

Plasma Cell-free RNA (cfRNA) Sequencing Enables Sensitive Detection of Lung Cancer

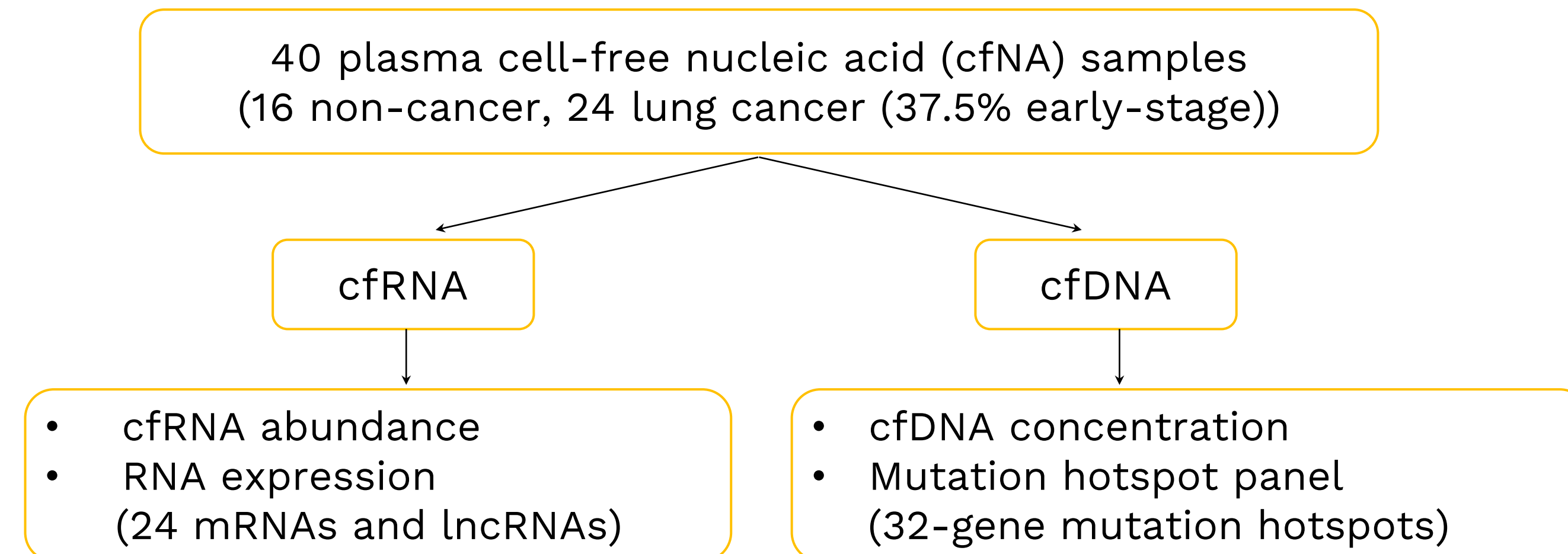
Yee Fang Hum¹, Jonathan Poh², Pannapa Pinweha¹, Chae Yin Cher¹, Jia Min Ho¹, Min-Han Tan², Kao Chin Ngeow¹

¹Lucence Diagnostics Pte. Ltd., Singapore, ²Lucence Health Inc, Palo Alto

BACKGROUND

- Plasma cell-free DNA (cfDNA) has been widely profiled in liquid biopsy assays, whereas cfRNA has remained relatively unexplored as an analyte.
- Plasma cfRNA can be interrogated to determine cancer-associated gene expression, and thus complements cfDNA-based liquid biopsy testing for potentially more sensitive and specific early cancer detection.
- We developed an ultrasensitive next-generation sequencing assay based on AmpliMark™ technology to assess the utility of cfRNA-based gene expression profiles for lung cancer detection.

METHODS



- Plasma cfNA samples from 40 patients with informed consent were analyzed.
- An AmpliMark™-based cfRNA expression panel incorporating molecular barcoding for error-suppression was designed to target 24 mRNA and long non-coding RNA (lncRNA) markers.
- cfRNA markers that are presumptively tumor-associated and absent in non-cancer controls are termed mirrored expression markers.
- cfRNA abundance was estimated as the geometric mean read counts of a set of housekeeping genes. RNA expression score was obtained by summing the relative normalized expression of all RNA targets.
- Matched cfDNA was analyzed with a 32-gene mutation hotspot panel to detect DNA mutations. DNA mutation score was obtained via summation of the normalized allele frequency of all cancer-associated mutations.

ACKNOWLEDGEMENTS

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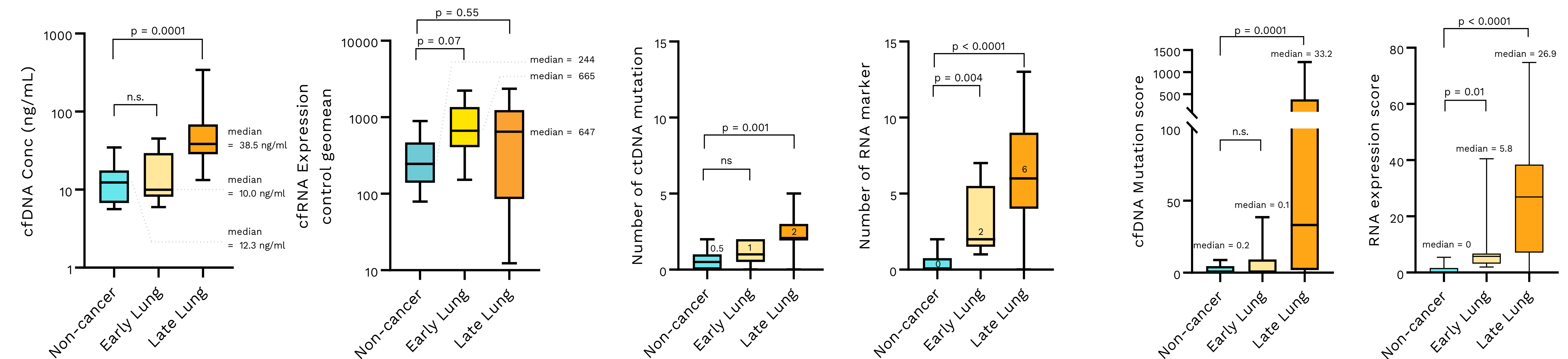
RESULTS

(A) cfRNA is enriched in early-stage lung cancer independent of cfDNA.

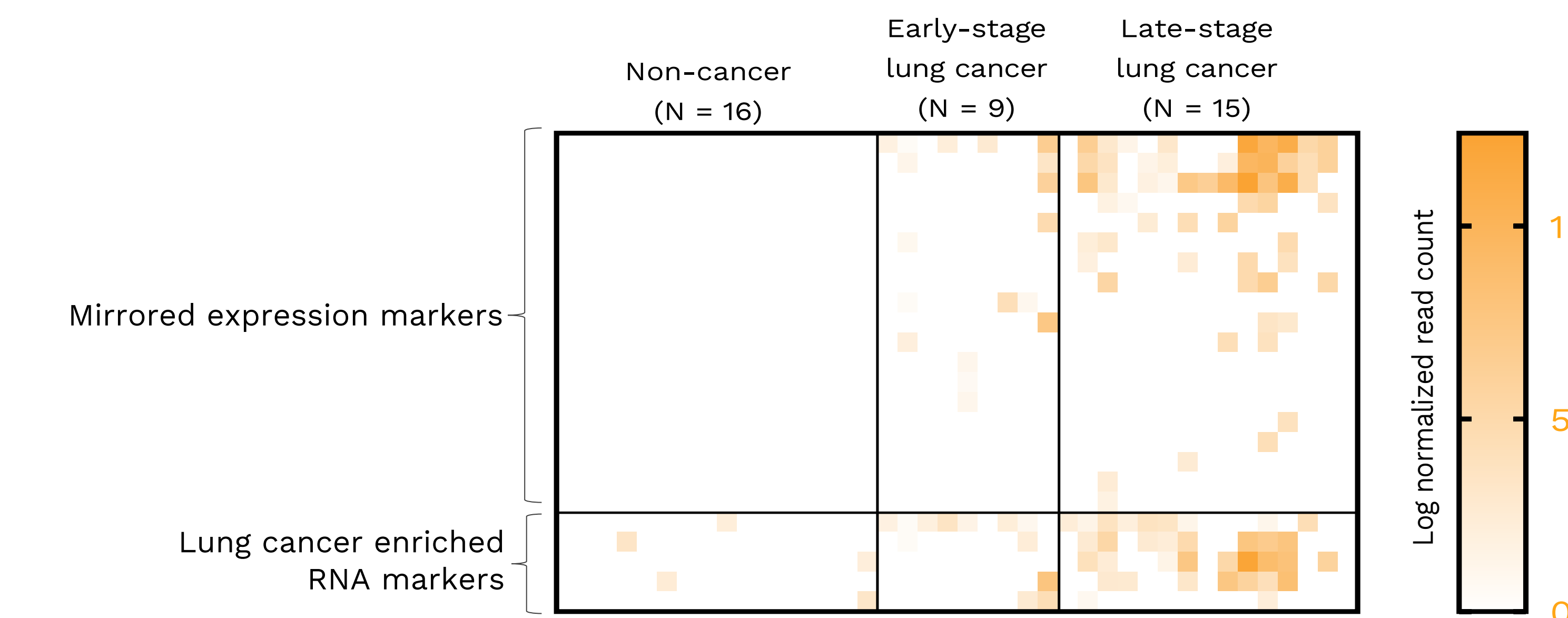
(i) cfRNA levels are elevated in early stages of lung cancer.

(ii) Positive cancer-associated cfDNA and cfRNA markers increase with cancer stage.

(iii) Expression of mirrored expression markers increases with lung cancer stage.



(B) Mirrored expression markers predict stage dependent lung cancer.



(C) Performance of mirrored expression markers in lung cancer prediction.

Lung Cancer Stage	Sensitivity (%)	Specificity (%)
Early-stage	77.8	
Late-stage	86.7	
All lung cancer cases	83.3	93.8

CONCLUSION

- We report that mirrored expression markers profiling provides an independent, and potentially earlier, measure of cancer signals compared to cfDNA profiling.
- Mirrored expression markers can be a reliable analyte to complement cfDNA-based technologies for sensitive and early detection of lung cancer.
- Further evaluation of this preliminary study is warranted to confirm the findings.