Study Name: PREEMPT CRC: Prevention of Colorectal Cancer Through Multiomics Blood Testing

NCT Number: NCT04369053

Date: April 5, 2021

PREEMPT CRC: Prevention of Colorectal Cancer Through Multiomics Blood Testing

PROTOCOL NUMBER:	**FRNM-004**
SPONSOR:	**Freenome Holdings, Inc.** **279 East Grand Avenue, 5th floor** **South San Francisco, CA 94080**
VERSION NUMBER:	**3**
VERSION DATE:	**05 April 2021**

Protocol Title: PREEMPT CRC: Prevention of Colorectal Cancer Through Multiomics Blood Testing

Study No: FRNM-004

Protocol Version: V3

Protocol Date: 05 April 2021

This study protocol was subject to critical review and has been approved by the appropriate institutional review board for each study site. The information contained in this protocol is consistent with:

The moral, ethical and scientific principles governing clinical diagnostic research as set out in the Declaration of Helsinki, principles of International Conference on Harmonization (ICH), and Good Clinical Practices (GCP) as described in 21 CFR parts 50, 54, 56 and 812, ISO 14155 and 20916 (Good study practices pertaining to clinical, and according to applicable local requirements).

DECLARATION OF THE INVESTIGATOR

PREEMPT CRC: Prevention of Colorectal Cancer Through Multiomics Blood Testing

The information contained in this protocol and all other information relevant to the study are the confidential and proprietary information of Freenome Holdings, Inc. (Freenome), and except as may be required by federal, state or local laws or regulation, may not be disclosed to others without prior written permission from Freenome.

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the regulations stated in the Federal Code of Regulations for Good Clinical Practices and International Conference on Harmonization guidelines and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Freenome or specified designees. I will discuss the material with them to ensure that they are fully informed about the study.

Principal Investigator Name (printed) Signature

_____ _____

Institution Name: _____

Date: _____

PROTOCOL SYNOPSIS

Title:	**PREEMPT CRC: Prevention of Colorectal Cancer Through Multiomics Blood Testing**
Background:	Currently, there are different methods available for colorectal cancer (CRC) screening, each with their own strengths and weaknesses. Colonoscopy (CS) is highly sensitive, but is inconvenient and invasive, and therefore less appealing. Non-invasive, stool-based approaches are more convenient, but are also less accurate (especially for early-stage disease and precancerous polyps), and require manipulating stools, which can be unpleasant. Consequently, despite being widely recommended, approximately one-third of age-eligible adults remain unscreened for CRC, and cancer is often detected too late for successful treatment. Freenome has developed a blood-based multiomics screening test to detect advanced colorectal neoplasia (ACN) that applies the power of artificial intelligence (AI) and machine learning (ML) to multiomics plasma profiling. In a prospective, multi-center clinical study, Freenome's test demonstrated high sensitivity (94%) and high specificity (94%) for early-stage (I/II) CRC. The availability of such a highly accurate blood-based test should help overcome some of the challenges with existing CRC screening methods. This study aims to validate the performance of Freenome's test and support its regulatory approval.
Sites:	Approximately 130 sites internationally.
Population:	Subjects between the ages of 45 and 85 (inclusive) within 30 days of enrollment who are at average risk of developing CRC. Enrollment will be stopped when a sufficient number of evaluable CRC cases have been diagnosed among the study population. Approximately 25,000 subjects will be enrolled, and an effort will be made to enroll approximately 70% of subjects in the 65-85 age group.
Study Design:	This is an international multi-center validation study. Subjects who meet eligibility criteria, provide informed consent, and provide a blood sample will be enrolled in the study. The blood collection must be done prior to undergoing bowel preparation for standard of care (SOC)

screening CS. Subjects will have the option to have blood collection done either at the study site or via mobile phlebotomy services, in which case the subject can have blood collected in their home or other location of preference. Subjects will have up to 50 mL of blood collected. De-identified blood specimens will be sent to Freenome or a designee for processing, testing, and storage. Specimens will be tested by laboratory personnel blinded to the results of the SOC screening CS. Subjects will undergo a SOC screening CS within 90 days of the blood collection. Subjects and their health care providers will be blinded to the results of Freenome's test, which will not be used in clinical management.

Objectives:

Primary:

- The primary objectives of this study are to determine the sensitivity (Sn) and specificity (Sp) of Freenome's test for colorectal adenocarcinoma using screening CS with histopathology as the reference method

Secondary:

- To evaluate the negative predictive value (NPV) of Freenome's test for CRC detection
- To evaluate the positive predictive value (PPV) of Freenome's test for CRC detection
- To evaluate the Sn of Freenome's test for advanced adenoma (AA)

Inclusion Criteria:

To be enrolled in this study, subjects must meet all of the following criteria:

1. 45-85 years of age (inclusive) within 30 days of enrollment (target ≥70% of subjects to be 65-85)
2. Willing to undergo a SOC screening CS within 90 days of blood collection
3. Considered by a physician or healthcare provider to be "average risk" for CRC

4. Able and willing to provide blood samples per protocol

5. Able to comprehend and willing to sign and date the informed consent document(s)

Exclusion Criteria: Subjects will be excluded from enrolling into the study if any of the following are met:

1. Family history

    1.1. At least 1 first-degree relative diagnosed with CRC before the age of 60 (Note: First-degree relatives include parents, siblings and offspring.)

    1.2. At least 2 first-degree relatives diagnosed with CRC at any age

    1.3. Known hereditary gastrointestinal cancer syndrome including but not limited to the following:

        1.3.1. Hereditary non-polyposis CRC syndrome (HNPCC) or Lynch Syndrome

        1.3.2. Familial adenomatous polyposis (FAP)

        1.3.3. Cowden's Syndrome

        1.3.4. Juvenile Polyposis Syndrome

        1.3.5. MUTYH-associated Polyposis

        1.3.6. Peutz-Jeghers Syndrome

        1.3.7. Serrated Polyposis Syndrome

2. Personal history

    2.1. CRC or colorectal adenoma

    2.2. History of any malignancy (except for non-melanoma skin cancer) at the time of enrollment, and for the 5 years preceding enrollment

2.3. Inflammatory bowel disease (IBD), including chronic ulcerative colitis (CUC) and Crohn's disease (CD)

2.4. Total colonic resection

2.5. Cystic fibrosis (CF)

2.6. CS in the 9 years preceding enrollment with the exception of an incomplete colonoscopy, or for a poor or inadequate bowel prep

2.7. Sigmoidoscopy or CT colonography in the 4 years preceding enrollment

2.8. Stool DNA testing in the 2 years preceding enrollment

2.9. Fecal occult blood testing (FOBT) or fecal immunochemical testing (FIT) in the 6 months preceding enrollment

2.10. Requiring an immediate or emergent colonoscopy for the investigation of symptoms

2.11. Solid organ or bone marrow transplantation

2.12. Blood product transfusion in the 120 days preceding enrollment

2.13. Any trauma or surgery requiring overnight inpatient hospitalization in the 30 days preceding enrollment

3. A medical condition which, in the opinion of the Investigator, should preclude enrollment in the study

4. Participated in or currently participating in a clinical research study in which an experimental medication has been administered during the 60 days up to and including the date of providing informed consent or may be administered through the time of the CS

5. Known to be pregnant

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LIST OF ABBREVIATIONS

AA	Advanced Adenoma
ACN	Advanced Colorectal Neoplasia
ACS	American Cancer Society
AE	Adverse Event
AI	Artificial Intelligence
AUC	Area under the curve
CD	Crohn's Disease
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CI	Confidence Interval
CRC	Colorectal Cancer
CRO	Contract Research Organization
CS	Colonoscopy
CTA	Clinical Trial Agreement
CUC	Chronic Ulcerative Colitis
DMARD	Disease-modifying anti-rheumatic drugs
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture

FAP	Familial Adenomatous Polyposis
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIT	Fecal Immunochemical Testing
FOBT	Fecal Occult Blood Testing
GCP	Good Clinical Practice
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
IBD	Inflammatory Bowel Disease
ICH	International Conference on Harmonization
ID	Identification
IRB	Institutional Review Board
mL	Milliliters
ML	Machine Learning
NAA	Non-Advanced Adenoma
NCI	National Cancer Institute
NEG	Negative
NPV	Negative Predictive Value
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SEER	Surveillance, Epidemiology, and End Results
Sn	Sensitivity
SOC	Standard of Care
Sp	Specificity
THC	Tetrahydrocannabinol
WGS	Whole Genome Sequencing

1. INTRODUCTION

1.1 Background

Colorectal cancer (CRC) is the fourth most frequently diagnosed cancer and the second leading cause of cancer death in the United States. In 2019, an estimated 101,420 new cases of colon cancer and 44,180 new cases of rectal cancer were expected to occur in the United States, and it was estimated that 51,020 people would die from CRC.[1] Screening for persons at average risk for CRC typically begins at age 50, and individuals at average risk have traditionally been defined as those aged ≥ 50 years without personal history of inflammatory bowel disease (IBD), adenomas, or CRC; without a family history of CRC or advanced adenomas (AAs); and without symptoms such as rectal bleeding.[2-4] Registry data from the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End Results (SEER) program indicated an increased incidence of CRC in African Americans prior to age 50 years,[5] which led to the recommendation in 2005 that CRC screening in African Americans begin earlier, at age 45.[3] More recently, epidemiologic reports suggest that the incidence of CRC may be increasing in adults aged < 50 years,[6-8] leading the American Cancer Society (ACS) in 2018 to recommend considering CRC screening in adults aged 45-49 years.[9] Accordingly, some large commercial payers (e.g., Aetna) have begun providing coverage for CRC screening in adults aged ≥ 45 years.

1.2 Study Rationale

Despite being widely recommended, more than one-third of age eligible adults remain unscreened for CRC,[10,11] and cancer is often detected too late for successful treatment, with nearly 60% of CRC cases detected after regional or distant metastases.[12] Currently, there are different methods available for CRC screening, each with their own strengths and weaknesses. Colonoscopy (CS) is highly sensitive, but is inconvenient and invasive, and therefore less appealing. Non-invasive, stool-based approaches are more convenient, but are also less accurate (especially for early-stage disease and precancerous polyps), and require manipulating stools, which can be unpleasant.[2,13]

Blood-based screening tests for cancer could address some of the aforementioned challenges and supplement or replace existing cancer screening methods. One area of interest is circulating cell-free DNA (cfDNA), which includes both tumor-derived DNA (circulating tumor DNA, or ctDNA) and DNA derived from non-tumor cells, such as hematopoietic and stromal cells. Different screening approaches using cfDNA are being explored, with some proposing that ctDNA-only based tests may enable sensitive and specific early detection of cancer.[14-17] However, ctDNA generally represents ≤ 0.01-1.0% of all cfDNA in early-stage cancer, and even lower in premalignant conditions, thus requiring clinically challenging volumes of blood (e.g., 150-300 mL).[18,19] These limitations are particularly important in early-stage cancer and precancerous conditions, when the tumor is small, and the shedding of tumor DNA into the blood may be minimal.

An alternative to detection based solely on ctDNA is to look more broadly at cfDNA—both tumor- and non-tumor-derived—and changes that early-stage cancer and precancerous lesions may induce that are detectable in blood. There is growing evidence of interactions between cancerous cells and other cells, including fibroblasts, platelets, and immune cells, especially within the tumor microenvironment. Moreover, exploring signals beyond cfDNA alone, using a multiomics approach that includes additional biomarkers (e.g., proteins, RNA, etc.), may be necessary to achieve the clinical performance required for a blood-based screening test.

In the case of CRC, given its molecular heterogeneity, and biological differences between early- and late-stage disease, detection may be better achieved using a multiomics approach that includes assessing the genome, methylome, transcriptome, and proteome in plasma. For example, proteins such as autoantibodies may enable detection of cancer at an early stage, before the development of clinical symptoms.[20,21] Conversely, measuring tumor-derived signals from the genome and/or methylome may allow detection of CRC at later stages, when genetic and epigenetic changes are more common. Therefore, combining different approaches may be necessary for detection of all stages of CRC, as well as precancerous lesions. Applying artificial intelligence (AI)/machine learning (ML) enables assessment of disease-related patterns from multiple signals and the identification of relevant, low-dimensional features that generalize to the screening population.[22,23]

Freenome's early data were based on a single-analyte approach, namely whole genome sequencing (WGS) of cfDNA. In brief, cfDNA alone was assessed for the detection of CRC from 546 individuals with CRC and 271 non-cancer controls (i.e., individuals without a current CRC diagnosis). In this cohort heavily weighted towards early-stage cancer (81% stage I/II), a mean area under the curve (AUC) of 0.92 (95% confidence interval [CI] 0.91-0.93) was achieved, with a mean sensitivity of 85% (95% CI 83-86%) at 85% nominal specificity.[24] More recently, this prototype single-analyte test was expanded to a multiomics test that demonstrated improved performance, achieving 94% sensitivity and 94% specificity for early-stage (I/II) colorectal adenocarcinoma in a multi-center prospective study that included average-risk screening and case-control subjects (n=574) and an analyte training set (n=17) drawn from a statistically-driven subset of AI-EMERGE® (NCT03688906).[25] These findings reinforce our belief that successful early detection of advanced colorectal neoplasia (ACN) will require such an ML-based multiomics approach, which also holds the promise of improving over time as additional real-world testing data becomes available.

Freenome's blood-based, multiomics test (hereafter referred to as "Freenome's test") for CRC screening will enable improved adherence versus current CRC screening efforts, which remain either invasive or stool-based. In addition, use of the test is expected to improve the identification of clinically-actionable disease, defined as disease (i.e., ACN) for which colonoscopic intervention has demonstrated health outcome benefit.[26,27] This in turn will improve the risk-benefit ratio for patients by increasing the likelihood that those patients directed by the test to undergo a diagnostic CS, with its attendant costs and complications, will benefit from it.

The purpose of the current study is to validate Freenome's test performance and support regulatory approval.

2. STUDY OBJECTIVES

2.1 Primary

- The primary objectives of this study are to determine the sensitivity (Sn) and specificity (Sp) of Freenome's test for colorectal adenocarcinoma, using screening CS with histopathology as the reference method.

2.2 Secondary

- To evaluate the negative predictive value (NPV) of Freenome's test for CRC detection
- To evaluate the positive predictive value (PPV) of Freenome's test for CRC detection
- To evaluate the Sn of Freenome's test for AA

3. STUDY DESIGN

3.1 Study Overview

This is an international multi-center study for the clinical validation of Freenome's blood test to detect ACN (i.e., CRC and AA). Subjects referred by a healthcare provider (HCP) for a standard of care (SOC) screening CS and providing informed consent are eligible to participate based on the study inclusion and exclusion criteria. Enrollment procedures should be completed within 30 days of signing consent. Should a patient fall outside of the 30-day window, these cases may be escalated to Freenome (or designee) for approval on a case by case basis.

- Subjects who provide informed consent, meet the eligibility criteria and provide the blood sample for this study will be enrolled. Subjects will undergo blood collection and will then have 90 days to complete a SOC screening CS. Maximum time allowed between enrollment and CS will be 90 days. Subjects will have the option to have blood collection done either at the clinical study site or by a study-provided mobile phlebotomy service, in which case the subject can have blood collected in their home or location of preference. Up to 50 mL of blood will be collected from each enrolled subject prior to the bowel preparation for CS. Blood specimens will be used for the clinical and analytical validation of Freenome's test. Blood specimens will be de-identified and sent to Freenome, or a designee, for processing, storage and testing. De-identified specimens and residual specimens will be banked and may be utilized for additional analytical validation testing by Freenome, to improve the current assay in the future, or to develop new assays. Specimens will be used for research purposes only. Since Freenome's test (and any future testing using residual specimens) is currently investigational, results will not be shared with Investigators or subjects.

Study duration is comprised of the time between enrollment and a single study visit for a SOC screening CS and should be no longer than 90 days.

Although no long-term follow-up is planned, subjects may also be contacted by site personnel up to three (3) years following the completion of the study to gather additional information (e.g., any new cancer diagnoses).

3.2 Population

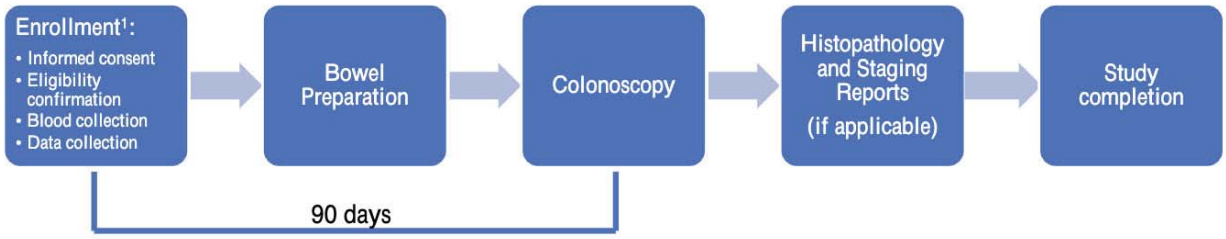
The study will enroll a representative sample of the population of subjects who are at average risk for the development of CRC and will undergo CRC SOC screening CS from sites across the U.S. and internationally.

3.3 Number of Subjects

Approximately 25 ,000 subjects will be enrolled. Of the 25 ,000 subjects, an effort will be made to enroll approximately 70% of subjects in the 65-85 years age group. Enrollment will be stopped when a sufficient number of evaluable cases of CRC have been diagnosed among the study population.

3.4 Study Schema

Figure 1. Study Schema



[1]Enrollment procedures should be completed within 30 days of signing consent

3.5 Schedule of Assessments

Table 1. Schedule of Assessments

Study Procedures	Enrollment	CS Visit (and additional reports)	Notes
Window	**30 days**	**90 days**	
Informed Consent	X		Enrollment procedures should be completed within 30 days of signing consent
Evaluation of Inclusion & Exclusion Criteria	X		
Medical History / Demographics	X		
Lifestyle Information	X		Including but not limited to: history of tobacco product use or history of and current information on alcohol consumption and drugs of abuse including recreational and prescribed, including THC.
Concomitant Medications	X		Concomitant medications: (limited to the medications listed in Appendix I) in the 30 days preceding enrollment
Weight and Height	X		Height and weight to be collected at enrollment; however, patients will have up until the CS visit to complete. Verbally reported measurements are acceptable.
Blood Collection	X		The blood collection should be done within 30 days of signing consent. Should a patient fall outside of the 30-day window, these cases may be escalated to Freenome (or designee) for approval on a case by case basis. The blood collection must be done prior to bowel preparation for the screening CS. Maximum allowed time between blood collection and CS will be 90 days.
Colonoscopy		X	Subjects will be enrolled and will then have 90 days to undergo a SOC screening CS following the blood collection. The CS report must be provided.
Histopathology Report		X	If any lesions are present from the CS report, the accompanying pathology report(s) must be provided.
Imaging Report, Relevant		X	Subjects diagnosed with CRC will require any imaging reports, post-surgery histopathology reports, and/or operative notes required to accurately stage the cancer.

Histopathology, or Operative Notes			
AEs/SAEs	X		Only blood collection related
Central histopathology review		X	H&E slide(s), FFPE blocks or digital image(s) from specific types of polyps resected or biopsied during the screening CS will be required to be sent for central pathology review. Only a subset of polyps will require such review and each site will be sent a list of cases to send for central review.

*CS: colonoscopy; SOC: standard-of-care; AE: adverse event; SAE: serious adverse event; THC: tetrahydrocannabinol

4. STUDY ELIGIBILITY

4.1 Inclusion Criteria

To be enrolled in this study, subjects must meet all of the following criteria:

1. 45-85 years of age (inclusive) within 30 days of enrollment (target ≥70% of subjects to be 65-85)
2. Willing to undergo a SOC screening CS within 90 days of blood collection
3. Considered by a physician or healthcare provider to be “average risk” for CRC
4. Able and willing to provide blood samples per protocol
5. Able to comprehend and willing to sign and date the informed consent document(s)

4.2 Exclusion Criteria

Subjects will be excluded from enrolling into the study if any of the following are met:

1. Family history
    1.1. At least 1 first-degree relative diagnosed with CRC before the age of 60 (Note: First-degree relatives include parents, siblings and offspring.)
    1.2. At least 2 first-degree relatives diagnosed with CRC at any age

1.3. Known hereditary gastrointestinal cancer syndrome including but not limited to the following:

1.3.1. Hereditary non-polyposis CRC syndrome (HNPCC) or Lynch Syndrome

1.3.2. Familial adenomatous polyposis (FAP)

1.3.3. Cowden's Syndrome

1.3.4. Juvenile Polyposis Syndrome

1.3.5. MUTYH-associated Polyposis

1.3.6. Peutz-Jeghers Syndrome

1.3.7. Serrated Polyposis Syndrome

2. Personal history

2.1. CRC or colorectal adenoma

2.2. History of any malignancy (except for non-melanoma skin cancer) at the time of enrollment, and for the 5 years preceding enrollment

2.3. Inflammatory bowel disease (IBD), including chronic ulcerative colitis (CUC) and Crohn's disease (CD)

2.4. Total colonic resection

2.5. Cystic fibrosis (CF)

2.6. CS in the 9 years preceding enrollment with the exception of an incomplete colonoscopy, or for a poor or inadequate bowel prep

2.7. Sigmoidoscopy or CT colonography in the 4 years preceding enrollment

2.8. Stool DNA testing in the 2 years preceding enrollment

2.9. Fecal occult blood testing (FOBT) or fecal immunochemical testing (FIT) in the 6 months preceding enrollment

2.10. Requiring an immediate or emergent colonoscopy for the investigation of symptoms

2.11. Solid organ or bone marrow transplantation

2.12. Blood product transfusion in the 120 days preceding enrollment

2.13. Any trauma or surgery requiring overnight inpatient hospitalization in the 30 days preceding enrollment

3. A medical condition which, in the opinion of the Investigator, should preclude enrollment in the study

4. Participated in or currently participating in a clinical research study in which an experimental medication has been administered during the 60 days up to and including the date of providing informed consent or may be administered through the time of the CS

5. Known to be pregnant

5. STUDY PROCEDURES

5.1 Informed Consent and Enrollment

Subjects must be able to provide informed consent prior to any study procedure. Subjects who provide informed consent, meet the eligibility criteria and provide the blood sample for this study will be enrolled.

5.2 Colonoscopy, Histopathology, and Imaging Reports

Each subject will undergo a SOC screening CS within 90 days of blood collection. Bowel preparation and CS will be performed according to SOC at each site. The endoscopist will record the quality of the bowel preparation after washing and suctioning. A CS that was limited in any way but wherein a CRC was identified or wherein a partially obstructing CRC was identified, will be included in the analyses.

For all non-CRC subjects, the following will apply:

A subject who has a bowel preparation rated as less than fair (i.e., poor or inadequate) will be considered unevaluable and therefore excluded from analysis of all primary and secondary endpoints. (Poor is defined as semisolid stool that could not be suctioned or washed away, and < 90% of mucosa is seen; inadequate is defined as needing repeat preparation or screening.)

A subject who has an incomplete CS will be considered unevaluable and therefore excluded from analysis of all primary and secondary endpoints. (A complete CS will be defined as reaching the cecum; reaching the junction between the small and large intestine if the cecum has been resected; or reaching the neo-cecum. Cecal intubation will be documented by photographic evidence and/or documentation of cecal intubation.)

A non-CRC subject who has (1) a poor or inadequate bowel preparation or (2) an incomplete CS may repeat the CS and continue as a subject for this study only if the repeat CS occurs within 90 days of the blood collection.

CS findings will be recorded on the CS reports in accordance with the standard of practice at each site. Histopathological results will also be conducted according to usual practice at each site. Results from the CS and, if performed, histopathology will be recorded in the clinical database. All results (e.g., CS, histopathology, and imaging reports) will be collected, de-identified, labeled with the subject ID and uploaded to a clinical study database. Personnel generating any of these clinical reports will remain blinded to the results of Freenome's test, and vice versa.

The following required information will be collected once available:

- CS report
- If applicable, any histopathology report(s)
- If applicable, any imaging reports or relevant histopathology or operative notes required for cancer staging

5.3 Central Pathology

Each patient will have their respective CS report and (if applicable) local histopathology report reviewed by a central pathologist in order to categorize the index lesion (if applicable) into the histopathological categories described below in section 7.2 (i.e., CRC, AA, NAA, and NEG). For all cases categorized as AA2.1, AA2.2, AA2.4, and AA2.5 and for 5% of NEG4.1, central pathology review will be required. The sites will send H&E slides, formalin-fixed paraffin embedded (FFPE) blocks or digital images for review of these specific cases. When the classification of the index lesion differs between the local histopathology result and the central pathology result, the case will undergo review by an additional central pathologist to adjudicate the final categorization of the index lesion. Instructions will be included in the central pathology manual.

H&E slides, FFPE blocks or digital images of the selected cases will be sent for central pathology review as detailed above. (H&E slides are preferred.) After analysis of the H&E slides or H&E slides made from FFPE blocks, slides and/or blocks will be returned to sites. Digital images will be stored in a secure site.

5.4 Data Collection

Refer to Section 3.5. Schedule of Assessments. The following information will be recorded in the electronic case report form (eCRF) for each subject after consent at enrollment.

- Medical History / Demographics
- Concomitant medications (specifically aspirin, estrogen, disease-modifying anti-rheumatic drugs (DMARDs), biological agents, and COVID-19 vaccinations) in the 30 days preceding enrollment, as listed in Appendix I
- Lifestyle information will be obtained either from the subject's clinical record or self-report, including but not limited to:
    - History of tobacco product use (i.e., cigarettes)
    - History of and current information on alcohol consumption and drugs of abuse including recreational and prescribed, including tetrahydrocannabinol (THC)
- Blood collection time and date
- Height and weight

5.5 Blood Specimen Collection and Storage

Blood specimens collected for this study will be separate from any specimens collected for SOC. Up to 50 mL of peripheral blood will be collected prior to the bowel preparation via standard phlebotomy. Subjects will have the option to have blood collected either at the study site or via mobile phlebotomy services, in which case the subject can have blood collected in their home or location of preference. The blood collection tubes will be labeled using supplied barcode labels which link to the unique study subject ID, as described in the study laboratory manual.

Refer to the study laboratory manual for complete instructions on blood collection procedures, labeling, handling, storage, and shipment.

Residual blood samples may be destroyed or, if the subject consents, archived for further research. Clinical data and samples will be kept in a manner that preserves the anonymity of subjects, using the subject ID as the only tracking information. Specimens will be stored for no more than 20 years in Freenome's biorepository or a similar commercial biorepository contracted by Freenome, and may be used for future research, which may improve the researchers' understanding of cancer or other diseases.

6. ADVERSE EVENTS

6.1 Adverse Event (AE) Definition

Due to the study design and the nature of the blood collection process, the Sponsor does not expect many adverse events (AEs) caused by or related to the blood collection.

Since the screening CS is being performed as SOC and is a routine part of patient care, subjects are at no increased risk because of participation in this study. Thus, while adverse events may be anticipated due to SOC screening CS, they are not regarded as study related.

For the purposes of this study, an AE is defined as any undesirable physical, psychological or behavioral effect experienced during participation in the study that is related to the blood collection for this study. As a result of blood collection, subjects may experience minor pain, discomfort, bruising at the venipuncture site, or lightheadedness.

6.2 Serious Adverse Event (SAE) Definition

A serious adverse event (SAE) is any AE that meets any of the following criteria:

- Death
- A life-threatening illness or injury
- Medical or surgical intervention to prevent permanent impairment of a body structure or body function
- Required inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

Definition of Terms

Life threatening: An AE is life threatening if the subject was at immediate risk of death from the event as it occurred (i.e., it does not include a reaction that if it had occurred in a more serious form might have caused death). For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug-induced hepatitis can be fatal.

Hospitalization: AEs requiring hospitalization should be considered SAEs. Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., elective surgery for a pre-existing condition that has not worsened) need not be considered AEs or SAEs. If anything untoward is reported during the hospitalization, that occurrence must be reported as an AE, either 'serious' or 'non-serious' according to the usual criteria.

In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. When in doubt about whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

Disability/incapacity: An AE is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to perform normal life functions.

6.3 Documenting Adverse Events/Serious Adverse Events

Sites and mobile phlebotomists must observe normal SOC practices to protect the subjects' health and safety during the specimen collection. For SAEs, at each occurrence, the event should be reported to the regulatory body (the Institutional Review Board, IRB) within 1 business day of Investigator knowledge of such event.

7. STATISTICS

If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final statistical analysis plan (SAP), a protocol amendment will not be issued, and the SAP will prevail.

The SAP will be finalized prior to the end of clinical database lock for the study. Any changes to the methods described in the final SAP will be described and justified in the clinical study report.

7.1 Evaluable Status

A subject may be considered unevaluable and therefore excluded from all primary and secondary analyses if any of the following criteria are met: (Note this list is not exhaustive. Refer to the SAP for details.)

- No Freenome test result (for example, resulting from errors in labeling, improper collection or handling, and poor specimen quality)
- Missing any of the required reports, i.e. CS and, if applicable, histopathology and imaging reports
- Incomplete CS, or no photographic evidence or documentation of cecal intubation (unless diagnosed with CRC)
- Bowel preparation quality rated as poor or inadequate (unless diagnosed with CRC)
- Subjects found to have occult IBD on CS

Unevaluable subjects will be assessed for bias as described in the SAP.

7.2 Histopathological Definitions

For this study, the index lesion is considered to be the most clinically significant lesion, and the hierarchy of clinical significance (and the categories of histopathological findings) is as follows:

1. CRC, all stages (I-IV)
2. Advanced adenoma (AA), including
    - 2.1 Adenoma with carcinoma in situ or high-grade dysplasia, any size
    - 2.2 Adenoma, villous growth pattern (≥ 25%), any size
    - 2.3 Adenoma ≥ 1.0 cm in size
    - 2.4 Serrated lesion, ≥ 1.0 cm in size (includes sessile serrated adenoma/polyp (SSA/P) with or without cytological dysplasia and hyperplastic polyps ≥ 1.0 cm)
    - 2.5 Traditional serrated adenoma (TSA), any size
3. Non-advanced adenoma (NAA)
    - 3.1 ≥ 5 adenomas, all < 1.0 cm in size, non-advanced
    - 3.2 <5 adenomas, < 1.0 cm in size, non-advanced
4. Negative (NEG)
    - 4.1 All SSA/P < 1.0 cm and HP < 1.0 cm not in sigmoid or rectum
    - 4.2 HP < 1.0 cm in the sigmoid or rectum
    - 4.3 Negative upon histopathological review
    - 4.4 No findings on colonoscopy, no histopathological review

Subjects will be placed into a category based on the most clinically significant lesion confirmed by histopathology. Subjects with histopathological findings in categories 3.1-3.2 above will be included in the Sp analysis as NAAs and 4.1-4.4 as NEGs. Those subjects in the NEG category will include those subjects with a CS showing no findings (and hence no histopathology submitted), non-neoplastic disease, and/or non-adenomatous (e.g., hyperplastic) polyps < 1 cm in diameter.

7.3 Study Endpoints and Test Performance Analyses

7.3.1 Co-Primary Endpoint Effectiveness Analysis

The co-primary endpoint effectiveness analysis for the blood test Sn for CRC cases (Sn_{CRC}) is to demonstrate whether the lower bound of the two-sided confidence interval for the point-estimate

of Sn_{CRC} meets or exceeds 65%. The co-primary analysis for Freenome's test Sn_{CRC} will be performed using an exact binomial test to compute the two-sided 95% confidence lower bound for the percentage of all Full Analysis Set (FAS) subjects identified with CRC from the CS and confirmed histopathology with a positive Freenome test result. The FAS set will include those study-eligible subjects who have an evaluable test result, and an evaluable CS result with confirmed histopathology findings.

The co-primary effectiveness analysis for test Sp for NAA and NEG cases ($Sp_{NAA+NEG}$) is to demonstrate whether the lower bound of the two-sided confidence interval for the point-estimate of $Sp_{NAA+NEG}$ meets or exceeds 85%. The co-primary analysis for Freenome's test Sp for NAA and NEG subjects in the FAS will be performed using an exact binomial test to compute the two-sided 95% confidence lower bound.

Both lower bound conditions must be achieved for the study to be declared a success.

The robustness of significant results from the analysis of the primary endpoint will be assessed by calculating the numbers of subjects whose test results would have to switch from positive to negative and vice versa to obtain a result that was just significant. All enrolled but excluded cases will be assessed for bias in the analyzed population by comparing the baseline demographics for those included versus those excluded. Including the excluded cases with various proportions of imputed results for the missing elements will be used to further demonstrate the level of robustness of the significant result from the FAS set alone.

7.3.2 Secondary Endpoints and Analyses

NPV of Freenome's test:

The analysis for Freenome's test NPV for CRC cases ($NPV_{Non-CRC}$) is to determine whether the lower bound of the two-sided CI for the point-estimate of $NPV_{Non-CRC}$ meets or exceeds the prevalence for Non-CRC in the study population. The analysis for Freenome's test $NPV_{Non-CRC}$ will be performed using an exact binomial test to compute the two-sided 95% confidence lower bound for the rate among FAS subjects with a test-negative result for those identified without CRC from CS and histopathology.

PPV of Freenome's test:

The analysis for Freenome's test PPV for CRC cases (PPV_{CRC}) is to determine whether the lower bound of the two-sided CI for the point-estimate of PPV_{CRC} meets or exceeds the prevalence of CRC in the study population. The analysis for Freenome's test PPV_{CRC} will be performed using an exact binomial test to compute the two-sided 95% confidence lower bound for the rate among FAS subjects with a test-positive result for those identified with CRC from CS and histopathology.

Sensitivity of Freenome's test for AA:

The effectiveness analysis for Freenome's test Sn for AA cases (Sn_{AA}) is to determine whether the lower bound of the two-sided CI for the point-estimate of Sn_{AA} meets or exceeds 25%. The analysis for Freenome's test Sn_{AA} will be performed using an exact binomial test to compute the two-sided 95% confidence lower bound for the percentage of all FAS subjects identified with AA from CS and histopathology with a test-positive result.

7.3.3 Sample Size Justification

The study should include a minimum of 48 evaluable adenocarcinoma cases meeting the requirements for inclusion in the analysis set for the co-primary Sn endpoint. Assuming a prevalence of 0.3 % CRC cases, the minimum number required in the FAS is approximately 48/0.3 % = 16,000 subjects. Allowing for a 30% unevaluable rate, this gives an enrollment of 22,857 subjects. A sample size of 48 CRC achieves 90.4% power to rule out a Sn of 65% using a one-sided exact test with a target significance level of 0.025. The actual significance level achieved by this test is 0.025 (PASS 2019, v19.0.2).

If 48 CRC cases are tested, then at least 38 need to be positive by Freenome's test to rule out a lower bound of 2-sided 95% CI (Clopper-Pearson method) of 65%. The lower bound of 38/48 CI is 65.1%.

7.3.4 Additional Analyses

Additional analyses include, for example, analysis of distribution of cancers by age, biological sex, race, and ethnicity: Using a chi square or Fisher's exact test of the observed incidence of CRC (or CRC and AA) relative to the expectation from SEER incidence data will help assess the representativeness of the study population.

Direct rate standardization will be used to project the Sn and Sp (for CRC and AA) of Freenome's test to the general population, males, females and subjects ≥ 65 years of age.

The distribution and receiver operating characteristic (ROC) curves for Freenome's test score among the FAS subject group separately will be included. The AUC of each curve will be presented with its estimated 95% CI. For the same groups, the Sn and Sp of subject test scores will be presented over the range of scores, with Youden's J and number needed to misdiagnose one (NNMD) superimposed on the figure.

More details of analyses will be described in the SAP.

7.3.5 Statistical Interim Analyses and Stopping Guidance

No interim data analyses of the study subjects' test results will be performed, so no planned sample size adjustment of the significance level due to interim analysis is needed.

Interim evaluation of test performance on samples other than those collected as part of this study protocol will be performed. Sample size adjustments may be made accordingly with appropriate supporting analysis and statistical justifications for those adjustments.

Enrollment will be stopped when a sufficient number of evaluable CRC cases have been diagnosed among the study population.

8. ETHICAL, LEGAL, AND ADMINISTRATIVE RESPONSIBILITIES

The procedures set out in this study protocol are designed to ensure that the study will be conducted according to the principles of International Conference on Harmonization (ICH)/GCP guidelines, as well as any local, regional, and country regulatory requirements.

8.1 Institutional Review Board

This protocol and consent form will be submitted to the governing IRBs. Before the study is initiated, the Investigator/Institution will obtain dated approval from the IRB for any study protocol/amendment(s), informed consent, and a statement from the IRB that they comply with applicable ICH/GCP requirements. The IRB approval will identify the protocol version as well as the documents reviewed.

8.2 Informed Consent

Subjects must be able to provide informed consent and meet all eligibility criteria prior to enrollment. Only subjects who meet all eligibility criteria will be enrolled in this study. No waivers of inclusion or exclusion criteria will be granted by the Investigator and/or Freenome or its designee for any subject enrolled in the study.

8.3 Data Monitoring Committee

The study will have a Data Monitoring Committee (DMC) that will operate independently from the Sponsor and the Principal Investigators. The primary responsibilities of the DMC are to determine whether the required number of events (e.g., evaluable subjects diagnosed with CRC) have been met to provide stopping guidance to the Sponsor. Details regarding DMC responsibilities, membership, schedule and format of meetings, format for presentation of data, access to interim data, method and timing of providing interim reports to the Sponsor, and other issues relevant to committee operations will be described in the DMC charter.

8.4 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures of a clinical protocol that is under the Investigator’s control and that has not been approved by the IRB. Upon discovery, the Principal Investigator is responsible for reporting protocol deviations to the IRB or other local authority, if required by reporting guidelines, using the standard reporting form. Protocol deviations and actions taken to resolve the deviations will be noted on the designated study documents.

8.5 Records Management

All study data will be recorded and maintained in a secure, validated electronic data capture (EDC) database. The Investigator and study site will permit study-related monitoring, audits, IRB review and regulatory inspection by providing authorized personnel from Freenome and its representatives, the IRB, the FDA and other appropriate regulatory agencies direct access to all study-related data as requested.

Direct access to examine, analyze, verify and reproduce any records, source documents or reports are important to the evaluation of a clinical study. Any party with direct access should take reasonable precautions to maintain the confidentiality of the study subjects and the Freenome's proprietary information.

All study documentation, including Informed Consent Forms and study logs, are to be kept by the Investigator's site in a secured location. Records should be maintained for a minimum of 5 years. The site should inform Freenome or its designee prior to moving records to an off-site location or destroying according to local practice. These records must be available for all types of study-related monitoring, audits, IRB review, and regulatory inspections. Access to these files is to be considered restricted and accessible only to the Principal Investigator(s), authorized study personnel, and legally required regulatory inspectors.

8.6 Source Documentation

All data obtained during this study should be entered in the eCRFs promptly. All source documents from which eCRF entries are derived should be placed in the patient's records. eCRF fields for which source documents will typically be needed include progress notes, CS, histopathology and/or imaging reports. Documentation of sample collection, storage and shipment will also be considered source documents for this study.

The eCRFs for each patient will be checked against source documents at the study site by the site monitor.

Instances of missing or uninterpretable data will be discussed with the Investigator for resolution.

8.7 Study Files and Record Retention

The Investigator is responsible for ensuring that the Investigator site file is maintained. The Investigator site file will contain, but is not limited to, the following information:

- A current, signed version of the protocol and any previous versions of the protocol (if applicable)
- Protocol amendment(s) (if applicable)

- Curricula Vitae of Investigator(s) where required by law; Investigators must also complete all regulatory documentation as required by the ICH/GCP and by local regulations
- Documentation of IRB approval of the protocol and any protocol amendment(s) (if applicable)
- All material correspondence between the Investigator, IRB, Freenome, and CRO relating to study conduct
- Delegation log/signature list of all staff completing eCRFs

8.8 Study and Site Closure

Freenome may close a study site or terminate the study at any time for any reason at its sole discretion. The study may also be terminated if an Investigator, Freenome, or Freenome's designee becomes aware of conditions or events that suggest a possible hazard to subjects if the study continues.

Conditions that may warrant termination of the study site or the study include, but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB or local health authorities, Freenome's procedures, or GCP guidelines
- Discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study
- Failure of a study site to enroll subjects at an acceptable rate
- Decision on the part of Freenome to suspend or discontinue development of the study intervention

Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a site closure visit has been performed.

9. DATA QUALITY ASSURANCE

All subject data relating to the study will be recorded on printed or electronic eCRFs unless transmitted to Freenome or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.

All data obtained during the study should be entered in the eCRFs promptly. All source documents from which eCRF entries are derived should be placed in the subject's medical records.

Freenome (or designee) will oversee the progress of the clinical study to ensure that the study is being conducted and recorded in accordance with the protocol, SOPs, and applicable ICH and GCP principles and has followed all the applicable regulatory requirements. During site initiation, appropriate study-specific training will be provided to the Investigator, co-Investigator (if applicable), and all site personnel involved in the study by the monitor. Instances of missing or uninterpretable data will be discussed with the Investigator for resolution. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

In accordance with ICH GCP guidelines, the Investigator must ensure provision of sufficient time, reasonable space, and adequate qualified personnel and other support for study monitoring visits. Moreover, regulatory authorities, IRBs, and/or Freenome's Quality Assurance group (or designee) may wish to perform additional source data checks and/or on-site audit inspections. The Investigator must provide direct access to source data documents upon request during study-related monitoring visits, audits, IRB review, and regulatory agency inspections, provided that such visits, audits, reviews and inspections must be performed giving due consideration to data protection and medical confidentiality. In addition, the Investigator will ensure that Freenome and/or Freenome's designee will receive the necessary support to complete these activities.

The CRF Completion Guidelines, to be developed during the initiation phase of the study, will provide detailed data entry instructions into the EDC system and guidance for query resolution. The Data Management Plan will include edit check specifications according to GCP standards and study-specific parameters.

The Investigator will make available for access all study-related records upon request from Freenome (or designee), clinical monitor(s), auditors, and/or IRB in accordance with applicable regulations. Audits may be conducted at any time during or after the study to ensure the validity and integrity of the study data.

Each study site will maintain an Investigator study file which should contain, at minimum, the protocol and any amendments thereto, the study laboratory manual, and any material correspondence with the IRB and Freenome (or its designee), and other study-related documents.

10. REGULATORY AND ETHICAL CONSIDERATIONS

10.1 Good Clinical Practice

The procedures set forth in this study protocol are designed to ensure that Freenome and Investigators abide by all applicable laws, regulations and guidelines, including ICH Good Clinical Practice (GCP) guidelines and international guidelines for ethical practices, including the Declaration of Helsinki (1989) and the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.

Before a subject is screened, the study protocol, including any amendments, the ICF, and any other relevant documents must be approved by the IRB, in accordance with applicable laws, regulations and guidelines.

It is the intent of Freenome to not permit any protocol modifications for this study. Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Freenome and may be implemented immediately, provided the IRB is notified within 5 days.

Any permanent change to the protocol must be handled as a protocol amendment. The amendment must be submitted to the IRB and the Investigator must await approval before implementing the changes. Freenome, or designee, will submit protocol amendments to the appropriate regulatory authorities for approval.

The Investigator will also be responsible for providing oversight of the conduct of the study at the site, including oversight of all personnel involved in the study, and adherence to all applicable laws and regulations, as set forth in the Clinical Trial Agreement (CTA).

Personnel involved in conducting this study will be qualified by education, training, and experience prior to performing their respective tasks.

The study will not use the services of study personnel against whom sanctions have been invoked or who have engaged in scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

11. CLINICAL STUDY REPORT AND PUBLICATION POLICY

Freenome is responsible for preparing and providing the appropriate regulatory authorities with clinical study reports according to the applicable regulatory requirements.

Freenome’s publication policy is discussed in the Investigator's Clinical Trial Agreement.

12. DISCONTINUATION

12.1 Subject Discontinuation/Withdrawal

There are no specific criteria for discontinuation from the study. Subjects may discontinue/withdraw from participation at any time. The Investigator may decide that a subject should discontinue/withdraw from the study. In all instances of subject discontinuation/withdrawal, the Investigator will ensure that all appropriate eCRF pages are completed for any subject who is withdrawn from the study, including the date of and reason for withdrawal from the study. The reason for withdrawal from the study will also be recorded in the clinical records.

12.2 Study Termination

Sites will conduct this study in compliance with the protocol and all applicable regulatory and legal requirements. Freenome reserves the right to terminate this study at any time for safety or administrative reasons, either in its entirety or at individual sites, by providing notification to the IRB or to the enrolling site at the time of termination.

13. CONFIDENTIALITY

All information generated or obtained in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from Freenome or its designee.

Identification of subjects and eCRFs shall be by study ID only. If required, the subject's full name may be made known to an authorized regulatory agency or other authorized official.

13.1 Subject De-Identification and Data Protection

Each subject will be de-identified by use of a unique study ID number that will be assigned sequentially in order of enrollment. From the time of number assignment, the specimen must not be labeled with any other identifier. This process must be conducted in accordance with institutional privacy requirements and may therefore vary by site.

Investigator and study center must maintain the anonymity of participating subjects. Each subject will be assigned a unique numerical subject ID number. Investigator and study center will include only the numerical subject ID on any eCRFs or other subject records or other documents that are transferred or submitted to Freenome or Freenome's designee during the course of the study and will redact the subject's name and all other personally-identifiable subject information from such documents.

14. REFERENCES

1. American Cancer Society. Cancer Facts & Figures. Atlanta, GA: American Cancer Society, Inc.; 2019.
2. Bibbins-Domingo K, Grossman DC, Curry SJ, et al. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2016;315:2564-75.
3. Rex DK, Boland CR, Dominitz JA, et al. Colorectal Cancer Screening: Recommendations for Physicians and Patients from the U.S. Multi-Society Task Force on Colorectal Cancer. The American Journal of Gastroenterology 2017;112:1016-30.
4. Provenzale D, Gupta S, Ahnen DJ, et al. NCCN Guidelines Insights: Colorectal Cancer Screening, Version 1.2018. Journal of the National Comprehensive Cancer Network: JNCCN 2018;16:939-49.
5. Howlader N NA, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA. SEER Cancer Statistics Review, 1975-2016. Bethesda, MD: National Cancer Institute; 2019 April.
6. Bailey CE, Hu CY, You YN, et al. Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. JAMA Surgery 2015;150:17-22.
7. Meester RGS, Peterse EFP, Knudsen AB, et al. Optimizing colorectal cancer screening by race and sex: Microsimulation analysis II to inform the American Cancer Society colorectal cancer screening guideline. Cancer 2018;124:2974-85.
8. Peterse EFP, Meester RGS, Siegel RL, et al. The impact of the rising colorectal cancer incidence in young adults on the optimal age to start screening: Microsimulation analysis I to inform the American Cancer Society colorectal cancer screening guideline. Cancer 2018;124:2964-73.
9. Wolf AMD, Fontham ETH, Church TR, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA: A cancer journal for clinicians 2018;68:250-81.
10. Shapiro JA, Klabunde CN, Thompson TD, Nadel MR, Seeff LC, White A. Patterns of colorectal cancer test use, including CT colonography, in the 2010 National Health Interview Survey. Cancer epidemiology, biomarkers & prevention: A publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2012;21:895-904.
11. White A, Thompson TD, White MC, et al. Cancer Screening Test Use - United States, 2015. MMWR Morbidity and Mortality Weekly Report 2017;66:201-6.
12. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA: A cancer journal for clinicians 2018;68:7-30.
13. Young PE, Womeldorph CM. Colonoscopy for Colorectal Cancer Screening. Journal of Cancer 2013;4:217-26.
14. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science Translational Medicine 2014;6:224ra24.
15. Aravanis AM, Lee M, Klausner RD. Next-Generation Sequencing of Circulating Tumor DNA for Early Cancer Detection. Cell 2017;168:571-4.
16. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Science Translational Medicine 2017;9.

17. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of Health and Social Behavior 1983;24:385-96.
18. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nature Reviews Cancer 2017;17:223-38.
19. Haque I, Elemento O. Challenges in Using ctDNA to Achieve Early Detection of Cancer. bioRxiv; 2017.
20. Lu H, Goodell V, Disis ML. Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer. Journal of Proteome Research 2008;7:1388-94.
21. Desmetz C, Mange A, Maudelonde T, Solassol J. Autoantibody signatures: progress and perspectives for early cancer detection. Journal of Cellular and Molecular Medicine 2011;15:2013-24.
22. Sima C, Dougherty ER. The peaking phenomenon in the presence of feature-selection. Pattern Recognition Letters 2008;29:1667-74.
23. Hua J, Tembe WD, Dougherty ER. Performance of feature-selection methods in the classification of high-dimension data. Pattern Recognition 2009;42:409-24.
24. Wan N, Weinberg D, Liu T-Y, et al. Machine learning enables detection of early-stage colorectal cancer by whole-genome sequencing of plasma cell-free DNA. BMC Cancer 2019;19:832.
25. Putcha G. Freenome Announces Encouraging Performance Data for Its Multiomics Blood Test to Detect Early-Stage Colorectal Cancer in a Prospective, Multi-Center Clinical Study. South San Francisco, CA, US: Poster published at the American Society of Clinical Oncology's Gastrointestinal Cancers Symposium (ASCO GI); 2020.
26. Zauber AG, Winawer SJ, O'Brien MJ, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. The New England Journal of Medicine 2012;366:687-96.
27. Corley DA, Jensen CD, Marks AR, et al. Adenoma Detection Rate and Risk of Colorectal Cancer and Death. 2014;370:1298-306.

15. APPENDICES

Appendix I Concomitant Medications for Data Collection

Concomitant Medications for Data Collection:*

Covid-19 Vaccination

Aspirin

NSAIDs

Estrogen

DMARDs

Biological Agents

*Concomitant medications to be collected in the 30 days preceding enrollment

Examples of commonly used DMARDs:

hydroxychloroquine (Plaquenil)

leflunomide (Arava)

methotrexate (Trexall)

sulfasalazine (Azulfidine)

minocycline (Minocin)

Examples of commonly used Biological Agents:

abatacept (Orencia)

adalimumab (Humira)

anakinra (Kineret)

baricitinib (Olumiant)

certolizumab (Cimzia)

etanercept (Enbrel)

golimumab (Simponi)

infliximab (Remicade)

rituximab (Rituxan)

sarilumab (Kevzara)

tocilizumab (Actemra)

tofacitinib (Xeljanz)

Appendix II Study Contact Information

Sponsor:	Freenome Holdings, Inc. 279 East Grand Avenue, 5th floor South San Francisco, CA 94080 [redacted]
Clinical Research Organizations:	[redacted]
Sponsor Laboratory:	Freenome Holdings, Inc. 259 East Grand Avenue South San Francisco, CA 94080 [redacted]

Appendix III Protocol Version History

Protocol Version	Effective Date	Reason for Change
V1.0	04Feb2020	Initial Release
V2.0	20Jul2020	See Summary of Changes
V2.1 (administrative note to file only)	02Oct2020	Amended Section 4.2: Exclusion Criteria 2.2 to clarify the intent to exclude patients who have any evidence of a malignancy in the five years preceding enrollment.
V3.0	05April2021	See Summary of Changes