Simultaneous Multi-cancer Detection and Tissue of Origin (TOO) Localization Using Targeted Bisulfite Sequencing of Plasma Cell-free DNA (cfDNA)

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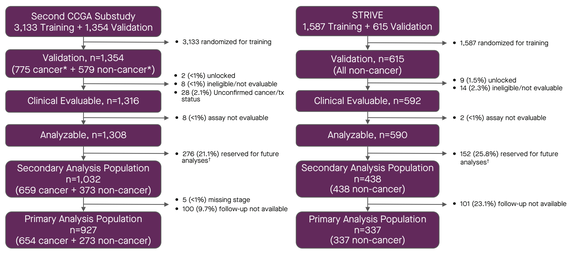
INTRODUCTION

- A noninvasive cell-free DNA blood test detecting multiple cancers at earlier stages (stages I–III) could decrease cancer mortality.
- For a multi-cancer test to be effective at population scale, it should:
  - Detect clinically significant cancers with a low false positive rate (ie, very high specificity [>99%]) to limit overdiagnosis;
  - Identify a specific tissue origin to direct appropriate diagnostic work-up for detected cancers.[1,2]
- In earlier discovery work, whole-genome bisulfite sequencing outperformed whole-genome and targeted sequencing approaches for multi-cancer detection across cancer stages at high specificity[3]; targeted methylation was selected for further assay development, including training and internal cross-validation.
- Presented here are data from a second pre-specified substudy of Circulating Cell-free Genome Atlas (CCGA; NCT02889978), in which a multi-cancer detection and tissue-of-origin (TOO) localization using targeted bisulfite sequencing of plasma cfDNA to identify methylomic signatures was validated in preparation for returning results in a clinical setting.

METHODS

- The primary analysis population used for this validation was comprised of 1,264 participants derived from the CCGA and STRIVE study populations (Figure 1); CCGA is a multi-center, case-control, observational study with longitudinal follow-up (15,254 participants enrolled: 56% cancer, 44% non-cancer) and STRIVE is a multi-center, prospective, cohort study enrolling women undergoing screening mammography (99,259 participants enrolled).
  - Importantly, to improve the resolution of the targeted high specificity (ie, >99%), non-cancer samples from the STRIVE study population were also analyzed.
- Previously, we presented cross-validated results from a training set analysis of 3,583 participants derived from CCGA and STRIVE (CCGA: 1,530 cancer, 884 non-cancer; STRIVE: 1,169 non-cancer participants).[4]

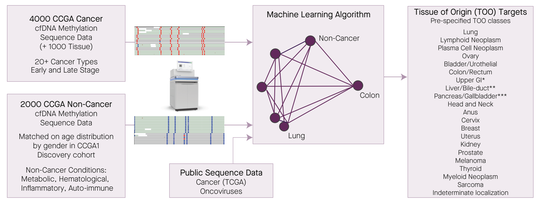
Figure 1. Detail of Validation Cohort from Second Substudy of CCGA



*At enrollment, prior to confirmation of cancer versus non-cancer status.
†Samples reserved for future analysis include, for example, a cohort of participants recruited from hematology clinics meant to understand cfDNA signal in premalignant or other hematologic conditions.*

- The validation set from the second substudy shown in Figure 1, was used to validate a trained and locked classifier for determining cancer versus non-cancer and TOO based on a targeted methylation sequencing approach.
  - Analysis followed a pre-specified statistical analysis plan, with clinical and assay data locked and blinded to each other.
- This validation set of 1,264 evaluable samples included 610 non-cancer samples (273 from CCGA and 337 from STRIVE), and 654 cancer samples (CCGA) from >50 cancers, which were grouped for reporting purposes; the pre-specified subset of cancers was: anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach (356 cancer [all stages]).
  - The list of pre-specified high detection rate cancer types (ie, those with sensitivity >50% across stages I–III in training) in the validation set versus the cross-validated training set[4] analyses differed by a single cancer type for consistency with the validation set TOO analysis; specifically, this resulted in the addition of bladder cancer and the removal of hormone-receptor negative breast cancer.
- Plasma cfDNA was subjected to a cross-validated targeted methylation approach that included high-efficiency methylation chemistry to enrich for methylation targets and subsequent machine learning classifier for determining cancer status and TOO (Figure 2).
  - Observed methylation fragments characteristic of cancer and TOO were combined across targeted regions and assigned a relative probability of cancer and of a specific TOO; precision was defined as the fraction of correct calls.
  - Classifier was trained and locked, including decision thresholds, targeting above 99% specificity with some allowance for statistical variability.

Figure 2. Methylation Database: Target Selection and Machine Learning Algorithm



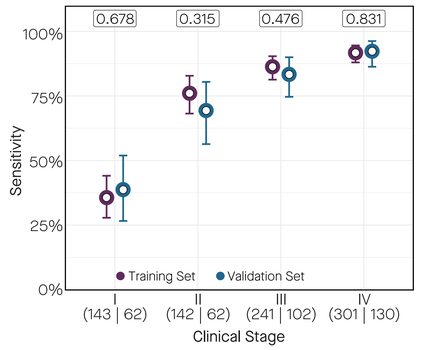
A large methylation sequence database of cancer and non-cancer was generated to enable target selection for a single test able to classify to cancer/non-cancer for multiple cancer types at high specificity and identify TOO.

*Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types. **Liver/bile duct includes liver and intrahepatic bile duct. ***Pancreas/gallbladder includes pancreas, gallbladder, and extrahepatic bile ducts.

RESULTS

- The trained classifier targeting specificity of >99% (see Methods) achieved specificity of 99.8% in the cross-validated training set and 99.3% in the independent validation set (P=0.095).
  - Therefore, assay performance reflected a consistent false positive rate of <1%.
- Importantly, the assay specificity and sensitivity were consistent between the cross-validated training set and independent validation set across stages (Figure 3), confirming that training data were not overfitted; this was also consistent for all cancer types.

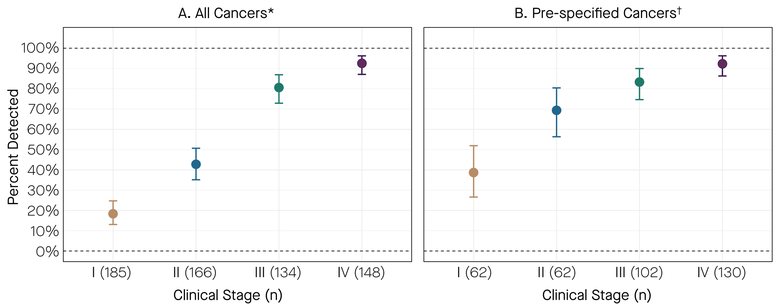
Figure 3. Stage-specific Performance Consistency Between Training and Test Sets



Plot reflects pre-specified list of cancer types: anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach. P values listed in the white boxes above (chi-squared test).

- At 99.3% specificity, the sensitivity (95% CI) for all cancer types was 55% (51–59%), and for the pre-specified cancer types was 76% (72-81%).
- At each stage, cancer detection for all cancer types combined (sensitivity [95% CI]) was 18% (13–25%) in stage I (n=185), 43% (35–51%) in stage II (n=166), 81% (73–87%) in stage III (n=134), and 93% (87–96%) in stage IV (n=148) (Figure 4).
- Among pre-specified high-signal cancer types, the stage-specific cancer detection was 39% (27–52%) in stage I (n=62), 69% (56–80%) in stage II (n=62), 83% (75–90%) in stage III (n=102), and 92% (86–96%) in stage IV (n=130).

Figure 4. Overall Cancer Detection by Stage



Stage-specific sensitivity (achieved with 99.3% specificity) for (A) all examined cancer types and (B) a pre-specified group of cancer types†.*

Plot excludes unstaged cancers: lymphoid leukemia, myeloid neoplasm, brain. †Includes anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach

- For the most common cancer types (that also have the most samples) breast, lung, and colorectal, cancer detection was 39% (30–50%; n=104), 66% (56–75%; n=111), and 77% (64–88%; n=53), respectively.

References

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Disclosures

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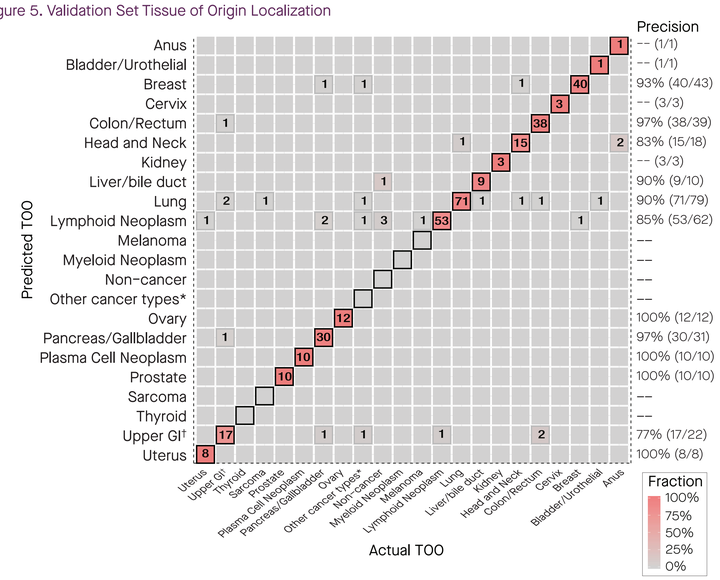
- Where a cancer signal was detected, cancer was localized to an anatomic site (ie, tissue type identified) for 96% (344/359) of cases; of these (and consistent with training set analyses), the TOO call was correct in 93% (321/344) of cases (Figure 5).

Figure 5. Validation Set Tissue of Origin Localization

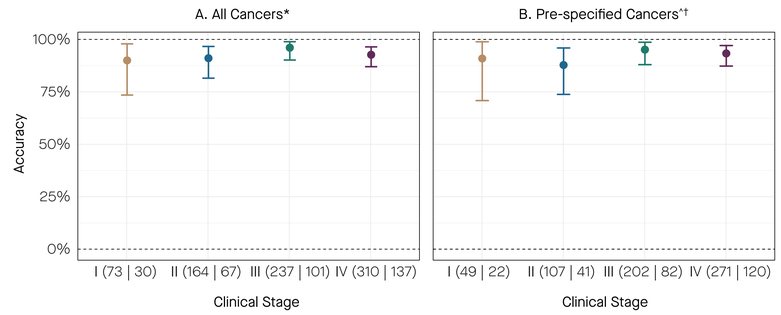


Agreement between the true (x-axis) and predicted (y-axis) TOO per sample. Precision reported for cancer types with ≥10 samples. Matrix excludes 21 cancers not expected to be staged (lymphoid leukemia, myeloid neoplasm, brain), 9 of which were detected (all lymphoid leukemia), and all 9 of which received the correct tissue of origin.

**Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types.*
***Other cancer types= skin cancer (not including basal cell carcinoma, squamous cell carcinoma, or melanoma), testis, seminoma, vagina, and vulva; these cancer types had too few samples to have a TOO trained.*

- TOO detection rates were similar across stage and slightly higher at each stage among the prespecified cancers compared to all cancers (Figure 6).

Figure 6. Consistently High Tissue of Origin Accuracy Across Stages



Stage-specific accuracy that includes localization to the top TOO calls for (A) all examined cancer types and (B) a pre-specified group of cancer types.†

**Plot excludes unstaged cancers. †Includes anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach.*

CONCLUSIONS

- Across stages, multiple deadly cancer types that currently have no screening paradigm were detected, and simultaneously accurately localized to a TOO, using methylation signatures in plasma cfDNA.
  - This was achieved with trained thresholds that resulted in a single, fixed, low false positive rate (<1%) in an independent validation set.
- Importantly, results in the independent validation set were indistinguishable from the training set, demonstrating robustness of machine learning classifier training, and no evidence of overtraining.
- This validation supports the feasibility of a single blood-based test that can simultaneously detect multiple cancers and supports further clinical development for the preparation of returning results.