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TITLE: PREPARE: PRevention using EPA against coloREctal cancer

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NCI-Supplied Agent(s): N/A
Other Agent(s): AMR101 (VASCEPA), manufactured by and obtained from Amarin Pharma, Inc.

Study Exempt from IND Requirements per 21 CFR 312.2(b).

Protocol Type / Version # / Version Date: Amendment 9 / Version 8 / 12/10/2021

SCHEMA

PREPARE: PRevention using EPA against coloREctal cancer

Adults (18-80 years) who have had a prior colonoscopy at Massachusetts General Hospital AND at least 1 adenoma was removed.

Participants have no diagnosis of inherited colorectal cancer syndromes (i.e. FAP), no fish oil use at any dose more than once per week w/in the last month, no medical contraindications, and are not pregnant/breastfeeding.

Determine Eligibility (see phone script)

Eligibility Confirmation
Provation review of most recent endoscopy report, and accompanying pathology report.

Registration in OnCore by the study staff
Investigating physician receives written informed consent from participant

Initial (Baseline/Pre-treatment) Visit
1. Lifestyle questionnaire; 2. Nutritional survey; 3. Flexible sigmoidoscopy (24 biopsies of normal colorectal mucosa, one stool sample); 4. Blood; 5. Urine

Intervention
Duration: minimum = 8 weeks, maximum = 12 weeks
AMR101, 8 capsules (4 grams) per day

Final (Post-treatment) Visit
1. Lifestyle questionnaire; 2. Nutritional survey; 3. Flexible sigmoidoscopy (24 biopsies of normal colorectal mucosa, one stool sample); 4. Blood; 5. Urine

Primary Endpoint:
Marine omega-3 fatty acid composition in tissue
Secondary Endpoints: Effects on colorectal cancer biomarkers:
a.) Microbiome alterations (stool); b.) Metabolomics (stool); c.) Gene expression by RNA-seq (tissue); Urinary PGE-M.

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1. OBJECTIVES

1.1 Study Design

Within the gastroenterology practice of Massachusetts General Hospital (MGH), we will conduct a prospective, single-arm clinical trial to measure the effects of daily 4-gram eicosapentaenoic acid (EPA), through treatment with AMR101 (VASCEPA, icosapent ethyl) on urine, stool and tissue biomarkers associated with colorectal cancer.

1.2 Primary Objectives

To measure the effect of daily 4-gram AMR101 treatment on fatty acid composition in colorectal tissue among individuals with a history of colorectal adenoma.

1.3 Secondary Objectives

To measure the effect of EPA treatment on the gut microbiome and metabolome, urinary prostaglandin metabolites (PGE-M) and gene expression profile of colorectal tissue among individuals with a history of colorectal adenoma.

2. BACKGROUND

2.1 Study Disease(s)

Colorectal cancer is the third most common cancer and third leading cause of cancer-related deaths in the U.S. In 2016, an estimated 95,270 new cases of colorectal cancer will be diagnosed and 49,190 people will die from the disease.[1] Age is strongly associated with colorectal cancer incidence, with more than a third of all colorectal cancer deaths occurring in the population aged 80 years or older. The death rates are higher in black populations and lowest in Asian individuals. Men have a slightly higher lifetime probability of receiving a colorectal cancer diagnosis (5.0%) compared to women (4.7%).[2]

Nationally, incidence rates have been declining over the past two decades; and at a slightly higher rate over the last 10 years compared to the 1990s. This is largely believed to be due to increasing use of screening methods and improved risk factor profile. Still the public health concern remains as colorectal cancer ranks third among all cancer deaths with only an approximate 65% five-year survival rate, necessitating further advances in cancer prevention.[2]

2.2 IND Agent(s)

N/A; Exempt.

This study will investigate the effects of standard-dose EPA treatment using the FDA-approved drug AMR101, and meets the five exemption criteria as laid out by 21 CFR 312.2(b):

- The drug product is lawfully marketed in the United States.
  - The drug AMR101 used for the study is FDA approved and lawfully marketed in the United States.
- The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
  - The end points of the study are mechanistic biomarkers related to EPA and are not used in any clinical context.
- In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
  - The study aims to investigate the potential anti-cancer mechanisms of EPA and is not intended to support any change in the advertising for the drug AMR101.
- The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
  - AMR101 used in the proposed study is made of ethyl esters of the omega-3 fatty acid, eicosapentaenoic acid (EPA), which are natural substances found in the oil of certain fish, and will be orally administered in a standard dose (4 g/day) in people who are being evaluated for colorectal cancer.
- The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
  - The protocol will be submitted to the Dana Farber/Harvard Cancer Center IRB, who will also review the informed consent documents.
- The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).
  - The study is not intended to promote or commercialize any drug product.

Our application for an IND exempt has been approved by the Division of Oncology Products 2 (DOP2) of the FDA (see the attached formal letter, IND 141418. Please note that the name of the study has been changed since receipt of the IND approval).

2.3 Other Agent(s)

AMR101

AMR101 (icosapent ethyl) is a prescription medicine for adults to lower blood levels of triglycerides. It is supplied as a liquid-filled gel capsule for oral administration. The standard dose is 4 g per day (administered as 8 half-gram capsules). Each 0.5-gram capsule of AMR101 contains 0.5 gram of icosapent ethyl, which is an ethyl ester of the omega-3 fatty acid eicosapentaenoic acid (EPA).

EPA possesses triglyceride-lowering and anti-inflammatory activities, and has been suggested to be beneficial for a variety of health outcomes in adults, including coronary heart disease,[3] stroke,[4] type 2 diabetes,[5] depression,[6] and inflammatory bowel disease,[7] although the data are not univocal.[8] Increasing evidence supports the anticancer effect of EPA.[9]

Besides EPA, AMR101 capsules also contain the following inactive ingredients: tocopherol, gelatin, glycerin, maltitol, sorbitol, and purified water.

2.4 Rationale

Colorectal cancer is the third leading cause of cancer-related deaths in the US.[1] Substantial data support the benefit of EPA for colorectal cancer prevention and treatment,[10] which may be mainly due to the immunomodulatory and anti-inflammatory activity of EPA.[11] Increasing data support that gut microbes are pivotal in integrating dietary cues with host immunity, and that disturbances in the gut microbiota may promote colorectal cancer by breakdown of intestinal immune homeostasis. Dietary fat composition is a major driver of the gut microbial community structure and EPA has been shown to enrich the gut bacteria that possess immunoprotective activities. These data together our hypothesis that EPA modulates the gut microbiota to preserve colorectalic immune homeostasis and suppress colorectal cancer. In the current study, we propose to experimentally test this hypothesis by assessing the effect of EPA supplement on the gut microbiota - host immune interaction.

2.5 Correlative Studies Background

2.5.1 EPA and colorectal cancer

Several lines of evidence support the benefit of EPA for colorectal cancer prevention and treatment.[10] In a meta-analysis of 3 prospective cohort studies and 8 case-control studies, high levels of EPA are associated with lower risk of colorectal cancer in a dose-dependent manner.[12] In an RCT of patients with familial adenomatous polyposis, EPA supplement of 2 g daily for 6 months reduces the number and size of polyps by 20-30%, an effect comparable to that by the FDA-approved prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX-2) inhibitors.[13]

2.5.2 Immune mechanisms underlying the anticancer effect of EPA

The anticancer effect of EPA may be related to its multifaceted immunomodulatory activity mediated by alterations in lipid raft structure and changes in fatty acid composition of cell membranes.[14] These changes alter gene expression and the activities of several key immune signaling pathways, such as PTGS2 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and modify downstream metabolite production, including a decrease in proinflammatory eicosanoids (e.g., prostaglandin E_2; PGE_2) and an increase in pro-resolving lipid mediators (e.g., resolvin and protectin).[14-19] Of note, PGE_2 has been suggested as one of the critical pathways that govern tumor-mediated immune dysfunction and contributes to a shift in the tumor microenvironment from anti-tumor responses to immunosuppressive responses.[20] On the other hand, increasing data suggest that EPA may revert the immune suppression mediated by Treg cells.[21,22] Treatment of Tregs with EPA decreases the immune suppressive activity by downregulating inhibitory precursors.[21] In experimental colorectal cancer models, fish oil exerts its antitumor effect by altering Tregs and their cytokine repertoire (e.g., reduction of IL-10 expression).[22] In an RCT of 60 patients with solid tumors, supplementation of EPA increased the

ratio of T-helper cells to T-suppressor cells in the peripheral blood of the malnourished patients, and prolonged the survival of all the patients.[23]

Our recent observational study indicated that high EPA intake was associated with lower risk of colorectal cancer that is infiltrated with a high density of FOXP3$^+$ T cells, but not tumors with low densities of FOXP3$^+$ T cells.[24] Similar benefit was observed when EPA and DHA were examined separately. Because FOXP3 is a prerequisite transcription factor for the immunosuppressive function of Treg cells, we further examined the *in vitro* effect of EPA on the suppressive activity of colorectal Treg cells against proliferation of T effector (Teff) cells.[24] Consistent with our human findings, we found that EPA treatment decreased the suppressive activity of FOXP3$^+$ Treg cells in a dose-dependent manner. Of note, the suppressive effect of EPA leveled off at around 50 uM, which is within the normal range of EPA in human plasma samples, highlighting the human relevance of these experimental findings.

2.5.3 Gut microbiome in relation to EPA and colorectal cancer

Increasing data suggest that the gut microbiota play an important role in colorectal cancer development and progression, possibly by influencing both local and systemic immune response.[25] Among all potential shaping factors, diet is particularly important for the gut microbial composition and function. Compared to other types of fat, EPA has been associated with enhanced intestinal microbiota diversity and EPA-rich diet ameliorates omega-6 fatty acids- or antibiotics-induced dysbiosis.[26-29] Animal studies indicate that EPA supplements increase the abundance of anti-inflammatory bacteria, such as lactic acid-producing bacteria (mainly *Lactobacillus* and *Bifidobacterium*), and decrease the abundance of immunosuppressive and pro-inflammatory bacteria, such as *F. nucleatum*, *Escherichia coli*, and *Akkermansia*. Administration of *Bifidobacterium* has been shown to improve patients' response to cancer immunotherapy,[30] whereas increased abundance of *F. nucleatum* has been linked to dampened antitumor immune response and poor prognosis of colorectal cancer.[31-33] Therefore, it is possible that the beneficial effect of EPA for colorectal cancer may be mediated by modulation of the gut microbial composition and function that in turn shape the host immune response.

3. PARTICIPANT SELECTION

Patients that meet the eligibility criteria will be identified through investigators during their routine clinical practice, supplemented by a periodic query of the MGH endoscopy (Provation) and pathology database. Patients also may be identified using Natural Language Processing clinical software that queries medical records. The MGH gastroenterologist that performed the procedure or saw the patient for outpatient care as follow-up to the patient's colonoscopy procedure will be notified and asked for permission to contact their patient. We plan to recruit patients who have undergone a colonoscopy by an MGH gastroenterologist either in the ambulatory clinic or for an endoscopic procedure. In some cases, these physicians may not be members of the study team. However, non-study physicians will not perform study-related procedures.

For appropriate patients, we will identify their preference for contact about research studies (Research Opportunities Direct to You [RODY] status) through queries of the Research Patient Data Registry (RPDR). Participants with a RODY YES response will be contacted by a letter (attached recruitment letter for RODY YES patients) from the study PI. Patients with a RODY

NO response or without RODY designation will be contacted by a different letter (attached recruitment letter for RODY NO patients). If the colonoscopy was performed at MGH, after an adenoma is resected from a patient at MGH, all gastroenterologists routinely contact their patients by mail with the results of their pathology. The recruitment letter will be included with the pathology results when possible, or it will be sent separately after the pathology report. If the colonoscopy and polypectomy were performed at another hospital, the MGH gastroenterologist will confirm the prior diagnosis of an adenoma through review of pathology reports. Their treating MGH gastroenterologist, regardless of whether the physician is study personnel, will sign the letter. If no response is received to this recruitment letter within two weeks (10 business days) a follow-up phone call or email will be placed by a research assistant/study coordinator (phone script/sample email attached). In some cases, the treating gastroenterologist may approach the patient following the colonoscopy that identified a polyp if he/she feels the patient would be a good candidate for the study. In these cases, the patients may not receive a recruitment letter as the primary method of contact. Instead, they will be directly contacted by phone or email (see call script/sample email) from a member of the research team prior to mailing the letter (if requested). Potential participants will have additional opportunities to ask any questions by contacting an investigator or a trained research coordinator. If the participant is interested in participating, eligibility will be assessed by the study coordinator (see script) and confirmed by the investigator through an additional check of the patients' medical record (see "Investigator Representation for Review of Protected Health Information Preparatory to Research" form). If eligibility is confirmed, the patient will be scheduled for their initial study visit and registered with OnCore by the study staff. The informed consent form will be mailed to the patient prior to the initial visit so that he or she can read it in detail, if requested by the participant. Eligible participants will provide written informed consent at the baseline visit prior to participation in the study. The consenting process will be performed by one of the trial's clinical investigators.

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Underwent screening or surveillance colonoscopy with removal of at least one adenoma;

3.1.2 Age 18-80 years.

This study will only include adult participants because colorectal carcinogenesis in children is more likely to be related to a cancer predisposition syndrome with distinct biological mechanisms compared with sporadic colorectal cancer in adults. Patients over age 80 will not be enrolled since the benefits and risks of AMR101 over the age of 80 have not yet been well-characterized.

3.1.3 The effects of AMR101 on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

3.1.4 Subjects must be able and willing to follow study procedures and instructions.

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

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Currently using or have used any fish oil supplement at any dose more than once per week within the last month.

3.2.2 Regularly consuming more than three servings of fish per week.

3.2.3 History of allergic reactions attributed to fish or compounds of similar chemical or biologic composition to omega-3 fatty acid.

3.2.4 Diagnosis of inflammatory bowel disease, liver or kidney disease, bleeding diathesis

3.2.5 Any prior diagnosis of gastrointestinal cancer (including esophageal, small intestine, colon, pancreatic), or any diagnosis of other cancers (with the exception of non-melanoma skin) in which there has been any active treatment within the last three years.

3.2.6 Known diagnosis of Familial Adenomatous Polyposis (FAP) or Hereditary Non-Polyposis Colorectal Cancer (HNPCC, Lynch Syndrome).

3.2.7 Any adenoma that was not completely removed during previous colonoscopy.

3.2.8 Known bleeding tendency/condition (e.g. von Willebrand disease) or history of peptic ulcer or gastrointestinal bleed requiring hospitalization, endoscopic complications, or contraindication to colonoscopy.

3.2.9 Current use of anticoagulant therapies, including Heparin, Warfarin, Dalteparin sodium, Bivalirudin, Argatroban, Lepirudin, Heparin Sodium, Heparin/Dextrose, and an unwillingness or inability to discontinue anticoagulants.

3.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.11 Inability or unwillingness to abstain from non-protocol use of fish oil supplements or to provide blood, urine or stool samples or colon biopsies during the study.

3.2.12 Participants who are receiving any other investigational agents.

3.2.13 Inability or unwillingness to swallow pills.

3.2.14 Pregnant or breastfeeding.

The effects of AMR101 on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately. Similarly, lactating women are excluded from this study because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with AMR101. Consequently, breastfeeding should be discontinued if the mother is enrolled on the study.

3.2.15 Known positive test for human immunodeficiency virus (HIV), hepatitis C virus, or acute or chronic hepatitis B infection.

Participants with these infections are ineligible because they are at increased risk of significant complications in the perioperative period, and because fresh tissue from patients with these infections cannot be harvested for research purposes, per institutional policy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women and Minorities

Women and minorities will be eligible for this study without alteration in eligibility criteria. Enrollment of these underrepresented populations to this trial will be encouraged.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

The study staff will register the participant in OnCore.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

N/A.

4.4 Registration Process for Other Investigative Sites

N/A.

5. TREATMENT PLAN

5.1 Treatment Regimen

After study registration, eligible participants will be assigned unique study identification numbers. The investigator will contact the MGH Research Pharmacy for drug dispensation. The MGH Research Pharmacy will dispense study capsules, obtained from Amarin Pharma (Bedminster, NJ), containing 0.5-gram of AMR101. The assigned dosage will not change over the course of the study unless dose reduction is indicated (See Section 6). The first dose of the study medication will be given to patients after the initial flexible sigmoidoscopy. Participants will be expected to take 4 capsules orally, twice a day, until the return for their final visit (minimum 8 weeks, maximum 12 weeks). The final visit, 8-12 weeks from the baseline visit, will be scheduled during or before the baseline visit. The MGH Research Pharmacy will assign the participant three bottles of AMR101 each containing 240 capsules (12 weeks, 8 capsules/day) and participants will return any unused capsules and the bottle to the study staff at their final visit. The bottle will be labeled with their participant ID. The participant number must be recorded on the drug dispensation form and in the participants case report form (CRF). These numbers are unique to each participant and must not be re-assigned. Remaining capsules will be counted as a measure of compliance, the number recorded and then the remaining capsules will be immediately destroyed. Weekly calls will be used to monitor adherence and adverse events. Patients who initiate fish oil supplements during the study will be withdrawn and an exit visit performed.

Compliance will be assessed based on drug diary and pills counts, as well as blood measurement of EPA levels. Reported adverse events and potential risks are described in Section 7. No investigational or commercial agents or therapies may be administered with the intent to treat the participants. The principal investigator will review subjects' data periodically throughout the duration of the study for quality, validity and integrity assurance and for adherence to the IRB

approved protocol and will address any data-related issues that might arise.

5.2 Pre-Treatment Criteria

N/A

5.3 Agent Administration

5.3.1 AMR101

Participants will self-administer eight AMR101 capsules by mouth (4 capsules twice a day), and will be instructed to keep a drug diary. The dose should be taken with food and a full glass of water. If a daily dose is missed, the dose should be skipped. A dose is considered missed if more than 24 hours has elapsed since the prior dose.

5.4 Definition of Dose-Limiting Toxicity (DLT)

N/A

5.5 General Concomitant Medication and Supportive Care Guidelines

Throughout the study, treating investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate supportive care. However, participants may *not* receive:

- Other investigational agents;
- Anticoagulant therapies, including Heparin, Warfarin, Dalteparin sodium, Bivalirudin, Argatroban, Lepirudin, Heparin Sodium, Heparin/Dextrose, and other anticoagulant therapies;
- Any supplements that contain EPA.

All prescribed and non-prescription concomitant medications that are ingested, applied, or injected on an ongoing basis from signing of the informed consent, as well as changes in such concomitant medications, and any new concomitant medication taken while the participant is on study, should be recorded in the medical record. Concomitant medications for medically significant adverse events, which are ongoing at the end of study treatment and considered related to study treatment, should be followed until the adverse event is resolved or considered stable**.**

5.6 Criteria for Taking a Participant Off Protocol Therapy

Duration of study drug administration will be at minimum 8 weeks and no more than 12 weeks from the initial visit. Duration of administration will equal the number of days between initial and final visit. Study drug administration will continue until the final visit or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of study drug

- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or compliance requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Participant self-administers any additional non-study fish oil supplements.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the eCRF. Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Overall PI, Dr. Andrew Chan at (617) 726-3212 or ACHAN@mgh.harvard.edu

5.7 Duration of Follow Up

Participants will be monitored closely until they complete the study. Participants who have completed the study will be those that have returned for the final visit and returned all necessary study materials including unused capsules, pill bottle and questionnaires. Participants will be followed for one month by phone after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Participant receives any concomitant medication or treatment included in the list in 5.5 describing medications or treatments the participant may NOT receive
- Participant demonstrates an inability or unwillingness to comply with the study regimen and/or compliance requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the eCRF.

The study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose reduction will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose reduction. A copy of the CTCAE version 5.0can be downloaded from the CTEP website
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

When the study staff is informed of a GI-related adverse event, dose reduction may be considered, according to the following dose de-escalation schema.

Dose Level	AMR 101 Dose
Initial Dose	4 grams/day, for the full course of the intervention
Level -1	2 grams/day, for the rest of the intervention period

Criteria for treatment reduction and suggested guidelines for the management of GI toxicities related to AMR 101 are summarized below. These general guidelines may be modified at the discretion of the investigator based on best clinical judgment at that time, but the reason for these reductions must be documented in the medical record. Any GI toxicities related to AMR 101 should be managed according to standard medical practice. These dose reductions are guidance for clinicians for use of this standard therapy.

Participants who report GI-related adverse events including but not limited to the adverse events described in the tables below will be offered dose reduction as well as the option to withdraw from the study.

Nausea	Management/Next Dose for AMR 101
≤ Grade 1	Reduce to dose level -1
Grade 2	Reduce to dose level -1
Grade 3	Reduce to dose level -1
Grade 4	Reduce to dose level -1

Vomiting	Management/Next Dose for AMR 101
≤ Grade 1	Reduce to dose level -1
Grade 2	Reduce to dose level -1
Grade 3	Reduce to dose level -1

Vomiting	Management/Next Dose for AMR 101
Grade 4	Reduce to dose level -1

Diarrhea	Management/Next Dose for AMR 101
≤ Grade 1	Reduce to dose level -1
Grade 2	Reduce to dose level -1
Grade 3	Reduce to dose level -1
Grade 4	Reduce to dose level -1

Constipation	Management/Next Dose for AMR 101
≤ Grade 1	Reduce to dose level -1
Grade 2	Reduce to dose level -1
Grade 3	Reduce to dose level -1
Grade 4	Reduce to dose level -1

Stomach pain	Management/Next Dose for AMR 101
≤ Grade 1	Reduce to dose level -1
Grade 2	Reduce to dose level -1
Grade 3	Reduce to dose level -1
Grade 4	Reduce to dose level -1

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

AMR101 may produce a hypersensitivity reaction in individuals with known hypersensitivity to shellfish/fish. Because AMR101 contains ethyl esters of omega-3 fatty acid, patients taking other anticoagulants may be at risk to develop a prolonged bleeding time; the risk of active bleeding is unknown.

7.1.1 Adverse Events List(s)

7.1.1.1 Adverse Event List(s) for AMR101

AMR101 is usually well tolerated. According to Micromedex, arthralgia was one of the most commonly reported adverse events associated with the use of AMR101 occurring in 2.3% (14/622) of patients compared with 1% (3/109) in placebo in pooled data from 2 double-blind clinical trials, which included patients with triglyceride values of 200 to 2000 mg/dL. An additional adverse effect from clinical studies was oropharyngeal pain. Mild to moderate GI-related adverse events

that include stomach pain, nausea, loss of appetite, constipation, or diarrhea have also been reported by participants on this and other studies.[13]

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
N/A					

7.4 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 AMR101

8.1.1 Description

The active ingredient of AMR101 is EPA ethyl ester (icosapent ethyl). The molecular formula is $C_{22}H_{34}O_2$, and its molecular weight is 330.51. Its structural formula is:

Icosapent ethyl is de-esterified during absorption into the active metabolite EPA. EPA is absorbed in the small interesting through the thoracic duct lymphatic system into the circulation.

The Tmax of EPA was about 5 hours following oral administration of icosapent ethyl. EPA is metabolized in the liver by beta-oxidation into acetyl Coenzyme A, which is converted into energy via the Krebs cycle. Cytochrome P450-mediated metabolism is a minor pathway of elimination. The total plasma clearance of EPA at steady state is 684 mL/hr. The elimination half-life of EPA is about 89 hours.

8.1.2 Form

The trial AMR101 (icosapent ethyl) capsules are manufactured and supplied by Amarin Pharma (Bedminster, NJ) as 0.5-gram amber-colored soft-gelatin capsules. The capsules will be ordered by and shipped to the MGH Research Pharmacy where they will be packaged in three pill bottles each containing 240 capsules, which are sufficient for 12-week supply where 8 capsules are taken daily.

8.1.3 Storage and Stability

AMR101 capsules should be stored at room temperature. When stored under this condition, it has been indicated that the product is stable and non-reactive.

8.1.4 Compatibility

N/A

8.1.5 Handling

N/A

8.1.6 Availability

AMR101 capsules are manufactured by Amarin Pharma, Inc. and commercially available in the US as VASCEPA (icosapent ethyl). Ordering and packaging of AMR101 will be handled by the MGH Research Pharmacy free of charge to the participants.

8.1.7 Preparation

AMR101 will be supplied by Amarin Pharma and packaged by the MGH Research Pharmacy in identical three bottles with a count of 240 capsules in each bottle.

8.1.8 Administration

AMR101 will be administered orally with 4 capsules twice a day at approximately the same times of day.

8.1.9 Ordering

AMR101 is a commercially available agent obtained from Amarin Pharma. It is the same agent

as that used for the EPA for Metastasis Trial 2 (EMT2), a randomized trial of omega-3 fatty acid among 448 patients with colorectal cancer liver metastases. AMR101 will be supplied by Amarin Pharma and stored in the MGH research pharmacy per institutional guidelines. The research pharmacist will be responsible for dispensing the study medication to participants.

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator (i.e., research pharmacist), must maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

At the end of the study, unused supplies of AMR101 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

9.1.1 Background

We hypothesize that EPA modulates the gut microbiota to preserve gut immune homeostasis and suppress colorectal cancer. The proposed study will assess the influence of EPA treatment on the tissue fatty acid composition, the gut microbiome and metabolome, gene expression in colorectal cancer tissue, and urinary PGE-M. In doing so, we aim to provide causality for the anticancer mechanisms of EPA established by other studies. We and others have put forth considerable efforts to determine measurable biomarkers implicated in the microbial and immune mechanisms linking EPA and colorectal cancer. A discussion of these biomarkers and their significance is provided below:

9.1.1.1 EPA in colorectal tissue

Increasing data suggest that the anticancer effect of EPA relates to its incorporation into the cell membrane phospholipids and alteration of the structure and function of the lipid rafts.[34,35] High intake of EPA results in increased incorporation of EPA and replacement of arachidonic acid (AA) in membrane phospholipids. This alteration has two consequences: 1) suppressed biosynthesis of AA-derived inflammatory eicosanoids in favor of EPA-derived prostanoids; 2) enhanced competition with omega-6 fatty acids for cyclooxygenases that leads to higher production of anti-inflammatory lipid mediators. Given the critical role of inflammation in colorectal cancer development and progression, EPA-induced shift from pro-inflammatory to anti-inflammatory metabolite profiles may protect against tumorigenesis.[36] Moreover, EPA can modify membrane lipid rafts, which are lipidic microdomains consisting mainly of sphingomyelin, cholesterol, and glycerophospholipids that hold many signaling proteins.[37] These

microdomains are involved in a myriad of cellular functions, such as signal transduction, membrane trafficking, neuronal differentiation, and entry of pathogens and toxins into the cells. EPA has been shown to target a number of lipid raft proteins, thereby modulating the signaling pathways critical for tumor growth and metastasis. In particular, given the role of lipid rafts as platforms for cell activation in the immune system,[38] animal and *in vitro* studies indicate that EPA can influence intracellular signaling in T lymphocytes by modification of the architecture of lipid rafts.[39-41] However, the relevance of these findings to the immunomodulatory effect of EPA in human cancer remains to be determined. Therefore, to establish the mechanistic foundation for testing downstream immune modulation, we will assess the effect of AMR101 treatment on the fatty acid composition in colorectal tissues as the primary endpoint.

9.1.1.2 EPA and the gut microbiome

Increasing data indicate that the gut microbiota mediates the health effects of dietary components by shaping the intestinal and systemic immune response.[42-45] As summarized in Table 2, both animal and human studies support the role of EPA in influencing the gut microbial composition and function. Compared to other types of fat, EPA have been associated with enhanced intestinal microbiota diversity and EPA-rich diet ameliorates ω-6 PUFAs- or antibiotics-induced dysbiosis.[26-29] Animal studies indicate that EPA supplements increase the abundance of anti-inflammatory bacteria, such as lactic acid-producing bacteria (mainly *Lactobacillus* and *Bifidobacteria*), and decrease the abundance of immunosuppressive and pro-inflammatory bacteria, such as *F. nucleatum*, *Escherichia coli*, and *Akkermansia*. Likewise, changes in the gut microbiota have been linked to CRC, with an enrichment of *F. nucleatum*, *Escherichia coli*, and *Enterobacteriaceae*; and a depletion of *Bifidobacterium* and *Streptococcus* in CRC patients.[46-54] Microbes may affect carcinogenesis by influencing DNA damage repair,[55-57] cell proliferation,[58,59] host metabolism,[60-62] and immune responses.[31,63-65] Moreover, recent data show that commensal bacteria, such as *Bifidobacteria*, may modulate tumor immune microenvironment and improve the efficiency of CTLA-4 or PD-L1 blockade.[30,66,67]

Table 2. Summary of EPA-related gut microbial changes in human and animal models and their link to host immunity and CRC

Bacteria	Mechanistic link to host immunity and CRC
Increased by EPA	
Lactobacillus[27,68]	Probiotics with anti-inflammatory and anticancer activity[16,69,70]
Bifidobacteria[12, 16, 17]	Probiotics with anti-inflammatory activity;[71-74] improve immunotherapy efficacy[30]
Butyrivibrio[75]	Produce short-chain fatty acids that have immunomodulatory and anticancer effect[60,76,77]
Decreased by EPA	
F. nucleatum[78]	Generate a tumor-permissive microenvironment and promote CRC[31,47,59,79]
Escherichia coli[78,80]	Some pathogenic strains are associated with inflammatory bowel disease and CRC[81,82]
Akkermansia[75,83]	Impair gut barrier integrity, induce inflammation and CRC[84,85]

9.1.1.3 EPA, microbiota, and stool metabolomics

Anaerobic gut bacteria, including some species of *Lactobacillus*, have been implicated in polyunsaturated fatty acid saturation, a detoxifying mechanism that transforms bacterial growth-inhibiting polyunsaturated fatty acids into less toxic fatty acids, such as hydroxyl fatty acids.[86-92] These microbial metabolites may help preserve intestinal barrier integrity, reduce oxidative stress, and lower inflammation.[93,94] Interestingly, *Lactobacillus* is selectively enriched by EPA (Table 2), suggesting a reciprocal mechanism by which gut microbes adapt to host dietary change with functional consequences for host health. Moreover, a cross-feeding effect has been noted between human *Bifidobacterium*, which produces lactate and acetate, and the butyrate-producing species, such as *Eubacterium rectale*, which convert lactate to butyrate.[95-97] Butyrate, a short-chain fatty acid, has potent anti-inflammatory[98] and potential anti-CRC properties.[61,99] In addition to microbial products, host cell metabolites can be shed into the gut lumen, including a series of EPA-derived eicosanoids (e.g., PGE_3, lipoxin, leukotriene [LT], thromboxane [TX], and hydroxyeicosatetraenoic acid [HETE]) and lipid mediators (including resolvin, protectin, and maresin) that possess anti-inflammatory activities and may mediate the anticancer effect of EPA.[100-105] Of note, some lipid mediators, such as resolvin, may alter the gut microbial composition by stimulating epithelial expression of microbial regulators, such as intestinal alkaline phosphatase (IAP),[106] a transmissible factor with lipopolysaccharides (LPS)-detoxifying activities.[78] Thus, stool metabolomics provides a critical tool to examine the products and functionality of the diet – gut microbiota – host interaction.

9.1.1.4 Gene expression profile – immune and inflammatory pathways

Pro-inflammatory cytokines such as TNF-α and IL-6 are central players in colon cancer through activation of the pro-inflammatory transcription factors NF-κB and STAT3.[107,108] EPA have multifaceted roles in immune regulation.[15] High EPA intake has been associated with lower circulating inflammatory markers (e.g., TNFα and IL6).[109,110] In a pilot study of women at high risk for breast cancer, EPA supplements decreased breast tissue expression of chemokine C-C motif chemokine ligand 2 (CCL2),[111] a cancer-promoting cytokine.[112] As described above, our prior study indicated that EPA might protect against colon cancer through downregulation of the immunosuppressive Treg cells.[24] However, despite these data, human data linking EPA to colorectal cancer-related immune and inflammatory pathways are lacking. Therefore, to fill this knowledge gap and extend our prior findings, we will examine the effect of EPA treatment on the gene expression profile of colorectal tissue. We hypothesize that EPA treatment reduces the gene expression of inflammatory cytokines and chemokines (e.g., TNFα, IL6 and CCL2).

9.1.1.5. Urinary PGE-M

A mechanism underlying EPA's immunomodulatory effect may be related to reduced synthesis of prostaglandin E_2 (PGE_2) by tumor epithelial cells and/or tumoral stromal cells.[35] PGE_2 promotes tumor cell survival and is higher in cancer cells than in normal cells.[113] PGE_2 contributes to a shift in the tumor microenvironment from anti-tumor to tumor-permissive.[20] PGE_2 may promote immunosuppression by inducing FOXP3 expression in naïve T cells[114-116] and inducing differentiation of myeloid-derived suppressor cells (MDSCs). Both animal[17,18,117,118] and human RCTs[14,119] indicate that EPA treatment reduces circulating and colonic tissue PGE_2 levels. A clinical trial showed that EPA treatment *reduced* urinary prostaglandin metabolites (PGE-M), a marker of systemic PGE_2 production, by 27.6% compared with a 27.9% *increase* in the placebo group.[120]

9.1.2 Study design

Using our gastroenterology practice population, we will implement a prospective clinical trial to measure the effect of EPA on tissue fatty acid composition, the gut microbiome and metabolome, gene expression profile, and urinary PGE-M. At MGH, we will target 80 individuals over a two year period. Eligible patients will have had a previous colonoscopy at MGH and had at least one adenoma removed during the previous procedure. Eligible patients must meet all eligibility requirements and none of the exclusion criteria as outlined in Section 3: Eligibility criteria.

9.1.2.1 Prior to the initial visit

No bowel preparation will be necessary for the procedure since the sigmoidoscope will only be advanced to the distal sigmoid colon.

9.1.2.2 Initial (baseline) Visit

At the initial visit, the study physician will obtain written, informed consent for the study as well as a standard clinical consent for a flexible sigmoidoscopy. Following OnCore registration, participants will be asked to complete with a study coordinator a brief lifestyle questionnaire as well as a nutritional survey on a research iPad via VioScreen™, a validated web-based dietary assessment tool developed by Viocare, Inc (See appendix for VioScreen security overview, the assessed food list, and sample screens).[121] Patients will undergo measurements of height, weight, waist and hip circumference and provide a blood and urine specimen. A study gastroenterologist will then perform a flexible sigmoidoscopy, advancing to the level of the distal sigmoid colon. No more than a total of 24 mucosal biopsies will be taken from the rectum and sigmoid and immediately placed in collection tubes. In the MGH GI Unit, we routinely perform endoscopic biopsies regardless of concurrent AMR101 use, a practice consistent with recommended guidelines.[122] A study of the safety of multiple endoscopic biopsies in research subjects from a National Institutes of Health series found that performing large numbers of endoscopic biopsies (mean number = 38.2 ± 15.6 biopsies per procedure) are "well tolerated and appears to have no more than minimal risk without appreciably increasing the risk of otherwise routine endoscopy."[123] Furthermore, there is no statistically significant association between risk of complications and the number of biopsies, type of procedure (flexible sigmoidoscopy vs. colonoscopy), colonic location of biopsy, operator, or polypectomy.[123] The number of biopsies is also consistent with our existing study protocols and has never been associated with any adverse events (See: "Endoscopy Protocol: Tissue Specific Immunity Against HIV-1"; PI: Kwon, Ragon Institute). We have estimated that 24 biopsies will be necessary to complete the proposed analyses. The RNAseq experiments proposed require a minimum of 4 pinch biopsies for each sample. The additional biopsies will be required for validation experiments based on the results of genomic and metagenomic analyses using targeted sequencing approaches. Due to the small number of cells obtained from pinch biopsies and the input requirements for these assays, we may use tissue culture techniques to expand cell populations. This will allow us to perform these comprehensive analyses without additional burden to the participants (i.e. increasing tissue yields by using larger or more biopsies). During flexible sigmoidoscopy, stool will be aspirated through the endoscope or using a Roth net and snap-frozen. . Following the visit, questionnaire data will be transferred to the Partners secure

REDCap electronic database system. Also, REDCap will be used to store data from the study including endoscopy data, medical history information, sample collection, and more.

Participants will be provided with $100 (US) compensation and free parking for up to 4 hours for this initial visit. The final visit will be scheduled with the patient at his or her convenience.

9.1.2.3 Final Visit

Participants will return for a second and final visit between 8 and 12 weeks from their initial visit. An abbreviated lifestyle questionnaire and a full nutritional survey via VioScreen will be administered to update information from the baseline visit. Participants will also provide blood and urine samples and undergo a second flexible sigmoidoscopy procedure with mucosal biopsies. A bowel preparation will not be necessary for the follow-up flexible sigmoidoscopy. Up to 24 mucosal biopsies will be taken, as described for the baseline visit. A stool specimen will also be collected, as previously described.

Participants will be provided with an additional $100 (US) compensation and free parking for up to 4 hours for this final visit. Total compensation will equal $200 (US) for successful completion with the study.

9.1.3 Collection and Processing of Biospecimens

Immediately following each flexible sigmoidoscopy, urine specimens will be aliquoted and blood specimens will be centrifuged into plasma and buffy coat. Stool specimens will be stored in a cryovial and immediately frozen. Colon biopsies will be immediately frozen for future RNA-seq analysis. Yield from these procedures typically exceeds requirements for RNA-seq. Thus, any excess colon tissue will be banked for future studies. All aliquots of stool, urine, plasma, buffy coat, and tissue will be frozen at -80°C until analysis. We may access FFPE blocks of polyps/adenomas removed during the participant's qualifying colonoscopy with accompanying pathology reports to correlate our findings with tissue-specific markers in the original adenoma.

Blood samples will be collected in two-6 mL EDTA/purple top tubes at each visit, stored on ice until processing and freezing (within 3 hours of collection). Immediately upon receipt, urine specimens will be aliquoted into 1.2 mL aliquots and stool samples will be homogenized, aliquoted into 100 mg aliquots, and frozen for long-term storage. Fresh tissue will be collected in the pathology laboratory immediately following resection, transported on wet ice and immediately flash frozen in liquid nitrogen. We may access archival formalin-fixed, paraffin-embedded (FFPE) blocks of any biopsies or tissue specimens collected during the participant's procedures that led to their diagnosis of colon cancer if the diagnostic procedures occurred at MGH. If the diagnostic procedures occurred at an external site, formal requests in writing will be made to the corresponding pathology department to release and ship tissue to study staff. We will request whole tissue blocks or 10-15 unstained slides. All samples will be transported to the laboratory of Dr. Andrew Chan and labeled with a study identification number, the date, and the type of specimen. Dr. Chan's laboratory will supervise all storage of coded specimens. All endpoint analysis will occur at the end of the trial as described for each endpoint below:

9.1.4 Tissue EPA Analysis (primary endpoint)

Fatty acid will be extracted from the biopsy tissues using the established protocol.[124] We will use gas chromatography-mass spectrometry to measure EPA content in to assess the incorporation of AMR101 into local tissue.

9.1.4.1 Collection of Specimens

Mucosal biopsy samples will be collected at both the initial and final visit.

9.1.4.2 Handling of Specimens

Mucosal biopsy specimens will be stored frozen and sent in batches to the Harvard T.H. Chan School of Public Health (HSPH) for fatty acid analysis.

9.1.4.3 Shipping of Specimens

Mucosal biopsy specimens will be sent on dry ice to Dr. Frank Sacks at the following address:

Dr. Frank Sacks
Department of Nutrition
Harvard T.H. Chan School of Public Health
665 Huntington Avenue
Boston, Massachusetts, 02115

9.1.4.4 Site performing study

Dr. Sacks Laboratory at HSPH.

9.1.5 Gut microbiome analysis

We will perform metagenomic and metatranscriptomic sequencing of microbial DNA and RNA on pre- and post-treatment stool samples to examine the biomolecular mechanisms by which gut microbial activity may be altered or respond to AMR101 treatment. Stool samples will be sent, coded, to the Broad Institute for processing and analysis.

9.1.5.1 Collection of Biospecimens

Stool will be collected using a Roth net or aspirated through the endoscope during flexible sigmoidoscopy at both the initial and final visit.

9.1.5.2 Handling of Biospecimens

An aliquot (~200 mg) of stool will be put in tubes provided by the Broad Institute and sent back for processing and analysis. The remaining stool will be frozen in a 15 mL conical tube at -80°C.

9.1.5.3 Shipping of Specimens

Stool specimens will be shipped on dry ice overnight to:

Broad Institute
Attn: BSP platform
301 Binney Street Lab 5076
Cambridge, MA 02142
617-714-8952

9.1.5.4 Site Performing Study

The Broad Institute

9.1.6 Stool metabolomics analysis

We will perform non-targeted global metabolomics and lipidomics analysis on pre- and post-treatment stool samples to examine the changes in the stool metabolite profile. Stool samples will be sent, coded, to Metabolon, Inc for processing and analysis. The global metabolomics analysis uses ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) and GC/MS platforms.[125-127] The lipidomics analysis uses the TrueMass™ Complex Lipid Panel that can provide data of both quantitative composition and complete speciation for over 1,110 individual lipid species.[128]

9.1.6.1 Collection of Biospecimens

As described in 9.1.5.1.

9.1.6.2 Handling of Biospecimens

An aliquot (~200 mg) of stool will be put in tubes provided by the Metabolon, Inc and sent back for processing and analysis. The remaining stool will be frozen in a 15 mL conical tube at -80°C.

9.1.6.3 Shipping of Specimens

Stool specimens will be shipped on dry ice overnight to:

Metabolon, Inc
617 Davis Drive, Suite 100
Morrisville, NC 27560
Phone: 919.572.1711
Fax: 919.572.1721

9.1.6.4 Site Performing Study

Metabolon, Inc

9.1.7 Red Blood Cell EPA measurement

EPA will be extracted from the baseline and post-treatment blood samples using the established protocol[129] and measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess circulating EPA levels. Red blood cell (RBC) membrane EPA content is widely accepted as the surrogate biomarker of EPA content in other tissues, including colon.[129-131]

9.1.7.1 Collection of Biospecimens

Blood samples will be collected at both the initial and final visits.

9.1.7.2 Handling of Biospecimens

Two blood samples in 6 mL EDTA/purple top tubes will be collected at each visit for a total of 24 mLs and stored on ice until processing.

9.1.7.3 Processing of Biospecimens

Blood will be spun in a centrifuge at 2000 x g for 20 minutes at 4°C within three hours of venipuncture (or in accordance with manufacturer instructions). After centrifugation, isolated red blood cells will be stored at -80°C until fatty acid extraction, buffy coat and plasma will be aliquoted and stored at -80ºC for future research purposes.

9.1.7.4 Shipping of Specimens

Blood samples will be processed and stored in the laboratory of Dr. Andrew Chan at MGH. A 1.2 mL aliquot of red blood cells will be shipped on dry ice overnight to Dr. Frank Sacks at the following address:

Dr. Frank Sacks
Department of Nutrition
Harvard T.H. Chan School of Public Health
665 Huntington Avenue
Boston, Massachusetts, 02115

9.1.7.5 Site Performing Study

Sacks Laboratory, HSPH

9.1.8 RNA sequencing of tumor tissue

We will perform RNA-seq analysis to profile gene expression in the mucosal tissue collected before and after AMR101 treatment using our validated platform (Illumina Transcriptome Capture protocol) at the Broad Institute.

9.1.8.1 Collection of Biospecimens

As described in 9.1.4.1.

9.1.8.2 Handling of Biospecimens

As described in 9.1.4.2.

9.1.8.3 Processing of Biospecimens

Tissue will be flash-frozen in liquid nitrogen within 30 minutes of collection and stored at -80ºC for future research purposes. At the conclusion of the trial, one portion will be processed to extract RNA using the RNAeasy micro kit. Total RNA or converted cDNA libraries will be stored at -80ºC prior to sending for sequencing.

9.1.8.4 Shipping of Specimens

RNA/cDNA samples will be sent on ice to the Broad Institute for RNA-seq analysis at the following address:

Broad Genomics
Attn: Genomics Platform
320 Charles St.
Cambridge, MA, 02141.

9.1.8.5 Site Performing Study

The Broad Institute

9.1.9 Analysis of Urinary PGE-M

We will use mass spectroscopy to measure PGE-M in single batches of pre- and post-treatment urine in the Eicasonoid core laboratory of Dr. Ginger Milne at Vanderbilt University.

9.1.9.1 Collection of Biospecimens

Urine samples will be collected at both the initial and final visit.

9.1.9.2 Handling of Biospecimens

Samples will be placed in a refrigerator within 2 hours of collection, and transferred to -80°C within 4 days of collection. Urine will be split into 1.2 mL aliquots in eppendorf tubes prior to freezing. Samples will be stored at -80°C until analysis.

9.1.9.3 Shipping of Specimens

A 1.2 mL aliquot of each urine sample will be shipped on dry ice overnight to Dr. Ginger Milne at the following address:

Attn: Ginger L. Milne, Ph.D.
Vanderbilt University Medical Center
561 Preston Research Building
Nashville, TN 37232-6602 USA

9.1.9.4 Site Performing Study

Eicasonoid Core Laboratory (Ginger Milne), Vanderbilt University

9.2 Laboratory Correlative Studies

N/A

9.3 Special Studies

N/A

10. STUDY CALENDAR

	Initial Visit	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Final Visit[a]
AMR101 Daily		Taken twice daily until final visit												Return unused drugs
Informed consent	X													
Demographics	X													X
Lifestyle Questionnaire	X													X
Nutritional Survey	X													X
Height	X													
Weight	X													
Waist & Hip Circumference	X													

Flexible Sigmoidoscopy	X													X
Tissue Biopsy Specimens Collected	X													X
Blood Samples	X													X
Urine Samples	X													X
Stool Samples	X													X
Drug Diary	X	Used daily until final visit												X
Drug Compliance Calls[b]		X	X	X	X	X	X	X	X	X	X	X	X	
Adverse event evaluation[b]		X	X	X	X	X	X	X	X	X	X	X	X	X

a: Final visit will be scheduled during the initial visit and will occur a minimum of 8 weeks and maximum of 12 weeks after the initial visit.
b: Once weekly, participants will be contacted by phone to monitor adherence to drug administration and check for adverse events.

11. MEASUREMENT OF EFFECT

This study only uses laboratory-based endpoints to measure the effect of AMR101 treatment. There are no clinically observable metrics that will be used as a primary or secondary endpoint or primary or secondary effect measure in this study. The primary efficacy endpoint is EPA levels in mucosal tissue. A detailed discussion of laboratory endpoints is included in section 9.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Primary Investigator and the study team will collect, manage, and perform quality checks on the data for this study using RedCap.

12.1.2 Responsibility for Data Submission

N/A

12.2 Data Safety Monitoring

The Primary Investigator and the study team will regularly review and monitor toxicity and accrual data from this study. The study team is composed of GI physicians, study coordinators, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the IRB.

The Primary Investigator will review study protocol periodically throughout the duration of the study for quality, validity and integrity assurance. Information to be provided to the Primary Investigator may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

N/A

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

This study is a single-arm clinical trial that will measure the effects of EPA treatment on specific biomarkers related to EPA incorporation, gut microbiota, gut metabolome, and immune response. In doing so, we aim to provide causality for the microbial and immune mechanisms that potentially mediate the beneficial effect of EPA for colorectal cancer prevention. We and others have put forth considerable effort to determine measurable biomarkers implicated in colorectal carcinogenesis. A comprehensive discussion of these biomarkers and their significance is provided in Section 9.

We will recruit 80 participants who recently underwent removal of their adenomatous polyps at MGH to conduct a single-arm trial of EPA (AMR101, 4 g/day) treatment for 2 months. The rationale for selection of this EPA preparation is because it 1) is FDA approved; 2) has good comparability across production lots; 3) has been previously used in several trials of EPA.[111,132] The rationale for selection of this dose is because it is 1) the prescription dose approved by FDA; 2) consistent with other RCTs of EPA in colorectal neoplasia;[13,120,133] and 3) can provide the best balance of efficacy and safety.

13.1 Study Design/Endpoints

This study is a single-arm trial of EPA treatment in patients with a history of colorectal adenoma.

The single-arm design is chosen for the following reasons: 1) Based on our prior experience, short-term dietary supplementation is unlikely to lead to substantial changes in lifestyle. 2) Any major lifestyle changes will be documented by the detailed lifestyle questionnaires and nutritional surveys administered at both the baseline (pre-intervention) and final (post-intervention) visits to allow for statistical adjustment in the analysis. Moreover, as described in the protocol, we will measure EPA content in red blood cells to further assess compliance and changes in omega-3 fatty acid intake. 3) Given the high dose (4 g/day) of highly purified and specially formulated omega-3 fatty acid used in the study (AMR101), even modest changes in lifestyle is unlikely to introduce significant confounding on the intervention effect of interest. For example, one serving (3 oz.) of Atlantic salmon only contains 1-2g of marine omega-3 fatty acids, and individuals have to consume at least 3 servings of salmon per day to achieve the intervention dose used in the study. 4) Given the fishy odor and oily appearance of the study drug, our MGH Research Pharmacy is unable to provide a visually identical, blinded placebo capsule for the study drug. Therefore, given all of these considerations as well as the biomarker-based nature of the study, we believe that the single-arm design is the best use of the limited resources for the study.

The primary aim is to assess the effect of AMR101 treatment on our primary efficacy endpoint, mucosal tissue EPA. A detailed discussion of the background of this biomarker is provided in Section 9.1.1.1. Briefly, we will determine the effect of AMR101 at standard dose on mucosal tissue EPA content through pre- and post-treatment comparison. The rationale for selection of this primary endpoint is to specifically test the effect of EPA on colonic tissue, which will build the mechanistic foundation for testing downstream endpoints within the local environment. Additional endpoints will include the gut microbiome, metabolome, urinary PGE-M and gene expression profile of mucosal tissue. Our study design for each of these endpoints is comprehensively discussed in Section 9. Statistical considerations relating to these endpoints are provided in the following sections.

13.2 Sample Size, Accrual Rate and Study Duration

Patients will be accrued in one stage with no early stopping rules. The total sample size is 80 patients and we expect accrual to be 6-7 participants per month and complete the enrollment process within one year. This accrual rate is based upon accruals under our recently completed IRB-approved protocol (Protocol # 14-496; Dr. Chan, PI) for a similar trial of aspirin in patients with history of colorectal adenoma. Each patient will be followed for at minimum 8 weeks and at maximum 12 weeks. This total sample size accounts for participant drop out, as endoscopy studies typical experience a drop-out rate of approximately 10%.

The sample size is based upon the primary efficacy endpoint of M3PUFA supplement at 4 g/d increasing mucosal MO3PUFA composition from pre- to post-treatment period by a mean of 3.0%, according to previous studies.[13,134] The null hypothesis is H_0: $\Delta_{post} - \Delta_{pre} = 0$ versus H_A: $\Delta_{post} - \Delta_{pre} \neq 0$, where Δ_{pre} and Δ_{post} are the measurement of mucosal MO3PUFA composition before and after the intervention, respectively. Based on prior studies, we assume the standard deviation (SD) of 9.0% for the paired difference in MO3PUFA measurement. With 80% power and the significance level of 0.05 in a two-sided paired t-test, we will require 73 participants to detect the mean change of 3.0% in MO3PUFA level from pre- to post-treatment period. To account for possible drop out

at 10%, we plan to enroll 80 patients.

Our accrual target rates for ethnic and racial minorities will likely be consistent with the rate at which we see these minorities in the gastroenterology practice at MGH. (See table on next page)

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	4	+	4	=	8
Not Hispanic or Latino	36	+	36	=	72
Ethnic Category: Total of all subjects	40	+	40	=	80
Racial Category					
American Indian or Alaskan Native	1	+	1	=	2
Asian	2	+	2	=	4
Black or African American	4	+	4	=	8
Native Hawaiian or other Pacific Islander	1	+	1	=	2
White	32	+	32	=	64
Racial Category: Total of all subjects	40	+	40	=	80

13.3 Stratification Factors

N/A

13.4 Interim Monitoring Plan

N/A

13.5 Analysis of Primary Endpoints

Analysis of mucosal tissue EPA (primary endpoint): Our primary biostatistician, Dr. Rosner, will supervise statistical analyses assessing the change in EPA levels in mucosal tissue after AMR101 treatment, using a paired t-test. In secondary analyses, we will use mixed linear regression models to adjust for duration of treatment and other major factors that may influence the gut microbiome, including aspirin use, antibiotic use, and major dietary and lifestyle patterns (e.g., fiber intake, physical activity, body mass index, smoking, and alcohol intake). A robust variance estimate will be used to eliminate any normality assumptions for the residuals.

13.6 Analysis of Secondary Endpoints

Microbiome analysis: Dr. Huttenhower will supervise the gut microbiome analysis. Stool specimens collected before and after treatment will undergo metagenomics and metatranscriptomics sequencing to assess the influence of EPA intervention on the gut microbial

composition and function. We will use the computational tools developed by Dr. Huttenhower, a leading authority in microbiome data analysis (http://huttenhower.sph.harvard.edu/biobakery). We will associate treatment status with the microbial operational taxonomic units (OTUs) determined by MetaPhlAn[135] using biomarker discovery tests: LEfSe (Linear Discriminant Analysis Effect Size),[136] a univariate class comparison method; and MaAsLin (Multivariate Analysis by Linear models),[137] a multivariate test that finds associations between clinical metadata and microbial abundance or function after adjusting for other covariates. Metatranscriptomic data will be analyzed using HUM[138]AnN,[139] a software package designed to efficiently and accurately define the abundance of microbial pathways within the community, and then linked to treatment status by linear mixed effects model. We expect to find that, after EPA treatment, study participants will have increased gut bacterial diversity, experience beneficial changes in microbiota composition, and demonstrate decreased transcriptional activity of inflammatory pathways that have been previously correlated with an increased risk of colon cancer.

Analysis of gene expression profiles: Dr. Huttenhower will supervise gene expression analysis. Tissues collected after treatment will undergo RNA-sequencing analysis to assess the influence of EPA intervention on gene expression profile of colon cancer. Samples are quantified and RNA quality is evaluated using Agilent's Bioanalyzer 2100. The percentage of fragments with a size greater than 200 nucleotides (DV_{200}) are calculated; samples with a DV_{200} score < 30% are included, as the likelihood of success is dramatically reduced. 100ng of RNA is used as the input for first strand cDNA synthesis using Superscript III reverse transcriptase (Life Technologies) and Illumina's TruSeq Stranded Total RNA Sample Prep Kit. Synthesis of the second strand of cDNA is followed by indexed adapter ligation. Subsequent PCR amplification enriches for adapted fragments. The amplified libraries are quantified using a Qubit assay (Life Technologies) and assessed for quality on an Agilent Technologies 2100 Bioanalyzer (DNA 1000 chip). 200ng of each cDNA library is combined into two 4-plex pools. Illumina's Coding Exome Oligos that target the exome are added and hybridized on a thermacycler. Following hybridization, streptavidin beads are used to capture probes that are hybridized. Two wash steps effectively remove any non-specifically bound products. These hybridization, capture, and wash steps are repeated to assure high specificity. A second round of amplification enriches the captured libraries. qPCR is performed on the pooled libraries and normalized to 2nM. The normalized, pooled libraries are loaded onto a HiSeq2500 for a target of 50 million 2x76bp paired reads per sample. RNA-Seq libraries will be analyzed using Tophat, Cufflinks[140] and other new statistical packages to identify consistent differences in the end-of-treatment changes in transcript levels between tissues before and after EPA treatment at pre-determined FDRs on the order of 0.01. Stringent tests for statistically significant differences in RNA levels between samples are built into the algorithms for RNA-seq analysis.

Analysis of stool metabolomics: We will use both the non-targeted Global Metabolomics and the TrueMass™ Complex Lipid Panel (Metabolon, Inc.) to examine the effect of intervention on the stool metabolite profile. The Global Metabolomics platform quickly and accurately identifies and quantitates more than 1,000 metabolites with less than 4.5% median process variability and is compatible with almost any sample type. The TrueMass™ Complex Lipid Panel provides absolute quantitation of 14 lipid classes, including principle phospholipid, sphingolipid and neutral lipid classes. It also provides molecular species concentrations and complete fatty acid composition of each lipid class, thereby offering unparalleled insight into the lipidome. The

resultant data will be imported into Surveyor Web Tools (Metabolon, Inc.) for intuitive data visualization and easier interpretation. We will also use the MetaboLync® Client Portal provided by Metabolon, Inc. to perform comprehensive, in-depth analysis, such as the heat map tool, the pathway enrichment tool, and the Pathway Visualizations Tool. The treatment effect on metabolites (log-transformed whenever needed) will be first assessed by paired t-test and then by linear mixed effects model that includes treatment status (pre/post-treatment), duration of treatment, and other major factors that may influence the metabolomics, including major dietary and lifestyle patterns (e.g., fiber intake, physical activity, body mass index, smoking, and alcohol intake).

Analysis of Urinary PGE-M: We will measure PGE-M (11a-hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostane 1,20-dioic acid) levels in baseline/pre-treatment and post-treatment urine samples using liquid chromatography-mass spectrometry (LC/MS) as previously described.[141] 0.5mL of each urine specimen will be stabilized by conversion to the *O*-methyloxime derivative and purified by C18 solid phase extraction with subsequent addition of the *O*-methyloxime derivatized deuterium-labeled internal standard (custom synthesis). Liquid chromatography (LC) will be performed on an Acquity BEH C18 column (2.0 × 50 mm, 1.7µm particle, Waters Corporation, Milford, MA, USA) connected to a Waters Acquity I-Class UPLC system and delivered to a Waters Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA).

13.7 Reporting and Exclusions

Participants who never start protocol therapy or do not return for final flexible sigmoidoscopy and sample collection will be considered inevaluable and will be excluded from all analyses.

13.7.1 Evaluation of Toxicity

N/A

13.7.2 Evaluation of the Primary Efficacy Endpoint

The primary endpoints will be determined for all patients who complete the final flexible sigmoidoscopy and sample collection, regardless of whether the patient complied with study drug use.[144]

13.7.3 Evaluation of the Secondary Endpoints

The secondary endpoints will be determined for all patients who complete the final flexible sigmoidoscopy and sample collection, regardless of whether the patient complied with study drug use.[144]

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome

data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A VIOSCREEN OVERVIEW

Product Background

VioScreen is a scientifically validated, dietary assessment tool that generates an accurate analysis of a subject's dietary behavior in about 20 minutes.

The assessment allows for the evaluation of subjects against a typical North American diet. This graphical online assessment consists of approximately 1,200 food images. Subjects are shown a set of foods within a food group from which they select only foods consumed at least once a month over the past three months. For each food identified, the system prompts the subject to select the frequency and portion size typically consumed. When needed, VioScreen branches to collect more relevant data. For example, if the subject lists "coffee," VioScreen prompts to see if sugar or milk is added and, if so, what kind of milk.

Subject responses are digitally stored in real time, verified for completeness, and analyzed to create an accurate and detailed nutritional profile with over 160 nutrient and food components, top foods with key nutrients, current and recommended eating patterns, Body Mass Index and Estimated Energy Requirement. Responses and analysis are immediately accessible to the researcher via VioScreen's administrative interface. This straight through process eliminates the need for lengthy and costly interviews or error prone paper-based questionnaire processing.

VioScreen's administrative interface is not just for data access, it makes the management of the entire process easy. Researchers are able to define organizations and protocols, assign user roles, import subject data, view results and export results to a standard file format for import to analytical software.

Hosting Environment & Backup

All data is stored in the latest version of Microsoft SQL Server database. The web server and database server reside on their own secure environments separated from other servers through a secure router implementation. All server access requires individual user level access via VPN, each user is required to update their password after 45 days, and all access is logged and reported. The application and database is backed up through highly secure cloud based services. The servers are cloud based and each server's operating system (Windows OS) is updated as new patches are released. HIPAA compliance is supported through the use of tools like Idera's SQL Server Compliance Manager for auditing sensitive data, monitoring alerts, and potential threat detection. Any unauthorized access to any environment or application is reported and tracked within the Windows OS.

System & Data Access

VioScreen is web-based application so there is nothing to install or manage. It has interactive data input and retrieval over the internet via transport layer security so the only requirement is an internet connection and access to www.vioscreen.com. All access to the application is secured through encrypted password access and each session is timed out after 15 minutes of inactivity

for both subjects and researchers.

Upon account setup new customers will receive a study administrator account. This account allows the study administrator to do many things including manage their account, set privileges, define protocols, create local administrators and create subjects. As part of the protocol definition, the study administrator is able to create subjects by a unique SubjectID to ensure participant confidentiality. All data is available in real-time to researchers via the export functionality available via the administrative functionality. Data is exported to a .csv format for easy import to any analysis software.

VioScreen Nutritional Assessment Food List

This document lists foods that are included as part Viocare's nutritional assessment. This is an electronic assessment that is administered online and includes integrated decision tree logic that prompts users to answer additional relevant questions (i.e. by choosing coffee, the system will ask if cream or sugar is used).

Food List

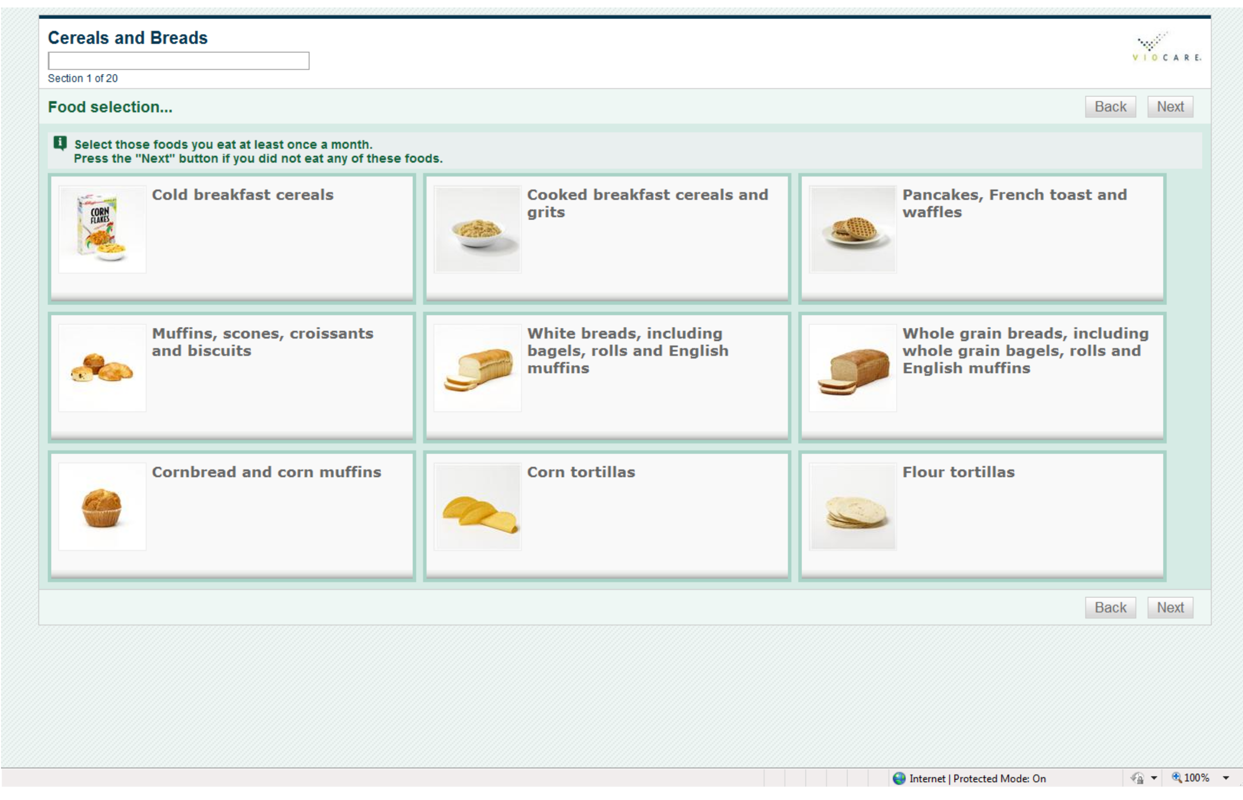
- Sweetened cereal (such as Apple Jacks®, Cocoa Puffs®, Fruit Loops®, Lucky Charms®, Trix®)
- Highly-fortified cereal (such as Total®, Product 19® and Smart Start®)
- Mostly or all whole grain cereal (such as Cheerios®, Grape-Nuts, Quaker Natural Granola®, Raisin Bran and Shredded Wheat)
- Standard cereal (such as Corn Flakes, Kellogg's Crispix® and Rice Krispies®)
- What type of milk or creamer did you put on cold cereals
- Whole grain or partial whole grain cooked cereal such as regular oatmeal (rolled oats), cream of brown rice, Roman Meal®
- Standard cooked cereals, such as cream of wheat, instant or quick-cooking oatmeal, cream of rice, grits, Malt-O-Meal®
- What type of milk or creamer did you usually use to cook the cereal?
- What type of milk or creamer did you usually put on cooked cereals?
- Pancakes, French toast and waffles
- Muffins, scones, croissants and biscuits
- White breads, including bagels, rolls and English muffins
- Whole grain breads, including whole grain bagels, rolls and English muffins
- Cornbread and corn muffins
- Corn tortillas
- Flour tortillas
- Eggs
- Egg Whites
- Tofu or soy breakfast sausage or other breakfast meat
- Bacon and breakfast sausage
- Tofu or soy cold cuts, hot dogs or deli meat substitutes
- Hot dogs and sausage, low or reduced fat
- Hot dogs and sausage, regular, not low fat
- Ham, turkey and low fat lunch meats

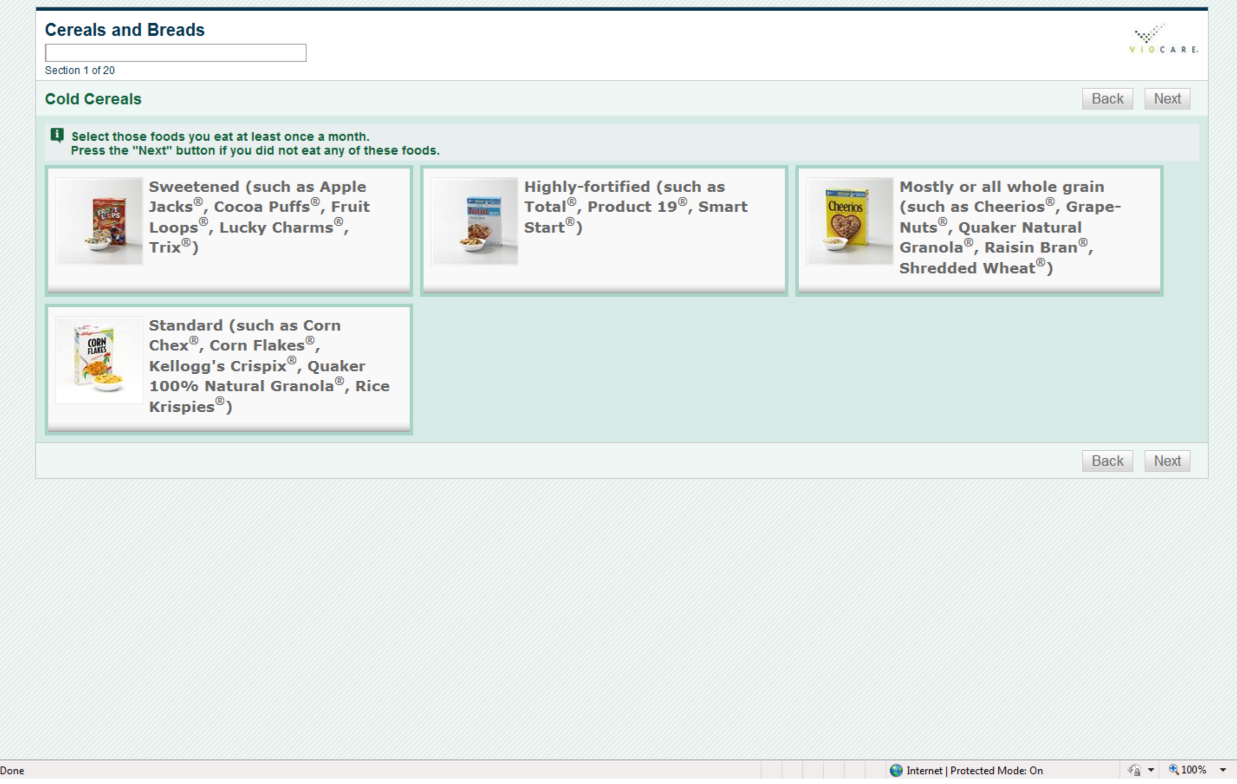
- Bologna, salami, Spam® and all other regular lunch meats
- Beef, pork, ham and lamb
- Veggie soy or tofu burgers or ground meat substitute
- Hamburgers, meatloaf and other ground meat
- Liver, chicken liver and organ meats
- Soy or tofu chicken or turkey
- Fried chicken, including nuggets and tenders
- Chicken and turkey, roasted, grilled, broiled or stewed
- Fried fish, fish sandwiches and fried shrimp and oysters
- Shrimp, oysters, crab and lobster
- White fish, broiled or baked, such as cod, halibut and snapper
- Dark fish, broiled or baked, such as salmon, trout and mackerel
- Canned tuna and tuna salad
- Packaged mixed dishes with soy or tofu
- Stew, pot pies, curries and casseroles with meat and chicken
- Chili with meat and beans
- Spaghetti, lasagna or other pasta with tomato and meat sauces (whole grain, regular)
- Spaghetti, lasagna or other pasta with tomato sauce (no meat) (whole grain, regular)
- Spaghetti and other pasta with oil, cheese or cream sauce, including macaroni and cheese
- Pizza (whole grain, regular)
- Asian-style (stir-fried) noodles and rice, such as chow mein, fried rice and Pad Thai
- Japanese noodles, such as Udon and Soba
- Burritos, tacos, tostados and quesadillas
- Enchiladas and tamales
- Tofu (all types), including low fat, flavored, marinated and smoked
- Tempeh (all types)
- Vegetable, minestrone and tomato soup
- Cream soups, such as chowders, potato and cheese
- Bean soups, such as pea, lentil and black bean
- Chicken noodle and other broth soups
- Miso soup
- Ramen noodle soup
- Cottage cheese and ricotta cheese (regular, low fat, nonfat)
- Soy cheese
- Sour cream (regular, low, nonfat)
- Cheese (regular, low, nonfat)
- Soy yogurt
- Greek Yogurt (sweetened, regular, low fat, nonfat)
- Yogurt (plain, sweetened, regular, low fat, nonfat)
- Salad greens (lettuce and spinach)
- Fresh tomatoes
- Carrots (raw)
- Green peppers and green chilies (raw)
- Red peppers and red chilies (raw)
- Avocado and guacamole
- Coleslaw

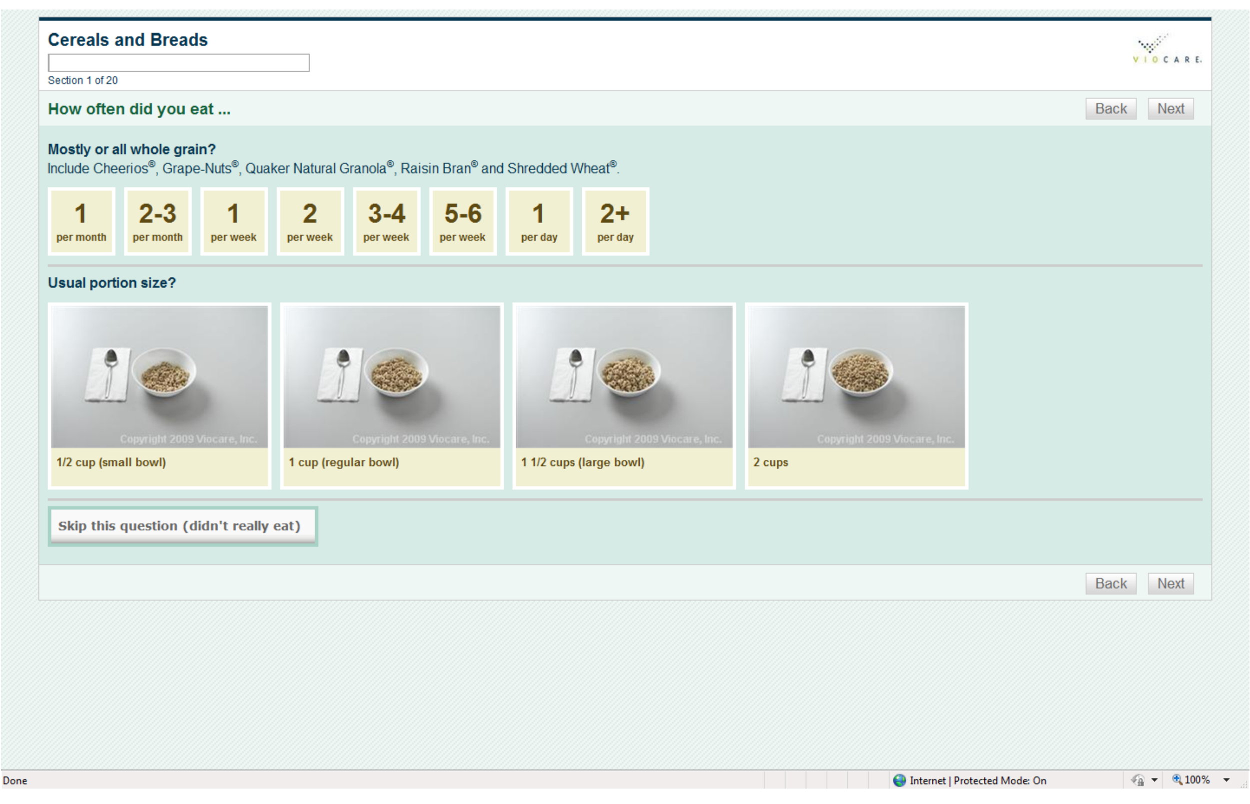
• Potato, macaroni and pasta salads
• Salad dressing
• Broccoli
• Cauliflower, cabbage and brussels sprouts
• Green beans and string beans
• Carrots (cooked)
• Green peas
• Corn and hominy
• Red peppers and red chilies (cooked)
• Green peppers and green chilies (cooked)
• Onions and leeks
• Summer squash and zucchini
• Winter squash, such as acorn and butternut
• Yams and sweet potatoes
• Kale, mustard greens and collards (cooked)
• Spinach, swiss chard and beet greens (cooked)
• French fries, fried potatoes and hash browns
• Potatoes (boiled, baked or mashed)
• Cooked soybeans or edamame
• Refried beans
• Baked beans, lima beans or all other beans, including chili made without meat
• White Rice
• Brown rice, cracked wheat and other whole grains
• Noodles as a side dish
• Cheese sauce and cream sauce
• Meat gravies
• Ketchup
• Salsa (as dip or on food)
• Mayonnaise and mayonnaise-type spreads
• Salt (in cooking or at the table)
• Soy sauce, tamari, teriyaki sauce, Szechwan sauce and natto
• Fresh garlic
• Apples, applesauce and pears
• Bananas
• Peaches, nectarines and plums
• Apricots - fresh or canned
• Apricots (dried)
• Raisins, prunes and other dried fruit (not apricots)
• Oranges, grapefruit and tangerines (not juice)
• Strawberries, blueberries and other berries
• Cantaloupe, melon and mango (in season)
• Watermelon and red melon
• Grapes, fresh
• Pineapple, fresh and canned
• Cherries, fresh
• Other fruit, including papaya, fruit cocktail or mixed fruit salads

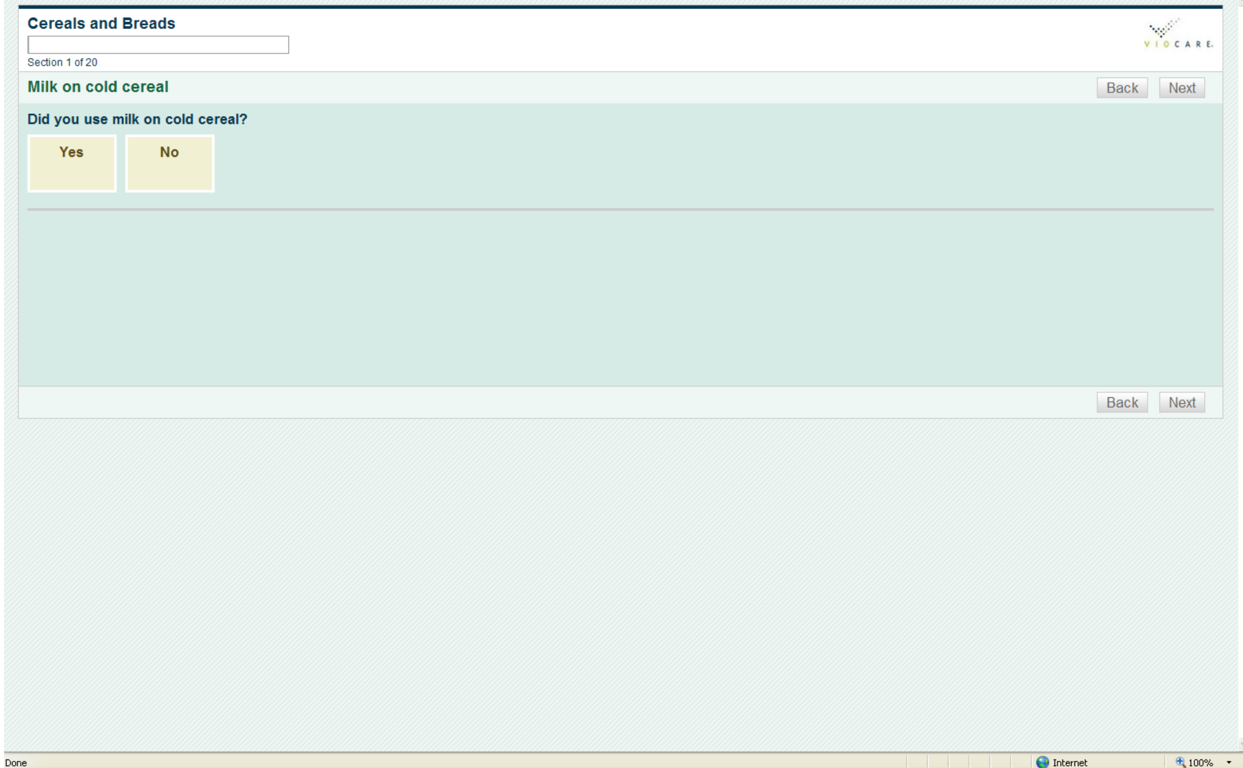
• Soy ice cream
• Other soy desserts such as cheesecake
• Low fat ice cream, frozen yogurt, sherbet or other low or nonfat frozen desserts
• Ice cream and milkshakes
• Pudding, custard and flan
• Doughnuts, pies and pastries
• Cookies or cakes
• Chocolate, candy bars, and toffee
• Life Savers, licorice, jelly beans or other hard candies
• Low or nonfat salty snacks such as pretzels and low or nonfat potato, tortilla and corn chips
• Regular potato, tortilla chips, corn chips and puffs
• Plain popcorn (no butter) or low fat microwave popcorn
• Microwave popcorn (Buttered and regular)
• Soy crackers
• Crackers, such as saltines and SnackWell's (low and nonfat)
• Regular crackers, such as Ritz and Wheat Thins
• Roasted soy nuts and soy nut butter
• Nuts, seeds, peanuts or peanut butter
• Whole grain crackers (low fat and regular)
• Sports bar and meal replacement bar containing soy
• Meal replacement drinks and shakes containing soy or soy protein [SOY]
• Granola bars and cereal bars such as Nutri-Grain Bars
• Sports or meal replacement bars such as Power Bars and Cliff Bars
• Meal replacement drinks and shakes such as Slim-Fast, Ensure and Carnation Instant Breakfast
• Milk as a beverage
• Latte, cappuccino, mocha or hot chocolate
• Coffee (not lattes, cappuccino or mochas)
• Sweet tea and bottled sweetened tea drinks
• Other teas, including black, green and herbal teas
• Sugar
• Diet soft drinks
• Regular soft drinks (not diet)
• Water (tap, bottled and sparkling)
• Tomato juice, V-8 and other vegetable juice
• Orange juice and grapefruit juice
• Other 100% fruit juice such as apple, grape and cranberry
• Fruit drinks fortified with Vitamin C, such as Hi-C, Fruitopia, and Kool-Aid
• Beer (all types)
• Red Wine
• White or rosé wine
• Liquor and mixed drinks

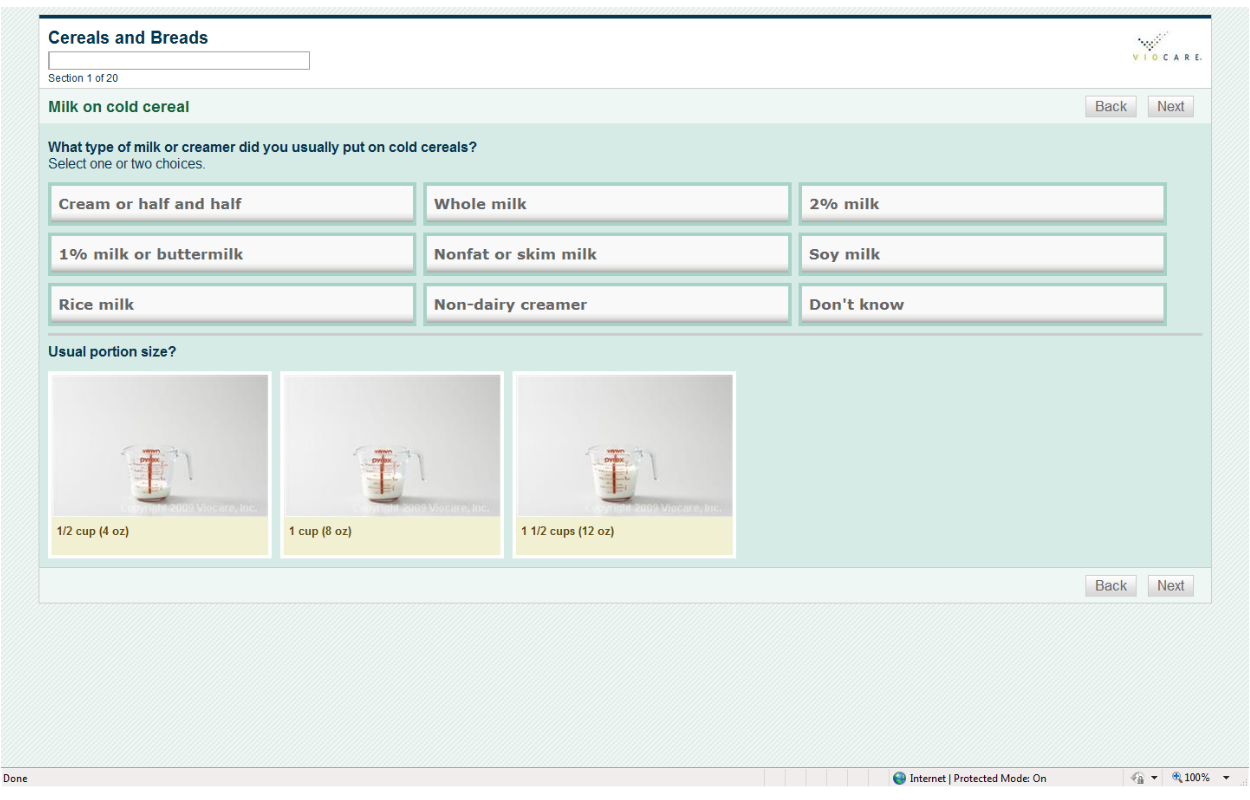
VioScreen Diet Assessment






Cereals and Breads
Section 1 of 20
How often did you eat ...
Back
Next
Mostly or all whole grain?
Include Cheerios®, Grape-Nuts®, Quaker Natural Granola®, Raisin Bran® and Shredded Wheat®.
1 per month
2-3 per month
1 per week
2 per week
3-4 per week
5-6 per week
1 per day
2+ per day
Usual portion size?
1/2 cup (small bowl)
1 cup (regular bowl)
1 1/2 cups (large bowl)
2 cups
Skip this question (didn't really eat)
Back
Next
Done
Internet | Protected Mode: On
100%


Cereals and Breads
Section 1 of 20
Milk on cold cereal
Back
Next
Did you use milk on cold cereal?
Yes
No
Back
Next
Done
Internet
100%


Cereals and Breads
Section 1 of 20
Milk on cold cereal
Back
Next
What type of milk or creamer did you usually put on cold cereals?
Select one or two choices.
Cream or half and half
Whole milk
2% milk
1% milk or buttermilk
Nonfat or skim milk
Soy milk
Rice milk
Non-dairy creamer
Don't know
Usual portion size?
1/2 cup (4 oz)
1 cup (8 oz)
1 1/2 cups (12 oz)
Back
Next
Done
Internet | Protected Mode: On
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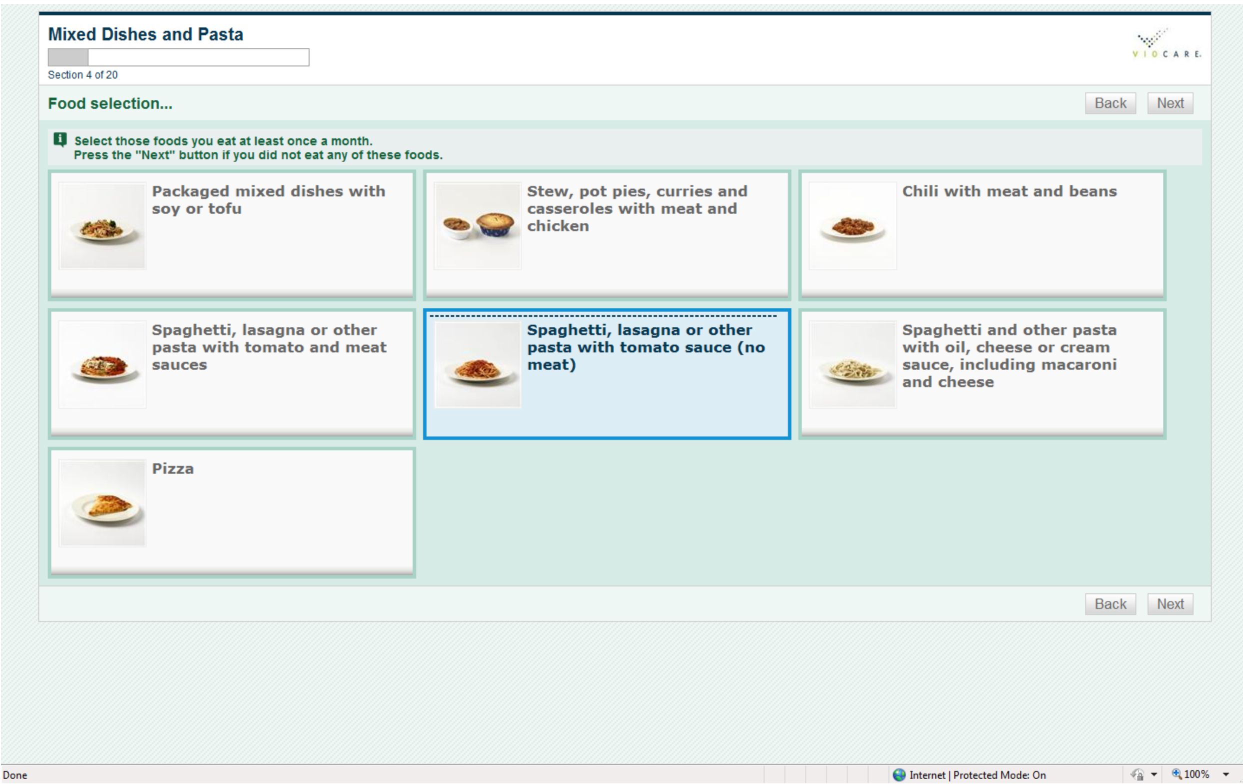
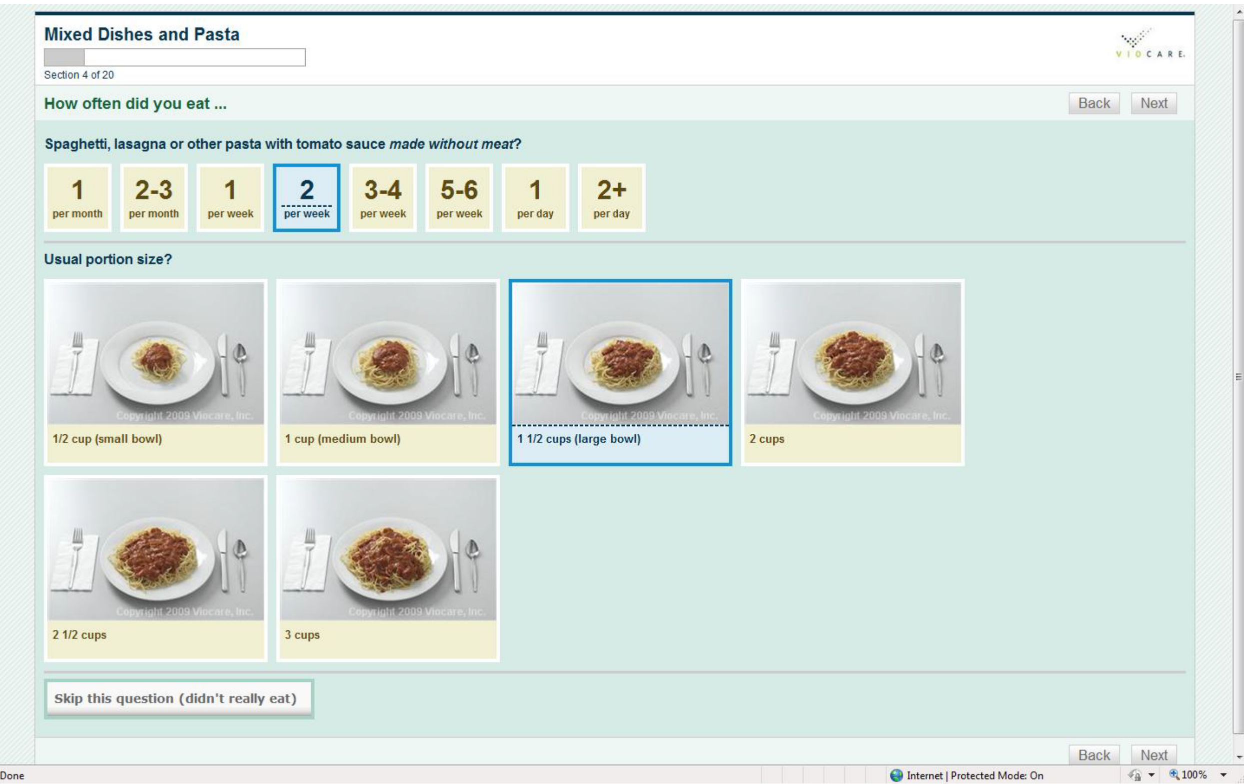

Mixed Dishes and Pasta
Section 4 of 20
Food selection...
Back
Next
Select those foods you eat at least once a month.
Press the "Next" button if you did not eat any of these foods.
Packaged mixed dishes with soy or tofu
Stew, pot pies, curries and casseroles with meat and chicken
Chili with meat and beans
Spaghetti, lasagna or other pasta with tomato and meat sauces
Spaghetti, lasagna or other pasta with tomato sauce (no meat)
Spaghetti and other pasta with oil, cheese or cream sauce, including macaroni and cheese
Pizza
Back
Next
Done
Internet | Protected Mode: On
100%


Mixed Dishes and Pasta
Section 4 of 20
How often did you eat ...
Back
Next
Spaghetti, lasagna or other pasta with tomato sauce made without meat?
1 per month
2-3 per month
1 per week
2 per week
3-4 per week
5-6 per week
1 per day
2+ per day
Usual portion size?
1/2 cup (small bowl)
1 cup (medium bowl)
1 1/2 cups (large bowl)
2 cups
2 1/2 cups
3 cups
Skip this question (didn't really eat)
Back
Next
Done
Internet | Protected Mode: On
100%