Multi-cancer early detection through evaluation of aneuploidy, methylation, mutation, and protein biomarkers in plasma

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Background

- Multi-cancer early detection (MCED) using a blood test represents a rapidly emerging, potentially transformative advance in preventive oncology.
- Given the complexity of carcinogenesis across organs, simultaneous analysis of multiple biomarkers has the potential to maximize clinical performance, particularly for early-stage tumors.
- Previously, we demonstrated the performance of blood tests that incorporated the detection of DNA mutations plus proteins[1,2] and combining DNA methylation and proteins, respectively[3,4].
- In the original abstract, we described the Training and Validation of 3 biomarkers (aneuploidy, DNA methylation, proteins) using stratified 5-fold cross-validation.
- In this presentation, we also included an independent test set and assessed the combination of 4 biomarkers (aneuploidy, DNA methylation, mutations, and proteins).

Methods

- To assess aneuploidy, we developed a modified version of the Repetitive Element AneupLoidy Sequencing System (REALSeqS).[5]
- DNA methylation testing was performed on a refined panel of markers using the Target Enrichment Long-probe Quantitative Amplified Signal (TELQAS) assay on bisulfite-converted cfDNA.
- A high-throughput platform was used to quantify six extensively-documented protein biomarkers.
- Mutation testing was performed using a modified version of the sequencing technology described by Cohen *et al.*[6]
- To assess biomarker performance, we designed a retrospectively-assembled, case-control feasibility study. The cancers were from all stages and up to 15 organ sites. The non-cancer control cohort was comprised of age-matched presumed-healthy individuals as well as an enriched fraction of samples from individuals with non-cancer diseases. Blood samples were collected in LBgard® tubes and obtained through different prospective collections and vendor sources.
- First, a training and validation set was analyzed including a total of 2386 samples. Twelve organ sites included in this set were breast (59), bladder (23), colon (61), esophageal (44), kidney (41), liver (40), lung (86), ovarian (30), pancreatic (64), prostate (49), stomach (28), and uterine (40). Three marker classes (aneuploidy, methylation, and protein) were tested **Fig. 1**.
- Second, an independent test set of 1132 samples was analyzed using the models, specificity, and thresholds defined in the training and validation set for the aneuploidy, methylation, and protein biomarkers. These 12 organ sites and samples numbers were included: breast (62), bladder (19), colon (87), esophageal (34), kidney (39), liver (18), lung (88), ovarian (27), pancreatic (37), prostate (25), stomach (31), and uterine (34). Three additional hematological cancers types were also included, namely Non-Hodgkin's lymphoma (38), multiple myeloma (14), and myelodysplastic syndrome (13). Furthermore, mutation analysis was performed using naive thresholds informed by a non-overlap subset. Calling stringency was also influenced by buffy gDNA availability **Fig. 1**.

Results

Figure 1. Diagram illustrating the full cohorts, analyzed samples, and sub-set analyses

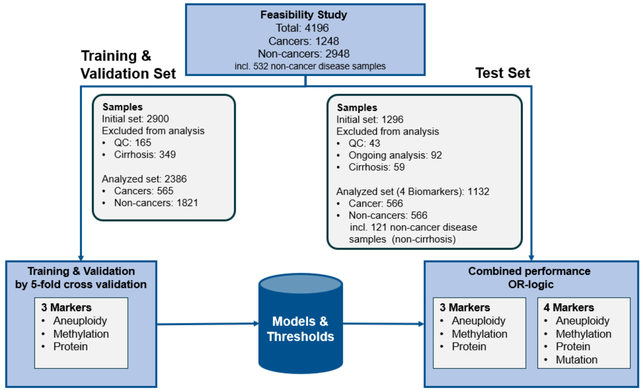

Figure 2. Sensitivities of Test Set by Stage for 3- & 4-Marker panel

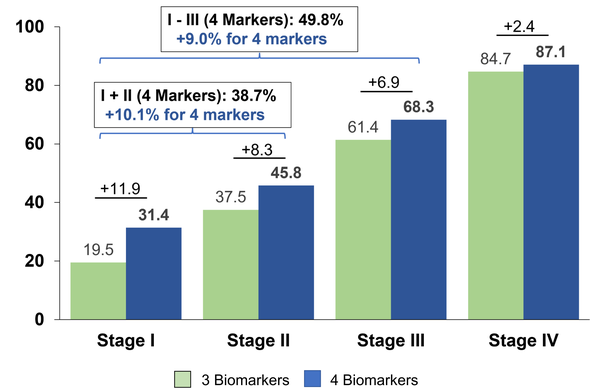

Table 1. Demographics and Clinical Data of Test Set

	Total Analyzed: 1132	
	Cancers: 566	Non-Cancers: 566
Age (mean, range)	67.9 (50-93)	62.4 (50-85)
Sex (n, %)		
Male	256 (49%)	138 (24%)
Female	310 (51%)	428 (76%)
Cancer Stage Distribution		
Stage I	118	–
Stage II	120	
Stage III	145	
Stage IV	170	
Myelodysplastic Syndrome Subtype	13	

- Training and Validation Set: 2,386 of 2,900 samples from the training and validation set were analyzed for 3 biomarkers. Using stratified 5-fold cross-validation, we found that the combined aneuploidy, methylation, and protein classes detected cancer across all 12 organ sites and all stages with a mean overall sensitivity of 52.6% (95% CI: 47.0%-58.2%) at mean specificity of 98.7% (95% CI: 98.3%-99.2%). Cirrhosis samples were not utilized for building the models and setting thresholds for the protein and methylation markers since the disease stages were beyond what would be expect in an average screening population and therefore would introduce a bias.
- Test Set: The same 3 markers were tested on an independent hold-out test set described in **Table 1**. The mean overall sensitivity was 53.4% (95% CI: 49.6%-57.8%) at mean specificity of 98.8% (95% CI: 97.9%-99.7%) **Table 2**. The inclusion of all 4 biomarker classes (aneuploidy, methylation, protein, and mutation) in the second sub-study resulted in a mean overall sensitivity of 61.0% (95% CI: 56.9%-65.0%). The addition of mutation testing increased sensitivity by 7.6% across all tumor sites and stages compared to the three-marker combination, while maintaining the specificity at 98.2% (95% CI: 97.1 – 99.4%) **Table 2**.
- 3 biomarker sensitivity by stage: Stage I 19.5%, Stage II 37.5%, Stage III 61.4%, and Stage IV 84.7% (**Fig. 2**). The cancer-specific sensitivities were lowest for prostate, myelodysplastic syndrome, and multiple myeloma, and highest for liver, esophageal, and stomach cancers.
- 4 biomarker sensitivity by stage: Stage I 31.4%, Stage II 45.8%, Stage III 68.3%, and Stage IV 87.1% (**Fig. 2**). The inclusion of mutations resulted in the greatest increase in sensitivity for Stage I/II cancer detection of 38.7% (average gain of 10.1%). The sensitivity of Stage I – III was 49.8% for all 4 markers. Mutations increased sensitivity by 9.0%. The cancer-specific sensitivities were lowest for prostate and kidney cancers and highest for liver, esophageal, and colorectal cancers.

Table 2. Performance of Test Set for 3- & 4-Marker panel

Analysis	Specificity %	Sensitivity %
3 Markers Aneuploidy Methylation Protein	**98.8** (95% CI: 97.9-99.7%)	**53.4** (95% CI: 49.6-57.8%)
4 Markers Aneuploidy Methylation Protein Mutation	**98.2** (95% CI: 97.2-99.3%)	**61.0** (95% CI: 56.9-65.0%)

Conclusions

- The combination of aneuploidy, DNA methylation, mutation, and protein biomarker classes provide complementary and highly-specific components for a multi-cancer early detection test.
- In a training and validation set as well as hold out test set, cancers from all organ types and stages could be detected.
- The addition of mutation data improved sensitivity across stages with the largest improvement in stage I and stage II cancer detection.
- Using a diverse set of analytes, we believe that a single blood test has the potential to robustly detect earlier-stage disease in multiple cancer sites.
- A larger, prospectively-collected case-control study is underway to further validate these results. This will be followed by a very large, prospective, randomized, interventional trial to demonstrate assay performance in an average risk population.

References

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