Early detection of cancer is critical for effective treatment and reduced mortality. In patients with cancer, a portion of cell-free DNA (cfDNA) in the bloodstream is tumor derived (ctDNA), providing an opportunity to analyze the cancer genome noninvasively. Although profiling somatic mutations from ctDNA has achieved significant success for cancer diagnosis and surveillance, the sensitivity remains low for early-stage diseases.

In a multi-center case-control study, we used a novel deep methylation sequencing technique called ELSA-seq to generate high-resolution maps of ctDNA from patients with cancers of lung, colorectum, or liver. To capture the highly diluted signals from early-stage tumors, a robust machine-learning classifier was built to identify cancer-associated signals and predict tissue of origin (TOO). Additionally, two somatic mutation profiling assays were evaluated in parallel in a pre-specified subset.

Study design: The study was conducted among 490 patients with lung cancer (LC, N=176), colorectal cancer (CRC, N=167), liver cancer (LHC, N=126), and 226 age-matched non-cancer controls. Surgery-eligible patients (stage I, II) consisted of 64% and 36%, respectively, for each cancer type respectively. Patients who were recognized to have anemia, acute infections, autoimmune diseases, or treated with neoadjuvant therapy were excluded from the study. The non-cancer controls were recruited with the criteria of showing no clinical symptoms or history of cancer at time of administration.

ELSIA-seq: The target panel covers 80,672 CpG sites, spanning genomic regions of around 1.5Mb. In total, 8312 co-methylation blocks were defined and used as features/markers to build the classification model.

HS-UMI: The target panel spans 188kb of human genome, including 158 genes that are frequently mutated in lung cancer. Both ctDNA and paired WBC were sequenced with an average depth of 35,000X.

do-PCR: doPCR (Bio-Rad) was used to absolutely quantify the copies of mutant and wild-type alleles in the sample. 30-50ng ctDNA input was required to reach 0.01-0.1% assay sensitivity.

The study consists of four sequential steps: target panel design, marker selection, model training and validation, and cross-platform evaluation. All samples were only used once at each step to avoid overfitting risks. To minimize batch effect, samples from case and control groups were mostly processed together.

A total of 716 patients were included and randomly assigned into the training or validation groups according to preplanned ratios.

In a pre-specified subset, 32 early-stage lung cancer patients and 21 non-cancer controls also had their ctDNA, white blood cells (WBC), and primary tumor samples analyzed by deep mutation sequencing (HM or HS-UMI), drop-in digital PCR (ddPCR) singly or in combination. To unbiasedly evaluate the performance of different assays, ELSA-seq was set at 98% training specificity, similar to the other two methods reported in previous studies.

**MATERIALS AND METHODS**

**TABLE1**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Normal Control</th>
<th>Target Cancer</th>
<th>Infrequent Somatic</th>
<th>Lung Cancer</th>
<th>Liver Cancer</th>
<th>Tumor Control</th>
<th>Lung Control</th>
<th>Breast Control</th>
<th>Colorectal Control</th>
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<tbody>
<tr>
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</table>

**FIGURE1**

**FIGURE2**

**FIGURE3**

**CONCLUSION**

- At overall specificity of 95% (215/226), ELSA-seq demonstrated robust detection rates of 65% (86/133), 82% (125/153), 89% (101/115), and 90% (84/93) respectively, highlighting the potential as a sensitive ctDNA profiling approach for early multi-cancer detection.
- ELSA-seq correctly predicted the TOO in 90% (117/126) of the cases in the validation set, offering an opportunity for decoding the ctDNA organ source.
- In a substudy, ELSA-seq identified nearly twice (27/32) as many patients as deep mutation sequencing (15/32) while reporting no false positives (0/21). It also required no prebiopsied tissues to detect ctDNA at AF as low as 0.02%.
- Large-scale prospective studies in high-risk populations and long-term follow-up will be needed for further evaluation.

**REFERENCES**

1. Burning Rock Biotech, Shanghai, China. 2. Shanghai Chest Hospital, Shanghai, China. 3. Changhai Hospital of Shanghai, Shanghai, China. 4. The 2nd Xiangya Hospital of Central South University, Changsha, China.

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