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Prognostic Significance of Blood-Based Multi-Cancer Detection in Cell-Free DNA: 4-Year Outcomes Analysis

Charles Swanton, MD, PhD[1]; Matthew Margolis, MS[2]; Earl Hubbell, PhD[2]; Kathryn N. Kurtzman, MD[2]; Stephannie Shih, PhD[2]; Oliver Venn, PhD[2]; Michael Seiden, MD, PhD[3]

Affiliations: [1]Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, London, UK; [2]GRAIL, LLC, Menlo Park, CA; [3]Gladius Therapeutics, Inc, Philadelphia, PA.

Disclosure Information

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Charles Swanton

I have the following relevant financial relationships to disclose:

- Employee of: Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute; Cancer Research UK; University College London
- Consultant for: Achilles Therapeutics; Bicycle Therapeutics; China Innovation Centre of Roche; Genentech; Medicxi; Metabomed (until July 2022); Roche-Ventana
- Grant/Research support from: AstraZeneca; Bristol Myers Squibb; Invitae; Ono Pharmaceutical; Personalis; Pfizer; Roche-Ventana
- Stockholder in: Achilles Therapeutics; Bicycle Therapeutics; Epic Bioscience, Relay Therapeutics
- Honoraria from: Amgen; AstraZeneca; Boehringer-Ingelheim; Bristol Myers Squibb; GlaxoSmithKline; Illumina; MSD; Novartis; Pfizer; Roche-Ventana
- Advisory Board member for: Achilles Therapeutics; Bicycle Therapeutics; GRAIL; Relay Therapeutics; Saga Diagnostics

My additional financial relationship disclosures are:

- GRAIL, LLC, Co-Chief Investigator of the NHS Galleri trial funded by GRAIL, previously held stock options in GRAIL; Achilles Therapeutics, Board member; AstraZeneca, Chief Investigator for the MeRmaiD 1 and 2 clinical trials, Steering Committee Chair; Invitae, collaboration in minimal residual disease sequencing technologies (company was previously Archer Dx Inc)
- Patent applications:
  - PCT/US2017/028013; PCT/EP2016/059401; PCT/EP2016/071471; US20190106751A1; PCT/GB2018/051912; PCT/GB2018/052004; PCT/GB2020/050221; PCT/EP2022/077987; PCT/GB2017/053289.

Detection of Cancer Signals May Lead to Earlier Diagnosis and Improved Survival

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The Multi Cancer Early Detection (MCED) test detects **a shared cancer signal associated with >50 cancer types**, most of which have no guideline-recommend screening.

Third (Validation) Substudy of the Case-Control **Circulating Cell-free Genome Atlas (CCGA) Study**[1] (NCT02889978)

99.5% Specificity

88.7% CSO Prediction Accuracy[a]

MCED Testing Process in CCGA

CSO, cancer signal origin.
[a]In true positives cases.
[1]Klein EA, et al. *Ann Oncol.* 2021;32(9):1167-1177.

How Do We Refine Our Understanding of Survival in Those With a Detected/Not Detected MCED Test Result?

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Previous analysis (Chen, et al)[1]

- *Assessed participant outcomes in the second (cross-validated) CCGA substudy*
- *Participants tested using an earlier version of the MCED test*
- *3-year longitudinal follow-up*

Conclusion: *Cancers not detected by the MCED test had better prognosis than cancers detected and SEER-based expected survival*

New analysis

- Assessed participant outcomes in the third CCGA (validation) substudy[2]
- Participants tested using a refined version of the MCED test
- 4-year longitudinal follow-up
- Used updated statistical model to account for confounding variation in cancer type and stage

CCGA, Circulating Cell-free Genome Atlas study; ctDNA, circulating tumor DNA; MCED, multi-cancer early detection SEER, Surveillance, Epidemiology, and End Results.

[1]Chen X, et al. Clin Cancer Res. 2021; 27(15):4221-4229. [2]Klein EA, et al. *Ann Oncol.* 2021;32(9):1167-1177.

Almost 70% of Participants Diagnosed With Cancer Were Alive After 4 Years

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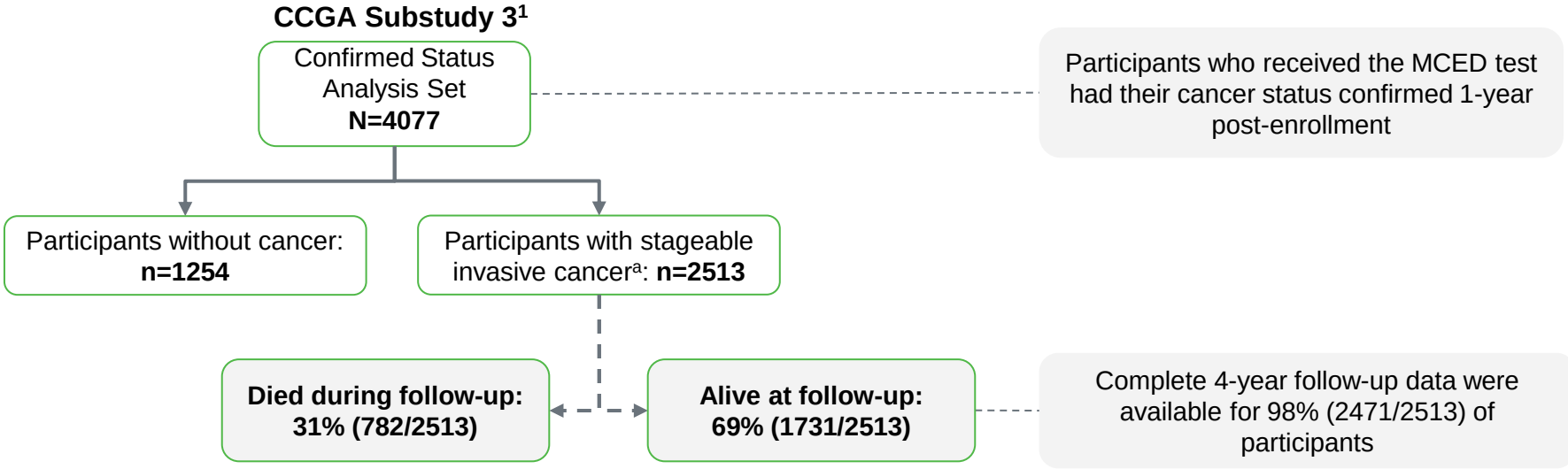

CCGA, Circulating Cell-free Genome Atlas; MCED, multi-cancer early detection.
[a]Excluding plasma cell cancers and non-staged cancers.
[1]Klein, et al. *Ann Oncol.* 2021;32(9):1167-1177.

A Higher Proportion of Those Diagnosed with Cancer Who Died During Follow-up Had Cancer Signal Detected

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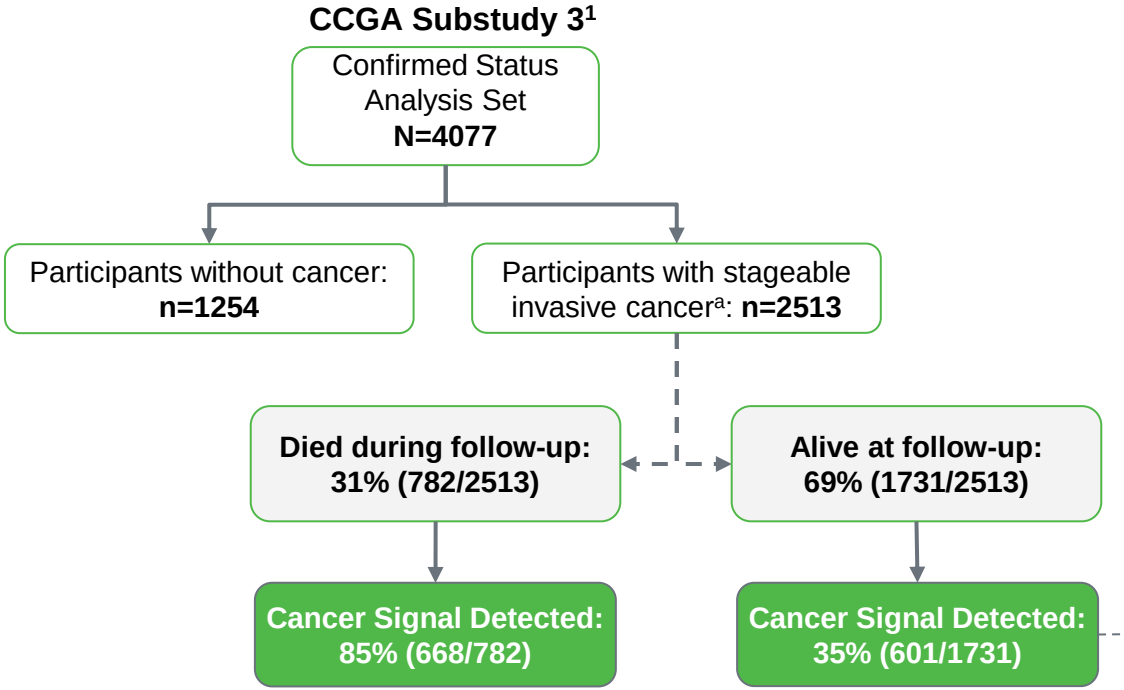

Do participants with a cancer signal detected fare worse?

To answer this question, we must understand expected survival rates

Raw odds ratio of 2.5 for survival of participants with cancer and a cancer signal detected

CCGA, Circulating Cell-free Genome Atlas; MCED, multi-cancer early detection.
[a]Excluding plasma cell cancers and non-staged cancers.
[1]Klein, et al. *Ann Oncol.* 2021;32(9):1167-1177.

Standardization to SEER Reference Removes Expected Variation from Known Predictors

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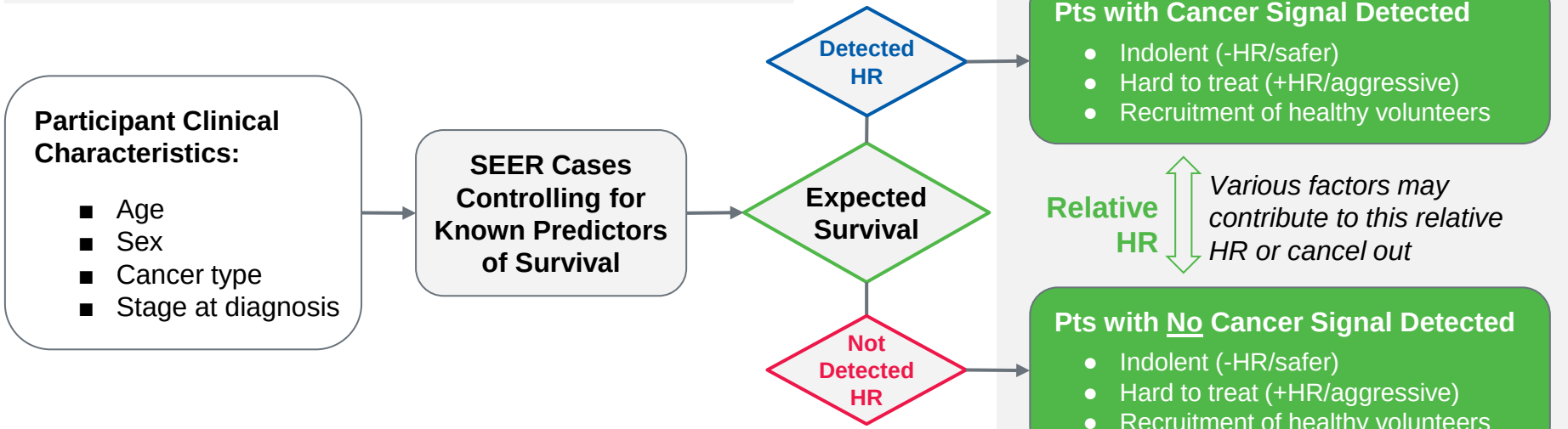
Observed survival[a] of participants who had 4-year longitudinal follow-up data was compared to **expected survival[b]** calculated from SEER reference data



HR, hazard ratio; Pts, participants; SEER, Surveillance, Epidemiology, and End Results.

[a]Defined as the time from the date of blood sample collection to the date of death or last date the participant was confirmed alive.

Survival Advantage for Cancer Signal Not Detected Remains After Removing Known Effects

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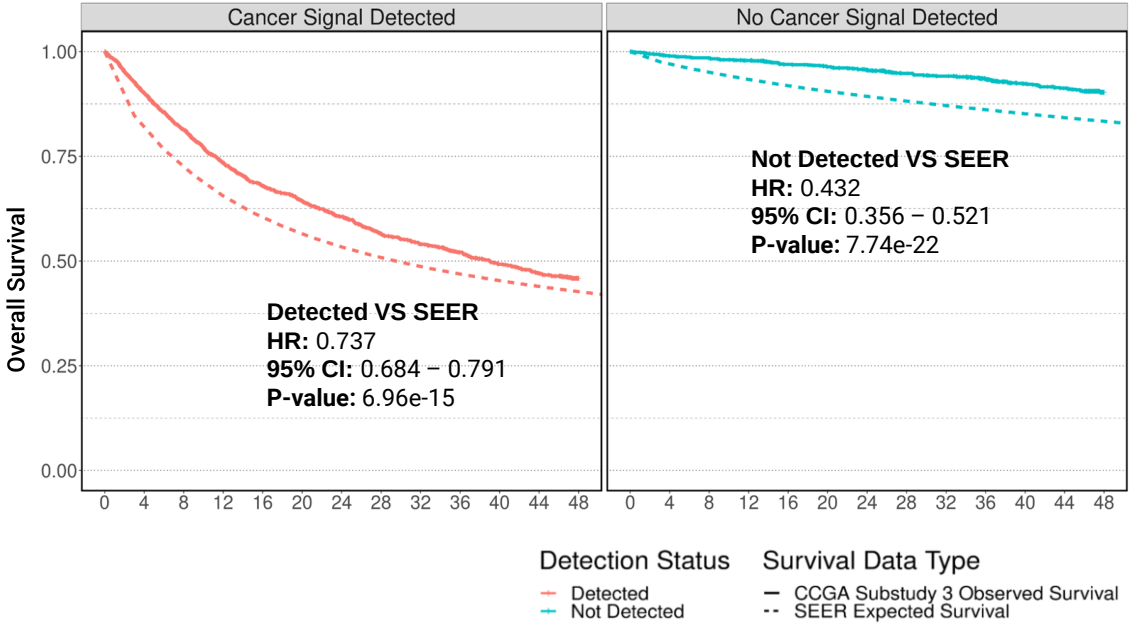
Observed Survival[a] vs Expected Survival by Signal Detection Status



Hazard Ratio for Cancer Signal Detection Groups After Controlling for Age, Sex, Cancer Type and Stage

	Hazard Ratio	95% CI	P-value
Not Detected vs Detected	0.585	0.474 – 0.712	2.22e-07

- Majority of variation results from known clinical factors (Expected 43% vs 83% [dotted lines: blue vs red, respectively])
- Both groups show improved survival vs reference suggesting healthy cohort relative to SEER

CCGA, Circulating Cell-free Genome Atlas study; CI,confidence interval; HR, hazard ratio; SEER, Surveillance, Epidemiology, and End Results.
[a]Defined as the time from the date of blood sample collection to the date of death or last date the participant was confirmed alive.

Most Important To Understand Prognosis at Early Stage When Treatment is Most Effective

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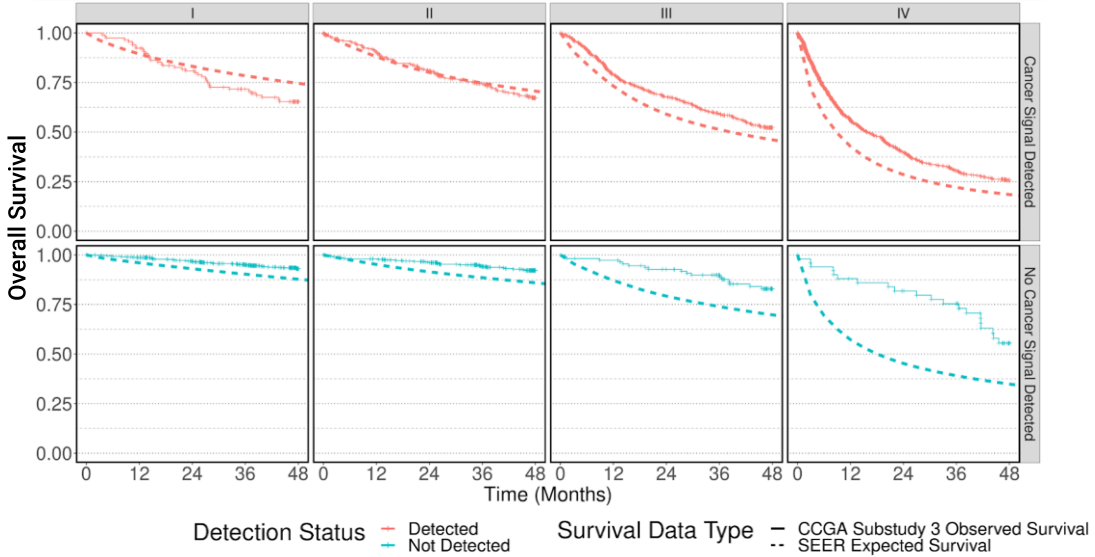
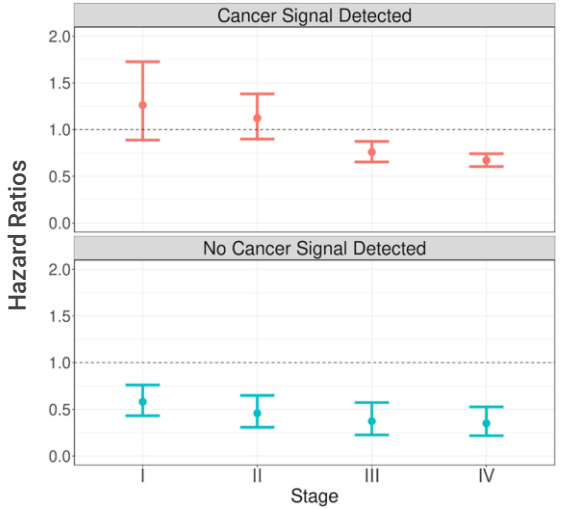
Observed Survival[a] vs Expected Survival by Stage and Signal Detection Status



Hazard Ratios by Stage for Cancer Signal Detection Controlling for Age, Sex, Cancer Type and Stage



- Stage I/II in Detected are serious cancers and are expected (~70-75%) to have worse survival than Not Detected (~87%)
  - Some change in prognosis from expected (HR >1, confidence interval overlaps 1, but upper bound <2)
- 2/3 of Detected individuals in stage I/II surviving 4 years (Kaplan-Meier estimate), Not Detected still unusually safe in stages I/II

CCGA, Circulating Cell-free Genome Atlas study; HR, hazard ratio; SEER, Surveillance, Epidemiology, and End Results.
[a]Defined as the time from the date of blood sample collection to the date of death or last date the participant was confirmed alive.

Potential For Future Refinement of Prognoses Using Estimates of Cancer Biology

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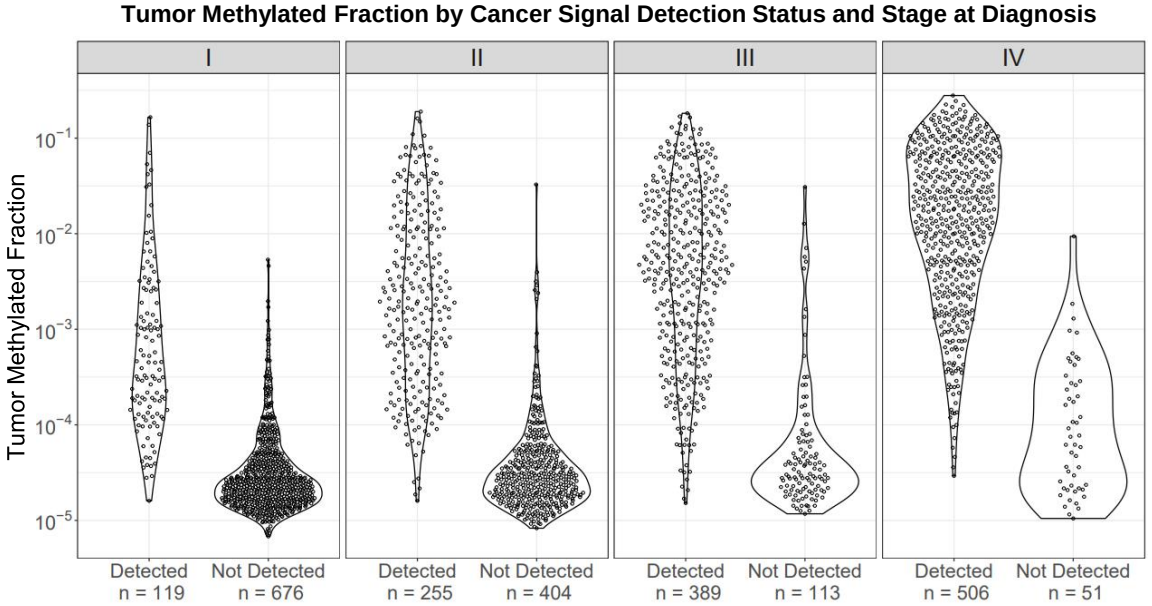
Tumor Methylated Fraction[1]:
A methylation-based, *biopsy-free* estimate of circulating tumor DNA within cell-free DNA

- Estimator designed to estimate fraction in known cancer cases, not robustly classify non-cancer vs cancer in screening

Cell-free DNA shedding may be connected to tumor growth rates and cell death[2,3]

- Tumor methylated fraction estimates increase in Detected with clinical stage[4,5]
- Not Detected estimates usually at background noise level



MCED, multi-cancer early detection.

[1]Melton CA, et al. *Cancers*. 2024; 16(1):82. [2]Bredno J, et al. *Am J Pathol.* 2022;S0002-9440(22)00211-5. [3]Avanzini S, et al. *Sci Adv.* 2020;6(50):eabc4308. [4]Jamshidi A, et al. *Cancer Cell*. 2022;40(12):1537-1549.e12. [5]Zhou X, et al. *Nat Commun.* 2022;13(1):7694.

4-Year Follow-Up Analysis Suggests That the MCED Test Preferentially Detects Fast-Growing Cancers

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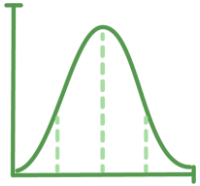
CCGA Substudy 3 Outcomes Analysis

MCED Testing of Participants **with Known Cancer**



Comparison of **Observed** to **Expected** Survival Removes Expected Variation in Survival

Survival Across Cancer Cases

- Most variation was due to clinical factors
  - Evidence of a healthy volunteer bias when compared to age, sex, and tumor matched controls
- Despite prognostic differences, 2/3 of Stage I-II Detected participants survive 4 years (K-M)
  - Hazard of detected signal could indicate a need for treatment escalation at earlier stages.

CCGA, Circulating Cell-free Genome Atlas study; K-M, Kaplan-Meier; MCED, multi-cancer early detection; SEER, Surveillance, Epidemiology, and End Results.

Ongoing Follow-up of CCGA Participant Outcomes Improves Understanding of Survival and MCED Detection

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Strengths

- Broad sampling of cancer types and stages, across multiple care systems
- SEER reference data used as a synthetic control to account for variation in cancer type and stage correlated with detection
- 4-year follow-up post blood draw
- Assessment of tumor methylated fraction links signal detection to underlying tumor biology

Limitations

- Non-interventional – Observational data diagnosed through usual care
- Complex cancer type and stage information with limited clinical covariates
- Healthy volunteer bias could impact observed survival
- Overall survival without cancer-specific causes of death

CCGA, Circulating Cell-free Genome Atlas study; MCED, multi-cancer early detection; SEER, Surveillance, Epidemiology, and End Results.

We acknowledge the contributions to this effort of the patients, staff, and healthcare providers who provided clinical data

Questions?

Appendix Slides

Cancer Signal Detection Varied by Cancer Type and Stage, Which Independently Affect Survival

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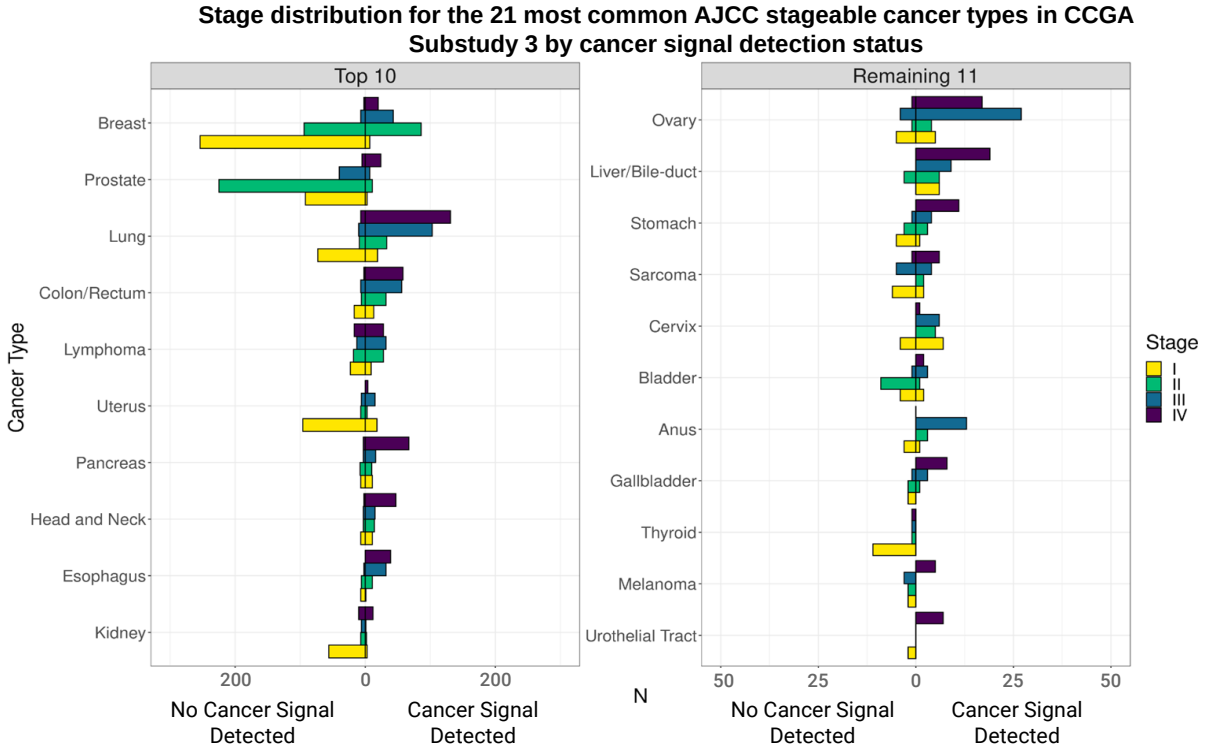

AJCC, American Joint Committee on Cancer; CCGA, Circulating Cell-free Genome Atlas study.