A multi-analyte blood test has the potential for robust sensitivity in detecting a broad range of cancer types and stages.

Previously, using retrospectively collected samples, we trained and independently assessed the performance of up to four different biomarker classes for the detection of cancers in a case-control study.1,2

The aim of this study was to further refine and assess calling algorithms, i.e., classifiers, for two of the four previously evaluated biomarker classes, methylation and protein, using samples from a multi-center, prospectively collected study: Ascertaining Serial Cancer patients to Enable New Diagnostic 2 (ASCEND 2).3

By measuring methylation and protein biomarkers that capture shared, cancer-associated signals, yet rely on different mechanisms of release into the circulation, the objective was to show that these two biomarker classes can detect a broad range of cancer types while maintaining high specificity.

**METHODS**

- For the methylation and protein classifier development a total of 6,354 blood samples (1,438 cancers and 4,916 non-cancers) collected in LBgard® tubes were selected from >11,000 subjects enrolled in ASCEND 2.
- The selection of the samples was based on plasma volume availability, maintenance of high specificity.
- Of the samples that were tested, additional exclusions were applied for clinical eligibility criteria, availability of validated clinical data prior to testing initiation, and demographic matching requirements between the training and testing set.
- Of the samples that were tested, additional exclusions were applied for training and the hold-out testing set, respectively.
- Tab. 1 shows the training and testing cohort demographics. Training and testing set tumor organ sites and stage distributions are depicted in Fig. 3.
- Methylation and protein measurements were performed as described previously.

**RESULTS**

- The generalizability of the combined methylation and protein classifier was evaluated by comparing the performance between the 5-fold CV set, the full training set, and the testing set all targeted to 98.5% overall specificity.
- Training set 5-fold CV and full training set achieved 98.9% (95% CI: 98.1-99.3%) specificity and 98.8% (95% CI: 98.3-99.2%) respectively. Testing set specificity achieved 98.5% (95% CI: 97.8-98.8%).
- Taken together, no significant bias was observed between full training set, CV set, and testing set performance, indicating good generalizability of the classifiers.
- Overall, training and testing sets have similar distributions within organ sites. The number of cancers per organ site was targeted to represent cancers with high, common, and rare incidence and is not reflective of intended use population.
- The data shown here is based on the 98.5% specificity target and uses the less complex model.
- The comparison of the unique collection site sensitivity (56.7%, 95% CI: 44.2-70.8%) and specificity (99.3%) were compared to all enrollment sites.
- A mini-holdout set was used to test multiple different models during the model selection process before evaluating two subsequent methylation–protein overarching classifier configurations in the test set, at 98.5% and >99.0% target specificity.
- The generalizability of the combined methylation & protein configuration demonstrated:
  - 50.9% sensitivity across all 21 cancer organ types, and 56.8% when breast and prostate cancers were excluded from the analysis.
- 54.8% sensitivity excluding cancer organ types with average-risk standard of care screening (i.e. excluding breast, prostate, cervix, colon and rectum).
- 63.7% sensitivity for the 6 most aggressive cancer organ types with the shortest 5-year survival rate (i.e. pancreas, esophagus, liver, lung and bronchus, stomach, and ovary).

**CONCLUSIONS**

As shown in Tab. 2, at a specificity of 98.5%, the combined methylation & protein configuration was demonstrated:

- 50.9% sensitivity across all 21 cancer organ types, and 56.8% when breast and prostate cancers were excluded from the analysis.
- 54.8% sensitivity excluding cancer organ types with average-risk standard of care screening (i.e. excluding breast, prostate, cervix, colon and rectum).
- 63.7% sensitivity for the 6 most aggressive cancer organ types with the shortest 5-year survival rate (i.e. pancreas, esophagus, liver, lung and bronchus, stomach, and ovary).

**REFERENCES**

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