, single-cell sequencing and TCR sequencing data. Interpretation of transcriptomic results, single-cell sequencing and TCR sequencing results, including minimal phenotype information needed to reproduce the primary analysis – such as associated phenotype data (e.g., clinical information), exposure data, relevant metadata, and descriptive information (e.g., protocols or methodologies used) – will be shared with the public and the scientific community. Individual-level phenotype data will include, at a minimum: sex, race, weight, height, and age. We will submit the sequencing and phenotype data after it has been cleaned and processed (i.e., once the QA/QC is complete and the analytical dataset is finalized). The data repository to be used will be Gene Expression Omnibus (GEO) or equivalents. Data will be made available with unrestricted access. We understand that following data submission, the data may be held for a period not to exceed six months. Following this period of exclusivity, or at the time of publication (whichever comes first), the data will be available for secondary research access without restrictions on publication (i.e., there will be no publication embargo). Data will be made available for general research use.

15. STATISTICAL CONSIDERATIONS

15.1 Study Design/Description

Ours is a phase Ib/II open-label study to evaluate the safety and immunogenicity of Nous-209 monotherapy as an immune-interception agent for participants with LS. The study will be conducted in two cohorts: Cohort 1 (initial vaccination, single-arm, non-randomized) and Cohort 2 (revaccination, randomized into two arms, Arm A and Arm B). Both chart review and the electronic database search will be applied to identify the potentially eligible participants diagnosed with LS who are undergoing standard-of-care lower GI endoscopy. Eligible and consenting participants will receive GAd20-209-FSP (prime) and/or MVA-209-FSP (boost) vaccination. Serial tissue biopsies and blood specimens will be collected for biomarker analyses.

For Cohort 1, we will apply Simon's minimax two-stage design and the response rate (i.e., the immunogenicity induction rate) defined by number of participants with immunogenicity among all participants treated will be estimated accordingly (Simon, 1989).

Cohort 2 will only open conditional on the trial continuing to Stage 2, with at least 16 participants having achieved immune response by Week 9.

15.2 Randomization/Stratification

For Cohort 1 of our study, there is no planned randomization or stratification.

For Cohort 2, all eligible participants will be randomized (1:1 ratio) to one of two intervention arms (revaccination schedules) as specified in Section 7.1. Furthermore, we will apply stratification according to

the level of peak vaccine-induced immune response measured at Week 9 per protocol under participation in Cohort 1. We will define "good responders" as those individuals with an overall T cell response (sum of T cell responses against the 16 peptide pools covering the vaccine sequence) that is equal to or above the median value calculated on 16 or more participants with evaluable response at Week 9 prior to randomizing the first participant in Cohort 2. We will further define "intermediate responders" as those individuals with an overall T cell response that is less than the median value calculated on 16 or more participants with evaluable response at Week 9 prior to randomizing the first participant in Cohort 2.

15.3 Sample Size

For Cohort 1, we plan to consent up to 60 individuals with the goal of enrolling 45 eligible participants to the study intervention [initial vaccination]. For Cohort 2, we plan to randomize 28 eligible participants from Cohort 1 to reach 24 evaluable participants in Cohort 2 (12 evaluable per treatment arm). It is expected that up to 15% of samples (4 samples) may not be evaluable, thus, our goal is to randomize 28 participants and reach 24 evaluable. We expect the total study duration to be approx. four years, to include approximately two years of accrual, one year to complete of all participant interventions, and approximately one year for biomarker and statistical analyses.

15.4 Primary Objective, Endpoint(s), Analysis Plan

The co-primary objectives of our study are to evaluate the safety/tolerability and immunogenicity of Nous-209 vaccination in healthy individuals with LS. Accordingly, our primary safety/tolerability endpoints will be the rates of grade 2/3 and symptom reactivity following initial vaccination with GAd20-209-FSPs (prime) and MVA-209-FSPs (boost) in Cohort 1, and following revaccination with GAd20-209-FSPs (prime) and/or MVA-209-FSPs (boost) in Cohort 2. For participants in Cohort 1, our primary immunogenicity endpoint will be the rate of reactivity to at least 1 of 16 synthetic FSP pools using the ELISpot assay. Our immunogenicity endpoint will be evaluated at Baseline and Week 9 (i.e., following the MVA-209-FSP boost vaccination). For participants in Cohort 2, our immunogenicity endpoint will be evaluated at Week 52 and at 8 weeks after the last boost with MVA-209-FSP, corresponding to Week 68 and Week 60, for arm A and B, respectively.

Cohort 1
For Cohort 1, we expect that the enrollment of 45 participants will provide **36 evaluable LS participants** for this clinical trial. The trial will be conducted using the Simon's minimax two-stage design, where the response rate (i.e., the immunogenicity induction rate) is defined by number of participants with immunogenicity among all participants treated (Simon, 1989). It is assumed that this vaccination regimen will elicit an immune response in at least 75% of the vaccinated participants (target response rate of 75%) based on previously published data indicating that all LS/MSI-H patients exhibited specific T-cell responses to at least one frameshift neoantigen peptides using ELISpot analysis after in vitro re-stimulation (36). Finally, we considered a response rate of 55% or lower as a failure, thus the hypothesis being rejected.

We have applied the Simon's two-stage minimax design assuming that the probability of accepting a "bad" vaccine (i.e., response rate≤55%) is 0.05 and the probability of rejecting a "good" vaccine (i.e., response rate ≥75%) is 0.20. In the first stage, 24 LS participants will be enrolled and accrual will be halted to fully evaluate immunogenicity in these patients; if 15 or fewer participants respond to the vaccine (i.e., with immunogenicity), the trial will be stopped and the vaccine will be declared as ineffective. If there are 16 or more responses, additional participants will be enrolled in the study to reach a total of 36 evaluable LS participants. By the end of the study, the new regimen will be rejected if the number of participants with

immunogenicity is less than or equal to 24 and will be accepted otherwise. The operating characteristics of the trial are given as follows. If the true response rate is 0.55 the probability of stopping the trial early is 83% and the expected sample size is 26.

As stated in Section 3.2, we assume an estimated attrition rate of 20%. Therefore, for Cohort 1, we plan to enroll a maximum of 45 participants in order to reach 36 evaluable LS participants for our co-primary endpoint of immunogenicity. Every effort will be made to minimize the attrition rate including but not limited to careful screening of eligibility criteria, close contact with patients after enrollment to monitor the adverse events, email/phone reminders of the follow-up visits.

Cohort 2

For Cohort 2, our aim is to establish an optimal Nous-209 revaccination schedule to ensure proper boosting of immune responses following initial vaccination (Cohort 1). We will evaluate the median overall T cell response detected at 8 weeks after the last boost with MVA-209-FSP (Week 68 and Week 60, for arm A and B, respectively) separately for both treatment arms. We will compare the fold increase in median overall T cell response (relative to Week 52, baseline for assessment of immunogenicity to re-vaccination in participants to Cohort #2) between treatment arms. The treatment arm associated with the highest fold increase in immune response will be considered the optimal schedule.. Previous studies in human shown still significant levels of T cell response detected at 4 weeks post-boost in participants vaccinated with similar Ad and MVA vaccines/regimen (47).

Cohorts 1 and 2

To address the potential missing data, we could apply multiple imputations as the secondary analysis. For example, under the missing at random assumption, imputations of the missing outcome variable can be achieved by regressing the outcome variable on medical demographic variables such as age, sex, race, BMI, and adverse event rate, etc. In addition, controlled multiple imputation procedures could be applied to combine pattern-mixture modeling with multiple imputation to provide a practical platform for sensitivity analysis to address the potential biased caused by treatment related dropouts.

Descriptive statistics such as mean, median, standard deviation (sd), frequency and range, as well as graphic presentations (e.g., boxplot) will be used to assess the distribution of all study-related variable of interest (e.g., medical and demographics variables) and report the data when appropriate. The rate of immunogenicity induced by Nous-209 will be reported along with its 95% exact (Clopper-Pearson) confidence interval. Immunogenicity will be defined as a significant increase in ELISpot activity comparing pre- and post-vaccination IFNγT cell numbers. Changes from baseline will assessed using a paired t-test or the Wilcoxon signed-rank test, if more appropriate.

If underlying distributional assumptions are not met, nonparametric tests (e.g., Wilcoxon signed rank test) and/or data transformation (e.g., logarithm) may be applied when appropriate. Time to event (e.g., time to adenoma) will be estimated by Kaplan-Meier method; log-rank test may be applied to evaluate the difference in time-to-event outcomes between different prognostic groups of participants (e.g., immunogenic vs non-immunogenic participants). Cox proportional hazards regression, when possible, will be employed to further evaluate the effects of important covariates on time-to-event outcomes while adjusting for clinical and demographic characteristics of interest (e.g., age, sex, BMI, tobacco use, etc.). Other statistical methods, when appropriate, may be utilized.

15.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives of our study are to evaluate the effects of Nous-209 vaccination on a) T cell immune profile and T cell receptor (TCR) repertoire in peripheral blood; b) TCR repertoire within histologically normal colorectal mucosa; c) tumor infiltrating lymphocyte (TIL) immune profile and TCR repertoire within colorectal adenomas; d) cytotoxicity of T cells on matched participant-derived colorectal adenoma organoids; e) the burden of colorectal adenomas/advanced neoplasia/carcinoma; f) burden of LS-related carcinomas; g) cell free DNA (cfDNA) mutation profiles and cfDNA burden in participants with LS; h) to correlate tobacco and alcohol consumption with the immune response to Nous-209 in trial participants; and i) assess the MMR and/or MSI status of polyps (and adjacent normal mucosa as control) detected in the baseline and end-of-the-study colonoscopy using different technologies such as immunohistochemistry, MSI analysis by PCR, or next-generation sequencing.

We will use two-sample t-test and general linear models for the comparison of continuous secondary endpoints (e.g., number of adenomas and advanced adenomas) among different groups of participants [e.g., participants displaying immunogenicity (immunogenic) vs. those without immunogenicity (non-immunogenic)]. One-sample binomial tests and one-sample t-tests will be utilized to assess the proportion and mean of specific outcomes of interest in this group of participants, respectively (e.g., proportion of adenomas, advanced adenomas and colorectal carcinomas, and mean sum of the maximum diameters of neoplasms). The occurrence of a neoplasm defined in three categories (adenoma, advanced adenoma, and CRC) will be compared to historical cohorts (e.g., CAPP-2 study placebo cohort).

Other analyses may be employed when appropriate.

15.6 Reporting and Exclusions

Regular clinical visits will be applied to monitor study participants during the intervention period. The non-compliance rates will be computed for both study cohorts. Complete case analysis will be performed as our primary analysis method in this phase Ib/II study. Multiple imputations will be applied whenever appropriate.

15.7 Evaluation of Toxicity

All enrolled participants will be considered evaluable for our toxicity and tolerability endpoints. We will describe the toxicity profile with respect to the types, attribution, and grade of toxicities.

15.8 Evaluation of Response

For our co-primary endpoint of immunogenicity following initial vaccination (Cohort 1), all enrolled participants who receive the MVA-209-FSP (boost) vaccine at Week 8 and undergo research blood collection at Week 9 will be considered evaluable, if the quality of collected PBMC allows measurement of immunogenicity. For our co-primary endpoint of immunogenicity following revaccination (Cohort 2), all enrolled participants who receive the MVA-209-FSP (boost) vaccine at Week 52 (in Arm B) or at Week 60 (in Arm A) and undergo research blood collection at 8 weeks post last MVA boost vaccine (Week 68 and Week 60, for arm A and B, respectively), will be considered evaluable, if the quality of collected PBMC allows measurement of immunogenicity.

For our secondary endpoints, all participants (with the possible exception of those who did not receive at least the GAd-209-FSP (prime) vaccine at Week 0) will be considered evaluable. Sub-analyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, sub-analyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported. For all measurements of response, the 95% confidence intervals should also be provided.

15.9 Interim Analysis

A Bayesian toxicity monitoring approach will be applied in this study. Expected local toxicities are erythema, swelling, pruritus, warmth and local pain and systemic toxicities are fever, arthralgia, myalgia, malaise, fatigue, abdominal pain, headache and nausea. Unacceptable toxicity is defined as any treatment related Adverse Event (AE) of grade 3 or higher including any death at least possibly related to the study treatment or any death within 30 days of receiving the study treatment unless clearly due to disease progression. Participants enrolled only in Cohort 1 will be monitored for AEs up to Week 52 (Month 12) visit. Participants enrolled in Cohort 1 and continuing in Cohort 2 will be monitored for AEs up to Week 68 visit using the same boundaries, including the AEs during the first 30 days of receiving the first and second study treatment, cumulatively. We will not include Grade 3 symptom reactogenicity towards the definition of AE as specified earlier as Section 13 Table 4. AEs following immunization will be considered following the guidance of the WHO and include: fatal or life-threatening adverse reactions, neuritis, convulsion, anaphylaxis, syncope, encephalitis, thrombocytopenia, vasculitis, Guillain-Barré syndrome and Bell's palsy.

We will consider the vaccination regimen unsafe if it results in an unacceptable toxicity rate of 30% or greater with 70% or higher probability. With a beta prior probability of toxicity with parameters (0.3, 0.7), consideration to stop the trial will be guided according to the stopping boundaries in Table 5. The operating characteristics for these stopping rules are summarized in Table 6. The boundaries and operating characteristics were generated using Bayesian Toxicity Monitoring, developed by the department of Biostatistics (https://ibl.mdanderson.org/BTM/).

Table 5. Stopping boundaries for toxicity monitoring

Cohort	Stop if number of Toxicity ≥
6	3
12	5
18	7
24	9
30	11
36	13

Table 6. Operating characteristics for the stopping rules for toxicity

True Toxicity Rate	Probability of Early Termination	Average No. of Participants Treated
0.1	0.018	35.5
0.2	0.155	31.9
0.3	0.490	24.1
0.4	0.832	15.4
0.5	0.975	9.9
0.6	0.999	7.4

Considering both Simon's two-stage design and toxicity monitoring and assuming independence between response and toxicity, operating characteristics indicate that the trial has a 99.6% chance of stopping early if the true toxicity rate is 50% when the true response rate is 55%. On the other hand, if the true response rate is 75% when the true toxicity rate is 10%, we will have an 13.7% chance of stopping the trial early.

Furthermore, as noted in Section 14.4, study treatment and enrollment will be paused pending a comprehensive safety evaluation by the DCP Medical Monitor and DSMB when any of the following death events occur:

- Any death at least possibly related to the study treatment
- Any death within 30 days of receiving the study treatment unless clearly due to disease progression

15.10 Ancillary Studies

No ancillary studies are planned.

16. REGULATORY AND ETHICAL CONSIDERATIONS

16.1 Required Documents

Besides the regulatory information that will be entered into the Registration and Credential Repository (see Section 5.1), the following documents are also required:

16.1.1 Documentation of Federalwide Assurance (FWA) number for the LAO and all Affiliate Organizations.

16.1.2 Signed Investigator's Brochure/Package Insert acknowledgement form

16.1.3 Delegation of Tasks Log form for the Lead Accruing Organization and all Accruing Sites signed by the Principal Investigator for each site and initialed by all study personnel listed on the form.

16.2 Informed Consent

All potential study participants will go through the Informed Consent process. The informed consent process may be conducted either in person or electronically (remotely). Electronic informed consent may be used to either supplement or replace paper based informed consent processes in order to best address the participant's need. Electronic means relating to technology having electrical, digital, magnetic, wireless, optical, electromagnetic, or similar capabilities; electronic informed consent process may be conducted via telephone or video conferencing, for example.

All potential study participants will be given a copy of the CIRB-approved Informed Consent to review. The Informed Consent may be provided on paper or electronically. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The signature may be provided on paper or electronically. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Individuals who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option should be included within the informed consent document.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, and the NCI CIRB. The NCI CIRB approves a model consent for each protocol. Each Signatory Institution inserts their CIRB-approved institutional boilerplate language into the model consent to create the CIRB-approved consent. If the model informed consent document is amended, Signatory Institutions must use the revised model informed consent document and insert their CIRB-approved institutional boilerplate language at the time the change becomes active.

The NCI CIRB is the IRB of record and is the only IRB authorized to approve changes to the protocol or informed consent document. Institutions may require additional oversight that involves the local IRB, but the local IRB is not responsible for any regulatorily-required IRB actions.

16.3 Collection of Regulatory Documents

Regulatory documents will be collected by the DCP regulatory contractor and reviewed for completeness and accuracy.

16.4 Other

This trial will be conducted in compliance with the protocol, the International Conference on Harmonisation's (ICH) Good Clinical Practice (GCP) guidelines, and the applicable regulatory requirements.

17. ROSTER MANAGEMENT

The LAO is responsible for establishing, maintaining, and monitoring all its members that participate in CP-CTNet studies. The LAO must have a "real-time," comprehensive, consolidated roster of all its members with their relevant Cancer Therapy Evaluation Program (CTEP) institution codes, associated investigators, and research staff. This roster information is used for determining compliance with monitoring requirements.

The LAO's organizational rosters will be managed by the CP-CTNet Roster Management System website (https://applications.prevention.cancer.gov/cp-ctnet) Requests to add memberships to a roster will be done via this website. All requests require that the following documents be uploaded:

- Consortium Letter of Commitment
- Site Letter of Commitment
- CV/NIH Biosketch

18. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for non-standard of care costs of this study. Study agent will be provided at no cost to the participant. There will be compensation for additional expenses associated with study visits, such as additional travel expenses, time missed from work or other expenses. The amount of compensation will be $50 provided at the time of study initiation (the first endoscopic procedure) and at the time of the study visits at Weeks 3, 8, 9, 24 and 52 (in Cohort 1), and additionally at Weeks 60 and 68 (in Cohort 2). The participants will also receive parking compensation for in person study related visits (at those centers where parking fees are charged). If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent,

immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage.

REFERENCES

1. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer. 2015;15(3):181-94. doi: 10.1038/nrc3878. PubMed PMID: 25673086.
2. Valle L, Vilar E, Tavtigian SV, Stoffel EM. Genetic predisposition to colorectal cancer: syndromes, genes, classification of genetic variants and implications for precision medicine. J Pathol. 2019;247(5):574-88. Epub 2018/12/26. doi: 10.1002/path.5229. PubMed PMID: 30584801; PubMed Central PMCID: PMC6747691.
3. Stoffel E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology. 2009;137(5):1621-7. doi: 10.1053/j.gastro.2009.07.039. PubMed PMID: 19622357; PubMed Central PMCID: PMC2767441.
4. Bonadona V, Bonaiti B, Olschwang S, Grandjouan S, Huiart L, Longy M, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA. 2011;305(22):2304-10. Epub 2011/06/07. doi: 10.1001/jama.2011.743. PubMed PMID: 21642682.
5. Dominguez-Valentin M, Sampson JR, Seppala TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genet Med. 2020;22(1):15-25. Epub 2019/07/25. doi: 10.1038/s41436-019-0596-9. PubMed PMID: 31337882.
6. The Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330. doi: 10.1038/nature11252 https://www.nature.com/articles/nature11252#supplementary-information.
7. Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and characterization of microsatellite instability across 18 cancer types. Nat Med. 2016;22(11):1342-50. Epub 2016/11/01. doi: 10.1038/nm.4191. PubMed PMID: 27694933.
8. Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. Nat Rev Immunol. 2017. doi: 10.1038/nri.2017.131. PubMed PMID: 29226910.
9. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217-21. Epub 2017/07/06. doi: 10.1038/nature22991. PubMed PMID: 28678778; PubMed Central PMCID: PMC5577644.
10. Yarchoan M, Johnson BA, 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer. 2017;17(9):569. doi: 10.1038/nrc.2017.74. PubMed PMID: 28835723.
11. Marty R, Kaabinejadian S, Rossell D, Slifker MJ, van de Haar J, Engin HB, et al. MHC-I Genotype Restricts the Oncogenic Mutational Landscape. Cell. 2017;171(6):1272-83 e15. doi: 10.1016/j.cell.2017.09.050. PubMed PMID: 29107334; PubMed Central PMCID: PMC5711564.
12. George JT, Kessler DA, Levine H. Effects of thymic selection on T cell recognition of foreign and tumor antigenic peptides. Proc Natl Acad Sci U S A. 2017;114(38):E7875-E81. doi: 10.1073/pnas.1708573114. PubMed PMID: 28874554; PubMed Central PMCID: PMC5617294.
13. Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. Lancet Oncol. 2017;18(8):1009-21. Epub 2017/07/12. doi: 10.1016/S1470-2045(17)30516-8. PubMed PMID: 28694034.
14. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology. 2008;134(4):988-97. Epub 2008/04/09. doi: 10.1053/j.gastro.2008.01.015. PubMed PMID: 18395080.
15. Ballhausen A, Przybilla MJ, Jendrusch M, Haupt S, Pfaffendorf E, Seidler F, et al. The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoediting during tumor

evolution. Nat Commun. 2020;11(1):4740. Epub 2020/09/23. doi: 10.1038/s41467-020-18514-5. PubMed PMID: 32958755; PubMed Central PMCID: PMC7506541.
16. Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res. 2014;2(7):616-31. doi: 10.1158/2326-6066.CIR-14-0027. PubMed PMID: 24942756; PubMed Central PMCID: PMC4082460.
17. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509-20. doi: 10.1056/NEJMoa1500596. PubMed PMID: 26028255; PubMed Central PMCID: PMC4481136.
18. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. Nat Rev Clin Oncol. 2019;16(9):563-80. Epub 2019/05/17. doi: 10.1038/s41571-019-0218-0. PubMed PMID: 31092901.
19. Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nature medicine. 2011;17(9):1128-31. doi: 10.1038/nm.2447. PubMed PMID: 21857654.
20. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nature medicine. 2014;20(10):1126-9. doi: 10.1038/nm.3702. PubMed PMID: 25194571.
21. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis. 2015;211(4):549-57. doi: 10.1093/infdis/jiu511. PubMed PMID: 25225676; PubMed Central PMCID: PMC4318920.
22. Quinn KM, Zak DE, Costa A, Yamamoto A, Kastenmuller K, Hill BJ, et al. Antigen expression determines adenoviral vaccine potency independent of IFN and STING signaling. J Clin Invest. 2015;125(3):1129-46. doi: 10.1172/JCI78280. PubMed PMID: 25642773; PubMed Central PMCID: PMC4362254.
23. Quinn KM, Da Costa A, Yamamoto A, Berry D, Lindsay RW, Darrah PA, et al. Comparative analysis of the magnitude, quality, phenotype, and protective capacity of simian immunodeficiency virus gag-specific CD8+ T cells following human-, simian-, and chimpanzee-derived recombinant adenoviral vector immunization. J Immunol. 2013;190(6):2720-35. doi: 10.4049/jimmunol.1202861. PubMed PMID: 23390298; PubMed Central PMCID: PMC3594325.
24. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine. 2010;29(2):304-13. doi: 10.1016/j.vaccine.2010.10.037. PubMed PMID: 21034824.
25. Ewer K, Rampling T, Venkatraman N, Bowyer G, Wright D, Lambe T, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. N Engl J Med. 2016;374(17):1635-46. doi: 10.1056/NEJMoa1411627. PubMed PMID: 25629663.
26. Colloca S, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med. 2012;4(115):115ra2. doi: 10.1126/scitranslmed.3002925. PubMed PMID: 22218691; PubMed Central PMCID: PMC3627206.
27. Barnes E, Folgori A, Capone S, Swadling L, Aston S, Kurioka A, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med. 2012;4(115):115ra1. Epub 2012/01/06. doi: 10.1126/scitranslmed.3003155. PubMed PMID: 22218690; PubMed Central PMCID: PMC3627207.
28. Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. Sci Transl Med. 2014;6(261):261ra153. Epub 2014/11/08. doi: 10.1126/scitranslmed.3009185. PubMed PMID: 25378645; PubMed Central PMCID: PMC4669853.
29. D'Alise AM, Leoni G, Cotugno G, Troise F, Langone F, Fichera I, et al. Adenoviral vaccine

targeting multiple neoantigens as strategy to eradicate large tumors combined with checkpoint blockade. Nat Commun. 2019;10(1):2688. Epub 2019/06/21. doi: 10.1038/s41467-019-10594-2. PubMed PMID: 31217437; PubMed Central PMCID: PMC6584502.
30. Leoni G, D'Alise AM, Cotugno G, Langone F, Garzia I, De Lucia M, et al. A Genetic Vaccine Encoding Shared Cancer Neoantigens to Treat Tumors with Microsatellite Instability. Cancer Res. 2020;80(18):3972-82. Epub 2020/07/22. doi: 10.1158/0008-5472.CAN-20-1072. PubMed PMID: 32690723.
31. Overman MJ, Leoni G, D'Alise AM, Cotugno G, Langone F, Capone S, et al. 1004P Initial results from a phase I study of Nous-209, an off-the-shelf viral vectored immunotherapy encoding 209 shared frame shift peptide neoantigens, with pembrolizumab, for the treatment of tumors with a deficiency in mismatch repair/microsatellite instability. Annals of Oncology. 2021;32:S850. doi: 10.1016/j.annonc.2021.08.1388.
32. Overman M, Fakih M, Le D, Shields A, Pedersen K, Shah M, et al. 410 Phase I interim study results of Nous-209, an off-the-shelf immunotherapy, with pembrolizumab, for the treatment of tumors with a deficiency in mismatch repair/microsatellite instability (dMMR/MSI). Journal for ImmunoTherapy of Cancer. 2021;9(Suppl 2):A441-A. doi: 10.1136/jitc-2021-SITC2021.410.
33. Finn OJ. The dawn of vaccines for cancer prevention. Nat Rev Immunol. 2018;18(3):183-94. Epub 2017/12/28. doi: 10.1038/nri.2017.140. PubMed PMID: 29279613.
34. Willis JA, Reyes-Uribe L, Chang K, Lipkin SM, Vilar E. Immune Activation in Mismatch Repair-Deficient Carcinogenesis: More Than Just Mutational Rate. Clin Cancer Res. 2019. Epub 2019/08/07. doi: 10.1158/1078-0432.CCR-18-0856. PubMed PMID: 31383734.
35. Chang K, Taggart MW, Reyes-Uribe L, Borras E, Riquelme E, Barnett RM, et al. Immune Profiling of Premalignant Lesions in Patients With Lynch Syndrome. JAMA Oncol. 2018. Epub 2018/05/02. doi: 10.1001/jamaoncol.2018.1482. PubMed PMID: 29710228.
36. Roudko V, Bozkus CC, Orfanelli T, McClain CB, Carr C, O'Donnell T, et al. Shared Immunogenic Poly-Epitope Frameshift Mutations in Microsatellite Unstable Tumors. Cell. 2020;183(6):1634-49 e17. Epub 2020/12/02. doi: 10.1016/j.cell.2020.11.004. PubMed PMID: 33259803.
37. Capone S, Brown A, Hartnell F, Sorbo MD, Traboni C, Vassilev V, et al. Optimising T cell (re)boosting strategies for adenoviral and modified vaccinia Ankara vaccine regimens in humans. npj Vaccines. 2020;5(1):94. doi: 10.1038/s41541-020-00240-0.
38. Nouscom. Investigator's Brochure, Nous-209 vaccine composed of GAd20-209-FSP and MVA-209-FSP (version 4.1).
39. Sheehy SH, Duncan CJ, Elias SC, Choudhary P, Biswas S, Halstead FD, et al. ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. Mol Ther. 2012;20(12):2355-68. Epub 2012/10/24. doi: 10.1038/mt.2012.223. PubMed PMID: 23089736; PubMed Central PMCID: PMC3519995.
40. Green CA, Scarselli E, Sande CJ, Thompson AJ, de Lara CM, Taylor KS, et al. Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci Transl Med. 2015;7(300):300ra126. Epub 2015/08/14. doi: 10.1126/scitranslmed.aac5745. PubMed PMID: 26268313; PubMed Central PMCID: PMC4669850.
41. Ewer K, Rampling T, Venkatraman N, Bowyer G, Wright D, Lambe T, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. New England Journal of Medicine. 2015;374(17):1635-46. doi: 10.1056/NEJMoa1411627.
42. Page K, Melia MT, Veenhuis RT, Winter M, Rousseau KE, Massaccesi G, et al. Randomized Trial of a Vaccine Regimen to Prevent Chronic HCV Infection. N Engl J Med. 2021;384(6):541-9. Epub 2021/02/11. doi: 10.1056/NEJMoa2023345. PubMed PMID: 33567193.
43. Maruvka YE, Mouw KW, Karlic R, Parasuraman P, Kamburov A, Polak P, et al. Analysis of somatic microsatellite indels identifies driver events in human tumors. Nat Biotechnol. 2017;35(10):951-9. Epub 2017/09/12. doi: 10.1038/nbt.3966. PubMed PMID: 28892075.
44. Han X, Zhang S, Zhou DC, Wang D, He X, Yuan D, et al. MSIsensor-ct: microsatellite instability

detection using cfDNA sequencing data. Brief Bioinform. 2021. Epub 2021/01/19. doi: 10.1093/bib/bbaa402. PubMed PMID: 33461213.
45. Jia P, Yang X, Guo L, Liu B, Lin J, Liang H, et al. MSIsensor-pro: Fast, Accurate, and Matched-normal-sample-free Detection of Microsatellite Instability. Genomics Proteomics Bioinformatics. 2020;18(1):65-71. Epub 2020/03/17. doi: 10.1016/j.gpb.2020.02.001. PubMed PMID: 32171661; PubMed Central PMCID: PMC7393535.
46. Niu B, Ye K, Zhang Q, Lu C, Xie M, McLellan MD, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. Bioinformatics. 2014;30(7):1015-6. Epub 2013/12/29. doi: 10.1093/bioinformatics/btt755. PubMed PMID: 24371154; PubMed Central PMCID: PMC3967115.
47. Bliss CM, Bowyer G, Anagnostou NA, Havelock T, Snudden CM, Davies H, et al. Assessment of novel vaccination regimens using viral vectored liver stage malaria vaccines encoding ME-TRAP. Scientific Reports. 2018;8(1):3390. doi: 10.1038/s41598-018-21630-4.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

ALCOHOL AND TOBACCO QUESTIONNAIRE INSTRUCTIONS

- Data collection will be required for all CP-CTNet studies.
    - Data will be collected at baseline and end of every study. Data may also be collected at follow-up visits as determined by each protocol. If you wish to collect additional information beyond these core elements, you may certainly do so. However, all studies need to collect the basic elements in the attached eCRFs.
    - The eCRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

ALCOHOL ASSESSMENT-- BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
	_____	_____	__ __/__ __/__ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.
When a number is requested in the response, please enter a whole number (i.e., "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since
   you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)

   ☐ Refused
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years

   ☐ Refused
   ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

   _____ years of age

   ☐ Refused
   ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

- ☐ Wine
- ☐ Liquor
- ☐ Beer
- ☐ Wine cool

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

ALCOHOL ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e., "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

☐ Refused

☐ Do not know/Not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e., "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line
for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus

☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

☐ Yes
☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

☐ Yes In total, for about how many years?_____ If less than 1, write "1."
☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
☐ No

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e., "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a whole number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____

☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other,
Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

☐ Smoked every day
☐ Smoked some days
☐ Did not smoke at all
☐ Don't know/not sure
☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

NATIONAL AND LOCAL RESOURCES TO HELP WITH ALCOHOL ABUSE AND ALCOHOLISM

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them.
https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

NATIONAL AND LOCAL RESOURCES TO HELP WITH QUITTING SMOKING

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Staying Smoke-Free for Good for smokers who have recently quit.
- Smoke-free for women, including pregnant women.
- Smoke-free information in Spanish
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov Web site.

APPENDIX C

COVID-19 ASSESSMENT INSTRUCTIONS

- Data collection will be required for all CP-CTNet studies.
    - Data will be collected end of every study. All studies need to collect the elements in the attached eCRFs.
    - The eCRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

CP-CT NET COVID-19 BASELINE ASSESSMENT

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE _____	VISIT DATE (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Instructions:
The following information is being collected for all Cancer Prevention Clinical Trials Network (CP-CTNet) studies. Only information from before study entry should be reported on this form.

Have you ever had a COVID-19 test?

- ☐ Yes
- ☐ No

If Yes, have you ever received a positive test result?

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer

Date of latest positive test: _ _ / _ _ / _ _ _ _
If positive, were you symptomatic?

- ☐ Yes
- ☐ No

Have you received a COVID-19 vaccine?

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer

If Yes, which vaccine did you receive for your first dose?

- ☐ Moderna
- ☐ Pfizer - BioNTech
- ☐ Johnson & Johnson
- ☐ AstraZeneca
- ☐ Other

If Other, specify: _____

Date of first vaccine dose: _ _ / _ _ / _ _ _ _

Have you received a second vaccine dose?

☐ Yes
☐ No

If Yes, date of second vaccine dose: _ _ / _ _ / _ _ _ _

If No, provide reason:

☐ Not yet due
☐ Second dose not required
☐ Other

If Other, specify: _____

Have you received a booster dose?

☐ Yes
☐ No

1. Date of first booster dose: _ _ / _ _ / _ _ _ _

   Which vaccine did you receive?

   ☐ Moderna
   ☐ Pfizer - BioNTech
   ☐ Johnson & Johnson
   ☐ AstraZeneca
   ☐ Other

   If Other, specify: _____

2. Date of second booster dose: _ _ / _ _ / _ _ _ _

   Which vaccine did you receive?

   ☐ Moderna

☐ Pfizer - BioNTech
☐ Johnson & Johnson
☐ AstraZeneca
☐ Other

If Other, specify: _____

Comments:
_____
_____

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX D

PARTICIPANT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Participant Name:

Diagnosis/Condition:

Study Doctor:

Study Doctor Phone #:

NCI Trial #:

Study Drug(S):
GAd20-209-FSP polyvalent vaccine;
MVA-209- FSP polyvalent vaccine

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX E

PARTICIPANT BOOKLET FOR REPORTING INJECTION SITE REACTIONS

Vaccine Report Card

Participant ID Number: _____

Injection Administration Date: _____

Note to Participant: If you have any questions or problems, please contact your study staff.

Name: _____

Telephone: _____

Email (optional): _____

Participant signature _____

Date report card returned to the study staff: _____

General Instructions

Temperature: Take your temperature orally around the same time each evening, using the thermometer provided, and record it in the space provided. Also record the time at which you took your temperature. If you recently drank very hot or cold liquids, wait 15 minutes before taking your temperature.

Injection Site Symptoms:

Pain and itching

Some people experience pain in the area where they were injected. Some people may also experience itching in the area where they were injected. By pain we mean that the place where you were injected hurts even when it isn't touched.

If you experience pain or itching at the injection site, use the following categories to describe how severe these symptoms were. If you don't experience one or more of these symptoms, mark the **NONE** box.

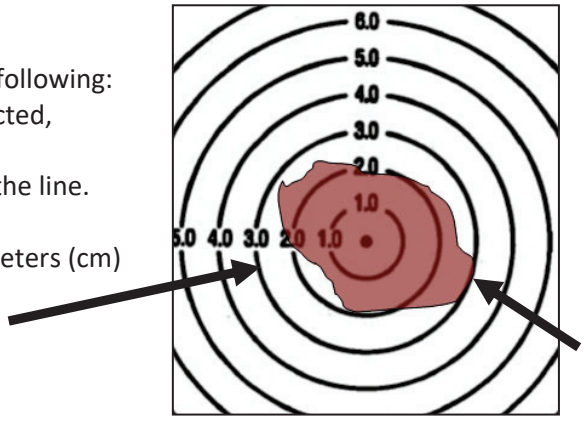
- **Mild**  I only had a little discomfort. I could still use my arm like always.
- **Moderate**  I noticed the discomfort and didn't use my arm as much as usual. I used over-the-counter pain medications to relieve the discomfort.
- **Severe**  I really noticed the discomfort. It kept me from doing something I wanted or had to do. Over-the counter pain medications didn't work.
- **Life-threatening** I had to go to the hospital or emergency room. *If this happens, please let your study team know right away.*

Redness, swelling, and bruising

Some people experience redness, swelling, or bruising in the area where they were injected. If you experience redness, swelling, or bruising, measure the area using the measuring tool you were provided, and record the measurement in the space provided.

To measure the area of redness, swelling, or bruising, please do the following:

1. Place the measuring tool over the area where you were injected, with the dot over the center of the area.
2. Select the circle where the longest part of the area touches the line.
3. If the area is between two circles, select the larger circle. For example, the area shown to the right measures 3 centimeters (cm) because it is touching the 3 cm line.



General and Other Symptoms and Medications

If you have additional symptoms that are not listed, or if you sought medical care for any reason from a health care provider (e.g., doctor's office, emergency room), or if you took any medications, these should be listed in the spaces provided. Please designate any symptoms as Mild, Moderate, or Severe.

- **Mild**  I only had minor discomfort. I went about my usual activities.
- **Moderate**  I noticed the symptom. It bothered me enough that I didn't do as much as I usually do. I used over-the-counter pain medications to relieve the discomfort.
- **Severe**  I really noticed the symptom. It kept me from doing something I wanted or had to do. Over-the counter pain medications didn't work.
- **Life threatening** I had to go to the hospital or emergency room. *If this happens, please let your study team know right away*.

Day 0: The Evening of the Injection **Date: _____/_____/_____**

Sometime during the evening on the day you were injected, fill out the information below. The items on this page refer to the time between when you were injected and 11:59 p.m. of today (Day 0). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 0). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 0). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 1: The Day after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 1). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 1). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 1). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 2: Two Days after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 2). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 2). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 2). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 3: Three Days after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 3). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 3). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 3). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 4: Four Days after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 4). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 4). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 4). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 5: Five Days after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 5). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 5). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 5). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____
_____

Day 6: Six Days after the Injection **Date: _____/_____/_____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 6). If any of the information changes after you fill out this page but before 11:59 p.m. tonight make any necessary changes below.

Temperature

Evening Temp.: _____ °C or °F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 6). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Unusually tired/feeling unwell	☐	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Nausea or vomiting	☐	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes the worst the symptom was until 11:59 p.m. tonight (Day 6). See **General Instructions** on page 2 for more information.

Symptom	None	Mild	Moderate	Severe	Life Threatening
Pain	☐	☐	☐	☐	☐
Itching	☐	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	____ cm at the longest part
Swelling	☐	____ cm at the longest part
Bruising	☐	____ cm at the longest part

Other Symptoms

If you experience symptoms other than the ones you've already described, write them in the space below according to the **General Instructions** on page 2.

Did you experience any other symptoms? ☐ **Yes** ☐ **No**

Symptom or Medical Event	Mild	Moderate	Severe	Life Threatening
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

Did you take any medications? ☐ **Yes** ☐ **No**
If yes, please list out the name(s) and dosage(s) below:

_____
_____
_____