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Randomized, Double-Blind, Placebo-Controlled Trial of MUC1 Vaccine in Patients with Newly Diagnosed Advanced Adenomas

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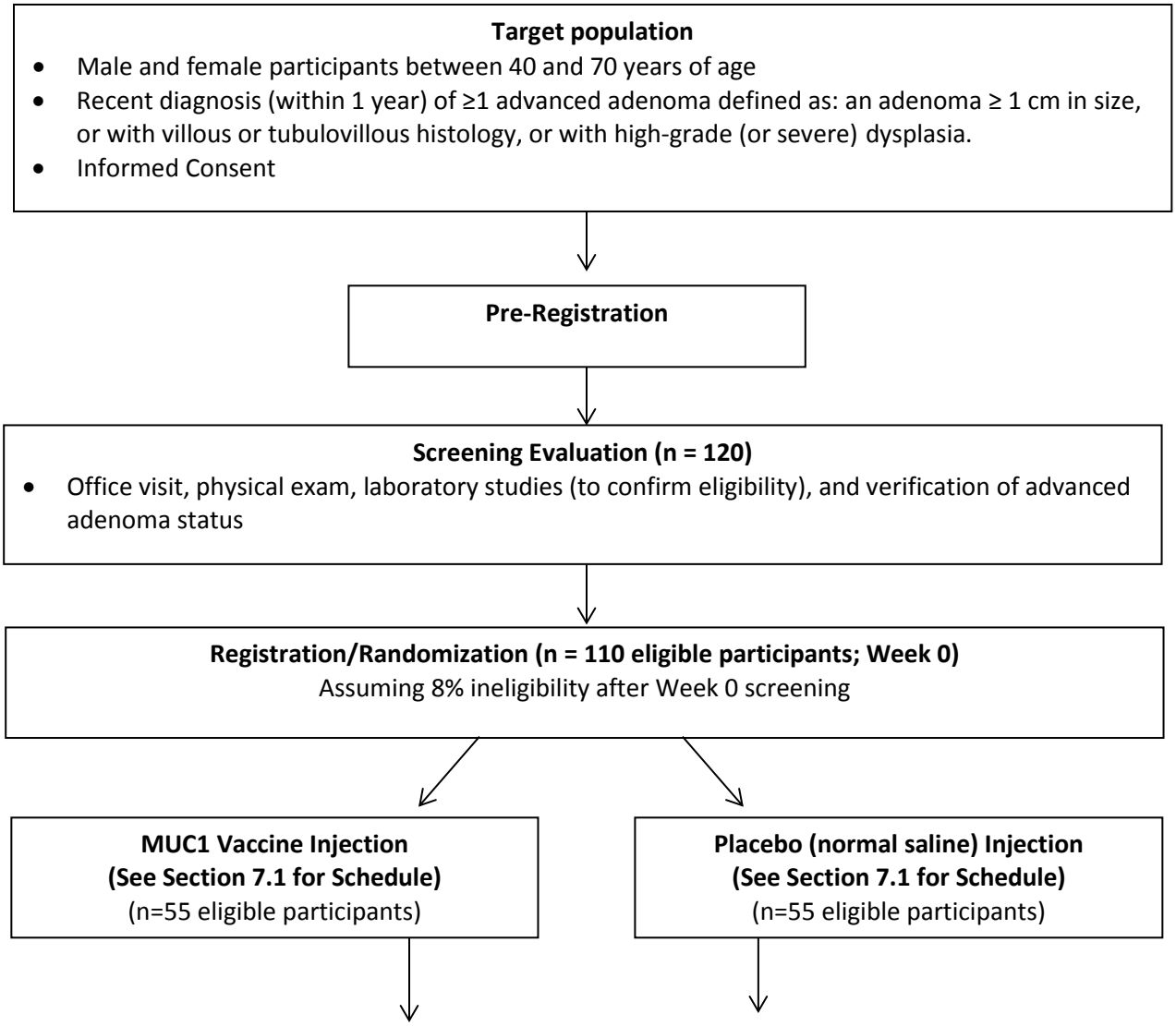
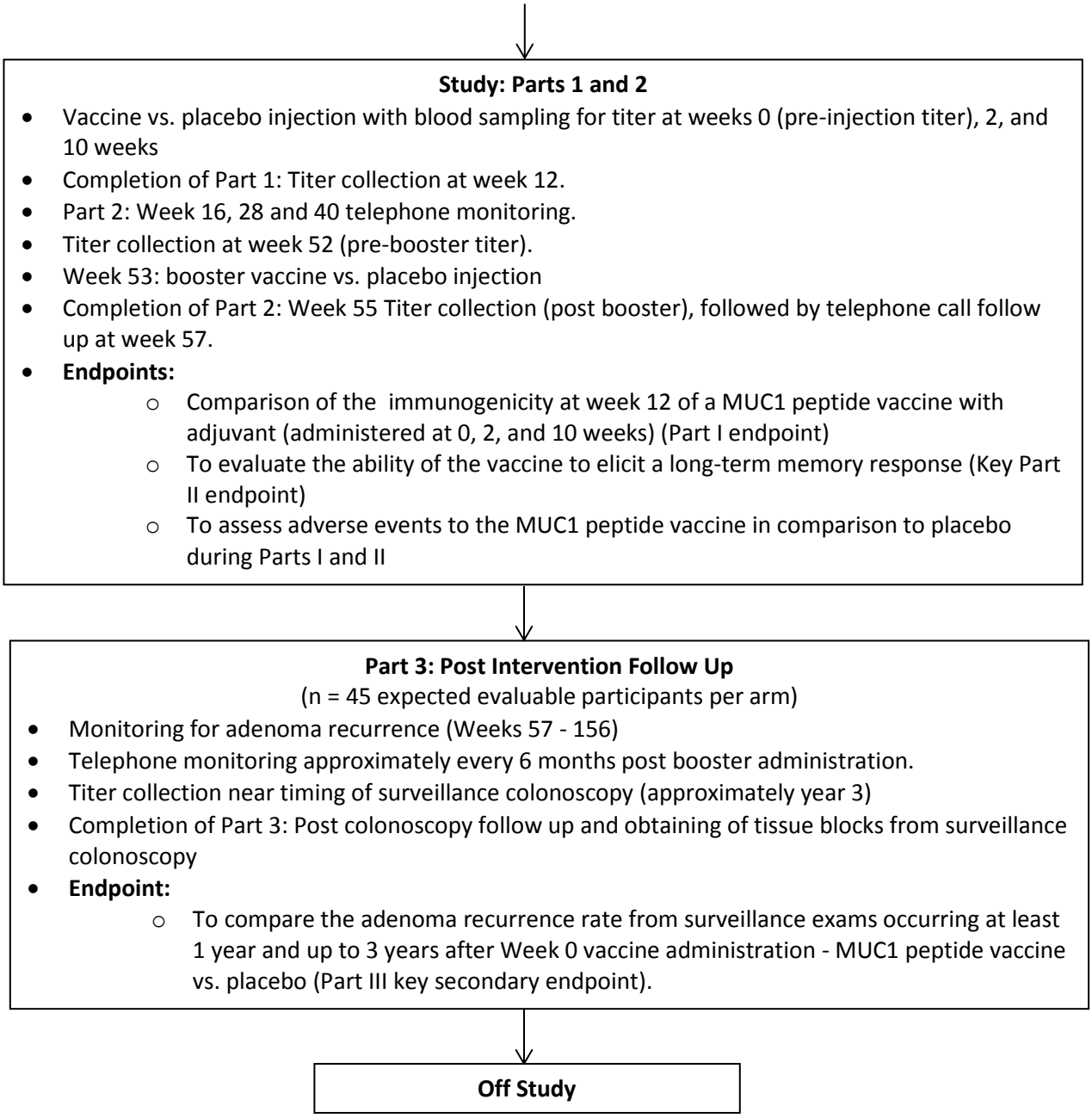
SCHEMA

Randomized, Double-Blind, Placebo-Controlled Trial of MUC1 Vaccine in Patients with Newly Diagnosed Advanced Adenomas




Study: Parts 1 and 2
• Vaccine vs. placebo injection with blood sampling for titer at weeks 0 (pre-injection titer), 2, and 10 weeks
• Completion of Part 1: Titer collection at week 12.
• Part 2: Week 16, 28 and 40 telephone monitoring.
• Titer collection at week 52 (pre-booster titer).
• Week 53: booster vaccine vs. placebo injection
• Completion of Part 2: Week 55 Titer collection (post booster), followed by telephone call follow up at week 57.
• Endpoints:
o Comparison of the immunogenicity at week 12 of a MUC1 peptide vaccine with adjuvant (administered at 0, 2, and 10 weeks) (Part I endpoint)
o To evaluate the ability of the vaccine to elicit a long-term memory response (Key Part II endpoint)
o To assess adverse events to the MUC1 peptide vaccine in comparison to placebo during Parts I and II
Part 3: Post Intervention Follow Up
(n = 45 expected evaluable participants per arm)
• Monitoring for adenoma recurrence (Weeks 57 - 156)
• Telephone monitoring approximately every 6 months post booster administration.
• Titer collection near timing of surveillance colonoscopy (approximately year 3)
• Completion of Part 3: Post colonoscopy follow up and obtaining of tissue blocks from surveillance colonoscopy
• Endpoint:
o To compare the adenoma recurrence rate from surveillance exams occurring at least 1 year and up to 3 years after Week 0 vaccine administration - MUC1 peptide vaccine vs. placebo (Part III key secondary endpoint).
Off Study

GLOSSARY

aa – Amino acid
AA – Advanced adenoma
ADL – Activities of daily living
AE – Adverse events
ADCC – antibody-dependent cell-mediated cytotoxicity
ANA – Antinuclear antibody
BAP – Biospecimens Accessioning and Processing
CACC – Colitis-associate colon cancer
CPN – Cancer Prevention Network
CRF – Case report form
CTL – Cytotoxic T-lymphocytes
DCP – NCI, Division of Cancer Prevention
dsRNA – double-stranded RNA
FAP – Familial adenomatous polyposis
FDA – Food and Drug Administration
HNPCC – Heritable nonpolyposis colorectal cancer
IBD – Irritable bowel disorder
IFNs – Interferons
IgG – Immunoglobulins
IND – Investigational new drug
IRB – Institutional Review Board
MDSC – Myeloid derived suppressor cells
MUC1 – Mucin 1 (high molecular weight type I transmembrane glycoprotein)
NCI – National Cancer Institute
NK – Natural killer cells
NF-kB – Nuclear factor kappa-light-chain-enhancer of activated B cells
OAS – Oligoadenylate synthetase
PBMC – Peripheral blood mononuclear cells
PO – Participating Organization
Poly-ICLC – Polyinosinic-Polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose (Hiltonol®)
PKR – Protein kinase receptor
SAE – Serious adverse events
T52 – Antibody titer at week 52
Th cells – T-helper cells
TLR – Toll like receptor
VNTR – Variable number of tandem repeats
WIWI – Was It Worth It Questionnaire

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1. OBJECTIVES

1.1 Primary Objective

The primary objective of this trial is to compare the immunogenicity at week 12 of a MUC1 peptide vaccine with adjuvant (administered at 0, 2, and 10 weeks) in participants with a history of an advanced adenoma, randomized to receive MUC1 peptide vaccine versus placebo (Part I endpoint).

1.2 Secondary Objectives

Secondary objectives which correlate with the planned parts of the study include:

- To evaluate the ability of the vaccine to elicit a long-term memory response. We will administer a booster injection of the vaccine or placebo at week 53. Anti-MUC1 antibody response will be assessed at week 55 and compared to the week 52 pre-booster vaccine level (T55/T52). (Part II key secondary endpoint).
- To compare the adenoma recurrence rate from surveillance exams occurring at least 1 year and up to 3 years after Week 0 vaccine administration – MUC1 versus placebo (Part III key secondary endpoint).
- To compare the adenoma recurrence rates between MUC1 and placebo by excluding the following types of adenomas. These adenomas are excluded since they may represent missed or residual adenomas from the baseline exam. These analyses are exploratory.
    - Participants with adenomas ≤ 5 mm,
    - Participants with adenomatous tissue which may represent residual adenoma at the site of the previous advanced adenoma,
    - Participants with adenomatous tissue detected in the same segment of the bowel as the previous advanced adenoma.
- To assess adverse events to the MUC1 peptide vaccine in comparison to placebo during Parts I and II.
- To assess patient reported injection site reaction events from the Vaccine Report Card.

1.3 Translational Objectives

- To compare the anti-MUC1 antibody titer at the time of surveillance colonoscopy for the purpose of evaluating the anti-MUC1 antibody response in relation to adenoma recurrence. Since surveillance colonoscopy is generally at 3 years after an advanced adenoma, this titer will typically be obtained at approximately week 156.
- To evaluate MUC1 expression on baseline advanced adenomas and on recurrent adenomas detected at surveillance colonoscopy. Based on current knowledge of MUC1 expression and function in tumor cells, MUC1 negative adenomas are not expected to grow as immune escape variants. This has never been tested before, and the trial will provide an appropriate setting to study this issue.
- To evaluate levels of circulating myeloid derived suppressor cells (MDSC) in the vaccinated and the placebo group and correlate with anti-MUC1 antibody levels and adenoma recurrence.
  To establish a biospecimen repository archive including live cells, plasma, and germline DNA for future immunologic (e.g. MUC1-specific T cells) and other assays (systems biology approach to detect differences between responders and non-responders), testing not currently accommodated within the budget of this trial.

2. BACKGROUND

Randomized controlled trials demonstrate that endoscopic removal of adenomatous polyps reduces cancer incidence[1, 2]. However, there are many more adenomas than cancers, adenomatous polyp

recurrence rates are high, and repeated colonoscopic surveillance to monitor and remove recurrent adenomas is invasive, expensive, and associated with medical risk. Furthermore, surveillance colonoscopy is frequently misapplied, with excessive colonoscopy in low risk individuals and inadequate colonoscopy in some high risk individuals [3]. Immunotherapy targeting antigens known to be aberrantly expressed on colon cancers and polyps offers the potential for a relatively non-invasive and non-toxic prevention strategy, and because of the specificity of the immune response and its long-term memory, offers the potential for prolonged protection. Data suggest that aberrant expression of tumor antigens is subject to immune surveillance. During evolution from premalignant lesion to tumor, tumor-specific antibodies and T cells can be identified that in many animal models and human studies have been correlated with better prognosis. In a large study in colon cancer, that has now been repeated numerous times and in different types of tumors, infiltration of primary tumors with T cells was the best predictor of increased survival, surpassing even the cancer stage predictive value [4]. MUC1 vaccines to boost immune responses have been tested in colon cancer patients with advanced metastatic disease with a clinical response between 10% and 14%[5]. While the immune responses induced were by and large weak due to the generally immunosuppressed state of patients with advanced disease, in 22 studies that measured vaccine induced immune responses, humoral immunity was seen in 59% of patients and cellular immunity in 44%[5]. The limited effect of vaccine therapy in established cancer is now known to be due to numerous immunosuppressive mechanisms that develop in the cancer patient over time, caused either by the tumor or the immunotoxicity of standard cancer therapy, or both. Drugs, such as ipilimumab, are under development or being employed to modulate the tumor microenvironment to favor vaccine efficacy. However, most of these drugs are highly toxic in addition to being very expensive.

An opportunity to bypass these barriers is presented by the discovery that many of the tumor antigens in cancer vaccines are also expressed on precursor lesions, e.g. adenomatous polyps as precursors to colon cancer, PanINs to pancreatic cancer and CINs to cervical cancer, suggesting that immunization of patients with pre-malignant lesions before the tumor and cytotoxic therapy suppresses the immune response is possible and may be preferred. Our hypothesis is that a vaccine that elicits or boosts immune surveillance early in the oncogenic process will prevent malignant disease by eliminating premalignant lesions and preventing their recurrence and their progression to cancer. We are testing this hypothesis in the best elucidated pathway of premalignant to malignant progression, adenomatous polyp to colon cancer. The antigen we propose is MUC1, a tumor-associated antigen abnormally expressed on polyps and colon cancer [6, 7], and the population for the planned intervention is individuals with advanced adenomas, a group at high risk for colorectal cancer, even years after initial diagnosis [8].

MUC1: Mucins are large glycoproteins that consist of an extended polypeptide core that is heavily glycosylated by *O*-linked carbohydrates. Mucin 1 (MUC1) is a high molecular weight (>200,000 dalton) type I transmembrane glycoprotein. MUC1 is produced by cells of epithelial origin and expressed on the apical surface of these cells as they form glands. MUC1 is expressed by normal epithelial cells but in low levels, polarized to the apical surface, and with extensive glycosylation. MUC1 is a cell surface mucin that is able to induce a specific immune response[9, 10]. When cells undergo malignant transformation they produce hypoglycosylated MUC1 that has lost luminal polarity and is expressed at a much higher level than on normal cells. These characteristics allow its recognition by the immune system resulting in low-titer anti-MUC1 antibodies and MUC1 specific cytotoxic T-lymphocytes (CTL). Patients with adenocarcinomas of the breast, pancreas, and large bowel have T cells and antibodies that are directed to MUC1 [11]. The naturally occurring weak response is thought to be secondary to the lack of induction of MUC1 specific T-helper (Th) cells [12]. A MUC1 vaccine that can boost that immune response, especially effector pathways that induce MUC1-specific Th cell responses, may be effective in controlling or

curtailing tumor growth. MUC1 is characterized by a large number of tandem repeats of 20 amino acids in the extracellular domain, which are quite immunogenic when underglycosylated, the form expressed on tumor cells [13, 14]. Recent work with T cells suggests that the immune response is directed to peptide and glycopeptide epitopes from the tandem repeat region exposed on the hypoglycosylated tumor form of MUC1. During neoplastic transformation, the amount of MUC1 produced by tumor cells increases and its glycosylation decreases thus increasing the number and density of epitopes that serve both as immunogens as well as targets of the immune response. The MUC1 molecule is also of interest because it binds β-catenin [15, 16] as well as intermediates in the *ras* signaling pathway [17] promoting cell transformation. Thus, immunization against this molecule may affect premalignant cells in several different ways. Cytotoxic T cells generated by a MUC1 vaccine may directly destroy premalignant cells, while the antibodies may be involved not only in direct destruction of cells through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement mediated lysis, but also by blocking signaling pathways that promote further neoplastic transformation.

Abnormal MUC1 expression has been a target for both active and passive immunotherapy of cancer in many pre-clinical and clinical studies[18-20]**.** Targeting a molecule that is critical for cancer progression and survival lessens the chance that MUC1 negative mutant cancer cells would arise. In a recent NCI workshop on tumor antigen prioritization, MUC1 was ranked as the overall #2 priority target (only slightly behind WT1) [21]. Its priority was a result of 25 years of preclinical studies in animal models and almost 20 years of clinical trials, primarily in the therapeutic setting.

MUC1 vaccines*:* MUC1 proteins, peptides, glycopeptides or DNA-based vaccines have been widely used with or without adjuvants, loaded on DCs, with cytokines and immune stimulatory molecules, or engineered in various viral vectors, for treatment of cancer. These have been administered to patients with advanced disease and compromised immune systems. Even though the immune response induced by the vaccines was infrequent and at low levels, both humoral and cellular responses have been reported. In a meta-analysis published in 2006 of all clinical trials of MUC1 colon cancer vaccines published since 1985, consisting of 32 studies involving 527 colorectal cancer patients and accounting for 108 publications, utilizing MUC1 antigens that varied from whole tumors to individual peptides with a variety of adjuvants, toxicity was mild in the vast majority of studies, and manifest primarily as injection site symptomatology and flu like symptoms [5]. At the University of Pittsburgh Cancer Institute we have run seven clinical trials using the unglycosylated VNTR 100mer MUC1 peptide representing 5 tandem repeats, a highly immunogenic form not found on normal epithelial cells. Trials in resected pancreatic cancer were performed under the condition of minimal residual disease. In the first trial we used MUC1 100mer peptide plus the SB-A2 adjuvant from SKB [22]. The vaccine was safe and induced IgG and T cells responses in some patients. Two of 16 patients were alive and disease free at 32 and 61 months of follow up and no toxicity was seen that could be attributed to the vaccine. The second Part I/II trial used MUC1 100mer-peptide-loaded on DC. The vaccine was well-tolerated and non-toxic in 12 patients enrolled. The patients were followed for over four years and four of the twelve patients were alive without evidence of recurrence at 5 years.

Two decades of clinical trials targeting MUC1in a variety of cancers in over 1200 patients were recently reviewed [23]. Ongoing trials registered at *clinicaltrials.gov* identify an additional 2000 patients planned for anti-MUC1 therapy [23]. The presence of anti MUC1 immunity in cancer patients favorably affects tumor development and prognosis[23].

MUC1 Peptide: Many types of vaccines have been tested by numerous investigators around the world incorporating a variety of MUC1 antigenic forms made immunogenic through the use of different adjuvants[23]. Other forms, such as endogenously expressed MUC1 peptides via a plasmid, synthetic mRNA or a virus vector comprise only a minority of MUC1 vaccines. Synthetic MUC1 peptides have ranged from 16mer to 105mer in length and, with very few exceptions, they have all been derived from the VNTR tandem repeat sequence. The synthetic MUC1 tandem repeat peptides have been conjugated to immunogenic proteins or substrates, such as keyhole limpet haemocyanin (KLH) or mannan, mixed with various adjuvants such as Bacille de Calmette et Guērin (BCG), partially purified mycobacteria cell-wall skeleton and monophosphoryl lipid A (MPL) (DETOX), Quillaja Saponaria extract (QS-21), incomplete Freund's adjuvant (IFA) and QS-21 and MPL (SB-AS2). The 3-D structure of the MUC1 tandem repeat using 20, 40 and 60aa-long peptides has been characterized [24] and the optimal number of repeats for induction of immune responses or detection of antibodies in ELISA assays has been determined [25]. The findings suggested that increasing numbers of tandem repeats elicited increasing titers of antibodies as well as detection levels of serum IgG in ELISAs. Attempts to synthesize long MUC1 peptides were successful first through manual synthesis and later also achieved through automatic synthesis. The optimal length was considered to correspond to 5 tandem repeats, 100aa, below which detection was weaker and above which no further benefit was seen. Thus the 100mer peptide became the vaccine antigen and the facility for production of the certified peptide for clinical use was established at the University of Pittsburgh.

Poly ICLC Adjuvant: Polyinosinic-Polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose (Poly-ICLC, Hiltonol®) is a synthetic, non-replicating double-stranded ribonucleic acid (dsRNA), with no specific genetic message. It acts as viral-mimic with broad innate and adaptive immune enhancing, vaccine adjuvant, antiviral and antiproliferative effects [25, 26]. These are mediated partly by its induction of a 'natural mix' of interferons (IFNs), cytokines, and chemokines, activation of natural killer [NK] cells, myeloid dendritic cells via TLR3 and MDA5, T4 and T8 cells, and activation of several nuclear and cytoplasmic enzyme systems (oligoadenylate synthetase [OAS], the dsRNA dependent protein kinase [PKR], RIG-I Helicase, and MDA5) that are involved in antiviral and antitumor host defenses. It has been shown to have broad gene regulatory actions as well. Poly-ICLC is currently being developed for the treatment of various disease indications in humans. Older studies in advanced cancer patients had shown a maximum tolerated dose of over 200 mcg/kg intravenously (IV), but Poly-ICLC has been administered intramuscularly (IM) 2 to 3 times weekly for years at doses of 10 to 50 µg/kg in patients with gliomas, multiple sclerosis or HIV/AIDS, and is well tolerated [27-29]. Three recent phase II multicenter trials of Poly-ICLC 20 mcg/kg IM 3 times weekly in over 180 patients with gliomas have confirmed safety and potential efficacy (see poly ICLC investigational brochure). Poly-ICLC is also being used for various cancer vaccine platforms in about two dozen, ongoing phase I/II clinical trials in patients with glioma, hepatoma, lymphoma, prostate, cervical, ovarian, breast, colon, and pancreatic cancers.

About 400 patients have been safely treated in those trials to date (see poly ICLC investigational brochure). The most common side effects of low-dose Poly-ICLC are temporary discomfort at the injection site and occasional transient malaise that lasts for several hours. Occasionally, patients on low-dose Poly-ICLC have developed a flu-like syndrome with fever, chills, and malaise. This syndrome typically resolves in 12 to 24 hours, responds to acetaminophen, and does not recur on subsequent dosing. Subcutaneous dosing, especially with vaccine, can also result in transient injection site erythema. Poly-ICLC has also been used nasally in a recent phase I randomized dose escalation trial in humans (N=50), and was well tolerated.

Vaccines for Prevention

Other than viral vaccines for cancers of known viral origin, vaccines for prevention of cancer have not received sufficient attention. Yet, there are many non-viral antigens that are differentially expressed between normal and cancer cells, such as MUC1, that are perceived by the immune system as foreign and to which immune responses can be safely generated through vaccination. In preclinical animal models of human cancer, these vaccine-elicited immune responses have prevented cancer development or cancer progression. These studies have served as the basis for the first clinical feasibility study of the MUC1 peptide vaccine in individuals with the history of advanced adenoma who are considered at increased risk for developing colon cancer[34]. This study showed that the vaccine consisting of 100mcg of the synthetic 100mer MUC1 peptide admixed with the TLR-3 agonist Hiltonol as adjuvant, induced anti-MUC1 IgG and long term immune memory thus confirming its immunogenicity. The study also showed that individuals with above normal numbers of circulating myeloid derived suppressor cells (MDSC) were prevented from responding to the vaccine. The randomized placebo-controlled trial described in this protocol will confirm the vaccine immunogenicity and its dependence on the MDSC and compare adenoma recurrence in vaccine responders vs. non-responders.

Colorectal Adenomas

MUC1 is overexpressed and hypoglycosylated on premalignant lesions such as adenomatous polyps, and its expression is significantly greater with higher degrees of dysplasia [7]. MUC1 is functionally important in the maintenance of the malignant phenotype. Thus, induction of an immune response against MUC1 in the setting of pre-malignancy, such as in patients with adenomatous polyps, may protect from polyp recurrence as well as from progression to cancer.

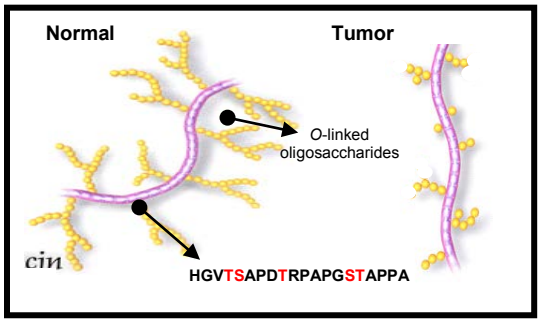

Figure1: Abnormal MUC1 is overexpressed and hypoglycosylated. Hypoglycosylated MUC1 exposes the disaccharide T antigen and the shorter monosaccharide Tn antigen attached to the protein backbone of MUC1 molecules.

Mouse model of MUC1 vaccine for colon cancer prevention

Mouse experiments have been performed over the years to test the immunogenicity, safety and efficacy of MUC1 vaccines. One of the best mouse models has been the MUC1 transgenic mouse that expresses human MUC1 under its own promoter and thus maintains tissue specific expression, including undergoing changes in expression during tumorigenesis. Most vaccines tested in human clinical trials showed both high immunogenicity and high efficacy in mouse models. It should be emphasized that this result was always achievable in the prevention setting and worked in the therapeutic setting only when the tumors were extremely small (e.g. 3-day tumors). Vaccination in the setting of a larger or metastatic tumor was never successful. Experiments in spontaneous mouse tumor models, though more labor intensive and of longer duration, are a good reflection of the immunosurveillance of the emerging tumor by the immune system and more closely resemble human disease than transplantable tumor models. A well-known model of human colon carcinogenesis is the Apc/MIN mouse that develops spontaneous adenomatous polyps that can develop into cancer. This mouse was crossed to the MUC1 transgenic mouse and both the polyps and the resulting tumors expressed the abnormal MUC1 and could be

targeted by a MUC1 vaccine. In the MIN mouse, a MUC1-based vaccine caused flattening of adenomas and significantly reduced the number of large adenomas [35]. Immunization was successful in generating a MUC1-specific immune response.

Two recent MUC1 vaccine studies in mouse tumor models of colorectal cancer were recently performed. In an IL-10-/- mice that spontaneously develop inflammatory bowel disease (IBD) and progress to colitis associated colon cancer (CACC), crossed with MUC1 transgenic mice, a MUC1 vaccine that induced humoral and cellular anti-MUC1 immunity reduced inflammation and prevented development of CACC [36]. The same result was obtained in a dextran sodium sulfate (DSS) model of CACC where the MUC1vaccine reduced chronic colitis, prevented development of dysplasia, and extended survival [37]. While this is a different route of colon cancer development in that these are inflammatory models, the model shows both efficacy and safety of the vaccine-induced anti-MUC1 immunity.

2.1 Study Disease: Advanced Adenomas

Advanced adenomas (AAs) are defined as pre-neoplastic adenomatous polyps that are either: 1) ≥ 1 cm in size, 2) demonstrate villous or tubulovillous histology, or 3) have severe or high grade dysplasia. Advanced adenomas occur in about 5-10% of patients undergoing screening colonoscopy. Individuals with AAs are a high risk group. Studies show that individuals having an advanced adenoma have a 2 fold or more risk of having a recurrent AA compared to individuals with non-advanced adenomas [38, 39], and more importantly, are at higher risk for subsequent colorectal cancer, even years later [8, 40]. Patients with AA are an appropriate group to focus on for prevention. Low levels of anti-MUC1 IgG can be detected in patients with advanced adenomas [41]. Most recently, we performed a pilot study of MUC1 vaccine in patients with a prior history of advanced adenoma, though not necessarily recently diagnosed advanced adenomas, and the vaccine was well tolerated with no significant toxicity [34].

2.2 Study Agent: MUC1 Vaccine (MUC1 peptide/Poly-ICLC)

A certified clinical grade 100-amino acid synthetic MUC1 peptide with the molecular structure of H_2N-$(GVTSAPDTRPAPGSTAPPAH)_5$-$CONH_2$, will be synthesized at the University of Pittsburgh Peptide Synthesis Facility. The adjuvant, toll like receptor (TLR) 3 agonist, Poly-ICLC (Hiltonol®), will be supplied by Oncovir Inc. (Washington, DC) in single-dose vials of 1mL solution containing 2mg poly-IC, 1.5mg poly-L-lysine, and 5mg sodium carboxymethylcellulose in 0.9% sodium chloride, adjusted to pH 7.6-7.8 with sodium hydroxide. The vaccine will consist of 100 micrograms (0.1mg) of the MUC1 100mer peptide dissolved in 50mcL (0.05mL) of sterile saline, admixed with 500mcg (0.5mg) of Hiltonol® in 250mcL (0.25mL), for a total injection volume of 300mcL (0.3mL).

2.3 Rationale

<u>Completed Feasibility study of MUC1 Vaccine at U. of Pittsburgh</u>
We enrolled 40 participants who received MUC1/Poly ICLC vaccine at 0, 2, and 10 weeks and antibody titers were evaluated prior to each vaccination and at week 12, 28 and 52 [34]. In the 39 participants that received all three initial doses of vaccine, 17 (44%) had over a two-fold ratio increase at week 12, ten participants had ratios over 4.5 fold, and another 4 participants had a ratio increase from 1.4 – 2.0. The kinetics of the response were similar, with peak antibody levels generally occurring after the third injection. As expected, circulating antibody levels tended to decline over the next year with clearance of the vaccine antigen.

Response to Booster vaccine

To test for the presence of a vaccine-induced T cell memory response, participants received a booster dose of vaccine at 1 year. Of the 16 participants who responded to the initial vaccination and received the booster, 12 (75%) had a two-fold ratio increase when comparing week 54 to week 52. Three of the 4 who didn't have a two- fold elevation at week 54 had maintained high levels of antibody at week 52, hence did not manifest a 2 fold ratio increase at week 54. Overall, the results suggest that responders established a T cell memory response to the MUC1 antigen.

Adverse Events

Over 150 vaccinations were administered in the pilot study. No adverse events above grade 1 were identified, and no unanticipated adverse events were observed. Most participants developed erythema with soreness at the injection site. Some developed short lived fever and muscle aches, which respond well to analgesics such as Tylenol. We had nearly 100% compliance with the study protocol. Participants were monitored with immunofluorescent ANA titers pre vaccination and at 1 year. No participants have developed a change in ANA titer and no new autoimmune diseases were observed.

Figure 2:

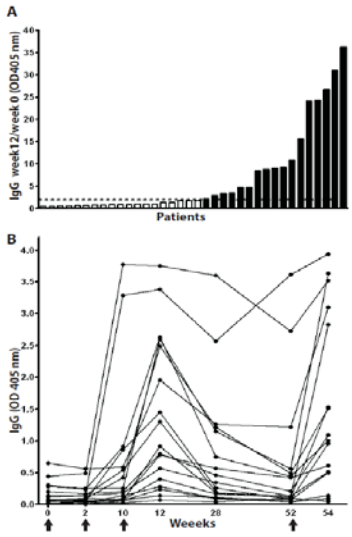

Figure 2A: Ratio response in MUC1 antibody.
Figure 2B: Kinetics of response to vaccination at 0, 2, and 10 weeks (arrows under x axis) and to booster vaccine at week 52.

Evaluation of Immune Non-Responders

Lack of response to vaccination was correlated with high levels of circulating myeloid-derived suppressor cells but not to circulating T regulatory cells.

2.4 Studies Planned in the Proposed Trial

Evaluation of anti-MUC1 antibody titer and MUC1 expression in adenomas in relation to adenoma recurrence

Anti-MUC1 IgG will be measured at w12 and w55 to measure the immune response to the vaccine in comparison to placebo, and at the time of the endpoint surveillance colonoscopy to examine the relationship between antibody titer and adenoma recurrence. In addition, sections of the qualifying advanced adenoma and of any recurrent adenomas will be obtained. Comparison of tumor associated

MUC1 antigen expression on the recurrent adenomas in relation to antibody response, and comparison among vaccine responders, non-responders, and the control group are planned.

Evaluation of anti-MUC1 antibody responses and pre-vaccination levels of circulating MDSC

Heparinized blood will be collected at week 0, 2, 10, and 12 and live PBMCs stored in DMSO and LN2 at -70 to -80^{o}C. We plan measurements of levels of circulating MDSC in all participants. We know from the feasibility study that higher levels of MDSC were associated with lack of generation of vaccine induced antibodies. We will confirm this observation in a larger number of individuals and will evaluate MDSC levels as a variable influencing adenoma recurrence. In the placebo group, we expect to have MDSC low and MDSC high individuals in whom we can determine if adenoma recurrence rates might correlate with MDSC.

Using samples stored in the biorepository, eventually, stored PBMCs will be used to evaluate MUC1 specific T helper and CTL responses. These studies are more time intensive and expensive and funding for them will have to be obtained through other sources or other NCI grants. We do not anticipate difficulty obtaining funding for sophisticated immune assessments on this unique collection of clinical samples among participants undergoing a randomized trial of immunotherapy in a prevention setting. Within this trial, we are using T dependent antibody responses and T dependent memory responses as a surrogate marker of vaccine elicited T cells.

Placebo Response

There are several important reasons to include a placebo group. We do not know the frequency of spontaneous acquisition of antibodies to MUC1, nor do we have data on the natural history of fluctuation in antibody level over time. A control group with which to compare the antibody response and a control group with which to compare adenoma recurrence is essential.

Exclusion of Individuals with Cancer

We are intentionally not enrolling participants with cancer (within the previous 5 years), even early stage cancer. By the time a cancer is present, the immune system has been exposed to and has attempted to cope with the lesion for some time. Attesting to the value of an intact immune system, the antibody response rates observed in our preliminary study far exceed that observed in previous trials of participants with cancer. Even in the setting of advanced adenoma, the pilot study showed signs of immunosuppression (higher levels of MDSC) in some patients. All cancer patients have elevated levels of these and other immunosuppressive cell populations.

How does the proposed trial differ from the completed feasibility study?

The completed study was performed to assess immune response, evaluate toxicity, and to trial recruitment, consent, and vaccine administration. The completed study was a resounding success in terms of enrollment, follow up, absence of adverse events, and immune response. The eligibility criteria included a history of advanced adenoma that could have been diagnosed years prior. The mean time from advanced adenoma diagnosis to vaccination in included participants was 824 days with a range up to 3500 days. As such, participants had interim colonoscopy exams prior to vaccination, and assessment of the effect of vaccine on adenoma recurrence is not possible. A significant difference in the proposed trial is the synchronization of vaccine administration with diagnosis of advanced adenoma such that a meaningful assessment of the vaccine on adenoma recurrence is attainable. A clinical endpoint is needed to advance immunotherapy in the prevention setting.

Laboratory Biomarkers

The most appropriate biomarker of vaccine efficacy is the immune response. We will measure the major T cell dependent anti-MUC1 antibody IgG isotype which requires MUC1 specific T cells to promote isotype switching by B cells from IgM. We will largely focus on anti IgG to MUC1, as that antibody isotype is easier to measure and more reproducibly induced. We will also measure MDSC levels to evaluate the relationship between MDSC and vaccine response. The biospecimen repository archive will include plasma and PBMC for immunologic and other assays, such as the frequency and quality of antigen-specific T cells induced by the vaccine or adenoma recurrence and changes in the serum cytokine and chemokine profiles. The tissue sections of adenomas will be also be useful in exploring the underlying hypothesis that vaccination can impair MUC1 expression and adenoma progression.

Conclusions and Implications

The robust antibody response obtained in our pilot trial in participants with a premalignant advanced adenoma challenges the assumption that tumor-associated antigens like MUC1 are subject to immune tolerance. Our data indicate that tumor or treatment-induced immune suppression was more likely responsible for ineffective immunization in cancer patients. The high level of antibody response in participants with pre-malignant disease attests to the promise of immunotherapy against tumor antigens as a means of prevention. Lack of toxicity will allow application of the vaccine in healthy as well as younger individuals with strong immune systems.

The proposed trial will add substantively to the pilot trial, because it offers **synchronization** of diagnosis and vaccine administration, and allows assessment of a clinical endpoint, adenoma recurrence. As the first randomized trial of immunoprevention, the trial offers a pioneering, innovative opportunity in cancer prevention research. The development of a biorepository will facilitate ancillary biomarker studies in this unique cohort and promise multiple opportunities for collaboration in and outside the NIH to make the trial even more productive over time.

3. SUMMARY OF STUDY PLAN

3.1 Study Parts

There are 3 Parts planned. Each Part will provide salient data on MUC1 immunoprevention, with continuation through Part III providing insight on the clinical efficacy of the vaccine.

1. Part 1 will include participant follow up to week 12 and will permit assessment of MUC1 vaccine response compared to placebo.
2. Part 2 will include participant follow up to week 56 and will permit assessment of MUC1 immune memory response to a booster dose of vaccine compared to placebo.
3. Part 3 will include participant follow up to week 156 and will permit assessment of the clinical endpoint of adenoma recurrence in participants with vaccine compared to placebo. Assessment of MUC1 expression on recurrent adenomas will allow assessment of whether vaccination can impair MUC1 expression and adenoma progression.

The vaccine is manufactured at the FDA certified peptide facility of the University of Pittsburgh. The vaccine will consist of 100 micrograms (0.1mg) of MUC1 100mer peptide dissolved in 50 microliters (0.05mL) of sterile saline, admixed with 500 micrograms (0.5mg) of the adjuvant poly-ICLC (Hiltonol®) in 250 microliters (0.25mL) volume, for a total injection volume of 300 microliters (0.3mL). Poly-ICLC is a

TLR3 agonist, a synthetic, non-replicating dsRNA with no specific genetic message, which acts as a viral-mimic and activator of diverse elements of both the innate immune response and of adaptive immunity. Participants randomized to placebo will receive an identically appearing injection of sterile saline.

3.2 Study Design

This is a randomized, double-blind, placebo controlled phase II trial designed to elicit immune responses to abnormal MUC1. A 1-year booster vaccine will be administered to assess the presence of T cell dependent immune memory. Participants will be randomized on a 1:1 scheme. The protocol will call for injections at week T0, T2, and T10, and a booster injection at week T53. Monitoring of the immune response will be at each time point prior to injection, T0, T2, T10 and at week T12, T52, T55 (post booster), and at the time of the endpoint surveillance colonoscopy up to T156, for a total of 7 measurements of MUC1 immune response. For details regarding study visits and assessments, see Section 7.

Up to 120 participants will be approached for pre-registration, and assuming an 8% ineligibility rate, approximately 110 participants will be randomized to either vaccine or placebo injections and followed up to 3 years for the primary and secondary endpoints. A dropout rate of up to 10% is assumed between baseline and year 1, and an additional 10% drop out rate is assumed between year 1 and year 3 (termination of follow-up).

Eligible participants will have had an advanced adenoma that was completely resected as reported by endoscopic assessment. Advanced adenomas will be defined as: 1) ≥ 1 cm in size; 2) demonstrates villous or tubulovillous histology; or 3) have severe or high grade dysplasia. We anticipate enrolling approximately 4 participants per month across all sites for the first 3-6 months, and then 8 participants per month across all sites until we reach the accrual target. Accrual is expected to be completed in 18 months. Total study duration for Part 1 is expected to be about 22 months, followed by an additional 12 months for Part 2 and 24 months for Part 3.

Intervention Arms: Participants meeting study criteria will be randomly assigned to receive one of two interventions according to the following intervention schedule:

- Muc1/poly ICLC 0.3 mL subcutaneous study vaccine in the upper thigh
- Normal saline injection 0.3 mL subcutaneous in the upper thigh

3.3 Vaccine and Clinical Endpoints

The response to the vaccine will be evaluated by monitoring changes in IgG anti-MUC1 antibody titer ratio; defined as t12/t0, where t0 is the “initial titer” measured prior to vaccination, and t12 is the “final titer” drawn at 12 weeks. For the primary endpoint, the titer measured 2 weeks after the 3rd vaccination, at week 12, will be compared to the baseline titer at week 0. Because IgG is more commonly induced and more likely to be detected because of the better quality of reagents, IgG levels will be the indicator of immungenicity. A titer ratio of ≥2 will be considered an immune response. The use of a titer ratio permits a uniform definition for an immunologic response regardless of the baseline level of MUC1 antibody. The titer ratio at week 12 will mark the close of Part 1 of the trial. The assessment of the response to the booster vaccine will be evaluated by examining the week 55 titer to the titer at week 52, prior to receipt of the booster vaccine. Once again, a titer ratio of ≥2 for the T55/T52 comparison will be considered a positive immune response.

The titer ratio of 2:1 as a measure of immune response was chosen in part from the infectious disease literature where this ratio is often employed as a marker of immune response. This ratio was commonly attained (44%) in our pilot study in participants with advanced adenoma [34]. A titer increase of ≤2 may also have clinical efficacy. We have no preliminary information to identify what titer ratio may be associated with a clinical endpoint. The measurement of the response to the booster vaccine and communication with the patient regarding response (week 55) will mark the close of Part 2 of the trial.

The week 156 endpoint will be adenoma recurrence. Adenoma prevention trials testing chemoprevention regimens have been performed for a variety of compounds ranging from beta carotene, calcium, folate, aspirin, DFMO, dietary modification, fiber supplementation, etc. Although there have been repeated calls for trials using other intermediate endpoints such as aberrant crypt foci, adenoma recurrence remains the mainstay in examining the efficacy of an agent in relation to colorectal cancer. The adenoma is a reproducible measure, with a reproducible, standardized nomenclature implemented by pathologists.

4. PARTICIPANT SELECTION

4.1 Pre-Registration

4.1.1 Pre-Registration Inclusion Criteria

4.1.1.1 History of at least one of the following conditions in the previous 12 months:
- Colorectal adenoma(s) ≥ 1 cm in maximal diameter
- Colorectal adenoma(s) with villous or tubulovillous histology
- Colorectal adenoma(s) with high grade (severe) dysplasia.

4.1.1.2 Presumptive evidence that all adenomatous lesions, including qualifying advanced adenoma, have been completely removed.

4.1.1.3 Age 40 - 70 years of age at time of registration/randomization. Note: Individuals under the age of 40 with advanced adenomas have a high risk of familial disease. Individuals over the age of 70 begin to show signs of impaired immune function due to age.

4.1.1.4 Ability to understand and the willingness to sign a written informed consent document.

4.1.1.5 Willingness to undergo screening tests and procedures.

4.1.1.6 Willingness to provide blood samples for toxicity monitoring and research purposes.

4.1.1.7 Not pregnant or nursing. Note: A negative (serum or urine) pregnancy test must be documented ≤ 7 days prior to registration/randomization for women of childbearing potential.

4.1.1.8 Willingness to employ adequate contraception through Week 53 of the study. Note: The effects of the MUC1 vaccine on the developing human fetus at the dose specified in this study are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal, barrier method of birth control, abstinence) prior to study entry and for the period of active vaccination (through Week 53). Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her physician immediately.

4.1.2 Pre-Registration Exclusion Criteria

4.1.2.1 History of any colorectal cancer

4.1.2.2 History of other malignancy ≤ 5 years prior to the Registration/Randomization evaluation, with the exception of basal cell or squamous cell skin cancer.

4.1.2.3 Presence of an active acute or chronic infection or uncontrolled illness including, but not limited to unstable congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.1.2.4 Acquired immunosuppressive diseases such as active HIV infection or congenital diseases of immunity.

4.1.2.5 History of heritable cancer syndrome (FAP, HNPCC).

4.1.2.6 History of auto-immune disease such as, but not restricted to, inflammatory bowel disease, systemic lupus erythematosis, rheumatoid arthritis, ankylosing spondylitis, scleroderma, multiple sclerosis, Hashimoto's thyroiditis, or Grave's disease.

4.1.2.7 Current or planned use of immunomodulators including: infliximab, 6-MP (mercaptopurine), methotrexate, cyclosporine, or other immunomodulatory drugs.

4.1.2.8 History of allergic reactions attributed to compounds of similar chemical or biologic composition to the study agent.

4.1.2.9 Pregnant women, because the teratogenic or abortifacient effects of the study agents remain incompletely defined.

4.1.2.10 Breastfeeding women, because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study agents.

4.1.2.11. Diagnosis of nonalcoholic steatohepatitis (NASH) and a NAFLD (nonalcoholic fatty liver disease) activity score (NAS) ≥5. NOTES and EXCEPTIONS: NAS is based on findings from a liver biopsy. Participants with NAS of ≤2 are eligible for enrollment. Participants with NAS of 3-4 must be discussed with the Principal Investigator and DCP before enrollment to consider other risk factors (i.e., obesity, alcohol intake). Participants with a prior diagnosis of NASH and no available NAS must be discussed with the Principal Investigator and DCP before enrollment to considered risk factors (i.e., obesity, alcohol intake). See Appendix F.

4.2 Registration/Randomization

4.2.1 Registration/Randomization Inclusion Criteria

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

4.2.1.2 Hemoglobin greater than 90% of the lower limit of institutional normal.

4.2.1.3 Normal marrow and organ function, obtained ≤ 28 days prior to Registration/Randomization, and defined as follows:

- Platelets ≥ 100 B/L (10^9/L)
- WBC > 2.5 B/L (10^9/L)
- AST (SGOT), ALT (SGPT), alkaline phosphatase, total bilirubin, BUN, and creatinine ≤ 1.5 x institutional upper limit of normal

4.2.1.4 ANA test result excludes overt autoimmune disease. Note: Test result may be reported in any of the following formats: ≤ 1:160, negative, or < 1.0.

4.2.2 Registration/Randomization Exclusion Criteria

4.2.2.1 Receiving any other investigational agent ≤ 3 months prior to Registration/Randomization, except innocuous agents with no known interaction with the study agent (e.g., standard dose multivitamins or topical agents for limited skin conditions).

4.2.2.2 Any use of oral corticosteroids ≤ 12 weeks prior to Registration/Randomization.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. Refer to the Recruitment, Retention, and Adherence (RR&A) Plan Template for details (Appendix B).

4.4 Recruitment and Retention Plan

Each Participating Organization will be required to develop and submit a study- and site-specific RR&A plan for the purposes of insuring equal access to the clinical trial by individuals of all genders, races, and ethnic groups and for attaining the organization's accrual target. Participating Organizations will use the RR&A Plan Template (Appendix B) as the basis for their plans. At a minimum, these plans will be reviewed annually.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported adverse events (AEs) and potential risks are described in Section 6.2. It should be noted that pharmacy personnel (or other appropriate personnel to whom this task is delegated) will not be blinded to the intervention assignment. Every effort should be taken to maintain the blinded nature of the study. For example, the time required to formulate and dispense the active injection should be no longer than the time to formulate and dispense the placebo injection.

5.1 Dose Regimen and Dose Groups

The vaccine will consist of 100 micrograms (0.1mg) of MUC1 100mer peptide dissolved in 50 microliters (0.05mL) of sterile saline, admixed with 500 micrograms (0.5mg) of the adjuvant poly-ICLC (Hiltonol®) in 250 microliters (0.25mL) volume, for a total injection volume of 300 microliters (0.3mL). Vaccine will be administered at weeks 0, 2, and 10 weeks. A booster vaccine will be administered at week 53 to establish the presence of T helper cell memory to the antigen. An identical-appearing placebo injection will be prepared using 0.9% NaCl.

5.2 Vaccine Administration

The vaccine will be administered by appropriate site personnel subcutaneously in the anterior thigh approximately 20 cm inferior to the inguinal ligament. The vaccine will be administered into the same thigh on all 4 occasions for vaccine delivery. The vaccine site may demonstrate erythema after administration. This erythema can persist even to the timing of the next vaccine dosing. Erythema is not a contraindication to repeat vaccination. However, at the discretion of the investigator, repeat vaccination may be delayed, but not to exceed the protocol limits. It is also possible to inject the vaccine

into the opposite thigh, if necessary. Preferred windows for visits and vaccine administration and sampling are provided in Section 7.1.

5.3 Run-in Procedures

Not applicable.

5.4 Concomitant Medications

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

If a study participant is prescribed corticosteroids or immunomodulators during the course of the study, the situation will be discussed among the treating physician, the study chair, and the medical monitor to determine whether or not the participant should be allowed to continue on study.

5.5 Contraindications

Contraindications to study participation include receiving other investigational agents, presence or occurrence of an active acute or chronic infection or undercurrent illness, use of immunomodulators pregnancy, or breastfeeding (as applicable). See Section 4.2 for details regarding initial eligibility.

5.6 Dose Modification

The vaccine will be administered in the protocol-defined dosage for all participants on all occasions.

5.7 Adherence/Compliance

5.7.1 All vaccine/placebo will be administered by appropriately trained study personnel at each site. Vaccine/placebo administration will be documented on the Intervention Administration CRF.

5.7.2 Individuals who receive the Week 0, Week 2, and Week 10 injections will be considered evaluable for the primary endpoint. Individuals who receive the Week 53 injection will be considered evaluable for the Part II key secondary endpoint. Individuals from whom data and specimens are collected for a subsequent clinical colonoscopy occurring at least 1 year (52 weeks) after the Week 0 injection will be considered evaluable for the Part III key secondary endpoint.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND # 15885, NCI, Division of Cancer Prevention)

MUC1 Peptide

The MUC1 peptide is composed of a 100 amino acids (aa). Representing five 20aa-long tandem repeats of the MUC1 VNTR region: H_2N-(GVTSAPDTRPAPGSTAPPAH)$_5$-$CONH_2$. The peptide is synthesized on a rapid multiple peptide synthesizer (Dupont NEN) in a dedicated facility under a strict and fully certified

procedure and using reagents with a defined expiration date. The peptide is highly hydrophilic and for testing as well as injection, it is dissolved in sterile saline. The peptide is tested for pyrogenicity and safety in guinea pigs, mice, and rabbits in accordance with an FDA approved protocol in a commercial facility. Once tested negative, it is aliquoted in small freezer vials containing one injection volume (100 mcg lyophilized peptide in 0.05 mL of sterile saline). Two percent of vials are sampled randomly for sterility testing in the University of Pittsburgh Medical center Microbiology Laboratories. Both 7 and 14-day sterility results are recorded. Peptide synthesis procedure, safety, pyrogenicity and sterility are contained in detail in Dr. Schoen's IND application to the FDA cross referenced by NCI, DCP. This peptide and all these procedures have been previously examined and approved by the FDA six times in six different IND applications. For administration as a vaccine, the peptide will be admixed with the POLY-ICLC adjuvant described above and below and injected subcutaneously as described above.

Poly ICLC

Poly-ICLC is supplied by Oncovir in vials containing 1 mL of 2 mg/mL opalescent white suspension. Poly-ICLC is withdrawn from the vial under sterile conditions and is to be administered intramuscularly, subcutaneously or intranasally as supplied. It can also be diluted with normal saline.

Each vial Poly-ICLC will be labeled with the following information:

- Drug Name
- Concentration
- Lot Number
- Date of Manufacture
- Manufacturer
- Investigational Use Statement

Low-dose Poly-ICLC administered IM or SC or intranasally has been well tolerated in clinical trials of patients with brain tumors, other cancers, multiple sclerosis, and in normal volunteers. Multiple additional trials have used it in combination with various cancer or HIV vaccines. The most common adverse events experienced in these studies have been transient discomfort at the injection site and transient malaise. Subcutaneous injection may result in a transient erythematous skin reaction. Preliminary findings from these trials suggest that low-dose Poly-ICLC may be effective in treating various tumors and further study is warranted.

Poly ICLC synthesis, procedure, safety, pyrogenicity, and sterility are contained in detail in the Oncovir IND and DMF to the FDA cross-referenced by NCI, DCP.

Placebo

The placebo injection will contain normal saline (0.9% NaCl) provided by each individual Participating Organization.

6.2 Reported Adverse Events and Potential Risks

The side effects listed in the table below are based on a variety of previous studies that were similar to this study in some ways but different in important ways (including stronger doses) that may have led to different side effects. In the previous study that was most similar to this study, no autoimmune disease or serious side effects were identified.

Possible Side Effects of the MUC1 – poly-ICLC vaccine:

Common, Some May Be Serious **In 100 people who receive the injection, more than 20 may have:**
• Redness at injection site (erythema) • Pain/soreness at injection site • Flu-like symptoms, which may include fever chills, headache, fatigue, muscle pain, and joint pain • Night sweats • Loss of appetite • Low albumin blood test result (a type of protein in the blood)

The redness and soreness generally resolve within a few days to a week. Generally, acetaminophen (Tylenol®) is sufficient for pain relief. The flu-like symptoms generally resolve within 12-24 hours. Acetaminophen (Tylenol®) generally helps with these symptoms if they occur.

Occasional, Some May Be Serious **In 100 people who receive the injection, 4 to 20 may have:**
• Low white blood cell count (cells that fight infections) • Low platelet count (cells that help the blood clot in order to stop bleeding) • Anemia (which may cause tiredness, or may require blood transfusion) • Abnormal liver function test results

Rare, Some May Be Serious **In 100 people who receive the injection, 3 or fewer may have:**
• Hives, shortness of breath, or an allergic reaction that is life-threatening • Immune reaction to his or her own tissue (autoimmune diseases) or if present, may be made worse.

6.3 Availability

MUC1 100mer peptide and Poly-ICLC will be supplied by the University of Pittsburgh and sent to each participating site's research pharmacy upon receipt of a completed CPN Study Agent Order Form. The vaccine/saline placebo injection will be prepared by each participating organization's pharmacy (or other appropriate designated personnel) within 8 hours prior to clinical administration. Normal saline for placebo injection will be provided by each participating organization. Study agent will be supplied by and shipped from:

Olivera Finn, Ph.D., E1040 Biomedical Science Tower, Pittsburgh, PA 15262
Telephone: 412-648-9816; Fax: 412-648-9378; E-mail: ojfinn@pitt.edu

6.4 Agent Distribution

Agent will only be distributed to participating organizations after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP, collection of all Essential Documents is complete (see DCP website for description of Essential Documents), and Drug Shipment Authorization has been confirmed.

6.5 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 using NCI Drug Accountability Record Form (DARF) or comparable institutional form. The investigator or designee is required to maintain adequate records of receipt, dispensing, and final disposition of study agent. 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.

After receipt of the final site close out visit report by the CPN Compliance Coordinator, any remaining study agent may be destroyed per institutional guidelines.

Forms required for study agent ordering, accountability, and return are available on the CPN website: http://cancerpreventionnetwork.org

6.6 Packaging and Labeling

Participating site pharmacy personnel will not be blinded to the study intervention. However, the pharmacy personnel will be required to label the injection supplied for each participant in such a manner as to not provide any information about intervention assignment to the site investigators. Each injection should be labeled according to institutional guidelines. The label should define the injection as MUC 1 100mcg/0.05mL w. poly-ICLC 500mcg/0.25mL or placebo 0.3mL. The label should also include the date of preparation, the route of administration, and the study name. The label may also include other information, such as the time of expiration, if required by local or state regulations.

6.7 Storage

The MUC-1 vaccine will be stored frozen at-70 to -80^{o} C until mixed for injection. MUC1 Vaccine will be shipped on dry-ice overnight to each site. Participating organizations are required to store the vaccine in a -70 to -80^{o} freezer upon receipt.

Poly-ICLC is stable at room temperature for brief periods (days). It is normally refrigerated at about 40^{o}F (2-8°C) but should not be frozen. Poly-ICLC will be shipped at ambient temperature overnight to each participating site. It is to be stored in the refrigerator upon receipt.

6.8 Pre-Registration and Registration/Randomization

6.8.1 Participant Pre-Registration

6.8.1.1 To pre-register a participant, access the Cancer Prevention Network (CPN) web page (https://cancerpr.ipower.com/member/MAY2013-01-01.shtml) and enter the remote pre-registration application. The remote pre-registration application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the CPN Registration Office at (507) 284-2753 between 8:00 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for remote pre-registration are available on the CPN web page (https://cancerpr.ipower.com/member/MAY2013-01-01.shtml) and detail the process for completing and confirming participant pre-registration. Prior to initiation of protocol screening, this process must be completed in its entirety and a CPN Participant ID number (PID) must be available as noted in the instructions. It is the responsibility of the individual pre-registering the participant to confirm the process has been successfully completed. Participant pre-registration via the remote system can be confirmed in any of the following ways:

- Contact the CPN Registration Office (507) 284-2753. If the participant was fully pre-registered, the Registration Office staff can access the information from the centralized database and confirm the pre-registration.
- Refer to "Instructions for Remote Pre-Registration" in section "Finding/Displaying Information about a Registered Participant" within the Remote Registration/Randomization User's Manual available for download from the CPN website.

6.8.1.2 At the time of pre-registration, the following will also be verified:

- IRB approval at the registering institution
- Participant eligibility (including existence of a signed informed consent document)
- Existence of a signed authorization for use and disclosure of protected health information (USA Institutions only).
- Study agent is available and Drug Shipment Authorization has been granted to the registering site.
- The following will also be recorded:
    - Participant has/has not given permission to collect and store blood and/or tissue for future use in research to learn about, prevent, treat, or cure cancer.
    - Participant has/has not given permission to collect and store blood and/or tissue for future use in research about other health problems (for example: Barrett's esophagus. causes of diabetes mellitus, Alzheimer's disease, and heart disease).
    - Participant has/has not given permission to send blood and/or tissue sample(s) to researchers at an outside institution.
    - Participant has/has not given permission to his/her doctor (or someone from the Cancer Prevention Network) to contact them in the future to ask them to take part in more research.

6.8.1.3 Baseline (screening) evaluations must be completed within the guidelines specified on the Schedule of Events (See Section 7.1).

6.8.1.4 Registration Office personnel will automatically register participants separately to the translational components of the study (See Section 13).

6.8.2 Registration/Randomization

6.8.2.1 Fax to 507-284-0885 a completed Eligibility Checklist (see CRF packet) to the CPN Registration Office between 8:00 a.m. and 4:30 p.m. Central time, Monday through Friday.

6.8.2.2 Randomization: For details, see Section 13.2.

6.8.2.3 Intervention on this protocol must commence at a CPN institution under the supervision of a CPN clinician.

6.8.2.4 Intervention cannot begin prior to Registration/Randomization and must begin ≤ 14 days after Registration/Randomization.

6.8.2.5 Pathology and colonoscopy reports confirming diagnoses must be sent ≤ 30 days post Registration/Randomization to the CPN QAS at CPN Operations Office, Lanmark 2, 200 First Street Southwest, Rochester, MN 55905; or FAX: 507-266-3722, Attention CPN QAS.

6.8.2.6 Once the intervention assignment has been ascertained by the CPN Registration Office, the appropriate blinded study agent information (MUC1 vaccine or placebo/saline injection) will be communicated to the designated data manager/nurse/pharmacist at the participant's Participating Organization. The name of this contact person is to be entered in the designated space on the eligibility checklist so the Registration Office personnel have it for each participant at the time of registering/randomizing the participant. Make sure this contact person will be available at the time of the registration so he or she can receive the information from the Registration Specialist. This contact person may not be involved in assessing adverse events or any other outcome measure and should not be the same person listed on page one of the eligibility checklist form as the person completing the form. The last page of the eligibility checklist form should provide the source of communication for the contact person, either fax or email, and the appropriate contact information.

6.8.2.7 Stratification Factors:

- Number of advanced adenoma on qualifying colonoscopy: ≥ 3 vs. < 3
- Gender: male vs. female

After the participant has been registered on to the study, values of the stratification factors will be recorded. Randomization will be performed by CPN personnel. The CPN electronic-based randomization system will be utilized to ensure that randomization of participants to appropriate study arms while balancing the stratification variables is carried out successfully.

6.9 Blinding and Unblinding Methods

There are two distinct situations in which it will be deemed appropriate to break the randomization assignment for participants enrolled onto this current trial:

- In the event of an *emergency* for an individual participant.
- Serious Adverse Event (SAE) that fulfills the criteria for expedited reporting to the FDA.

In these situations, Participating Organization personnel may assume the participant is on active agent and call the CPN Registration Office within one business day to receive unblinding information. The study name/number, participant identifier, and participant initials will be required to break the randomization code. The Participating Organizations are responsible for notifying the medical monitor at DCP, NCI of the unblinding event.

6.10 Agent Destruction/Disposal

At the completion of investigation and after submission of the site final close out visit report by the CPN Compliance Coordinator, all unused study agent will be destroyed on site according to institutional SOPs.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Parts I and II													
Study Visit	**Pre-Registration**	**Screening/ Baseline[1]**	**Registration/ Randomization Week 0[2]**	**Week 2**	**Week 4 (+/- 7 days)**	**Week 6 (+/- 7 days)**	**Week 10**	**Week 12**	**Weeks 16, 28, and 40 (+/- 7 days)**	**Week 52**	**Week 53 Booster**	**Week 55**	**Week 57 (+/- 7 days)**
Informed Consent	X												
History and Physical		X								X			
Vital Signs		X		X			X			X		X	
Baseline Symptoms		X											
Concomitant Medications		X		X	X	X	X	X	X	X	X	X	X
CBC w/5-part diff., platelets		X		X			X			X		X	
Blood chemistry panel (potassium, sodium, chloride, bicarbonate, creatinine, serum glucose, BUN, total bilirubin, alk phos, and uric acid)		X								X			
Antinuclear antibody (ANA)		X								X			
AST/SGOT; ALT/SGPT		X		X			X	X		X		X	
Research blood[3]		X[3]		X[3]			X[3]	X[3]		X[3]		X[3]	
Obtain medical record release, if applicable		X											

Study Visit	Pre-Registration	Screening/ Baseline[1]	Registration/ Randomization Week 0[2]	Week 2	Week 4 (+/- 7 days)	Week 6 (+/- 7 days)	Week 10	Week 12	Weeks 16, 28, and 40 (+/- 7 days)	Week 52	Week 53 Booster	Week 55	Week 57 (+/- 7 days)
Obtain colonoscopy reports and specimens[4,5]		X											
Pregnancy test[6]		X	X	X			X				X		
Vaccination (MUC1/POLY-ICLC or placebo)			X	X[7]			X[8]				X[9]		
Adverse Events			X	X	X	X	X	X	X	X	X	X	X
Phone call					X	X			X				X
QOL Questionnaire(s)			X[11]								X[11]	X[11]	

Part III		
Study Visit/Event	**Weeks 88, 104, 130 (+/- 10 days)**	**Week 156 (Year 3)[12]**
Update medical/surgical history		X
Phone call	X[10]	
Concomitant medications	X	X
Research blood[3]		X[3]
Obtain medical record release, if applicable		X
Obtain colonoscopy reports and specimens		X
QOL Questionnaire		X[11]

1. Baseline evaluations must occur after pre-registration and must be completed ≤ 28 days prior to registration/randomization.
2. Week 0 injection must be administered ≤ 14 days after registration/randomization. The date on which the injection is administered will become the "zero" time point from which all subsequent time points will be measured.
3. Research blood (blood for immunology assays and other endpoints) must be collected M-Th only and shipped overnight for next day analysis.
4. Operative notes, endoscopy reports, and/or pathology reports must be reviewed locally to confirm that the candidate meets at least one of the entry criteria specified in inclusion criterion 4.1.1.1. Participants will need to registered/randomized within 1 year from the initial polyp removal, unless evidence of an advanced adenoma is identified at the subsequent colonoscopy, in which case the timing for eligibility will start anew. Note: if

participants are scheduled for early repeat colonoscopy, such as within 12 months of the colonoscopy that identified the advanced adenoma, to insure complete removal of the qualifying lesion, they should be enrolled after that repeat colonoscopy insures that no residual adenoma is present.

5. Endoscopy report and/or pathology report confirming qualifying advanced adenoma diagnosis as well as the fact that all polyps and adenomas have been removed must be submitted by FAX or email to the CPN Quality Assurance Specialist (QAS) within 30 days after registration/randomization.
6. Pregnancy test (serum or urine) will be performed for female participants of childbearing potential at baseline/screening and prior to each injection (Week 0, 2, 10, and booster vaccine). Week 0 pregnancy test does not have to be repeated if ≤ 7 days since baseline/screening test. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.
7. Week 2 injection may not occur less than 14 days after Week 0 injection. It may occur up to 28 days after the Week 0 injection.
8. Week 10 injection should occur 7-10 weeks (ideally 8 weeks) after Week 2 injection.
9. Week 53 booster should ideally occur as soon as possible after the week 52 monitoring test results are available. It may occur up to 4 weeks after the Week 52 visit.
10. Telephone contact will be made to monitor concomitant medications, possible adenoma recurrence, and the timing of follow up surveillance colonoscopy.
11. The Risk, Regret, and Symptom Questionnaire (Appendix D) will be administered before the Week 0 injection, after the Week 53 injection, and at the Week 156 visit. The Was It Worth It (WIWI) Questionnaire (Appendix C) will be administered during the Week 55 visit.
12. The timing of the follow up surveillance colonoscopy will vary among individuals and institutions. Ideally, the participants will return at Week 156 (+/- 4 weeks) to update their medical/surgical history, concomitant medications, and immune response. If any clinical surveillance colonoscopies are scheduled between Weeks 52 and 156, it would be ideal to gather the updated med/surg history and concomitant medications within 2 weeks before the scheduled colonoscopies. If this is not possible, it will not be considered a protocol deviation. These data should still be gathered and the timing noted. Reports and specimens from the colonoscopy should be gathered within 30 days after the colonoscopy. Colonoscopy and pathology reports must be submitted by FAX or email to the CPN Quality Assurance Specialist (QAS) within 30 days after the date of the procedure.

Summary of Parameters for Visit Timing

Week/Visit	Window	Ideal timing
0	Within 28 days of screening visit	within 1 week
2	No earlier than 2 weeks and up to 4 weeks from week 0 injection	2 weeks from week 0 injection
10	7 – 10 weeks from week 2 injection	8 weeks from week 2 injection
12	10 – 21 days after week 10 injection	2 weeks from week 10 injection
52	51 to 54 weeks from week 0 injection	52 weeks
53	As soon as possible after week 52 monitoring tests return	Within 4 weeks of week 52 visit
55	10 – 21 days from week 53 injection	2 weeks
156	Within 4 weeks of any planned colonoscopy	2 weeks before colonoscopy

Note: If the visit/event takes place within the window specified above, it will not be considered a deviation. However, for consistency, every effort should be made to schedule visits and events according to the "ideal timing" column. The scheduling of subsequent injections should be based on timing of previous injection. If sampling or injections are delayed, they should still occur (even after colonoscopy for week 156 titer, for example), and timing should be noted.

7.2 Baseline Testing/Prestudy Evaluation

After participants have been informed about the study and have signed the informed consent document, they will be pre-registered and assigned a unique participant identification number (PID). They will then undergo initial screening tests and procedures. The order in which these tests and procedures is conducted is flexible within the designated study timelines and should be consistent with good clinical practice and institutional policies and procedures. These tests and procedures include a physical exam, documentation of medical/surgical history, documentation of any existing baseline symptoms, allergies, and concomitant medications, and baseline blood tests. Baseline blood tests include CBC with 5-part differential, potassium, sodium, chloride, bicarbonate, creatinine, serum glucose, BUN, total bilirubin, alkaline phosphatase, uric acid, AST/SGOT, ALT/SGPT, and antinuclear antibody (ANA). Blood will also be drawn at baseline for the immune assay (IgG assay). Authorization to obtain data and specimens from the clinical colonoscopies will be obtained.

If participants are eligible and willing to participate, they will be registered to the study and randomized to one of two intervention arms (vaccine versus placebo/saline injection). Values of the stratification factors will be recorded and the CPN electronic-based randomization system will be utilized to ensure that randomization of participants to appropriate study arms, while balancing the stratification variables, is carried out successfully.

7.3 Evaluation During Study Intervention

Vaccine or placebo/saline will be administered at weeks 0, 2, and 10, with a booster at week 53. Females of childbearing potential will be required to document negative pregnancy test prior to each injection. Immune response will be tested 2, 10, 12, 52, 55, and 156 (Year 3). Adverse events will be evaluated at regular time points after each injection (See Section 11.1 for details).

At the Week 2 and Week 12 visits, if any erythema or induration injection site reactions are still present, the participant's measurement of the diameter will be compared with the study team's measurement for the purpose of validating the participant-reported measurement.

Blood tests to monitor toxicity will be conducted at weeks 2, 10, 12, 52, and 55. At weeks 2, 10, and 55, CBC with 5-part differential, AST/SGOT, and ALT/SGPT will be performed. At week 12, AST/SGOT and ALT/SGPT will be performed. Possible adverse events and concomitant medication information will be captured at each visit and by telephone at weeks 4, 6, 16, 28, and 40. All visits will involve informing the participant of the results of previous blood tests.

Participants will undergo an evaluation at week 52, including a physical exam, blood tests for immune response and toxicity monitoring, discussions of the results of previous tests, discussions of possible adverse events and concomitant medications.,. The following blood tests will be performed: CBC with 5-part differential, potassium, sodium, chloride, bicarbonate, creatinine, serum glucose, BUN, total bilirubin, alkaline phosphatase, uric acid, AST/SGOT, ALT/SGPT, and antinuclear antibody (ANA).

Risk, Regret, and Symptom Questionnaires will be administered prior to the Week 0 injection and at weeks 53 and 156. The Was It Worth It (WIWI) Questionnaire will be completed at Week 55.

7.4 Evaluation at Completion of Study Intervention

The final blood tests for immune response and toxicity monitoring will take place at Week 55. Those results will be provided, and discussions of adverse events and concomitant medications will take place at Week 57 by telephone,

7.5 Post-intervention Follow-up Period

For the purpose of monitoring adenoma recurrence and concomitant medications, contact by telephone will be made approximately every six months until Week 156 (Year 3) or the time of reported adenoma recurrence. Reports and specimens from any clinical colonoscopies will be obtained. Immune response will be tested. See Section 7.1 for details.

7.6 Methods for Clinical Procedures

7.6.1 Vaccine injection procedure details
See Section 5.2.

7.6.2 Colonoscopy data and specimens
Data will be abstracted from operative and pathology reports from clinical surveillance colonoscopies to confirm initial eligibility (Section 4.1.1.1) and to document any recurrence or new diagnosis. Permission will be obtained, if applicable, from each participant to obtain paraffin blocks and/or unstained slides from any specimens removed during the clinical surveillance colonoscopies.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint for this study is to compare the ratio of the week 12 to week 0 IgG levels between the MUC1 vaccine and placebo. It is hypothesized that this ratio will be increased in patients treated with the MUC1 vaccine as compared to patients receiving the placebo. All randomized participants who are evaluated at week 0 and week 12 and have IgG values from those time points will be evaluable for the primary endpoint of this study using a modified intent-to-treat principle. If we are missing the data for Week 0 or Week 12, we will be missing data for the analysis since the primary endpoint will compare the ratio of the Week 12 to Week 0 IgG levels between the MUC1 vaccine and the placebo.

The measurement of vaccine-induced anti-MUC1 IgG will be determined by Enzyme-Linked Immunosorbent Assay (ELISA). See Section 10.2.2.

8.2 Secondary and Translational Endpoints

The secondary endpoints will include the following:

- The Part I key secondary endpoint will be a comparison of the proportion of patients with at least a 2-fold increase in the IgG ratio between the 2 arms at 12 weeks.

- The Part II key secondary endpoint will assess the booster response at week 55 vs. week 52 for the vaccine as compared to placebo.
- The Part III key secondary endpoint will compare the adenoma recurrence rate from surveillance exams occurring at least one year and up to three years after Week 0 vaccine administration – MUC1 peptide versus placebo.

In addition to these key powered endpoints for the 3 different parts, we will also assess the following additional secondary endpoints:

- Comparison of adverse events between the MUC1 vaccine and placebo during Part 1 and 2.
- Comparison of the adenoma recurrence rates between MUC1 and placebo by excluding the following types of adenomas. These adenomas are excluded since they may represent missed or residual adenomas from the baseline exam. These analyses are exploratory.
    - Participants with adenomas ≤ 5 mm,
    - Participants with adenomatous tissue which may represent residual adenoma at the site of the previous advanced adenoma,
    - Participants with adenomatous tissue detected in the same segment of the bowel as the previous advanced adenoma.
- Assessment of participant-reported injection site reaction events from the Vaccine Report Card.

In addition, we will assess the following translational endpoints:

- To compare the anti-MUC1 antibody titer at the time of surveillance colonoscopy for the purpose of evaluating the anti-MUC1 antibody response in relation to adenoma recurrence. Since surveillance colonoscopy is generally at 3 years after an advanced adenoma, this titer will typically be obtained at approximately week 156.
- To evaluate MUC1 expression on baseline advanced adenomas and on recurrent adenomas detected at surveillance colonoscopy. Based on current knowledge of MUC1 expression and function in tumor cells, MUC1 negative adenomas are not expected to grow as immune escape variants, but this has never been tested before and the trial will provide an appropriate setting to study this issue.
- To evaluate levels of circulating myeloid derived suppressor cells (MDSC) in the vaccinated and the placebo group and correlate with anti-MUC1 antibody levels and adenoma recurrence (See Section 10.2.2).
- To establish a biospecimen repository archive including live cells, plasma, and germline DNA for future immunologic and other assays, for testing not currently accommodated within the budget of this trial.

Where appropriate, secondary and translational analyses will be based on all randomized participants (i.e. adverse event analyses) or defined participant subgroups.

8.3 Off-Agent Criteria

Participants may stop taking the study agent due to: completion of the planned intervention period, development of an adverse event or serious adverse event, inadequate agent supply, noncompliance, use of concomitant medications, medical contraindication, refusal, ineligibility (see Section 8.4), major

treatment violation (see Section 8.4) or alternative treatment. Participants will continue to be followed, if possible, for safety according to the intended schedule of events (see Section 7).

Participants discontinuing the planned intervention prematurely will be encouraged to complete the Post-Intervention Evaluation tests and procedures as appropriate (if participant does not refuse, is not lost to follow-up, or unless it is clinically contraindicated). See Section 8.4 for further details as to data submission for participants deemed Ineligible after starting treatment or classified as a Major Treatment Violation (i.e., protocol requirements regarding intervention during the first week post-randomization were severely violated). In such cases, all data and all on-study materials, collected up until the point of confirmation of ineligibility or major violation status must be submitted.

8.4 Off-Study Criteria

Participants may go "Off-Study" for the following reasons: development of an adverse event or serious adverse event, death, lost to follow-up, participant withdrawal, physician decision, protocol violation, completion of study, or other (with detailed comments provided). Reason(s) will be noted in the participant's research records, with the primary reason clearly identified. The participant will be classified as (Off Study/Off Agent). Data submission and follow-up after participants are determined to be a screen failure or "Off-Study/Off-Agent" for specific situations is noted below:

Ineligible: A registered participant is deemed ineligible if the participant does not satisfy each and every eligibility criterion for study entry.

- If participants received study intervention, on-study materials and all data up until the point of confirmation of ineligibility will be submitted.
- If participants did not receive study intervention, on-study materials must be submitted. No further data submission is necessary. No follow-up is required.

Major Treatment Violation: A registered participant is deemed as being in major treatment violation by the coordinating center, if the participant's very first treatment/intervention administration is so grossly administered in error, that the participant's data can no longer be used for the primary endpoint. These cases are typically rare.

- On-study material and all data up until the point of confirmation of a major violation must be submitted.

Cancel: A registered participant is deemed a cancel if he/she refuses the study before any study intervention is given.

- On-study material must be submitted. No further data submission is necessary. No follow-up is required.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time. The FDA also has the authority to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

The MUC1 ELISA is an established assay that has been used to monitor many clinical trials as well as IgG response in large cohorts of patients on other studies. It has been validated as highly reproducible. The set-up includes positive and negative control anti-MUC1 antibodies that allow comparison of results between different studies. Nevertheless, in this trial, each patient will also be his/her own control as we will compare post-vaccination titers with pre-vaccination baseline. All time points will be assayed simultaneously and multiple times. See Section 10.2.2 for details.

9.2 Comparable Methods

This study will employ procedures that have been validated previously for clinical use.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Heparinized whole blood collected at all locations will be shipped to the University Of Pittsburgh School Of Medicine. Dr. John McKolanis will receive and record the specimens, and he will also be responsible for processing the blood into plasma and PBMC. PBMC will be frozen at -70 to -80^{o}C in human serum with 20% DMSO and plasma will be frozen at -20^{o}C. Dr. McKolanis will run ELISA assays for anti-MUC1 IgG and FACS analysis of % MDSC. Approximately 50% of plasma and 25% of the live cells will be shipped to the Biorepository at the Mayo Clinic for storage and later release when requested for additional biomarker studies.

Unstained slides from tissue blocks of the qualifying advanced adenoma and for adenomas detected at the time of surveillance colonoscopy that determines adenoma recurrence (baseline and recurrent adenomas) will be collected at each site and shipped to the Mayo Clinic. They will then be batched and shipped to the University of Pittsburgh for subsequent immunostaining and other assays.

10.2 Collection, Handling, and Shipping Procedures

10.2.1 Specimen Kits and Shipping Instructions

Research blood kits will be provided by BAP Kit Building (Biospecimen Accessioning and Processing Core Facility). Collection, handling, and shipping instructions will be included with each kit. Participating Sites may obtain research blood kits by faxing the Supply Order Form to the number provided (found in the Forms Packet). At least two weeks should be allowed to receive the shipping kits. Kits will be sent via FedEx® Ground at no additional cost to the participating institutions. They will not be forwarded by FedEx® rush delivery service unless the participating institution provides their own FedEx® account number. CPN will not cover the cost for rush delivery of kits.

Because charges are incurred for all outgoing kits, a small, but sufficient, supply of specimen collection kits should be ordered prior to participant entry.

- ALL sections of the requisition form and specimen collection labels must be completed and legible.
- Blood specimens should be sent over night via FedEx® on cold packs **Monday - Thursday ONLY**.
- All samples must be shipped to the address provided on the specimen shippers (See Section 10.2.2).

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.2.2 Research Blood Specimens

Research blood specimens will be collected according to the table in Section 7.1. Blood specimens must be collected **M-Th only** and shipped overnight for next day analysis. Detailed instructions for collecting, processing, labeling, and shipping the specimens can be found in the instructions provided in each blood specimen kit.

Blood specimens will be sent to:

Dr. John McKolanis, E1000-17B Biomedical Science Tower
200 Lothrop Street, Pittsburgh, PA. 15261
Phone: 412-648-8561 or 412-648-9817; Fax: 412-383-8098; Email: mckolani@pitt.edu

For tracking purposes, participating organizations will telephone or email the following two individuals to inform them that specimens are being sent to the University of Pittsburgh.

1. **Dr. John McKolanis,**
   Email: mckolani@pitt.edu
   Cell phone: 412-508-7671
2. **Roxann Neumann, Biospecimens Resource Manager**
   Email: Neumann.roxann@mayo.edu
   Telephone: 507-538-0602

Upon receipt of the overnight shipment or immediately after collection, heparinized blood will be centrifuged over a density gradient (Ficoll) to separate the plasma and PBMC. Plasma will be collected, aliquoted, and stored at -20^{o}C. PBMC will be washed several times, aliquoted, slowly frozen to -70 to -80^{o}C in human serum with 20% DMSO and stored in the vapor phase of liquid nitrogen.

Measurement of the vaccine-induced anti-MUC1 IgG will be determined by the **E**nzyme-**L**inked **I**mmunosorbent **A**ssay (ELISA) that was established in Dr. Finn's laboratory and successfully used to measure anti-MUC1 responses in humans and mice. The procedure is as follows: Immulon 4 (Thermo-Fisher Scientific, MA) microtiter plates are coated overnight at 4ºC with 1µg of synthetic MUC1 100mer peptide (vaccine antigen) dissolved in 0.9% Dulbecco phosphate buffered saline (PBS). Corresponding control plates receive PBS but no antigen. The plates are washed three times with and incubated with 2.5% bovine serum albumin (BSA) in PBS (PBS-BSA) to fully coat the microtiter plate wells with protein and block non-specific binding. PBS-BSA is removed and plasma diluted in PBS-BSA added to the wells. After one-hour incubation at room temperature the plates are washed five times with PBS with 0.1% tween-20 (Sigma-Aldrich, MO), and alkaline phosphatase-conjugated anti-human IgG secondary antibody (Sigma-Aldrich) in PBS-BSA added. Following a one-hour incubation the plates are washed five times and the substrate, p-nitrophenyl phosphate (Sigma-Aldrich), added to each well. The reaction is

terminated after one hour by adding 0.5M NaOH. The results are read at optical density (OD) 405nm on a spectrophotometer. The OD values from the control wells containing no antigen are subtracted from the OD values in test wells coated with peptide. Every sample is assayed multiple times at multiple dilutions, in at least triplicate wells. The results can be expressed wither as OD values or control antibody of a known titer, included in the assay, is used to convert the OD values to titers.

For detecting Myeloid-Derived Suppressor Cells (MDSC), PBMC will be thawed and stained with APC-labeled mouse anti-human CD11b antibody (clone: ICRF44, BD Biosciences), PE-Cyanine 7 (PE-Cy7) labeled mouse anti human CD14 antibody (clone: M5E2, BD Biosciences), PE -labeled mouse anti human CD33 antibody (clone: WM53, BD Biosciences) and FITC-labeled mouse anti human HLA-DR antibody (clone: G46-6, BD Biosciences). MDSC will be defined by flow cytometry as $CD11b^{+}$ $CD33^{+/low}$ $HLA\text{-}DR^{-/low}$ cells.

10.2.3 Tissue Specimens

Paraffin blocks and/or unstained slides from the polyps/adenomas removed during routine colonoscopy, pre- and post-intervention, will be requested from the institution at which the procedure was performed.

Tissue paraffin sections will be deparaffinized by baking overnight at 59°C. Endogenous peroxidase activity will be eliminated by treatment with 30% H2O2 for 15 min at room temperature. Antigen retrieval will be performed by microwave heating in 0.1% citrate buffer. Nonspecific binding sites will be blocked with Protein Blocking Agent (Thermo-Shandon Corp). Sections will be immunostained with anti-MUC1 antibody HMPV, which recognizes all forms of MUC1 by binding the epitope APDTR in the VNTR region in a glycosylation-independent manner (BD Pharmingen) and anti-MUC1 antibody VU-4H5 (Santa Cruz Biotechnology), which recognizes the epitope APDTRPAP in the VNTR region of hypoglycosylated MUC1 (Santa Cruz Biotechnology). Staining will be performed by the avidin-biotin peroxidase complex (ABC) method with a commercial kit (Vectastain ABC kit, Vector Laboratories). Color development will be performed by 3,3'Diaminobenzidine (DAB) kit (BD Pharmingen).

Institutions that are unable to submit paraffin blocks may send five 5-micron unstained slides from each advanced adenoma as an alternative. Paraffin blocks and/or slides must be labeled with the participant ID number, specimen type, specimen number, and date of collection. Every effort must be made to make certain the specimen number on the block and/or slide labels corresponds to the polyp or adenoma number reported on the Colonoscopy Report CRF.

Send blocks/slides to:

CPN PC Office (Study MAY2013-01-01)
RO_FF_03_24-CC/NW Clinic
200 First Street Southwest, Rochester, MN 55905

10.3 Tissue Banking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI.

At study completion, remaining frozen biologic specimens will be labeled (study number, participant ID number, specimen type, specimen number, date of collection) batched, and shipped (overnight, M-Th only) for banking/long term storage to:

Biospecimens Accessioning and Processing (BAP) Freezer
ST SL-39, 150 Third Street Southwest, Rochester, MN 55902

At study completion, remaining paraffin blocks and slides will be labeled (study number, participant ID number, specimen type, specimen number, date of collection) batched, and shipped for banking/long term storage to:

CPN PC Office (Study MAY2013-01-01)
RO_FF_03_24-CC/NW Clinic
200 First Street Southwest, Rochester, MN 55905

NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS AND DEVIATIONS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

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. 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.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6.2, Pharmaceutical Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events and Deviations

11.1.1 Reportable Adverse Events (AEs)

All AEs that occur after the informed consent is signed and baseline assessments are completed must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- System Organ Class (SOC)

- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

All AEs, including injection site reactions, will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below. AEs will be reported and evaluated by use of the standard Adverse Events case report form (CRF).

CTCAE v4.0 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.

ADL
*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.

11.1.3.2 Injection Site Reactions

Injection site reactions, a subset of all AEs, will also be reported by use of a participant-completed Vaccine Report Card, captured on a separate CRF, and reported to NCI, DCP. This report will not be part of the MDS. The following guidelines will be used to evaluate the injection site reaction:

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
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

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

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

11.1.6 Reporting Deviations

A protocol deviation is any noted variance or difference in the conduct of the study from the written protocol plan. All deviations will be reported by completing the DCP Deviation Reporting form available on the CPN website: https://cancerpr.ipower.com/member/Deviations.shtml

The deviation reporting form will be completed in Word format and submitted by email to the DCP medical monitor (see Section 11.2.2.2 for contact information).

11.2 Serious Adverse Events

11.2.1 DEFINITION: Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/files/clinical-trials/SAE_form.doc.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Luz Maria Rodriguez, MD, FACS
National Institutes of Health, National Cancer Institute
Division of Cancer Prevention
9609 Medical Center Drive, Room 5E228
Bethesda, MD 20892
Phone: 240-276-7039
Fax (with cover sheet, Attn: Dr. L. Rodriguez): 240-276-7848
Email (preferred): rodrigul@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.3 The Lead Organization and all Participating Organizations will FAX or email written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form.

The written SAE reports will also be FAX'ed or emailed to Dr. Robert Schoen, Study Principal Investigator and CCS Associates, DCP's Regulatory Contractor.

Robert Schoen, M.D.
Email: rschoen@pitt.edu
Fax: 412-648-7080

DCP's Regulatory Contractor, CCS Associates:
Email: safety@ccsainc.com
Fax: 650-692-4410

Participating Sites will also FAX this form to the CPN Operations Office within 48 hours of learning of the event at fax 507-284-9628. See the Data Management Plan (see CPN web site) sections "Data Collection" and "Data Entry and Processing" for further instructions as SAEs must also be recorded on the participant's appropriate case report forms.

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission, per CFR 21 Section 312.32

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE
Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs that are determined to be at least possibly related to study intervention will be followed according to institutional standards of care until resolved.

12. STUDY MONITORING

12.1 Data Management

The Mayo Clinic Cancer Center database will be the database of record for the protocol and subject to NCI and FDA audit. Minimum Data Sets will be submitted to DCP per contract requirements. Please see 2012 CPN Master Data Management Plan.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens for data entry into the Mayo Clinic Cancer Center database. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval.

12.3 Source Documents

A source document is any document, form, or record where *specific participants'* data are first recorded. FDA [21 CFR 312.62 (b)] requires that the investigator "...prepare and maintain accurate case histories

designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational agent or employed as a control in the investigation." Among many other items, source documents include:

- Inpatient and outpatient medical records
- Progress notes
- Consults
- Nursing notes
- Pathology reports
- Radiology reports
- Medicine/radiation administration records
- Surgical reports
- Laboratory reports
- Admission forms
- Flow sheets that are signed and dated
- Protocol or study road maps
- Appointment books
- Participant diaries/calendars
- Participant-completed symptom diary
- Participant-completed Vaccine Report Card.

12.4 Data and Safety Monitoring Plan

The Master DSMP, applicable to all studies within the CPN Consortium provides detailed information regarding data and safety monitoring for this study. The trial will be monitored closely for occurrence of adverse events by the study team and by the Mayo Clinic Cancer Center Data Safety Monitoring Board (DSMB). The DSMB will be consulted regarding whether or not accrual should be suspended to allow for investigation in the occurrence of severe adverse events, particularly for those that are possibly, probably, or definitely related to the study agent.

12.5 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.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as IRB 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.

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

A CRADA/CTA agreement is not needed as, the contact between DCP and Mayo along with the Mayo subcontract with the University of Pittsburgh delineate each party's' responsibilities.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a randomized Phase II double blind placebo controlled trial designed to assess the effects of the MUC1-1 peptide with poly-ICLC adjuvant vs. placebo. There will be 3 "Parts" to the study to allow periodic monitoring and the opportunity to evaluate whether to proceed after each part. They key endpoints from each part are listed below:

Part I: The ratio of the week 12 to week 0 IgG levels will be calculated and compared between the MUC1 vaccine and placebo (primary endpoint, see 13.4).
Part II: Assessment of the booster response at week 55 vs. week 52 (T55/T52) for the vaccine as compared to placebo (key secondary endpoint, see 13.5)
Part III: Comparison of the adenoma recurrence rate from surveillance exams occurring at least one year and up to three years after Week 0 vaccine administration – MUC1 peptide versus placebo. All patients will be followed for this endpoint (key secondary endpoint, see 13.5)

In addition to these key powered endpoints for the 3 different parts, we will also assess the following additional secondary endpoints:

- Comparison of adverse events between the MUC1 vaccine and placebo during part 1 and 2 (13.7).
- To assess patient –reported vaccine-related side effects from the Vaccine Report Card.
- Comparison of the proportion of patients with at least a 2-fold increase in the IgG ratio between the 2 arms at 12 weeks (13.5).
- Comparison of the adenoma recurrence rates between MUC1 and placebo by excluding the following types of adenomas. These adenomas are excluded since they may represent missed or residual adenomas from the baseline exam. These analyses are exploratory.
    - Participants with adenomas ≤ 5 mm,
    - Participants with adenomatous tissue which may represent residual adenoma at the site of the previous advanced adenoma,
    - Participants with adenomatous tissue detected in the same segment of the bowel as the previous advanced adenoma.
- Assessment of participant-reported injection site reaction events from the Vaccine Report Card (Appendix E).

In addition, we will assess the following translational endpoints (see 13.10):

- To compare the anti-MUC1 antibody titer at the time of surveillance colonoscopy for the purpose of evaluating the anti-MUC1 antibody response in relation to adenoma recurrence. Since surveillance colonoscopy is generally at 3 years after an advanced adenoma, this titer will typically be obtained at approximately week 156.
- To evaluate MUC1 expression on baseline advanced adenomas and on recurrent adenomas detected at surveillance colonoscopy. Based on current knowledge of MUC1 expression and function in tumor cells, MUC1 negative adenomas are not expected to grow as immune escape variants, but this has never been tested before and the trial will provide an appropriate setting to study this issue.
- To evaluate levels of circulating myeloid derived suppressor cells (MDSC) in the vaccinated and the placebo group and correlate with anti-MUC1 antibody levels and adenoma recurrence.
- To establish a biospecimen repository archive including live cells, plasma, and germline DNA for future immunologic and other assays, for testing not currently accommodated within the budget of this trial.

There are limited Quality of Life (QOL) data on participants who participate in chemoprevention trials and we intend to create a databank of QOL information by administering the "Was It Worth It" (WIWI) questionnaire at trial completion across multiple trials. We will seek to evaluate participant perception of their experience in trial participation once we have a reasonable amount of information (large enough sample size). Since participants who participate in these chemoprevention trials are high risk but otherwise healthy, the WIWI tool would help answer simple questions about participants' assessment of whether or not participation in this trial was "worth it."

In addition to the WIWI Questionnaire, participants will be asked to complete a pre-vaccine questionnaire at baseline (prior to the Week 0 injection) and a post-vaccine questionnaires at Week 53 (after the Week 53 booster injection) and approximately Week 156. These questionnaires were used in the Pilot study of this MUC1 vaccine and are designed to gather information about perceived risk, regret, and symptoms. The original copies of these questionnaires will be placed in the participants' study files. Copies should be sent electronically to: Lyn Robertson DrPH, RN, MSN, University of Pittsburgh; Email: robertsonlk@upmc.edu. Questions should be forwarded to Dr. Robertson by email or telephone: 412-647-8588.

Information from these risk assessments will only be used as supplementary data to explain for any potential confounding factors and not for any analytical purposes.

13.2 Randomization/Stratification

Participants will be randomized in a 1:1 fashion to one of two arms (MUC1 vs. placebo), using a dynamic allocation procedure called the Pocock-Simon [42], which balances the marginal distributions of the stratifications factors across the intervention groups and has been shown to be able to accommodate a large number of factors (10-20) without difficulty[43]. For this study we will use the following stratification factors: number of adenomous polyps removed >=3 vs. < 3 and gender.

13.3 Accrual and Feasibility

The study design requires a total of 120 participants for screening evaluation with approximately 110 moving forward to randomization (55 total participants per arm) due to anticipated screen failures of approximately 8% prior to randomization. We further expect up to 10% of participants to drop out or otherwise become non-evaluable by 1 year; this rate will be reflected in power calculations for parts 1 and 2. An additional 10% expected drop out or exclusion rate between 1 and 3 years is expected, reflected in the power calculations for part 3. We expect to enroll a minimum of 1 participant per month for each of the participating CPN sites, for an overall accrual rate of 8 participants s per month. With this accrual rate and with a 3-6 month ramp up for participating organizations, we plan to complete accrual in about 18 months. Accounting for patient follow-up, data entry, and analysis, the part 1 portion of the study is expected to be completed in about 22 months, followed by an additional 12 months for part 2 and an additional 24 months for part 3. The entire study duration is expected to take approximately 62 months (5.1 years), if we complete all 3 parts of the study.

13.4 Primary Objective, Endpoint, Analysis Plan

The primary objective for this study is to compare the ratio of the week 12 to week 0 IgG levels between the MUC1 vaccine and placebo. It is hypothesized that this ratio will be increased in patients treated with the MUC1 vaccine as compared to patients receiving the placebo. Prior data from a pilot study of 39 patients that were treated with the MUC1 vaccine[37] showed a mean IgG ratio of 6.46 with a standard deviation of about 10. Assuming equal standard deviations (i.e. 10) across the MUC1 and placebo groups, and assuming up to a 10% drop-out rate by year 1 (presumably less by 12 weeks), retaining at least 50 evaluable participants per arm yields at least 86% power to detect an increase in the mean IgG ratio from 1 to 6.5 for placebo vs. MUC1 (effect size = 0.55), using a 1-sided t-test with a significance level of 0.05. If the data are not normally distributed, we will use the Wilcoxon Rank-Sum test for this primary endpoint analysis. If the vaccine shows a significant increase in the 12-week immune response rate as compared to placebo, we will proceed to Part 2.

13.5 Secondary Objectives, Endpoints, Analysis Plans

As discussed previously, there will be key powered secondary endpoints for this study across all 3 parts.

1) Immune response at 12 weeks (Part 1): The key secondary endpoint for Part 1 will compare the proportion of patients that have at least a 2-fold increase in the IgG ratio (week 12/week 0) between MUC1 and placebo. Based on the pilot study[37], we hypothesize that around 40% of vaccinated participants will demonstrate a 2 fold increase in MUC1 titer at week 12 compared to around 10% for the placebo arm. Assuming a drop-out rate of up to 10% by 1 year (presumably less by 12 weeks), retaining at least 50 evaluable participants per arm provides 94% power to detect an increase in the 12-week immune response rate from 10 to 40%, assuming a 2-sided significance level of 0.05 (nQuery Advisor 6.01).

2) Booster Response at 1 year (Part 2): The key secondary endpoint for Part 2 will assess the booster response at week 55 vs. week 52 for the vaccine as compared to placebo. Based on the pilot study, we expect 75% of week-12 responders will respond with a doubling of MUC1 antibody levels, or about 35% of the overall sample. We expect the 1-year response rate to be around 10% in the placebo group. Of the 110 randomized participants (55/arm), we expect that up to 10% will drop out by 1 year, leaving

approximately 100 participants evaluable for this analysis (50/arm). With 50 evaluable participants per arm, we would have 92% power to detect an increase in the 1-year immune response rate from 10 to 35%, assuming a 1-sided significance level of 0.05 (nQuery Advisor 6.01). We will also compare the actual IgG ratio values between the 2 treatment arms as well. If the booster vaccine shows a significant increase in the 1-year immune response as compared to placebo and a response rate that exceeds 15%, we can proceed to Part III.

3) Adenoma recurrence rate at 3 years (Part 3): Part 3 will evaluate the adenoma recurrence rate at least one year and up to 3 years. The adenoma recurrence rate at 3 years in participants with an adenoma is generally around 40-50% [33]. Assuming an additional 10% drop out rate between years 1 and 3, we will have 90 participants (45/arm) evaluable for this endpoint, which will provide >80% power for the different effect sizes shown in the table below (1-sided alpha = 0.05).

# Evaluable	Effect Size (delta)	Power	1-sided alpha
90 (45/arm)	50 vs. 25%	79%	0.05
90 (45/arm)	45 vs. 20%	82%	0.05
90 (45/arm)	40 vs. 15%	85%	0.05

4) As an exploratory analysis, we will also evaluate the adenoma recurrence rate from surveillance exams occurring at least 1 year and up to 3 years after Week 0 vaccine administration between MUC1 and placebo by excluding adenomas ≤ 5 mm, adenomatous tissue that may represent residual adenoma at the site of a previous adenoma, and adenomatous tissue detected in the same segment of the bowel as the previous advanced adenoma. These adenomas are excluded since they may represent missed or residual adenomas from the baseline exam. These analyses are exploratory.

5) Participant-reported injection site reaction information will be collected by use of a participant-completed Vaccine Report Card (Appendix E). Descriptive statistics will be used to summarize these data and compare the data between study arms.

13.6 Reporting and Exclusions

All randomized participants who are evaluated at week 0 and week 12 and have IgG values from those time points will be evaluable for the primary endpoint of this study using the intent-to-treat principle. We plan to over randomize assuming 10% drop out within 1 year to ensure an adequate sample size in the primary analysis cohort. There will be no imputation for missing data. A summary and listing of all major protocol violations will be provided. All details will be given in the final study report and/or manuscript. Participants lost to follow-up will be censored on the last date of assessment (or contact) and as appropriate for analyses that are dependent upon length of study participation.

13.7 Evaluation of Toxicity (secondary endpoint)

All participants will be evaluable for adverse events (AEs) from the time of their first dose of MUC1 or Placebo during parts 1 and 2 of the study. To evaluate the AE profiles associated with each arm, the maximum grade for each type of adverse event will be recorded for each participant and frequency tables will be reviewed to determine the overall patterns. The number and severity of adverse events

(overall and by arm) will be tabulated and summarized across all grades. Grade 3+ adverse events will be similarly described and summarized separately. As per NCI CTC Version 4.0, toxicities are defined as adverse events that are classified as either possibly, probably, or definitely related to the interventional agent. Overall toxicity incidence, as well as toxicity profiles will be explored and summarized within and across the MUC1 and placebo arms. Frequency distributions, graphical techniques, and other descriptive measures will form the basis of these analyses. In addition, we will review all adverse event data that are graded as 3, 4, or 5 and classified as either "unrelated or unlikely to be related" to the study intervention in the event of an actual relationship developing.

In addition, we will assess patient reported vaccine-related side effects from the Vaccine Report Card as an exploratory analysis. We will summarize the results descriptively and compare the results between the 2 arms.

13.7.1 Adverse Event Stopping Rule

The trial will be monitored closely for occurrence of adverse events by the study team and by the Mayo Clinic Cancer Center Data Safety and Monitoring Board (DSMB) using the adverse event (AE) stopping rule specified below during the first 2 parts of the study (i.e. first 57 weeks):

Adverse Event Stopping Rule: The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual after any temporary suspension or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team in consultation with the Mayo DSMB may also choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e., an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy any of the following criteria for each arm separately:

- If at any time 2 of the initial 10 treated participants or 20% of all participants (i.e. when accrual is greater than 10 participants) have experienced a grade 3 or higher adverse event.)
- Each grade 5 event will be reviewed on a case by case basis in a real time fashion to determine whether study accrual should be suspended. We will also review all grade 4 adverse events regardless of attribution to monitor the emergence of any previously unrecognized treatment related adverse event.

13.8 Evaluation of Response

All randomized participants who are evaluated at week 0 and week 12 and have IgG values from those timepoints will be evaluable for the primary endpoint of this study using the intent-to-treat principle. All conclusions regarding efficacy will be based on all participants who completed both week 0 and week 12 and have IgG values from those timepoints as well. Subanalyses 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 (i.e. the primary endpoint), the 95% confidence intervals will also be provided.

13.9 Interim Analyses

Part I: The Part 1 primary endpoint analysis will be the first interim analysis. If the MUC1 vaccine is shown to significantly increase the IgG ratio as compared to placebo, we will move into the Part 2 portion of the study. This is defined in detail in Section 13.4.

Part II: If the booster vaccine shows a significant increase in the 1-year immune response as compared to placebo and the response rate exceeds 15%, we can proceed to Part III.

Study enrollment and implementation will not be halted for these analyses unless the results indicate that the study should not proceed to the next Part.

13.10 Ancillary Studies

We will assess the following translational endpoints for this study.

- To compare the anti-MUC1 antibody titer at the time of surveillance colonoscopy to evaluate the anti-MUC1 antibody response in relation to adenoma recurrence. Since surveillance colonoscopy is generally at 3 years after an advanced adenoma, this titer will typically be obtained at approximately week 156.
- To evaluate MUC1 expression on baseline advanced adenomas and on recurrent adenomas detected at surveillance colonoscopy. Based on current knowledge of MUC1 expression and function in tumor cells, MUC1 negative adenomas are not expected to grow as immune escape variants, but this has never been tested before and the trial will provide an appropriate setting to study this issue.
- To evaluate levels of circulating myeloid derived suppressor cells (MDSC) in the vaccinated and the placebo group and correlate with anti-MUC1 antibody levels and adenoma recurrence.
- To establish a biospecimen repository archive including live cells, plasma, and germline DNA for future immunologic and other assays, for testing not currently accommodated within the budget of this trial.

Analysis of these translational endpoints**:**
Laboratory measures will be correlated with participant outcome (i.e., adverse events, IgG levels, etc.) and with each other as well. Cut-points will be determined based on previously defined and accepted standards. Descriptive statistics and simple scatter plots will be generated to review the continuous biomarker data. In addition, for continuous biomarker values, the actual and % change in the level of each of the biomarkers from baseline to post-baseline time points will be explored within each arm using Wilcoxon signed rank tests, and paired sample t-tests. Comparisons between MUC1 and placebo will be performed using a two-sample t-test or Wilcoxon rank sum test, as appropriate. All categorical variables will be analyzed using chi-square tests or Fisher's exact test. For all translational endpoints, any notable statistical result will be viewed as an impetus for further study rather than as a definitive finding in and of itself.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form.

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. 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 IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. 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 study agent(s) will not be released to a participant who has not signed the Informed Consent document. Participants 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 and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to the DCP Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
2001 Gateway Place. Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions: regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

No expenses will be incurred by the study participant and/or their insurance carrier. This does not include any injuries or illnesses the participant may have related to their participation on the study. In the event of an injury or illness, the study participant and/or their insurance carrier will be responsible for all expenses related to the injury or illness. Participants may be provided remuneration for their participation in the study, at the discretion of the local Institutional Review Board.

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Study Title: MAY2013-01-01 "Randomized, Double-Blind, Placebo-Controlled Trial of MUC1 Vaccine in Patients with Newly Diagnosed Advanced Adenomas"

Short Title: MUC1 Vaccine Trial for the Prevention of Polyps and Adenomas

Instructions to Participating Sites: This is a template designed to provide you with the information you will need for your institution's informed consent document. Please paste applicable information into the format required by your Institutional Review Board (IRB). Text in bold, other than the section headings, is required. You are welcome to add any text required by your IRB and/or institution. Please submit your draft consent document to the CPN Operations Office for review prior to submission to your IRB. Please remove these instructions (blue text) prior to submission.

Introduction

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. *You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.*

You are being invited to take part in this research study because you have a history of advanced adenomatous polyps. Adenomatous polyps are growths in your colon that may turn into cancer over time.

Why is this study being done?

The MUC1/poly-ICLC vaccine is being tested in persons with a history of advanced adenomatous polyps, which are growths that may develop into colorectal cancer. The primary purpose of this study is to compare the safety and effects of the MUC1/poly-ICLC vaccine with placebo and to see if the vaccine can prevent the recurrence of adenomatous polyps.

How many people will take part in the study?

About 120 people will be screened at 8 different medical centers around the United States and in Puerto Rico. We estimate that about 110 people will take part in the study.

What will happen if I take part in this research study?

Before you begin the study, you will need to have the following exams or procedures to find out if you can be in the study. These procedures are not part of your standard medical care.

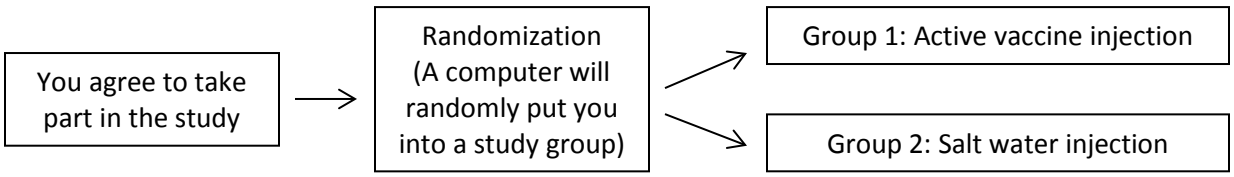
Before the study

Procedures to determine if you are eligible to take part in a research study are called "screening or baseline procedures." For this research study, the screening/baseline procedures, which may take up to 2 hours, include:

- You will be asked to read, understand, and sign this consent form.
- You will be asked to provide information about your past medical history, you will undergo a physical examination (including pulse rate, temperature, blood pressure, height, and weight), and you will be asked about current symptoms or illnesses.
- You will be asked about drug allergies and all medications you are taking.
- Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm for eligibility and research. Blood will also be drawn and tested several times during study to measure changes that might have been caused by the injection.
- A blood or urine pregnancy test, if you are woman able to become pregnant
- Pregnant women, women who are currently breast-feeding, and women considering a pregnancy will not be allowed to participate.
- Men considering fathering a child are also not allowed to participate.
- All procedures will be performed as an outpatient

During the study

If you are eligible for the study and wish to participate you will be "randomized" into one of two study groups. Randomization means that a computer program will be used to place you into one of the two study groups by chance. Neither you nor your doctor can choose the group you will be in and neither you nor your doctor will know which group you've been assigned to. One group will be injected with the MUC1/poly-ICLC vaccine on 4 occasions: Weeks 0, 2, 10, and Week 53. The other group will be injected with a similar looking injection called a placebo. The placebo injection will contain salt water.



Instruction to Participating Sites: If your IRB/REB requires it or if you believe it would be helpful for your study participants, you may create a study calendar or chart.

After the screening visit and placement into one of the study groups, you will be asked to return to the outpatient study site for the following schedule:

<u>Week 0</u>

- You will be asked to complete some brief questionnaires. These will take 15-30 minutes.
- You will receive 0.30 cc (1/20 of a teaspoon) of MUC1/poly-ICLC study vaccine or 0.30cc (1/20 of a teaspoon) of salt water. The injection will be administered subcutaneously (SQ) (under the skin) in the upper thigh with a thin, tiny needle (like the one used for a tuberculosis test). The site of

injection will remain the same for all injections. If you are a woman able to have a child, a pregnancy test will be performed prior to each study injection.
- You will be given a Vaccine Report Card along with instruction for completing it and reporting any reaction to the study injections.

Week 2 and week 10
- Your heart rate, temperature, and blood pressure will be checked, and medications will be reviewed and recorded.
- You will be asked questions about any side effects you may have experienced after your study injection. Your first Vaccine Report Card will be collected, and a new one will be provided.
- Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm and tested to see if there are any changes that might have been caused by the study injection. You will also be given the results of the blood tests used to monitor your health, performed at your previous visit.
- If you are a woman able to have a child, a pregnancy test will be performed.
- The vaccine or placebo injection will be administered subcutaneously (SQ) in the same upper thigh after a review of the routine laboratory testing results.

Week 12
- You will be asked questions about any medications you may be taking and any side effects you may have experienced after your study injection.
- Your Week 10 Vaccine Report Card will be collected.
- Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm and tested to measure changes that might have been caused by the study injection.

Week 52
- You will undergo a medical history, physical examination (including heart rate, temperature, blood pressure, height, and weight), review of previous test results, and current symptoms or illnesses
- You will be asked about drug allergies and all medications you are taking. Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm and tested to measure changes that might have been caused by the study injection.

Booster vaccine injection: Week 53
- If you are a woman able to have a child, a pregnancy test will be performed prior to study injection.
- You will be given another Vaccine Report Card to record any reactions to the booster injection.
- You will be asked to complete some brief questionnaires very similar to the one you completed at the beginning of the study.
- The fourth and last study injection will be administered.

Week 55 (about 2 weeks after booster vaccine)
- Your heart rate, temperature, and blood pressure will be checked, and any medications will be reviewed and recorded.
- You will be provided with the results of the blood tests done at your previous visit.
- You will be asked questions about any side effects you may have experienced after your study injection, and your Vaccine Report Card will be collected.
- You will be asked to complete a “Was It Worth It” Questionnaire.

- Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm and tested to measure changes that might have been caused by the study injection.

Week 4, 6, 16, 28, 40, 57

- You will be contacted by phone between clinic visits by a research coordinator. At each telephone interview, you will be asked questions about any side effects you may be having, any medications you may be taking, and your overall health.

Phone calls approximately every 6 months following booster injection

Patients with advanced adenomas typically undergo repeat colonoscopy 3 years after initial detection of an advanced adenoma. However, clinicians performing colonoscopy may bring participants back earlier than the typical 3-year interval. You will be contacted by phone about every 6 months after the booster injection at week 53 to monitor your health, ask about any medications you are taking, and to check on any planned colonoscopy exams. Please notify study personnel if and when a colonoscopy exam is scheduled.

At the time of your next colonoscopy (approximately 12-36 months, possibly longer, after beginning the study)

- Your heart rate, temperature, and blood pressure will be checked and any medications used will be reviewed and recorded.
- Approximately, 80 cc (4 tablespoons) of blood will be collected from a vein in your arm and tested to measure any changes that might have been caused by the study injection.
- A medical release to acquire the colonoscopy report and pathology findings will be obtained, if needed.
- A medical release to obtain tissue specimens from polyps found at subsequent colonoscopy (if any) will be requested, if needed, from the pathology department where the tissue blocks reside. These tissue blocks will be used for research study.
- You will be asked to complete one final questionnaire about your experience in this study.

After Study Follow Up

Study personnel may contact you in the future to see if you are interested in participating in any additional research studies.

How long will I be in the study?

You will be in the study through the time of your next colonoscopy depending upon when your next colonoscopy occurs. The timing of your next colonoscopy will be up to your regular doctor. It may be three years, but it could be more or less than that. Even if you do not finish the study, your doctor will continue to watch you for side effects.

Can I stop being in the study?

Yes. You can decide to withdraw at any time. Tell the study doctor if you are thinking about stopping or decide to stop. The investigators will discuss withdrawal from the study with you.

The study doctor may stop you from taking part in this study at any time if he/she believes stopping is in your best interest; if you do not follow the study rules; or if the study itself is stopped.

What are the possible risks, side effects, and discomforts of this research study?

You may or may not have side effects while on the study. Everyone taking part in the study will be watched carefully for side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. In some cases, side effects can be serious, long lasting, or may never go away.

You will be asked to keep track of any symptoms and possible side effects using a Symptom Diary and a Vaccine Report Card. The study team will provide instructions on how to complete these, and they will be reviewed at each study visit.

Most of the information in the below table is based on a variety of previous studies that were similar to this study in some ways but different in some important ways that may have led to different side effects. In the study that was most similar to this study, no autoimmune or serious adverse reactions were identified.

Common, Some May Be Serious **In 100 people who receive the injection, more than 20 may have:**
• Redness at injection site (erythema) • Pain/soreness at injection site • Flu-like symptoms, which may include fever, chills, headache, fatigue, muscle pain, and joint pain • Night sweats • Loss of appetite • Low albumin blood test result (a type of protein in the blood)

The redness and soreness generally resolve within a few days to a week. The flu-like symptoms generally resolve within 12-24 hours. Acetaminophen (Tylenol®) generally helps with these symptoms if they occur.

Occasional, Some May Be Serious **In 100 people who receive the injection, 4 to 20 may have:**
• Low white blood cell count (cells that fight infections) • Low platelet count (cells that help the blood clot in order to stop bleeding) • Anemia (which may cause tiredness, or may require blood transfusion) • Abnormal liver function test results

Rare, Some May Be Serious **In 100 people who receive the injection, 3 or fewer may have:**
• Hives, shortness of breath, or an allergic reaction that is life-threatening • Immune reaction to his or her own tissue (autoimmune diseases) or if present, may be made worse.

Risks of the blood tests

Bruising, soreness, or rarely, infection may occur as a result of the needle sticks to obtain blood from your vein.

Risks of the questionnaires

There are some questions on the study questionnaires that may request sensitive information or make you uncomfortable. You are welcome to skip any questions that you do not wish to answer.

Reproductive risks

You should not become pregnant or father a baby while on this study because we do not know the effect that the drugs in this study may have on an unborn baby. Women should not breastfeed a baby while on this study. You should use adequate birth control methods while on this study if you are at risk for becoming pregnant or impregnating someone. Check with your study doctor about what kind of birth control methods to use and how long to use them. For more information about risks and side effects, ask your study doctor.

Effects of other drugs you may be taking

It is important to tell the doctor or study staff if you are taking any prescription medications including prescription steroids, over the counter drugs, or herbal supplements. We will need this information to make sure that there is no interaction with the study agent.

Are there benefits to taking part in the study?

There may or may not be direct medical benefit to you. At this time, it is not known whether or not the MUC1**/**poly-ICLC vaccine can prevent or delay adenomatous polyp recurrence.

What other choices do I have if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

1. You may choose to have the usual approach which is periodic colonoscopy to monitor for polyps without being in a study. Please remember that participation in this research study does not take the place of the colonoscopy.
2. You may choose to take part in a different study, if one is available.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of this study?

You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of procedures that are part of the research study. The sponsor will pay all costs associated with the research study. You or your insurer will be billed for any routine, standard care services not connected to the research study. You will be responsible for any usual co pays, insurance, or deductibles. Follow up colonoscopy exams are part of your standard clinical care. They are not part of the study, and the study does not pay for these tests. Before you take part in this study, you should contact your insurer to find out if the cost of these tests will be covered. You will have to pay for any costs not covered by your insurance.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at: http://www.cancer.gov/clinicaltrials/learning/insurance-coverage

You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Are there any costs or payments?

You will not be paid for taking part. However, you may receive some funds to defray some of the cost of participating (e.g., parking, child care). If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

Note to participating organizations: You may provide whatever payments to the participants are allowed by your Institutional Review Board. Payments to each participant may not exceed a total of $400. You are allowed to break this into smaller payments for each visit and, if appropriate, provide that detail in this section.

Will my medical information be kept private?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, we will do our best to make sure that any information that is released will not be able to identify you. There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

- The National Cancer Institute (NCI) and other government agencies, such as the Food and Drug Administration (FDA), involved in keeping research safe for people.
- Regulatory agencies within and outside the United States.
- The Institutional Review Board.

The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

What happens if I am injured as a result of taking part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from the institution conducting the study.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study. In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form or release the institution from liability for negligence.

Please note: This section of the informed consent document is about additional research studies that are being done with people taking part in the main study. You may take part in these additional studies if you want to. You can still be a part of the main study even if you say "no" to taking part in any of these additional studies.

About Using Your Blood and Tissue for Research

We are drawing your blood and requesting specimens from your colonoscopy exams as part of the study. We would like to keep the left over blood and tissue for future research. If you agree, this blood and tissue will be kept and may be used in research to learn more about cancer and other diseases.

Your blood and tissue may be helpful for research whether or not you have cancer. The research that may be done with your blood and tissue is not designed specifically to help you. It might help people who have cancer and other diseases in the future.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

The researchers ask your permission to store and use your samples and health information for medical research. The research that may be done is unknown at this time. Storing samples for future studies is called "biobanking". The Biobank is being run by the Cancer Prevention Network and supported by the National Cancer Institute.

Your samples and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be kept until they are used up.

Qualified researchers can submit a request to use the materials stored in the Biobank. A research committee at the Cancer Prevention Network and the National Cancer Institute will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.

Neither you nor your study doctor will be notified if/when research is conducted using your samples.

Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

Things For You To Think About

The choice to let us keep the leftover blood and tissue for future research is up to you. ***No matter what you decide to do, it will not affect your care***.

What If I Change My Mind?

If you decide now that your blood and tissue can be kept for research, you can change your mind at any time. If you change your mind, please contact *<Participating Organization Investigator >* at *<Institution Address>* and let them know that you do not want your blood and tissue to be used. Then any blood and tissue that remain will no longer be used for research. Samples or related information that have already been given or used by researchers will not be returned.

Benefits

The benefits of research using blood and tissue include learning more about what causes cancer and other diseases, how to prevent them, and how to treat them. You may not benefit from taking part.

Risks

There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

Here are just a few of the steps the researchers will take to protect your privacy:

1. When your samples are sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only.
2. The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and Cancer Prevention Network staff with access to the list must sign an agreement to keep your identity confidential.
3. Researchers to whom the Cancer Prevention Network sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.

4. Information that identifies you will not be given to anyone, unless required by law.
5. If research results are published, your name and other personal information will not be used.

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No" and initial each line. If you have any questions, please talk to your doctor or nurse, or call our research review board at the phone number provided. No matter what you decide to do, it will not affect your care.

1. My blood and tissue may be collected and stored for future use in research to learn about, prevent, or treat cancer.

Yes No Initials_____

2. My blood and tissue may be collected and stored for future use in research to learn about, prevent or treat other health problems (for example: Barrett's esophagus, causes of diabetes, Alzheimer's disease, or heart disease).

Yes No Initials_____

3. My blood and/or tissue may be sent to researchers at outside institutions.

Yes No Initials_____

4. My study doctor or someone from the Cancer Prevention Network may contact me in the future to ask me to participate in future clinical trials.

Yes No Initials_____

What If I Have More Questions?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ *<name>* at _____ *<telephone number>.*

For questions about your rights while taking part in this study, call the _____ <*name of institution*> Institutional Review Board (a group of people who review the research in order to protect your rights) at _____ <*telephone number*>. *[Note to Local Investigator: Contact information for participant representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions may be listed here.]*

Where can I get more information?

You may visit the NCI website at http://cancer.gov for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.clinicaltrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

*[Note to Informed Consent Authors: the above paragraph **(bold text)** complies with the new FDA regulation found at 21 CFR 50.25(c) and must be included verbatim in all informed consent documents. The text in this paragraph cannot be revised.]*

You will get a copy of this form. If you want more information about this study, please ask the study doctor.

Signature

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies where I circled "yes."

Participant _____

Date _____

APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	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.
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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

Recruitment and Retention Plan Template

Instructions

NCI, DCP requires a site and study-specific recruitment, retention, and adherence (RRA) plan for each cancer chemoprevention study. Each participating organization should tailor the study RRA plan template to meet the needs at their local site. The below RRA plan outline is intended as a tool to assist site investigators and recruitment coordinators in the RRA planning process.

Black/Bold font indicates required language

Instructions for specific information Participating Organizations should insert into each section are in blue italics. These can be deleted when the site-specific RRA plan is complete. When complete, please submit your RRA Plan to the CPN Operations Office for review.

I. Pre-initiation phase (Developing the Recruitment, Retention, and Adherence Plan)

A. Determine protocol staff assignments *(It may be helpful to specify by job title who on the site team will fill each of the roles described below)*

1. The site recruitment coordinator, who may be the study coordinator, will have primary responsibility for coordinating all aspects of recruitment for this study, including but not limited to, reviewing databases, medical records, and previous studies' participants lists for potentially eligible study participants of both genders, all races, and all ethnic groups; contacting these individuals to assess interest and initial eligibility (in whatever manner is most appropriate for the study and the institution); setting up appointments for screening and informed consent (including checking investigators' schedules for appointment and procedures); and maintaining contact with study participants throughout their participation to insure compliance with the protocol.
2. Alternate recruiters and back up personnel will be assigned to assist as needed.
3. The site PI and site co-investigators (all listed on the study-specific FDA form 1572) will:
    a. Assist in the initial assessment of eligibility
    b. Meet with the site recruitment coordinator regularly to monitor recruitment and accrual activity as well as progress of individuals on study
    c. Review the informed consent document with the study participant
    d. Perform all examinations and procedures required per protocol
    e. Determine/confirm eligibility
    f. Provide information and training to other departmental personnel as needed to maintain an appropriate level of recruitment and consistent implementation of the protocol.

B. Review protocol design and its impact on recruitment

1. Evaluate feasibility of sample size:
    a. Availability of target population considering demographics

b. Availability of target population considering eligibility criteria
c. Possible effects of placebo control arm
d. Possible effects of participant access to study drug if it is FDA-approved
e. Possible effects of potential toxicity
f. Possible effects of complicated entry criteria and burdensome protocol procedures

2. Calculate availability of eligible participants based on:
    a. Consultation with potential participants
    b. Review of patient lists within each practice. If appropriate and necessary, contact referring physicians for permission to contact their patients.
    c. Review of the literature
    d. Calculate the number of eligible enrolled participants versus number of eligible participants who chose not to enroll per site
    e. Estimate accrual rate (# of participants per month)
3. After a pre-determined period of time,* (if timeframe for achieving overall recruitment goal is unrealistic), considerations for protocol modifications include the following:
    a. Broadening eligibility criteria (e.g. time frames for completion of tests and procedures, lab values)
    b. Simplifying protocol
    c. Decreasing sample size
    d. Extending recruitment period
4. Considerations for improving access to this study by women and minorities:
    a. Having multiple sites conduct the study will offer a more diverse population of potential participants.
    b. Convert consent form and have translator available for non-English populations

C. Identify and contact referral sources

1. Survey potential referral doctors, obtain letter of commitment if possible. Cultivate working relationship with non-oncology specialists who provide care related to the study target organ (e.g. Gastroenterologists, Internists, etc.).
2. Non-physician referrals - Enlist endorsement and cooperation to promote enrollment:
    a. Community centers e.g. Jewish Community Centers (JCC)
    b. Retirement communities
    c. Senior centers
    d. YMCAs, public libraries, etc.
    e. Health clubs
    f. Churches
    g. Local corporations
    h. Alliances with disease specific organizations, such as patient advocacy groups, support groups, and charitable organizations
3. Keep referring physicians updated and engaged using tools such as:
    a. Letters to referring physicians

b. Quick fact sheets and cards
c. Study information posted in physician work areas

Participating Organizations: Section D should be left in your site's plan for informational purposes.

D. Determine and document metrics for evaluation of recruitment and retention performance

1. Estimated total accrual per site will be 10 per site, with an average of 1-2 participants registered per site per month.
2. Keep site study teams updated and engaged.
    a. Recruitment strategies will be discussed at all the study coordinator teleconferences to discuss general effectiveness as well as other approaches. Modified or alternative plans will be implemented if recruitment or retention is lagging.
    b. Weekly accrual reports will be generated by the CLO Statistical Programmer Analyst and sent to all appropriate study personnel. These will be summarized and posted on the CPN website.
    c. Each site will submit monthly screening and recruitment activity reports, which will be summarized and forwarded to DCP study personnel. These will be reviewed at least monthly by the CPN recruitment coordinator and discussed at all study coordinator teleconferences.
    d. CLO will keep open communication with study sites, sending out a monthly memo, scheduled study coordinator and PI teleconferences, and discussing logistical problems. The CLO will be available to assist with resolution of any identified problems.
3. After initial activation of the study at Mayo and University of Pittsburgh, overall accrual will be evaluated quarterly. If by a predetermined period of time* after initial activation at a given site, there have been no participants accrued, the CLO will have a teleconference with the site PI and team to discuss issues/obstacles. A timeframe, i.e. six months, will be determined during which the site team must implement its action plan. If recruitment activity is determined to be unsatisfactory at this point, the site will be asked to close to accrual.

*** The time points at which accrual milestones must be met will be determined collaboratively between the Study Chair, CLO Personnel, and the DCP Medical Monitor.**

Participating Organizations: Please revise the section E below to describe your site's specific plans to train study team member and provide any pertinent study updates to them. The Study Coordinator Memo mentioned above will be sent to the lead Study Coordinator. Plans should be described to distribute the memo and/or any protocol-related material to all pertinent site study team members.

E. Train staff

1. Training of participating site personnel by CLO
    a. Site personnel will be provided with the protocol and protocol-related documents in accordance with CPN and DCP SOPs

b. At or shortly after activation of each site, an orientation webconference will be held to provide training in protocol implementation, participant registration, CRF completion, etc.
c. Regular teleconferences will be held with all sites to discuss study implementation, questions, and issues.
d. The CPN website will be used to house the Study Coordinator Workbook, resources (such as FAQ lists), and all approved protocol-related materials.
e. Twice monthly, the CLO will distribute a Study Coordinator Memo to all participating sites. This memo will announce formal distributions of protocols and related materials, current accrual, and other pertinent study information.

2. The staff at each Participating Organization will be informed and trained by the site PI and Study Coordinator in accordance with institutional policies and procedures. This training must be documented for the study files, to be reviewed by the CPN Compliance Coordinator during monitoring visits. This includes, but is not limited to:
    a. Educating all participating staff regarding the details of protocol implementation and all study procedures
    b. Insuring consistent implementation of the protocol
    c. Improving the likelihood that all potentially eligible participants will be identified
3. Resources provided by the CLO
    a. Study coordinator and PI teleconference will be held throughout the study every 4-6 weeks or as needed.
    b. CLO Newsletter will be distributed twice yearly
    c. Recognition of high recruiters and sharing of their specific strategies
    d. Draft recruitment, retention, and adherence materials that can be modified by each Participating Organization for review by local IRB prior to use at each Participating Organization. These may also include public services announcements, use of social media, such as Twitter, and videos to upload to YouTube®.
    e. Buddy system among site study coordinators
    f. Holiday cards with approved study-specific news for distribution to enrolled participants (after appropriate approvals are received)

Participating Organizations: Please add any additional information necessary to Section F below to adequately describe your site's plans for implementing appropriate informed consent process and creating a positive clinical trial experience for your participants.

F. Each participating site will promote comfortable and pleasant clinic environment/experience in accordance with institutional policies and procedures. The following are recommended:

1. Supply driving directions to schedulers.
2. Coordinate well-organized clinic flow.
3. Assure user-friendly test scheduling, immunization administration, etc.

4. Negotiate (if possible, insist upon) flexible appointment times (i.e., more than one day per week).
5. Plan to allow ample time for participants with the clinical trial staff for the entire informed consent process.
6. Schedule periodic meetings between the clinic coordinator and the protocol staff.

Participating Organizations: Please describe in section G any participant compensation or remuneration, if applicable. Please also describe the time and/or funds that will be devoted to participant recruitment, retention, and adherence.

G. Budget:
The overall budget for this study will involve a standard amount for each participant accrued to the study. Partial payments will be made for participants who are determined to be screen failures. All payments will be made when all of the data (case report forms) up to the point of going off study are submitted. The site subcontracts do not specify how the per-patient reimbursement is to be allocated. However, the following should be considered at each site as appropriate to successful implementation of the study:

1. Staff time to implement recruitment and retention plan
2. Recruiter transportation to community outreach events
3. Participant compensation/remuneration
4. Recruitment and retention tools

H. Determine and list site-specific recruitment and retention strategies based on evaluation of protocol, target population, clinic, and referral sources.

1. Describe general strategies such as:
   a. Regularly contacting referral sources
   b. Advertisements and public relations
   c. Newspaper
   d. TV and radio
   e. Internet, social networking sites such as Pinterest, Twitter, and Facebook
   f. Direct mailings
   g. Investigator interviews
   h. Patient education lectures
   i. Investigator or coordinator educational sessions to relevant community organizations
   j. FAQ documents
   k. Mass media and press releases
   l. Partner with other studies
   m. Identify Spokespersons
   n. Volunteers

2. *Describe recruitment strategies for special populations, such as women and minorities, such as:*
    a. *Perform cultural assessment of local communities*
    b. *Consider centralized minority coordinator*
    c. *Consider matched ethnicity recruitment coordinator*
    d. *Have translator available*
    e. *Minority community liaison*
    f. *Meet with minority community leaders*
    g. *Go to community meetings*
    h. *Establish relationships with churches*
    i. *Special needs participants*
    j. *Large print documents*
    k. *Arrange transportation*

Participating Organizations: Revise Section II (below) to describe your actual recruitment strategies. Describe specifically how you will identify potential participants, screen potential participants, and begin the informed consent process.

II. Active recruitment phase (Implementing the Recruitment Plan)

1. Implement strategies as determined during pre-initiation phase of plan
2. Consider non-compliance and retention potential
    a. Exclude participants unlikely to comply and stay on study – unless you can provide compensatory support and follow-up
    b. Known history of non-compliance
    c. Socially unstable
    d. Expressed difficulty with, and numerous objections to, protocol requirements
    e. Cavalier attitude toward protocol
    f. Verbalized a minimization of cancer risk
    g. Verbalized desire for “active immunization only”
    h. Consider multiple objections as red flag indicating possible retention/compliance problems
3. Contact potentially eligible participants and pre-screen for initial eligibility.
4. Assess eligibility of individual potential participants
5. Build supportive relationship with participant
6. Involve each participant’s social network/support system in decision-making
7. Clarify any misconceptions
8. Offer to help resolve manageable participants’ logistical problems
9. Continuously evaluate the site recruitment plan
    a. Maintain screening log and reasons for ineligibility or non-enrollment. Data concerning screening, recruitment, and resulting accrual will be forwarded from the Participating

Organizations to the CLO on a monthly basis. Timeliness and compliance with this requirement will be monitored by the CLO.

b. Each site should consider modifying recruitment plan if recruitment is lagging. If necessary, ask the CLO for advice and assistance.

Participating Organizations: Revise Section III below to describe the activities you will employ to maintain contact with the study participants while on study and insure compliance with events, procedures, and study medication. Please also describe any activities that you do to maintain a good relationship with them so that they might, if eligible, be willing to participate in future clinical trials. The activities listed are ideas and suggestions.

III. Retention and adherence phase – Be proactive

1. Maintain communication with referring physicians re: participant progress
2. Establish and maintain rapport among staff during follow-up
    a. Newsletters or other communication tools
    b. Adequate staff compensation
    c. Staff recognition/awards
3. Establish and maintain rapport and communication with participants
    a. Identify and track red flags for possible attrition (but do not offer support that cannot be maintained):
        i. adverse effects/events (review protocol carefully, be prepared to respond to AE reports, inform participants as to what can be expected)
        ii. missed appointments
        iii. frequent appointment time changes
        iv. major personal or family events
        v. health deterioration
        vi. loss of support system
    b. Do not promise support that cannot be maintained
    c. Maintain current contact info for each participant
    d. Enlist support of participant's social network
        i. Transportation
        ii. Encouragement
        iii. Protocol compliance
    e. Provide remuneration/compensation for expenses incurred as budget and local IRB regulations permit:
        i. Parking, meals, time lost from work, transportation, child care
    f. Ensure pleasant clinic visits
        i. Limit waiting room time and provide refreshments, if possible
        ii. Coordinate assessments (e.g., blood work, etc.) with visits
        iii. Flexible scheduling

iv. Toll-free numbers
v. Ensure consistent staff contact person and access to PI
vi. Establish and communicate schedule for contact with participant
vii. Consider retention tools, such as calendars, newsletters, appointment cards, reminder cards and phone calls, certificates of appreciation, holiday cards

g. Track participant withdrawals and reasons for withdrawal and communicate this information to the CLO

Participating Organizations: Section IV describes the activities of the CLO. It should be left in your site's plan for informational purposes. Additionally, you are encouraged to describe any analysis or evaluation of recruitment that will occur at your site.

IV. Evaluation Phase

1. During the study
    a. The CLO Recruitment Coordinator will continuously review study recruitment and adherence
    data from all tracking documents. These will be summarized and forwarded on a monthly basis.
    b. The CPN Operations Committee will review the monthly reports and discuss possible revisions, protocol modifications, site closures, and other activities as needed.
    c. All discussions, recommendations, conclusions, and/or lessons learned will be documented with the possibility of publication in mind.
    d. Successful strategies will be shared with other participating organizations during teleconferences and via the Study Coordinator Memo.
2. After the study
    a. Historically it has been difficult to reach recruitment goals across all cancer prevention studies. In order to explore the obstacles sites are facing, the CLO will distribute a short survey to all participating sites at the study completion. The survey will target: site issues, inclusion/exclusion issues, time issues, CPN support, education and training.

APPENDIX C

Was It Worth It Questionnaire

Visit type (Time point):* ☐ Week 55 or early termination

Participating in a clinical trial / research study is a personal choice and an individual experience. We would like to get your feedback on your experience in this research study. Please respond to the following questions as indicated.

Directions: Please answer each question by circling Y (for yes), N (for no), or U (for uncertain).

Was it worthwhile for you to participate in this research study?	Y	N	U
If you had to do it over, would you participate in this research study again?	Y	N	U
Would you recommend participating in this research study to others?	Y	N	U

Directions: Circle one response

Overall, did your quality of life change by participating in this research study?

It improved It stayed the same It got worse

Overall, how was your experience of participating in this research study?

Better than I expected The same as I expected Worse than I expected

If there was one thing **that could have been done to improve your experience in this research study, what would it be?**

_____

_____

_____

Would you like to talk to someone about your concerns (circle one response)? Yes No

_____ _____

Signature Date

APPENDIX D

Pre-Vaccine Survey

Visit type (Time point):* ☐ Week 0
Participant ID: _____
Visit Date: _____

The purpose of these questions is to help us to understand more about how individuals who are at risk for developing colorectal cancer make the decision to participate in a vaccine study.

Circle the answer or number that best describes your answer

1. What do you think is your chance of developing colorectal cancer?
(Circle 0 for no chance and 10 for great chance)

No chance	0	1	2	3	4	5	6	7	8	9	10	Great chance

2. Which of the following people did you talk to about the vaccine study? (Circle all that apply)

Spouse or significant other Family Physician Parent Friend Clergy

Other, please specify: _____

3. What was the response of the person/s you talked to about the study? (Circle all that apply)

a. Spouse or significant other	Supportive	Neutral	Non-Supportive
b. Family Physician	Supportive	Neutral	Non-Supportive
c. Parent	Supportive	Neutral	Non-Supportive
d. Friend	Supportive	Neutral	Non-Supportive
e. Clergy	Supportive	Neutral	Non-Supportive
f. Other, specify: _____	Supportive	Neutral	Non-Supportive

4. How often, prior to receiving the vaccine, did you think that you should have a colonoscopy?

Yearly 2 years 3 years 4 years 5 years 10 years

Other, please specify (Please write your answer):

5. How important to your decision was it that you might get the following benefits from the vaccine:

	Not at all					Very
a. Decrease the number of polyps you develop	0	1	2	3	4	5
b. Prevent you from developing polyps	0	1	2	3	4	5
c. Prevent you from developing colorectal cancer	0	1	2	3	4	5
d. Decrease the frequency that you have colonoscopies	0	1	2	3	4	5

Pre-Vaccine Decision Regret Scale

Visit type (Time point):* ☐ Week 0
Participant ID: _____
Visit Date: _____

Please reflect on the first decision you made about participating in the MUC1 vaccine trial for individuals with advanced colorectal adenoma after talking with your study doctor or a member of the research team. Please show how strongly you agree or disagree with these statements by circling a number from 1 (strongly agree) to 5 (strongly disagree) which best fits your views about your decision.

	Strongly Agree	Agree	Neither Agree Nor Disagree	Disagree	Strongly Disagree
1. It was the right decision	1	2	3	4	5
2. I regret the choice that was made	1	2	3	4	5
3. I would go for the same choice if I had to do it over again	1	2	3	4	5
4. The choice did me a lot of harm	1	2	3	4	5
5. The decision was a wise one	1	2	3	4	5

Decision Regret Scale (copyright) AM O'Connor, 1996 University of Ottawa

Perceived Stress

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please circle the number that best represents how often you felt or thought a certain way by circling a number from 1 to 5.

	Never	Almost Never	Sometimes	Fairly Often	Very Often
1. How often have you felt that you were able to control the important things in your life	1	2	3	4	5
2. How often have you felt confident about your ability to handle your personal problems?	1	2	3	4	5
3. How often have you felt that things were going your way?	1	2	3	4	5
4. How often have you felt difficulties were piling up so high that you could not overcome them?	1	2	3	4	5

Pre-Vaccine Symptom Inventory

Visit type (Time point):* ☐ Week 0
Participant ID: _____
Visit Date: _____

Below is a list of problems and complaints that people sometimes have. Read each item carefully, and select one of the numbered answers that best describes how much discomfort that problem has caused you in the past month, including today. Please circle the number to the right of the problem. Answer each question by circling the answer as indicated. If you change your mind, erase your first circle completely.

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Nervousness or shakiness inside	0	1	2	3	4
2. Faintness or dizziness	0	1	2	3	4
3. Pains in heart or chest	0	1	2	3	4
4. Suddenly scared for no reason	0	1	2	3	4
5. Feeling lonely	0	1	2	3	4
6. Feeling blue	0	1	2	3	4
7. Feeling no interest in things	0	1	2	3	4
8. Feeling fearful	0	1	2	3	4
9. Nausea or upset stomach	0	1	2	3	4
10. Trouble getting (catching) your breath	0	1	2	3	4
11. Numbness or tingling in parts of your body	0	1	2	3	4
12. Feeling hopeless about the future	0	1	2	3	4
13. Feeling weak in parts of your body	0	1	2	3	4
14. Feeling tense or keyed up	0	1	2	3	4
15. Spells of terror or panic	0	1	2	3	4
16. Feeling so restless that you couldn't sit still	0	1	2	3	4
17. Feelings of worthlessness	0	1	2	3	4

Pre-Vaccine Health

Visit type (Time point):* ☐ Week 0
Participant ID: _____
Visit Date: _____

The survey below asks for your views about your health. This information will help better understand how you feel and how well you are able to do your usual activities. Answer each question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say that your health is:
   ☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?
   a. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
   ☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all
   b. Climbing several flights of stairs?
   ☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

3. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?
   a. Accomplished less than you would like? ☐ Yes ☐ No
   b. Were limited in the kind of work or other activities? ☐ Yes ☐ No

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?
   a. Accomplished less than you would like? ☐ Yes ☐ No
   b. Didn't do work or other activities as carefully as usual? ☐ Yes ☐ No

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and around the house)?
   ☐ Not at all ☐ A little bit ☐ Moderately ☐ Quite a bit ☐ Extremely

6. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

a. Have you felt calm and peaceful?

☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

b. Did you have a lot of energy?

☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

c. Have you felt downhearted and blue?

☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

7. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

☐ All of the time ☐ Most of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

Post-Vaccine Survey

Visit type (Time point):* ☐ Week 53 ☐ Week 156 or early termination
Participant ID: _____
Visit Date: _____

The purpose of these questions is to help us to understand more about how individuals who are at risk for developing colorectal cancer make the decision to participate in a vaccine study. Please circle the response that best describes your answer.

1. What do you think your chance of developing colorectal cancer is since you have been vaccinated? (Circle 0 for no chance and 10 for great chance)

No chance	0	1	2	3	4	5	6	7	8	9	10	Great chance

2. What do you think your chance of developing colorectal cancer would have been if you had not received the vaccine?

No chance	0	1	2	3	4	5	6	7	8	9	10	Great chance

3. To what extent do you think that the vaccine will provide you with protection (immunity) against the development polyps?

No chance	0	1	2	3	4	5	6	7	8	9	10	Great chance

4. If you feel the vaccine will provide protection (immunity) against the development of polyps, how long do you think the protection will last?

No immunity Weeks Months Years Lifetime

5. Do you think that the vaccine will provide you with protection (immunity) against developing colorectal cancer?

No chance	0	1	2	3	4	5	6	7	8	9	10	Great chance

   a. If so, how long do you think the vaccine will provide protection (immunity) against the development of colorectal cancer?

      Lifetime Weeks Months Years

   b. How often do you think that you should have a colonoscopy now that you have received the vaccine?

   Yearly 2 years 3 years 4 years 5 years 10 years Other

   If other, please specify: _____

6. Would you have been vaccinated if the following individuals were not supportive?

a. Spouse or significant other	Yes	No
b. Family Physician	Yes	No
c. Parent	Yes	No
d. Friend	Yes	No
e. Clergy	Yes	No
f. Other, specify: _____	Yes	No

7. Would you get vaccinated if you had to pay for the vaccine? ☐ Yes ☐ Maybe ☐ No

8. How far would you travel to receive the vaccine?

   Less than 10 miles Over 10 miles Up to 100 miles Anywhere

9. Would you get vaccinated if you were told that the potential side effects for the vaccine were unknown?

   ☐ Yes ☐ Maybe ☐ No

10. Would you get vaccinated if you were told that the safety of the vaccine was unknown?

    ☐ Yes ☐ Maybe ☐ No

Post-Vaccine Decision Regret Scale

Visit type (Time point):* ☐ Week 53 ☐ Week 156 or early termination
Participant ID: _____
Visit Date: _____

Please reflect on the first decision you made about participating in the MUC1 vaccine trial for individuals with advanced colorectal adenoma after talking with your study doctor or a member of the research team. Please show how strongly you agree or disagree with these statements by circling a number from 1 (strongly agree) to 5 (strongly disagree) which best fits your views about your decision.

	Strongly Agree	Agree	Neither Agree Nor Disagree	Disagree	Strongly Disagree
1. It was the right decision	1	2	3	4	5
2. I regret the choice that was made	1	2	3	4	5
3. I would go for the same choice if I had to do it over again	1	2	3	4	5
4. The choice did me a lot of harm	1	2	3	4	5
5. The decision was a wise one	1	2	3	4	5

Decision Regret Scale (copyright) AM O'Connor, 1996 University of Ottawa

Post-Vaccine Perceived Stress

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please circle the number that represents how often you felt or thought a certain way.

	Never	Almost Never	Sometimes	Fairly Often	Very Often
1. How often have you felt that you were able to control the important things in your life	1	2	3	4	5
2. How often have you felt confident about your ability to handle your personal problems?	1	2	3	4	5
3. How often have you felt that things were going your way?	1	2	3	4	5
4. How often have you felt difficulties were piling up so high that you could not overcome them?	1	2	3	4	5

Post-Vaccine Symptom Inventory

Visit type (Time point):* ☐ Week 53 ☐ Week 156 or early termination
Participant ID: _____
Visit Date: _____

Below is a list of problems and complaints that people sometimes have. Read each item carefully, and select one of the numbered descriptors that best describes how much discomfort that problem has caused you in the past month, including today. Please circle the number to the right of the problem. Please do not skip any items. If you change your mind, erase your first circle completely.

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Nervousness or shakiness inside	0	1	2	3	4
2. Faintness or dizziness	0	1	2	3	4
3. Pains in heart or chest	0	1	2	3	4
4. Suddenly scared for no reason	0	1	2	3	4
5. Feeling lonely	0	1	2	3	4
6. Feeling blue	0	1	2	3	4
7. Feeling no interest in things	0	1	2	3	4
8. Feeling fearful	0	1	2	3	4
9. Nausea or upset stomach	0	1	2	3	4
10. Trouble getting (catching) your breath	0	1	2	3	4
11. Numbness or tingling in parts of your body	0	1	2	3	4
12. Feeling hopeless about the future	0	1	2	3	4
13. Feeling weak in parts of your body	0	1	2	3	4
14. Feeling tense or keyed up	0	1	2	3	4
15. Spells of terror or panic	0	1	2	3	4
16. Feeling so restless that you couldn't sit still	0	1	2	3	4
17. Feelings of worthlessness	0	1	2	3	4

Thank you!

Post-Vaccine Health

Visit type (Time point):* ☐ Week 53 ☐ Week 156 or early termination
Participant ID: _____
Visit Date: _____

The survey below asks for your views about your health. This information will help better understand how you feel and how well you are able to do your usual activities. Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say that your health is:
   ☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?
   a. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
   ☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all
   b. Climbing several flights of stairs?
   ☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

3. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?
   a. Accomplished less than you would like? ☐ Yes ☐ No
   b. Were limited in the kind of work or other activities? ☐ Yes ☐ No

4. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?
   a. Accomplished less than you would like? ☐ Yes ☐ No
   b. Didn't do work or other activities as carefully as usual? ☐ Yes ☐ No

5. During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and around the house)?
   ☐ Not at all ☐ A little bit ☐ Moderately ☐ Quite a bit ☐ Extremely

6. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

   a. Have you felt calm and peaceful?

   ☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

   b. Did you have a lot of energy?

   ☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

   c. Have you felt downhearted and blue?

   ☐ All of the time ☐ Most of the time ☐ A good bit of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

7. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

   ☐ All of the time ☐ Most of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

Thank you!

APPENDIX E

Vaccine Report Card

Dear Study Participant,

Thank you for your willingness to participate in this clinical trial of the MUC1 vaccine. This is one of the first cancer prevention vaccine trials, and we want to carefully document the vaccine experience.

In this booklet you will find a "vaccine report card" to record symptoms. Please review these instructions carefully. If you have any questions about the report card, ask your study team.

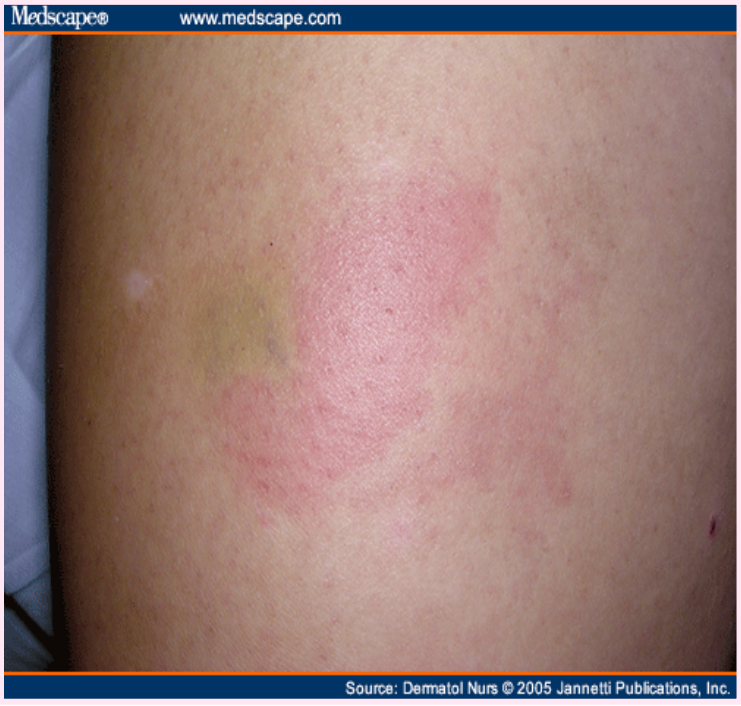
When to record symptoms:
The day of your first vaccine injection will be considered Day 0.

- On Day 2, please record symptoms you have experienced since you left your study visit. If you took any medications to relieve symptoms, please write them down. If the symptoms have disappeared, write down the date on the report card in the column labeled "date symptoms disappeared." Once a symptom disappears, you do not need to continue recording anything about that symptom.
- On Day 7, write down all of the symptoms you experience from Day 3 to Day 7.
- On Day 14, write down all of the symptoms you experience from Day 8 to Day 14.
- If you are not scheduled to return for a study visit on or around Day 14, please send this booklet back to the study team by mail, fax, or email. Your study team members will provide an envelope, fax number, or email address for you.

What to record:
The pilot studies have shown us that some, but not all people receiving the vaccine will have reactions such as redness at the injection site, swelling, warmth or itching, and pain or tenderness. Not everyone experiences these symptoms. Some people will have no symptoms.

If there are any other symptoms you experience and want to document, please write them in the text box on the back page of this booklet.

About Injection Site Reactions



Measuring the size of the redness (erythema):
This picture shows redness of the skin. To measure this, use a tape measure or ruler to measure the maximum distance from one side of the red patch to the other in centimeters. It might be helpful to measure it across the red patch both from side to side and from top to bottom. Record the longest distance.

Pain and Tenderness:
You may experience pain at the injection site. There are two possible types. One is general muscle pain or achiness at the site that is present even when you're not touching the area, such as with movement or even sitting. The other type is tenderness, which is pain when you touch the injection site. Please record these separately.

Swelling or Induration:
Swelling or induration is firmness, like the bump that may be experienced after a mosquito bite. The easiest way to measure it is to use your fingertip to feel the edge of the firmness, and then make a mark with an ink pen at one edge. Make another mark at the opposite edge. It might be helpful to measure and mark the swelling or induration both from side to side and from top to bottom. To measure this, use a tape measure or ruler to measure the maximum distance between the two marks in centimeters. Record the longest distance.

Warmth and itching:
Document when warmth or itching begins and when these symptoms go away.

_____ _____

Your Signature **Date**

_____ _____

Study Coordinator Signature **Date**

MAY2013-01-01," Randomized, Double-Blind, Placebo-Controlled Trial of MUC1 Vaccine in Patients with Newly Diagnosed Advanced Adenomas"

Vaccine Report Card

Participant ID _____ Date of Injection _____

Instructions: Please complete this "report card" at 2 days, 7 days, and 14 days after your injection. Bring it to your next study visit or return it to the study team by mail, fax, or email.

Possible side effects	Date Symptom Appeared	2 days after injection Date _____	7 days after injection Date _____	14 days after injection Date _____	Date Symptom Disappeared
Redness at the injection site		□ None □ Yes. It measured _____ centimeters	□ None □ Yes. It measured _____ centimeters	□ None □ Yes. It measured _____ centimeters	
Pain at the injection site <u>without</u> touching		□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	

Possible side effects	Date Symptom Appeared	2 days after injection Date _____	7 days after injection Date _____	14 days after injection Date _____	Date Symptom Disappeared
Tenderness (Pain at the injection site <u>with</u> touch)		□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	□ None □ A little, but it didn't interfere with normal activity □ Some, required OTC* medications for more than 24 hours or limited normal activity □ A lot, required prescription medications, interfered with normal activity □ Required ER visit or hospitalization	
Swelling/ Induration**		□ None □ Yes. It measured _____ centimeters	□ None □ Yes. It measured _____ centimeters	□ None □ Yes. It measured _____ centimeters	
Itching at site		□ None □ Yes	□ None □ Yes	□ None □ Yes	
Skin warmth at site		□ None □ Yes	□ None □ Yes	□ None □ Yes	

* OTC – Over the counter medications such as Tylenol® or ibuprofen.

** Firm, raised swelling.

If you experience any other symptoms that you would like to report, please make note of them on this page. Be sure to write down the date the symptom appeared and the date the symptom disappeared. If you took any medications for the symptom or visited a doctor or hospital for the symptom, please inform the study team.

APPENDIX F

NAFLD Activity Score

The Non-Alcoholic Fatty Liver Disease (NAFLD) Activity Score (NAS) is a validated score that is used to grade disease activity in patients with NAFLD. The NAS is the sum of the biopsy's individual scores for steatosis (0 to 3), lobular inflammation (0 to 2), hepatocellular ballooning (0 to 2), and fibrosis (0 to 4). An NAS of 1 or 2 corresponds to NAFL, 3 to 4 corresponds to borderline NASH, and a score ≥5 corresponds to NASH. (See "Histologic scoring systems for chronic liver disease", section on 'Nonalcoholic fatty liver disease'.)

Please note that for this protocol, the calculation of the NAS applies only for the determination of eligibility for individuals with a diagnosis of Non-Alcoholic Steatohepatitis (NASH).