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Prognostic Significance of Blood-based Cancer Detection in Plasma Cell-free DNA (cfDNA): Evaluating Risk of Overdiagnosis

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Geoffrey R. Oxnard,[1] MD; Xiaoji Chen,[2] PhD; Eric T. Fung,[2] MD, PhD; Ting Ma,[2] MS; Jafi Lipson,[2] MD; Earl Hubbell,[2] PhD; Kathryn N. Kurtzman,[2] MD; Shilpen Patel,[2] MD; Rita Shaknovich,[2] MD, PhD; Zhao Dong,[2] MS, ScD; Hai Liu,[2] PhD; Jacqueline D. Brooks,[2] MS; David Spigel,[3] MD; Michael V. Seiden,[4] MD; Alexander M. Aravanis,[2] MD, PhD; Eric A. Klein,[5] MD; Anne-Renee Hartman,[2] MD; Minetta C. Liu,[6] MD

[1]Dana-Farber Cancer Institute, Boston, MA; [2]GRAIL, Inc., Menlo Park, CA; [3]Tennessee Oncology, Nashville, TN; [4]US Oncology Research, The Woodlands, TX; [5]Cleveland Clinic, Cleveland, OH; [6]Mayo Clinic, Rochester, MN.

BACKGROUND

- Early detection and intervention can alter the course of cancer, improve patient outcomes, and reduce cancer mortality.[1–5]
- Prognosis in screen-detected cancers is generally better than in cancers detected outside of screening paradigms.[6]
- However, effective screening paradigms exist for only a subset of common cancers, and compliance with screening recommendations is variable.[7–11]
- Plasma cfDNA is promising for detection of multiple cancer types across stages from a single blood draw.[12]
  - High circulating tumor cfDNA levels are generally associated with later stage cancers and worse prognosis.[13,14]
- An effective cfDNA-based multi-cancer detection tool ideally should detect clinically significant cancers across stages, with a low limit of detection, at very high specificity, and determine tissue of origin, to provide a potential clinical benefit to patients.[15,16]
  - Such a detection tool may reduce false positive rates and avoid unnecessary testing,[15–17] and may minimize detection of indolent cancers, avoiding overdiagnosis and overtreatment.
- CCGA is a prospective, multicenter, longitudinal, observational study for the development of a noninvasive assay for cancer detection (**Figure 1**).
  - 15,254 participants have been enrolled (56% cancer, 44% non-cancer).

Figure 1. CCGA Study Design

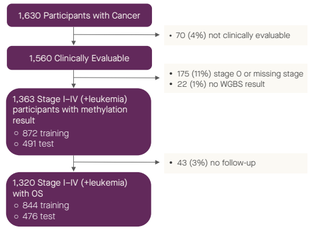
- Here, we report longitudinal follow-up (overall survival) in patients with cancer detected and not detected by the whole-genome bisulfite sequencing (WGBS) methylation assay from the first CCGA substudy to evaluate the prognostic significance of cancer detection using cfDNA.

METHODS

Sample Processing

- Plasma cfDNA samples (**Figure 2**) were subjected to WGBS (30X), whole-genome sequencing (WGS, 30X), and targeted sequencing (60,000X) as part of a previously-reported Circulating Cell-free Genome Atlas (CCGA; NCT02889978) substudy.
  - The assays detected clinically significant cancers (ie, cancers with >50% 5-year cancer-specific mortality), as well as cancers that lack screening paradigms, at high specificity.[12]
  - Methylation patterns were identified as most informative[12] (see also **Poster 3049**) and least subject to confounding signal from clonal hematopoiesis.[18,19]
- This exploratory analysis evaluated overall survival (OS) of training and test set participants (pts) with cancer (20 cancer types, stages I-IV) detected by the methylation assay versus those not detected.
- Univariate and multivariate analyses were performed using Cox proportional hazards to assess the association of overall survival (OS) with: cancer detected versus not detected (set at 98% specificity) by the methylation assay, cancer type, clinical stage (IV vs I-III), diagnosis method (symptom- vs screen-detected), sex, age, and histologic grade.

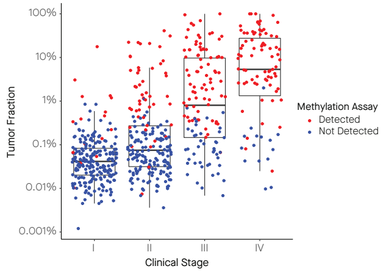
Figure 2. Participants with Cancer in Training and Test from the First CCGA Pre-specified Substudy were Included in the Survival Analysis



RESULTS

- Tumor fraction is related to detection by the methylation assay (**Figure 3**).

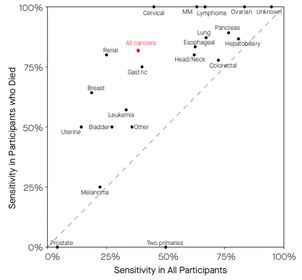
Figure 3. Box Plot Depicting Clinical Stage (x-axis) Versus Tumor Fraction (y-axis) for All Cancers



Cancer detected (red) or not detected (blue) by the methylation assay is indicated.

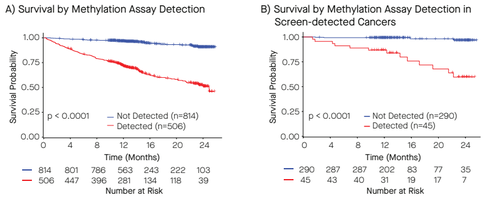
- The median follow-up duration was 12.9 months.
- Sensitivity was higher in participants with cancer who died during follow-up (82%) than in the overall cohort of participants with cancer (38%) (**Figure 4**).
  - Of 1,320 participants with cancer with at least one year of follow-up, and with a result on the methylation assay, 506 (38%) had cancer detected.
    - Among the 1,320 participants with cancer, 230 (17%) participants died during follow-up.
  - By comparison, in the 230 participants with cancer who died during follow-up, 188 (82%) had cancer detected by the methylation assay (**Figure 4, "All Cancers"**).
  - This suggests that cfDNA-based detection using this methylation assay may be an indicator of prognosis.

Figure 4. Sensitivity in All Participants with Cancer, and in Participants with Cancer who Died, During the Follow-up Period



- Participants with cancer detected by the methylation assay had worse survival than participants with cancer not detected (**Figure 5A**).
- The methylation assay appeared more predictive of survival than detection by existing screening paradigms (**Figure 5B**).
- Additionally, survival in participants with cancers detected outside of recommended screening modalities had lower survival compared to those screen-detected cancers (**Figure 5C**), suggesting a potential benefit in cancers detected outside of screening.

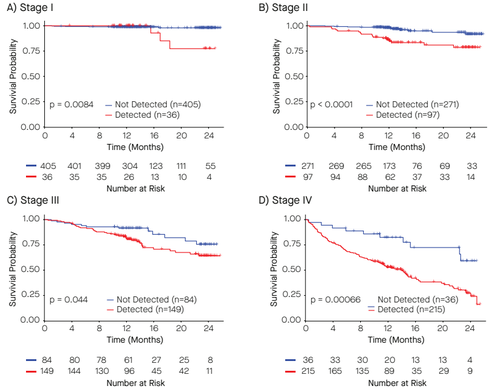
Figure 5. Survival by Methylation Assay Detection Overall, in Screen-detected Cancers, and by Screen- versus Clinical Presentation-detected Cancers



(A) Kaplan-Meier curve depicting overall survival for participants with cancers detected (red) versus not detected (blue) by the methylation assay. (B) Kaplan-Meier curve depicting overall survival for participants with cancers with USPSTF-recommended screening modalities (breast, cervical, colorectal, lung) detected by screening prior to enrollment, that were detected (red) or not detected (blue) by the methylation assay. (C) Kaplan-Meier curve depicting overall survival for participants with cancers with USPSTF-recommended screening modalities (breast, cervical, colorectal, lung) detected by clinical presentation prior to enrollment, that were detected (red) or not detected (blue) by the methylation assay.

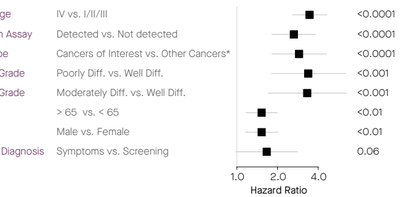
- Detection by the methylation assay was prognostic regardless of clinical stage (**Figure 6**).

Figure 6. Survival by Methylation Assay Detection in (A) Stage I, (B) Stage II, (C) Stage III, and (D) Stage IV Cancers



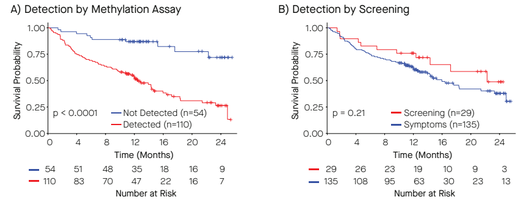
- Univariate analysis identified numerous factors associated with survival, including detection by the methylation assay (HR: 8.3; p<0.001).
- In multivariate analyses, detection by the methylation assay was identified as prognostic (HR: 2.6; p<0.001), while accounting for other covariates including clinical stage (also prognostic in multivariate analysis [HR: 3.4; p<0.001]) (**Figure 7**).
- Detection by the methylation assay may be more prognostic than detection by screening in participants with lung cancer pts (**Figure 8**).

Figure 7. Multivariate Cox Proportional Hazards Regression Model to Identify Factors Associated with Survival



*Hazard ratios and 95% confidence intervals are indicated by black boxes and gray lines, respectively. P-values are indicated. *Cancers of interest include: hormone receptor-negative breast, colorectal, esophageal, gastric, head and neck, hepatobiliary, lung, lymphoma, multiple myeloma, ovarian, pancreas. Other cancers include: bladder, hormone receptor-positive (or not specified) breast, cervical, leukemia, melanoma, prostate, renal, thyroid, uterine.*

Figure 8: Detection by the Methylation Assay may be More Prognostic than Detection by Screening in Lung Cancer



(A) Kaplan-Meier curve depicting overall survival for participants with lung cancer detected (red) versus not detected (blue) by the methylation assay. (B) Kaplan-Meier curve depicting overall survival for participants with lung cancer detected by screening (red) or by clinical presentation (blue). Cervical, colorectal, and breast cancers not depicted due to small sample sizes.

- An optimized targeted methylation approach was selected for further development; results from a cohort of ~2,300 additional samples from the CCGA study are reported in full in **Poster 3049**.

CONCLUSIONS

- Cancers detected using a methylation assay had a worse prognosis than cancers not detected, independent of clinical stage.
- Methylation-based cancer detection carried comparable prognostic significance to clinical stage.
- Detection using this methylation assay was more prognostic than detection by screening, and more prognostic than detection by clinical presentation, suggesting a benefit in asymptomatic as well as symptomatic patients.
- Together, this suggests that this cfDNA-based methylation approach preferentially detects higher risk cancers, may avoid some of the overdiagnosis that has been seen with some existing cancer screening methods, and could inform how a cfDNA-based test is developed and integrated into clinical care.

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Corresponding author: Geoffrey_Oxnard@dfci.harvard.edu

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