**Word Count (including section headings):** 1,146

**References:** 7

**Clinical Procedures**

Bowel preparation procedures and colonoscopy were performed according to the standard of care at each clinical site. No dietary or medication restrictions were required for the mt-sDNA test. Source documentation included the quality of bowel preparation (modified Aronchick scale1), cecal intubation, colonoscope withdrawal time, and colonoscopy findings with histopathology results for any excised or biopsied lesion. Only the most advanced colorectal epithelial lesion (index lesion) and its location (proximal or distal) was used to categorize participants for the analysis (**Supplemental Table 1**), with the larger or more histologically-advanced finding among multiple findings designated as the index lesion. The proximal colon was defined as the splenic flexure and all segments proximal to it, an insertion depth of >60 cm, or any part described by the phrase “right colon” or “proximal colon”. The distal colon was considered to include all other segments, an insertion depth of ≤60 cm, or any part described by the phrase “left colon” or “distal colon”. Subjects with a poor-quality bowel preparation (after washing and suctioning) were allowed to repeat the colonoscopy if the endoscopist believed it was in the subject’s best interest clinically (poor-quality was defined as large amounts of debris with <90% of the surface examined). Participants repeated the colonoscopy only if the second procedure fell within the 90-day window.

All biopsy and surgical specimens underwent histopathological analysis at the laboratory typically used by each study site. Polyps with high grade dysplasia or 25% or more villous elements in adenomas measuring less than 1 cm, as well as sessile serrated or hyperplastic polyps measuring 1 cm or larger, were re-reviewed centrally by a gastrointestinal pathologist who was blinded to mt-sDNA test results. Diagnostic disagreements were resolved by consensus of at least two central pathologists. A colonoscopy that identified a CRC or advanced precancerous lesions was included in the analyses, even if limited by extent of examination or quality of bowel prep. Participants whose lesions could not be confirmed by histology (e.g., due to no biopsy or lost biopsy during retrieval) were excluded from the study since they could not be categorized for the primary or secondary analyses.

**Mt-sDNA Sample Processing & Laboratory Procedures**

All stool samples were delivered by express shipping to Exact Sciences Laboratories (Madison, WI) for processing. Incoming samples were inspected for acceptability per the laboratory’s standard operating procedure. For samples not collected according to the instructions for use or if there was no valid test result (e.g., “no result obtained”, or “sample could not be processed”), a repeat sample was requested. Valid mt-sDNA test results were recorded as either “positive” or “negative”. If a valid sample result could not be obtained for a subject at T0 after multiple attempts, the subject was withdrawn from the study and an additional participant was enrolled.

All accepted samples were collected per Exact Sciences mt-sDNA-based Colorectal Cancer Screening Test instructions for use2. A positive mt‑sDNA test result was based on the FDA-approved logistic regression algorithm threshold score of ≥183; the algorithm is published as supplemental material3,4. The mt-sDNA test is a qualitative, dichotomously-reported test, the component values of which are not reported separately2. The test includes molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions, mutant KRAS, and ß-actin (a control gene for DNA quantity), as well as an immunochemical assay for human hemoglobin, none of which have individually reported thresholds or cutoffs.

*Exploratory analysis*. The primary analysis was estimated on non-missing data. However, to explore the impact of missingness when verification of disease is potentially biased, two exploratory analyses were performed. First, the potential mechanisms of missingness were assessed by constructing a Test Ignorance Region (TIR), which provides the range of performance statistics across all possible incidence rates of disease (ranging from 0 to 1) in those with missing disease verification5. The TIR was evaluated with respect to two different aspects of missingness. Missing completely at random (MCAR) assumes that missingness was not dependent on any patient-specific factors; under MCAR, one assumes that the estimates are unbiased. However, with the presence of verification bias, it was unlikely that MCAR would be an appropriate assumption. Alternatively, missing at random (MAR) assumes that the prevalence of disease in the unverified subset is the same as observed in the verified subset. Performance estimates under the assumption of MCAR and MAR were plotted against the TIR to determine the acceptability of the corresponding mechanism of missingness.

Second, missing data on mt-sDNA outcome and CRC/advanced precancerous lesion occurrence were imputed using other available observed study data. Based on exclusion patterns, demographic data such as age, sex, race, and ethnicity were considered for imputing missing data6,7 to estimate the effects of missing data on the primary endpoint, and to determine if the year 3 mt-sDNA result was significantly related to a colonoscopy-detected CRC or advanced precancerous lesion. Imputations considered age (50-59, 60-69, 70+ years), race (White, other), and mt-sDNA T3 results (negative, positive) to infer the percent of true positive, true negative, false positive, and false negative findings using simulation to generate 25 independent random samples. Since there were no year 3CRC cases for this analysis, advanced precancerous lesion at year 3 represent the true state while the positive mt-sDNA tests represent positive tests. This analysis assumed that data were missing at random and was performed to predict colonoscopy outcome for the subjects with unverified disease after a valid mt-sDNA test result using a logistic model (0=categories 3-6, 1=categories 1-2 (CRC/advanced precancerous lesion).

**RESULTS**

**Sensitivity and Robustness Analyses**

We considered the effect of the Stage IV CRC detected at month 22 on baseline findings. Assuming this cancer was missed at baseline does not change the PSI at year 3. The subject did not have a year 3 mt-sDNA test but had the mt-sDNA missed the CRC at year 3, the PSI for CRC would be -0.21%, a non-significant finding (P=0.63).

To assess the reliability of the year 3 PSI estimate for advanced precancerous lesions, two common patterns of missing data (naïve and corrected) were examined. The MCAR estimates (using N=591, naïve assumption) fell outside the TIR with estimated sensitivity of 34.9%, specificity of 81.1%, and a PSI of 9.3% (cited above); the MAR estimate fell within the TIR (using N=1,036, corrected assumption), with a sensitivity of 26.2%, specificity of 86.6%, and PSI of 9.4%, consistent with the observed 9.3%. The MAR is therefore a more reliable estimate.

Additional imputation analyses were performed to account for the 400 subjects who did not undergo colonoscopy in year 3. The imputations resulted in a median number of CRC/advanced precancerous lesions increases in those with a positive year 3 mt-sDNA test result from 22 of 125 (17.6%) to 28 of 152 (18.4%), with a range of increase from 15.8% to 22.4%. Those with a negative mt-sDNA test result increased from 41 of 497 (8.2%) to 78 of 884 (8.8%), with a range of increase from 7.8% to 11.2%.

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