Abstract 243
Tumor Area and Microscopic Extent of Invasion to Estimate Circulating Tumor DNA Fraction in Plasma and Detectability of Colorectal Cancer

BACKGROUND
- Circulating Cell-free Genome Atlas (CCGA): A prospective, multicenter, observational, case-control study with longitudinal follow-up to support the development of a plasma circulating cell-free DNA (cfDNA)-based multi-cancer early detection test.
- We previously reported that a targeted methylation (TM) assay and machine-learning classifier detected over 20 cancer types, including breast, lung, colon, prostate, cervical, lymphoid, plasma cell neoplasms, ovarian, bladder, gastrointestinal, liver, pancreatic, head and neck, renal, urologic, uterine, kidney, melanoma, thyroid, myeloid, and sarcoma, with a single fixed false positive rate of 1%, and simultaneously determined tissue of origin with >90% accuracy.1
- Previously we showed that the fraction of cfDNA fragments from tumor versus normal cells (tumor fraction, TF) was a stronger predictor of cancer detection than clinical stage and was an equivalent predictor of survival.2
- Here, we used colorectal cancer (CRC) as one example to understand the biophysical determinants of TF in solid tumors.

METHODS
- Blood samples were prospectively collected from participants with newly diagnosed colorectal cancer and from participants without a diagnosis of cancer (Figure 1).3
- Specimens in the first CCGA substudy were subjected to a prototype whole-genome bisulfite sequencing (WGBS) assay with a machine-learning classifier that predicted cancer or non-cancer. Samples in the second CCGA substudy were subjected to a TM assay with a newly trained machine-learning classifier that predicted cancer or non-cancer, with tissue of origin predicted for samples with cancer signal detected (Figure 2).4
- Tumor fraction was determined by comparing variants detected in a targeted sequencing assay of cfDNA from these tissues to variants from a whole-genome sequencing (WGS) assay of matched, macrodissected formalin-fixed, paraffin-embedded tumor samples, while using a white blood cell WGS assay to control for hematopoietic contamination.2
- To predict tumor fraction for non-metastatic colorectal adenocarcinoma, we constructed a simple physical model (TF = m * TSA) that assumes a linear relationship (slope, m) with tumor surface area (TSA) and interacts with microscopic invasion beyond the subserosa (Figure 3).
- The Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study is a prospective, multicenter, observational, case-control study with longitudinal follow-up to support the development of a plasma circulating cell-free DNA (cfDNA)-based multi-cancer early detection test.
- The model included tumor surface area (TSA) to represent the number of tumor cells and access to circulation (microinvasion for CRC) of solid tumors may strongly affect the shedding of cfDNA into the bloodstream.

RESULTS
- For the WGBS assay, the classifier score for CRC cases increased approximately linearly with log(TF) before saturation for high tumor fractions (Figure 4A). The model predicted m = 3.818 x 10^-3 mm² for tumors that invaded beyond the subserosa (p < 0.001); this was 4.4 times higher than estimates for tumors below the subserosa (m = 0.866 x 10^-3 mm²).
- TF distributions over clinical stage I, II, and III (Figure 4B), while TF distributions were clearly separated by microscopic tumor extension (pathological T stage) alone (Figure 3C).
- TF distributions were overlapped for clinical stages I, II, and III (Figure 4A), while TF distributions were clearly separated by microscopic tumor extension (pathological T stage) alone (Figure 3C).
- With a given extent of microinvasion (shallow or deep), TF can be modeled to increase linearly with TSA (Figure 4A).
- The model trained on the WGS assay predicted CRC detection in the TM assay with an area under the curve of 0.944 (Figure 5).6

CONCLUSIONS
- Higher cfDNA TF was significantly associated with cancer detection.
- The strong relationship between cfDNA WGBS classifier scores and TF in the first substudy implies that TF might be impacted by training a classifier on cfDNA assay results.
- A model using TSA (number of tumor cells) and microinvasion (bloodstream access) predicted TF.
- Tumors not penetrating the subserosa had low cfDNA shedding into the bloodstream that likely limited detection.
- As a greater extent of tumor penetration may be associated with higher-risk disease, these results suggest that the classifier may preferentially detect higher-risk cancers.
- Careful examination of pathologic features of CRC suggest that size (TSA for CRC) and access to circulation (microinvasion for CRC) of solid tumors may strongly affect the shedding of cfDNA into the bloodstream.

Figure 1. Study Design and CONSORT Diagram

Figure 2. Detection of Cancer Increases With Increasing TF

Figure 3. TF Distribution by Clinical Stage (A) and Extent of Microinvasion (B)

Figure 4. TF Model Including Both Tumor Size and Microinvasion in the First CCGA Substudy

Figure 5. Receiver Operating Characteristic Curve Based on Model TSA and Microinvasion in the Second CCGA Substudy

References
2. Oxnard GR, et al. ASCO Breakthrough Meeting 2019; Abstract 44.
4. May 7 - 11, 2019; Cold Spring Harbor, NY.
5. FPR 1.00 0.75 0.50 0.25 0.00 0.01 0.02 0.03 0.04 n=1,628 n=74 n=7 n=57 0.01 0.02 0.03 0.04 n=1,531 n=29 39 (74%) stage III cases. n=22 of 39 (59%) stage II cases. n=11 of 18 (61%) stage I cases, and 17 of 30 (57%) stage II cases. The TM-implicated TF estimation n=57 Valid/Validation— TF, tumor fraction.
6. The model trained on the WGS assay predicted CRC detection in the TM assay with an area under the curve of 0.944 (Figure 5).6

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