

# A 3-year Observational Study of Persons With a Negative Colonoscopy and Positive Multi-target Stool DNA Test

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## Introduction

We studied whether persons with a positive multi-target stool DNA (mt-sDNA) test but a negative colonoscopy were associated with a higher incidence of colorectal cancer or non-colorectal aerodigestive cancer 3-4 years after testing than would be predicted in the overall population using the U.S. Surveillance Epidemiology and End Results (SEER) program data.

- mt-sDNA is FDA approved for colorectal cancer (CRC) screening of average-risk persons age ≥50 years and is recommended by the U.S. Preventive Services Task Force. Here, we report the 3-4 year follow-up of DeeP-C pivotal study<sup>1</sup> participants who were mt-sDNA positive but colonoscopy negative (false positive). The study objective is to determine whether these discordant results were followed by a later diagnosis of CRC or non-colorectal aerodigestive cancer.

- The DeeP-C pivotal study was a prospective, cross-sectional 90 site, 10,000 person study evaluating the performance of mt-sDNA using colonoscopy as the reference method. Participants with negative colonoscopy findings and positive mt-sDNA tests were considered to have discordant results.

- DNA biomarkers are released into stool from cells shed from the colorectal epithelium and epithelial lesions. [Figure 1] A proportion of patients normally have age related elevated background biomarker levels of methylated DNA and hemoglobin that can lead to a positive mt-sDNA, which can affect test specificity.

- mt-sDNA quantitates 11 stool biomarkers that can be associated with CRC and pre-malignant lesions (9 DNA markers (2 methylated genes and 7 KRAS mutations); one DNA reference gene (beta-Actin); one fecal hemoglobin (FIT) marker). [Figure 2] A composite marker level score is calculated by an algorithm and provides a single patient result of positive or negative.

- Previously, case control studies showed that non-colorectal etiologies could be associated with 1.6 cases per 1000 mt-sDNA positive patients per the FDA Cologuard Statement of Safety and Effectiveness (SSE)<sup>2</sup>.

## Methods

This is an observational study using medical records review and direct patient interviews to ascertain the incidence of CRC and non-colorectal aerodigestive cancers in patients found to be mt-sDNA positive but colonoscopy negative compared to the incidence reported in the U.S. SEER data.

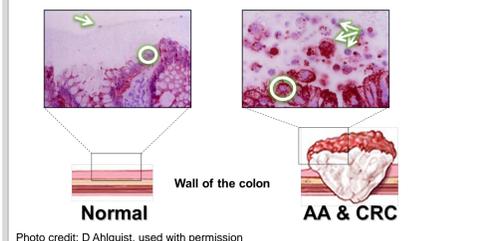
- There were 187 DeeP-C participant medical records with negative colonoscopies of which 150 had negative and 37 had positive mt-sDNA tests. Participants with <3 years of follow-up records were contacted by telephone.
- Follow-up interval was the time between DeeP-C informed consent (IC) and medical records review. DeeP-C colonoscopy was performed within 90 days of IC.
- Negative colonoscopies included non-advanced adenomas and/or hyperplastic polyps, or no findings requiring biopsy.
- Outcomes were based on aerodigestive cancer incidence compared to the incidence in the U.S. SEER program.

## Results

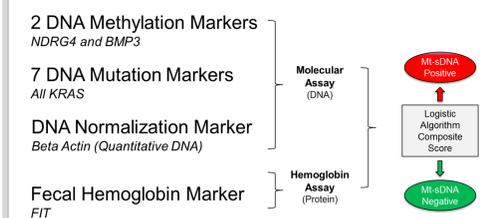
None of 37 (0/37) participants who were mt-sDNA positive but colonoscopy negative were diagnosed with CRC or non-colorectal aerodigestive cancer after mean of 4.1 (3.6 - 4.8) years of follow-up. [Table 2]

- Demographics of subjects with positive or negative mt-sDNA results were similar. [Table 1]
- Of 150 participants with negative mt-sDNA, 142 were alive and cancer free with 3.1- 5.1 yrs. of follow up (mean 4.2, median 4.3 yrs.), 3 died (gangrenous gall bladder, complications of lung transplant for interstitial lung disease, and mesothelioma at 0.8, 2.3 and 3.1 years, respectively), and 5 were lost to follow-up (3 immediately post DeeP-C colonoscopy, one each at 0.6 and 1.7 yrs.
- Ten participants with a negative mt-sDNA test and a negative colonoscopy procedure repeated colonoscopy 2-4 years post DeeP-C IC, 8 of whom had negative findings while 2 had non-advanced colorectal neoplasia (single small non-advanced adenoma).
- All 37 participants with a positive mt-sDNA test and a negative colonoscopy procedure were alive with 3.6 - 4.8 years of follow-up (mean 3.9, median 4.1). One had a low-grade parotid mucoepidermoid cancer diagnosed 2.9 years post DeeP-C. Three of these participants repeated colonoscopy 2, 3, and 4 years post Deep-C IC, which were: negative, had non-advanced adenomas (3), and negative, respectively. [Table 2]
- The comparable incidence rate of aerodigestive cancers in CRC screening eligible persons is 1953 per 100,000 person years in U.S. SEER data.

**Figure 1.** DNA biomarkers in stool are released from colonic epithelial cells (circles). Low background levels of age related methylated DNA biomarkers can be released from the scant exfoliating non-neoplastic apoptotic epithelial cells (arrow, Normal, below). Abundant cells exfoliate from CRC's (triple arrow, below) though less so from advanced adenomas (AA, not shown). This elevates levels of stool DNA biomarkers, especially from CRCs, which manifest escape from apoptosis.



**Figure 2. Multi-target Stool DNA Test**  
Biomarkers with algorithm generate a single qualitative result



Imperiale TF et al. N Engl J Med. 2014;370(14):1287-1297

**Table 1. Demographics and Colonoscopy Findings: Colonoscopy Negative Participants (N=187)**

Characteristics	mt-sDNA Positive Colonoscopy Negative 37 (20%)	mt-sDNA Negative Colonoscopy Negative 150 (80%)
Sex		
Male	17 (46%)	68 (45%)
Female	20 (54%)	82 (55%)
Race		
Caucasian	33 (89%)	132 (88%)
Other	4 (11%)	18 (12%)
Ethnicity		
Hispanic/Latino	1 (2.7%)	5 (3.3%)
Age range, years, at time of Informed Consent		
50-54	7 (19%)	12 (8%)
55-59	5 (14%)	45 (30%)
60-64	5 (14%)	22 (15%)
65-69	7 (19%)	19 (13%)
70-74	7 (19%)	30 (20%)
75-79	4 (11%)	12 (8%)
80-85	2 (5.4%)	10 (6.7%)
Follow up interval (years)		
Range	3.6-4.8	3.1-5.1
Median	3.9	4.2
Mean (+/- SD)	4.1 (0.78)	4.3 (0.72)
<b>Negative Colonoscopy Findings</b>		
Non advanced adenoma		
1 or 2: >5, <10 mm	2 (5.4%)	8 (5.3%)
≥3; <10 mm	1 (2.7%)	2 (1.3%)
1 or 2; ≤5 mm	9 (24%)	32 (21%)
No colorectal neoplasia	25 (67%)	108 (72%)

**Table 2. Current Study - Participants with a Negative Colonoscopy<sup>A</sup> from the Geenen DeeP-C Pivotal Study<sup>B</sup> Site**

mt-sDNA Result	N (187)	Average Follow up period, yrs	Alive	Aerodigestive Cancer	Deceased <sup>C</sup>	Lost to follow-up
Negative	150	4.3 (0.25 - 5.1)	142	0	3	5
Positive	37	4.1 (0.25 - 4.8)	37	0	0	0

**Table 3. Previous Study - Participants with a Negative Colonoscopy<sup>A</sup> from the Hilsden DeeP-C Pivotal Study<sup>B</sup> Site (Hilsden et al DDW 2016)**

mt-sDNA Result	N (716)	Average Follow up period, yrs	Alive	Aerodigestive Cancer	Deceased <sup>D</sup>	Lost to follow-up
Negative	598	3.9 (0.25 - 5.0)	592	1	6 <sup>(a)</sup>	0
Positive	118	4.0 (0.25 - 5.0)	116	1	2 <sup>(b)</sup>	0

**Table 4. Combined Data from Tables 2. and 3.**

mt-sDNA Result	N (903)	Average Follow up period, yrs	Alive	Aerodigestive Cancer	Deceased	Lost to follow-up
Negative	748	4.0 (0.25 - 5.1)	734	1	9	5
Positive	155	4.0 (0.25 - 5.0)	153	1	2	0

- A. Absence of CRC or AA is considered a negative colonoscopy  
 B. Multi-target stool DNA testing for Colorectal Cancer Screening, Imperiale et al, N Engl J Med 2014;370:1287-97  
 C. Cause of death (yrs post Cologuard): gangrenous gall bladder (10 mos), complications lung transplant (2.3), mesothelioma (3.1)  
 D. Cause of death (yrs post Cologuard): (a) - Lung (3.8), Leukemia (3.9), Non-cancer (10 mos) not coded (1.6 3.4, 4.1); (b) - Pancreatic cancer (6 mos), Leukemia (2.3)

## Discussion

This study's findings are consistent with two other reports<sup>3,5</sup> showing the low incidence of CRC or non-colorectal aerodigestive cancer in mt-sDNA positive and colonoscopy negative participants after a mean of 4 years of follow-up.

The composite incidence rate of aerodigestive cancers in mt-sDNA positive and colonoscopy negative participants combined from two study sites [Table 4] was approximately 161 (95% CI: 137 - 188) per 100,000 person years, which is substantially lower than that seen in the U.S. SEER data of 1953 per 100,000 person years in the general comparable population.

- Previously reported data from a second, larger DeeP-C study site<sup>3</sup> [Table 3] with similar demographics was combined with our data from this study [Table 4] and the combined data demonstrate similar findings.
- The expected cumulative incidence rate for aerodigestive cancers based on the U.S. SEER program<sup>4</sup> data set is 1953 per 100,000 person years<sup>4</sup>.
- The combined incidence per 100,000 person years of aerodigestive cancers in DeeP-C participants from 2 sites [Table 4] with discordant mt-sDNA and colonoscopy results (Pos/Neg) is 161 (95% CI: 137 - 188) and for those with concordant negative mt-sDNA and colonoscopy results (Neg/Neg) is 33 (95% CI: 23 - 46).
- An additional report<sup>5</sup>, which combined mt-sDNA case control patients and DeeP-C participants from three other DeeP-C study sites also demonstrates that the cumulative incidence of non-colorectal aerodigestive cancers in mt-sDNA positive and colonoscopy negative patients is well below the U.S. SEER rate.
- Limitations:
  - Relatively small study size.
  - Cohort comprised only participants at average risk of CRC.

## Conclusions

- None of 37 (0/37) mt-sDNA positive and colonoscopy negative patients developed an aerodigestive cancer after 3.6 - 4.8 years of follow-up.
- This study and two previous reports demonstrate the incidence of aerodigestive cancers in mt-sDNA positive and colonoscopy negative patients is less than the expected U.S. SEER incidence of 1953 per 100,000 person years in the general comparable population.
- The empiric data to date are consistent with the case control data included in the FDA Cologuard SSE demonstrating approximately 1.6/1000 mt-sDNA positive cases could be related to non-colorectal sources.
- The data from this study, two previous reports, and the FDA Cologuard Statement of Safety and Effectiveness do NOT support further evaluation of well prepared and well examined otherwise asymptomatic mt-sDNA positive and colonoscopy negative persons for CRC or non-colorectal aerodigestive cancers based solely on discordant results.

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## References

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