Abstract 3249

The development of a tissue-agnostic genome-wide methylome enrichment assay for lung cancer

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BACKGROUND

- Lung cancer remains the leading cause of cancer-related death in the United States. Currently, up to 38% of patients are ineligible for tumor-informed molecular residual disease (MRD) tests due to insufficient tissue, often resulting from risks associated with invasive procedures, small biopsy sizes (e.g., FNAs), low tumor purity, competing demands for tissue (e.g., PD-L1 staining), or complete response to neoadjuvant treatment[1,2]
- Methylation based analyses of plasma circulating tumor DNA (ctDNA) have emerged as industry leading approaches for MRD testing.
- In resectable non-small cell lung cancer (NSCLC) there are several potential applications for ctDNA based approaches to aid in treatment escalation or de-escalation decisions after surgical resection. In addition, ctDNA has the potential to be used to monitor for recurrence before clinical or radiographic presentation and potentially improve outcomes in these patients.
- This study evaluates a novel tissue-agnostic genome-wide methylome enrichment platform based on cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq).

METHODS

Study Design and Patient Cohort

- This is a pilot study to assess whether generating MRD test results on banked early-stage lung cancer samples is feasible and to correlate MRD test results with recurrence-free survival (RFS).
- 136 samples from 24 patients with early-stage lung cancer were available in this cohort. 1 patient was excluded due to recurrence greater than 4 years from the last available blood draw timepoint. The demographics and clinical characteristics of the 23 patients are listed in Table 1.
- Blood collections for each patient occurred prior to surgery, after surgery and at intervals before recurrence or last clinical follow-up.
- Patients were assessed for recurrence as part of standard of care. The analysis of RFS excludes 4 patients due to a single post treatment timepoint that was MRD not detected.

Statistical Methods

- The analysis considers multiple MRD tests over a surveillance period following surgery. RFS is compared between detection groups using a log-rank test and Kaplan-Meier curves.
- The hazard ratio (HR) is estimated using a Cox proportional hazards model.

METHODS (CONTINUED)

Genome-Wide Methylome Enrichment Platform

- Samples were analyzed with a bisulfite-free, non-degradative genome-wide DNA methylome enrichment platform based on cfMeDIP-seq, using 5-10 ng of cell-free DNA (cfDNA) extracted from plasma (Figure 1).
- ctDNA was quantified using a within sample normalized ratio of ctDNA specific signal to background noise across informative regions.

Figure 1. Genome-Wide Methylome Enrichment Platform Enables High Specificity for Recovery of Methylated cfDNA
Utilizes cfMeDIP-seq

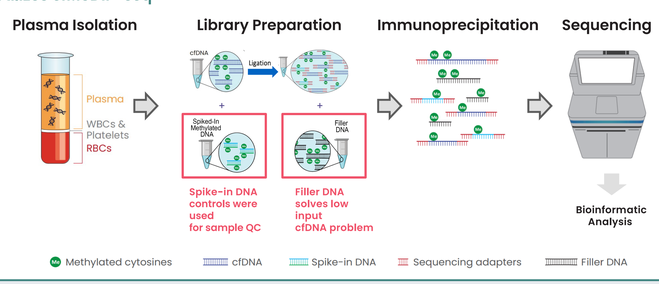


Table 1. Cohort Demographics and Characteristics

		Overall (N=23)*		NR (N=9)		R (N=14)	
Age	Mean (SD)	72.4	(7.2)	72.6	(8.6)	72.3	(6.4)
Sex	Female	12	(52.2%)	5	(55.6%)	7	(50.0%)
	Male	11	(47.8%)	4	(44.4%)	7	(50.0%)
Stage	Stage I	20	(87.0%)	9	(100.0%)	11	(78.6%)
	Stage II	3	(13.0%)	0	(0.0%)	3	(21.4%)
Smoking Status	Former	19	(82.6%)	7	(77.8%)	12	(85.7%)
	Never	4	(17.4%)	2	(22.2%)	2	(14.3%)
Histology	Non-small cell - adenocarcinoma	21	(91.3%)	8	(88.9%)	13	(92.9%)
	Non-small cell - sarcomatoid carcinoma	1	(4.3%)	0	(0.0%)	1	(7.1%)
	Non-small Cell - Squamous Cell	1	(4.3%)	1	(11.1%)	0	(0.0%)
Race	Asian	3	(13.0%)	1	(11.1%)	2	(14.3%)
	White	18	(78.3%)	7	(77.8%)	11	(78.6%)
	Other	1	(4.3%)	0	(0.0%)	1	(7.1%)
	Unknown	1	(4.3%)	1	(11.1%)	0	(0.0%)

NR = Non-recurrence, R = Recurrence
* 1 patient was excluded due to recurrence >4 years after last blood draw

RESULTS

- The Kaplan-Meier plot shown below compares RFS between MRD detected and MRD not detected groups. Statistically significantly worse RFS was observed in the MRD detected group with hazard ratio being 3.58 (1.00, 12.89) and log-rank P-value = 0.038 (Figure 2).

Figure 2. Recurrence-Free Survival (Surveillance Set)

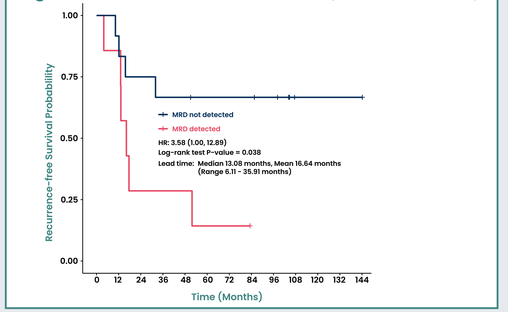
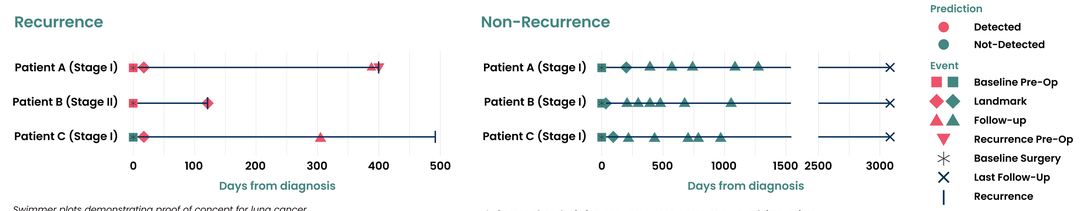


Figure 3. Early-Stage NSCLC Representative Cases



Swimmer plots demonstrating proof of concept for lung cancer recurrence detection and monitoring.

Swimmer plots depicting non-recurrence cases at assayed time points.

CONCLUSION

- MRD detection with a tissue-agnostic genome-wide methylome enrichment platform in patients with lung cancer after curative intent treatment correlates strongly with RFS.

Future Studies

- Additional studies are forthcoming, with a focus on a validation analysis to demonstrate the potential clinical use case and test performance for this tissue-agnostic methylome based MRD detection test.

ACKNOWLEDGMENTS

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- Sequencing was completed by the Princess Margaret Genomics Centre, Toronto, Canada.

References

1. Khan S, et al. *JCO.* 2024.
2. Aggarwal C., et al. *JAMA. Oncol.* 2019.
3. Shen Y., et al. *Nature.* 2018.
4. Liu G. *Ann Oncol.* 2025.