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

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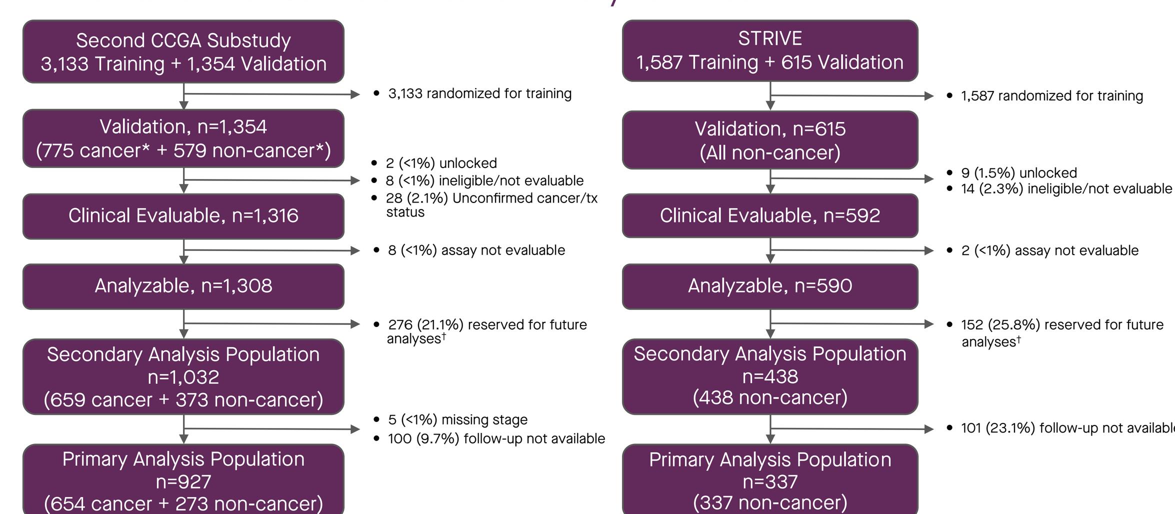
INTRODUCTION

- 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]

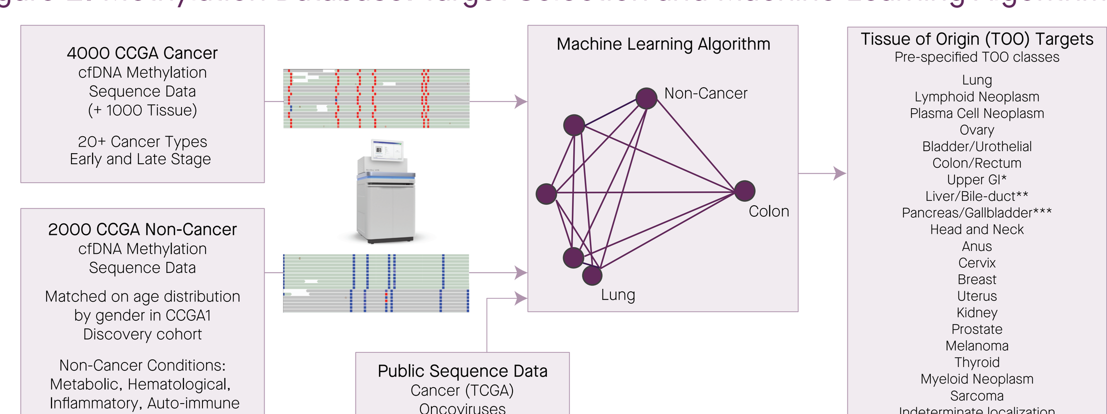
Figure 1. Detail of Validation Cohort from Second Substudy of CCGA



*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 >20 tumor types; the pre-specified subset of cancer types 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



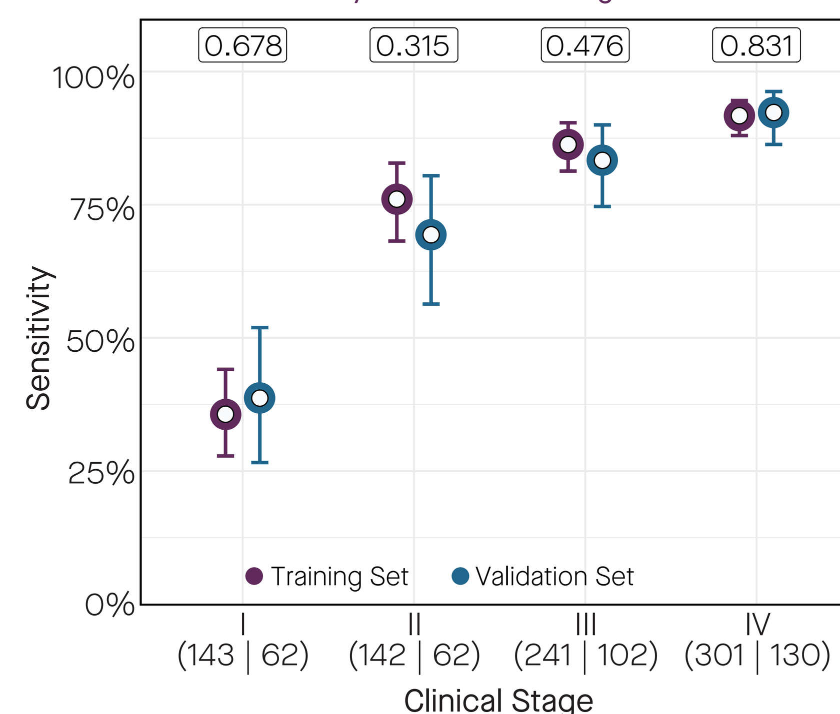
A large methylation sequence database of cancer and non-cancer was generated to enable target selection for a single test able to classify to cancer/non-cancer for multiple cancer types at high specificity and identify TOO.

*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 (>20) cancer types.

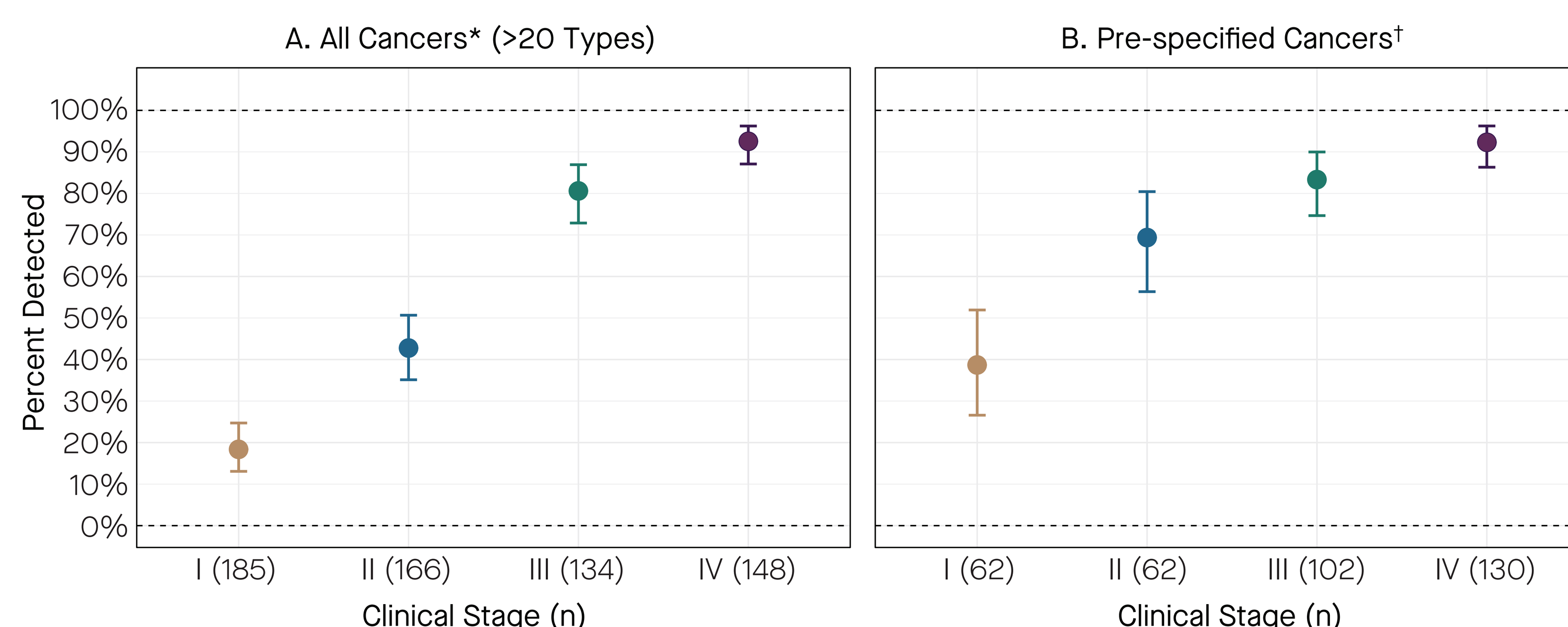
Figure 3. Stage-specific Performance Consistency Between Training and Test Sets



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



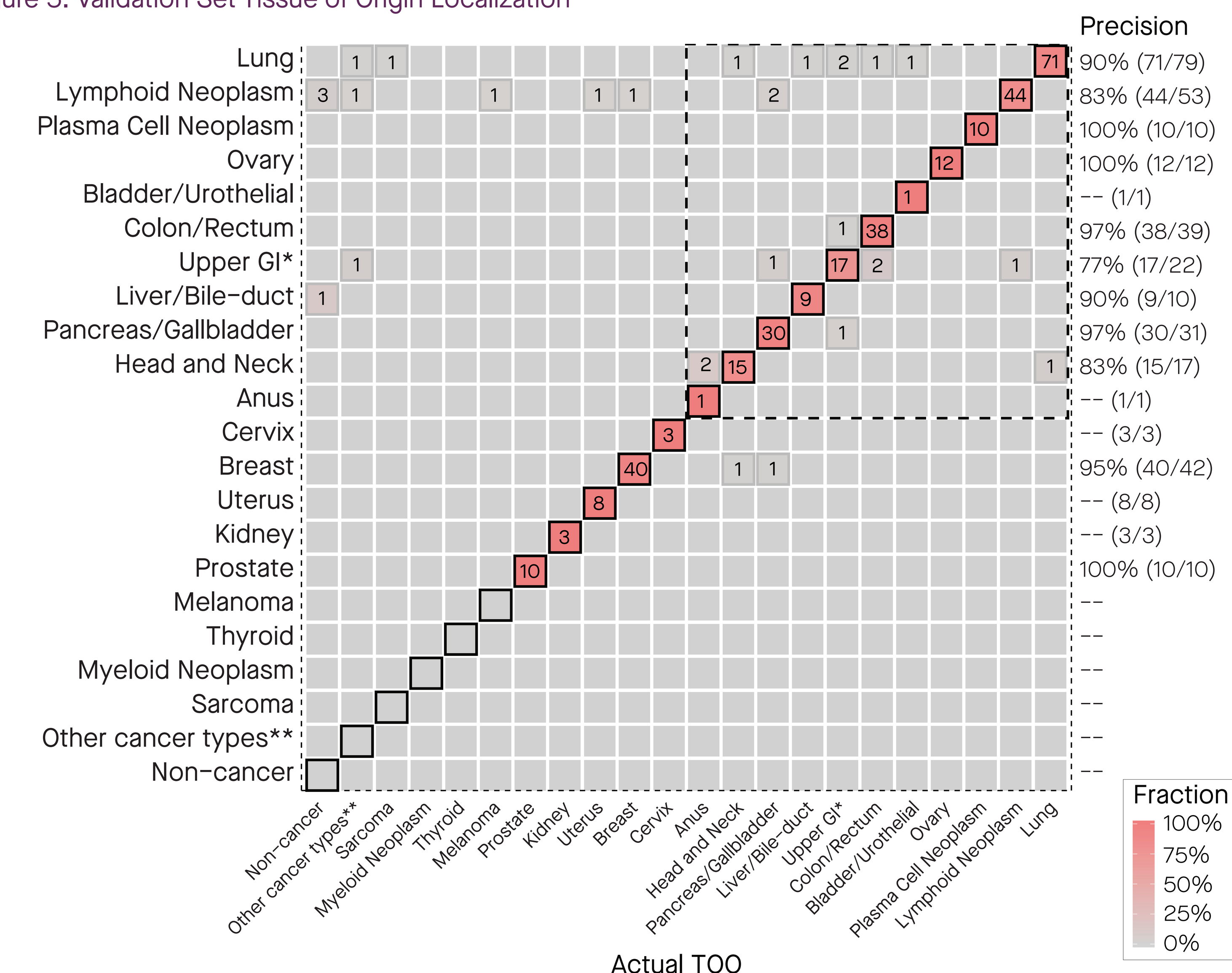
Stage-specific sensitivity (achieved with 99.3% specificity) for (A) all examined cancer types and (B) a pre-specified group of cancer types†.*

*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.

- Where a cancer signal was detected, cancer was localized to an anatomic site (ie, tissue type identified) across >20 distinct tumor types 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

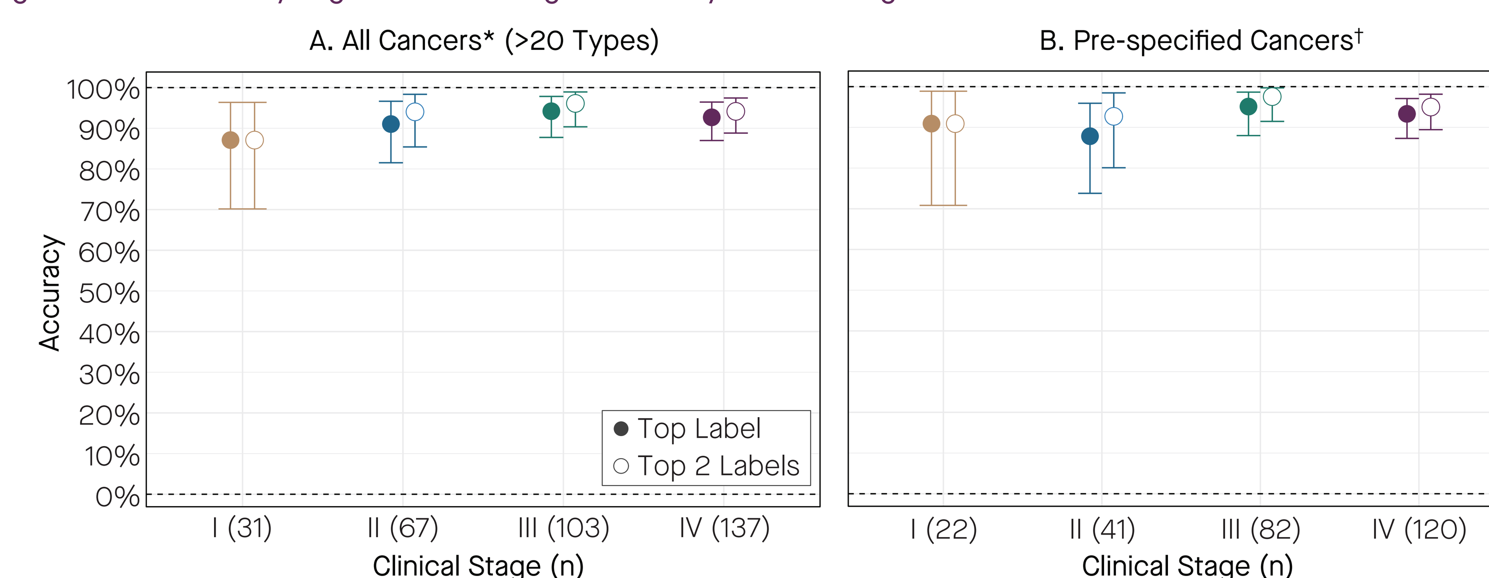


Agreement between the true (x-axis) and predicted (y-axis) TOO per sample. Precision reported for cancer types with ≥10 samples. Matrix excludes 21 cancers not expected to be staged (lymphoid leukemia, myeloid neoplasm, brain), 9 of which were detected (all lymphoid leukemia), and all 9 of which received 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).
- When considering localization to the top two TOO calls, 95% of samples assigned a TOO were correct and this was consistent across stages.

Figure 6. Consistently High Tissue of Origin Accuracy Across Stages



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

*Plot excludes unstaged cancers (9 cases of lymphoid leukemia). †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.

References

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Disclosures

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