SCIENCE BEHIND THE STUDY
Elizabeth G. Phimister, Ph.D., *Editor*

Cell-free DNA for Colorectal Cancer Screening

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Colorectal cancer is a common cancer in many parts of the world. Of interest, then, is a report in this issue of the *Journal* by Chung et al.,[1] who used a noninvasive assay of cell-free DNA (cfDNA) in plasma to screen for colorectal cancer in an average-risk population in the United States. The findings are also discussed in an editorial by Carethers.[2]

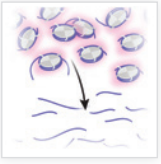
WHAT IS CFDNA?

This substance is a composite of extracellular DNA molecules found in bodily fluids, including plasma and urine. Plasma cfDNA has attracted most of the attention in the field and is made up of DNA molecules released from various tissues in the body, hence providing a source for noninvasive sampling that may provide insight into physiologic and pathologic processes. Short fragments of DNA molecules make up the majority of cfDNA. The fragmentation patterns of cfDNA have been found to carry diagnostic information, the study of which is now referred to as fragmentomics (see Key Concepts).[3] The epigenetic signature of the tissue of origin is carried by cfDNA.[3,4]

HOW IS CFDNA ASSAYED?

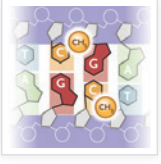
This substance can be assayed with the use of methods that are sensitive enough to detect low concentrations of DNA, including the polymerase-chain-reaction assay and DNA sequencing. DNA methylation is the most common epigenetic signature assayed in cfDNA. To analyze the DNA methylation status of a piece of cfDNA, the cfDNA is most commonly subjected to a chemical or enzymatic process that converts the epigenetic signature into a genetic change, which can then be detected with the use of DNA sequencing. Another approach is to treat the cfDNA with a reagent that binds or cleaves it, depending on whether it is methylated. (Chung et al. used this approach.) A more recent development is direct detection of methylation by single-molecule sequencing of the cfDNA.[5]

Key Concepts



Fragmentomics

The characteristics of a large number of DNA fragments. Fragmentomics involves the measurement and analysis of fragment lengths and the characteristics of the ends of the fragments (e.g., having a blunt or jagged end or having a specific sequence at the end, called an "end motif") and the patterns of the different lengths and fragment characteristics. There is a correlation between methylation and fragment end motif. DNA fragments from circulating tumor DNA, which can be in the blood of a person with cancer or precancer, are shorter, have more jagged ends, and have a different distribution of specific end motifs than the circulating free DNA in the blood from normal cells, which is present in the blood of all persons regardless of whether they have a tumor. Fragmentomics is also used in the study of cell-free DNA in the plasma of pregnant persons and transplant recipients.



DNA methylation

The process by which a methyl group, consisting of one carbon and three hydrogen atoms, is added to DNA. Methylation of a gene's promotor (a regulatory region upstream of the coding sequence) can suppress the expression of that gene.

Adenomatous colon polyp

An abnormal growth of colonic mucosal cells (polyp) that is benign, but with time, may develop into a malignant neoplasm.

HOW IS CFDNA USED NOW?

During pregnancy, fetal cfDNA is found, together with fragments of maternal cfDNA, in the plasma of the pregnant person: assay of the fetal cfDNA is the basis of noninvasive prenatal testing for fetal chromosomal aneuploidy and monogenic diseases.[6] Another application currently in development is the detection of cfDNA after solid-organ transplantation: the presence of cfDNA derived from the grafted organ in the plasma of the recipient may be used as a marker for graft rejection.[7,8]

An expanded illustrated glossary is available at NEJM.org

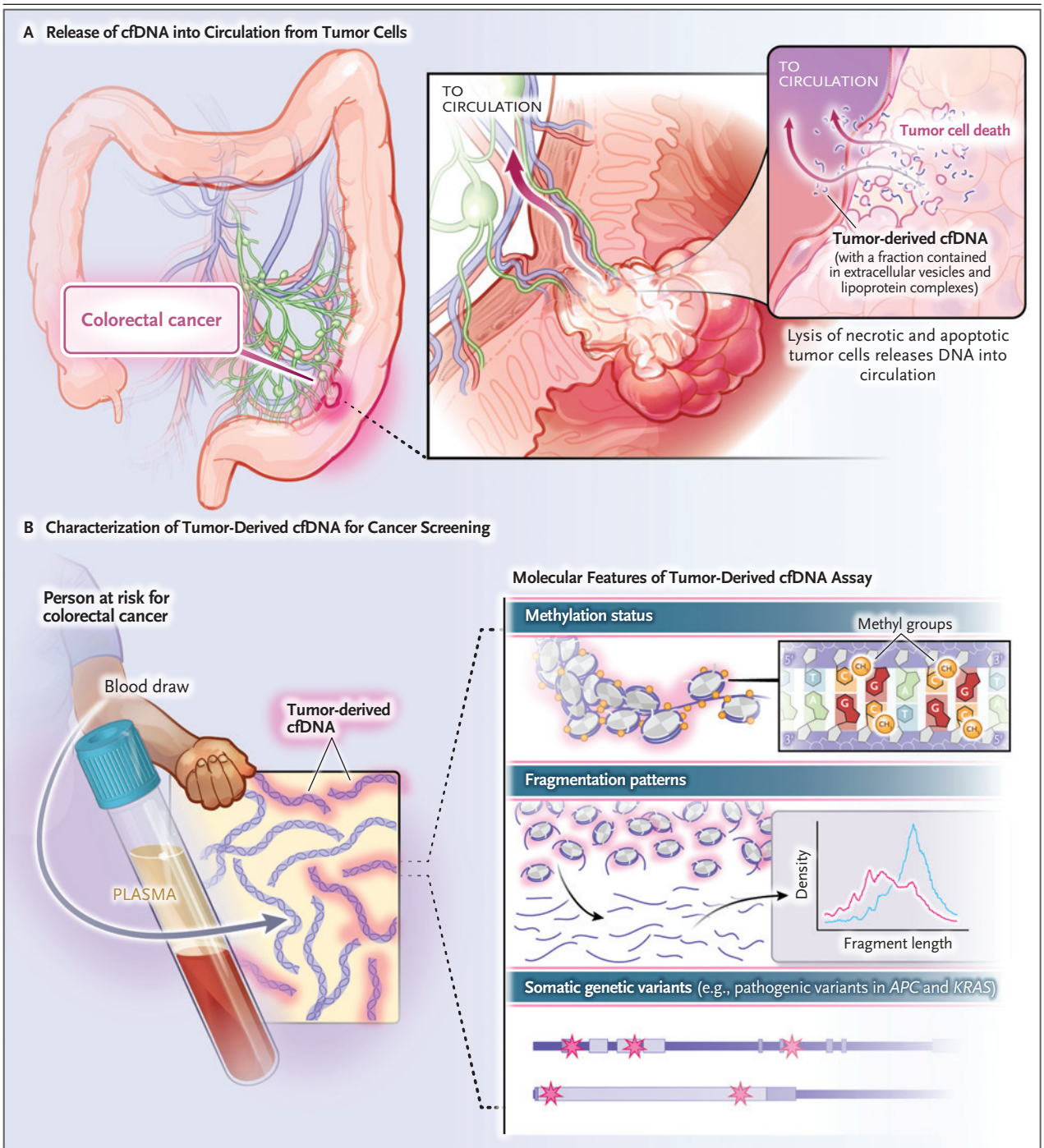


Figure 1. Screening for Colorectal Cancer with the Use of Circulating Cell-free DNA (cfDNA).

Chung et al.[1] studied the use of a blood-based cfDNA test for the screening of colorectal cancer. As shown in Panel A, tumor cells from a colorectal cancer release DNA into the bloodstream. As shown in Panel B, circulating tumor-derived DNA is present in the plasma fraction of a blood sample obtained from a screened person. After DNA extraction, cfDNA is analyzed for DNA methylation status, fragmentation patterns, and specific somatic variants in *APC* and *KRAS*. Quantitative scores from the methylation status and fragmentation patterns, as well as qualitative assessment of the presence or absence of mutations, are combined in a logistic-regression model to produce a single integrated score, which indicates abnormal (test is positive) or normal (test is negative) status. The model had previously been trained with the use of an independent set of samples for predicting colorectal cancer.

Tumor-derived DNA can also be found in plasma cfDNA obtained from patients with cancer,[9] a finding that underpins the experimental use of plasma cfDNA for cancer screening, monitoring, and prognostication.

AND THE RESULTS OF CHUNG ET AL.?

Chung et al. used a commercially available assay (called the Shield test) to analyze the plasma cfDNA of 7861 persons with a mean age of 60 years. The test characterizes and then integrates three types of information about the person's cfDNA: methylation status, aberrant fragmentation patterns, and the presence or absence of somatic pathogenic variants in the genes *APC* and *KRAS* (Fig. 1). Using this test, they detected colorectal cancer with a sensitivity of 83%, advanced neoplasia with a specificity of 90%, and advanced precancerous lesions (including advanced adenomatous colon polyps and sessile serrated lesions) with a sensitivity of 13%. The specificity for the detection of advanced neoplasia was inversely correlated with age.

WHAT'S NEXT?

Although Chung et al. demonstrated the feasibility of using plasma cfDNA to screen for colorectal cancer, the relatively low sensitivity for the detection of advanced precancerous lesions is a limitation. Moreover, colonoscopy not only detects such lesions with high sensitivity but also permits their immediate removal. The noninvasiveness (relatively speaking) of the plasma cfDNA assay, though, is a feature that seems likely to result in greater uptake than colonoscopy: further work is warranted to determine whether this is true and whether the cost–benefit ratio would justify its implementation. Also of note are fecal tests (which are truly noninvasive), such as the one reported by Imperiale et al. in this issue of the *Journal*.[10]

Chung et al. used an assay of DNA methylation status and of fragmentation patterns and a qualitative test (present or absent) of somatic DNA variants. However, the relative contributions of each of these components to the end result is opaque and may vary across patients and populations: further investigation is warranted. The possibility of a reduction in test specificity with age, speculated to be dependent on age-related changes in DNA methylation, also merits further investigation. The possibility that false positive results might arise from persons with non–colorectal cancer tumors represents another avenue for investigation. Indeed, aberrant methylation and fragmentomic profiles have been reported for multiple tumor types.[11]

According to Chung et al., the manufacturer of the test recommends a 3-year interval between cfDNA-based screenings for colorectal cancer. It would be crucial to examine the scientific rationale behind such a recommendation. A study of plasma cfDNA as a basis for screening for nasopharyngeal carcinoma showed that persons with a positive cfDNA test but without an immediately identifiable cancer were at increased future risk for cancer.[12] If a similar phenomenon occurs in persons at risk for colorectal cancer, perhaps the interval for repeating cfDNA screening or scheduling of conventional, invasive screening could be tailored according to the results of previous cfDNA testing.

Disclosure forms provided by the author are available with the full text of this editorial at NEJM.org.

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Maternal RSV Vaccine — Weighing Benefits and Risks

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Respiratory syncytial virus (RSV) poses a substantial burden to the health of infants. An estimated 1.4 million RSV-associated hospitalizations and 45,700 RSV-attributable deaths occur worldwide each year in infants younger than 6 months of age.[1] In the United States, RSV is the leading cause of hospitalization among infants, with 2 to 3% of infants younger than 6 months of age hospitalized for RSV infection annually.[2] Recently, two agents to protect young infants from severe RSV disease have become available. In July 2023, the Food and Drug Administration (FDA) approved nirsevimab, a long-acting monoclonal antibody,[3] for use in infants; 1 month later, the FDA approved the first RSV vaccine, which is based on the RSV prefusion F protein (RSVPreF; Abrysvo, Pfizer), for use in pregnancy.[2]

This issue of the *Journal* includes a report of a phase 3 trial by Dieussaert et al.,[4] who evaluated the effects of a candidate maternal RSV vaccine (RSVPreF3-Mat) on severe RSV-associated disease in young infants. Data suggest that the vaccine was efficacious; however, the trial was halted early because of a higher incidence of preterm birth in the vaccine group than in the placebo group (6.8% [237 of 3494 infants] vs. 4.9% [86 of 1739 infants]; relative risk, 1.37; 95% confidence interval [CI], 1.08 to 1.74; P=0.01). An imbalance in the risk of neonatal death in the two trial groups was also seen — a finding that was probably attributable to a higher incidence of preterm birth in the vaccine group than in the placebo group — but the imbalance was not significant (relative risk, 2.16; 95% CI, 0.62 to 7.56; P=0.23). The development of RSVPreF3-Mat was subsequently discontinued.

The difference in the incidence of preterm birth between the vaccine and placebo groups was primarily seen in low- and middle-income countries (relative risk, 1.56; 95% CI, 1.17 to 2.09) as compared with high-income countries (relative risk, 1.04; 95% CI, 0.68 to 1.58). The difference was observed only during a particular period, with the greatest difference having occurred during the wave of infections due to the B.1.617.2 (delta) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); however, no relationship was identified between preterm birth and the report of coronavirus disease 2019 (Covid-19) by the maternal participants or evidence of SARS-CoV-2 infection during pregnancy. The difference between the vaccine and placebo groups was seen for all three pathways to preterm birth in pregnant persons (premature preterm rupture of membranes, preterm labor, and provider-induced preterm birth), and the time between vaccination and preterm birth varied from weeks to months, which made it difficult to identify a potential mechanism for preterm birth.

Ultrasonography during the first trimester pregnancy — the most accurate method to establish or confirm gestational age[5,6] — was not performed in 45% of the pregnancies (146 of 323) that resulted in preterm birth (see the Supplementary Results section in the Supplementary Appendix, available with the full text of the article by Dieussaert et al. at NEJM.org). However, misclassification of gestational age would be expected to occur similarly in the vaccine and placebo groups owing to randomization and thus would not explain the between-group difference in the risk of preterm birth. The detection of fetal growth restriction and being small for gestational age also relies on the accurate assessment of gestational age; in the current trial, both events were less frequent in the vaccine group than in the placebo group (relative risk, 0.57 [95% CI, 0.34 to 0.97] and 0.78 [95% CI, 0.65 to 0.95], respectively).

Although the FDA-approved maternal RSV vaccine is bivalent and RSVPreF3-Mat is monovalent, the vaccines are otherwise similar. The bivalent maternal RSV vaccine was studied in a phase 3 randomized clinical trial, in which pregnant persons received the vaccine or placebo