Noninvasive longitudinal monitoring of residual disease in chemotherapy-treated colorectal cancer patients

Abstract number 4788

Alison Tang*, Rebecca Gupte*, Victoria Cheung*, Tao Qing*, Austin Cauwels, Emily Leff, Kimberly Walter, Ehsan Tabari, Alex Lovejoy, Cheng-Ho Jimmy Lin
Freenome, 279 East Grand Avenue 5th Floor, South San Francisco, CA
** These authors contributed equally*

INTRODUCTION

- Monitoring of cancer minimal or molecular residual disease (MRD) shows great promise in assessing therapy response and improving patient outcomes
- Aberrant DNA methylation patterns are a hallmark of cancers, and robust signals can be detected by sensitive plasma circulating tumor DNA (ctDNA) assays

OBJECTIVES

- We present a methylation-based approach for longitudinal monitoring of tumor burden that is tumor-naive, i.e., with no reliance on prior characterization of tumor molecular characteristics
- We assess our approach in a cohort of colorectal cancer (CRC) patients receiving chemotherapy with or without additional targeted agents

METHODS

- We identified genomic regions where cell-free DNA (cfDNA) methylation was related to early stage CRC
- We developed a methylation disease burden score by quantifying methylation in these regions (data not shown)
- We trained a model on an independent cohort of CRC and healthy donor plasma cfDNA samples to identify a threshold for classifying disease burden as positive and negative
- Our developed method's C_{95} limit of detection for model-relevant methylation is 0.007%
- We leveraged this model to detect CRC in our longitudinal samples
- Patients have associated Response Evaluation Criteria in Solid Tumors (RECIST) statuses of complete/partial remission (CR/ PR) or progressive/ stable disease (PD/SD) based on imaging (**Table 1**)

Table 1. CRC cohort information categorized by RECIST 1.1 patient groups

RECIST at therapy start		CR, PR	PD, SD
Age at diagnosis (range)		59.7 (41-87)	64.9 (44-88)
Early Stage	Stage I	0	1
	Stage II	4	0
Late Stage	Stage III	15	6
	Stage IV	11	20
Unknown Stage		0	1
Sex		18M, 12F	17M, 11F

- Longitudinal blood samples were collected from patients while on treatment, which averaged 4.9 months (median samples per patient=5; **Figure 1**)

Figure 1. Study analysis workflow

- We generated plasma cfDNA-derived libraries for deep methylation sequencing, targeting regions relevant to CRC

RESULTS

- We computed a disease burden score for each sample and related these scores to clinical response, scaling the scores for better resolution for visualization
- We grouped patients by their disease burden trajectories, finding that many track with disease trends as confirmed with imaging. Each line is a patient; circles are blood draws (**Figure 2**)
- Group definitions for remission throughout: predominantly low scores; eventual progression: increase in scores; no remission: positive calls throughout and consistent scores; eventual remission: decrease in scores and/or flip to negative call
- 58/65 (89%) time points in remission throughout were negative ctDNA calls and all had remission RECIST
- Using this classifier to check for residual disease at the end of treatment, we detected disease in only 3/16 complete responders (19%) but in 35/41 non-responders (85%)

Figure 2. Aggregated disease burden trajectories show patterns of treatment response

- In patients with varied therapy responses, we successfully detected residual disease in plasma prior to the tumor imaging responder/non-responder assessment. Blood draws with associated RECIST are indicated on top; vertical lines indicate therapy window; triangles denote that the patient is alive at the latest follow-up (**Figure 3**)
- Our method identified burgeoning disease up to 5 months before imaging

Figure 3. We detect residual disease in plasma prior to positive RECIST results

- We observed that apart from imaging, a positive prediction from cfDNA at the start of therapy (a patient's T0 blood draw) can effectively stratify patients' survival (**Figure 4**)

Figure 4. Plasma disease burden at T0 stratifies patients by their survival probability

KEY FINDINGS AND CONCLUSIONS

- Methylation signals in cfDNA are an effective approach for quantifying disease burden, identifying disease in 35/41 non-responders to treatment
- Our results suggest improved sensitivity relative to RECIST, identifying disease up to 5 months before imaging, and highlights the potential of using noninvasive blood tests for continuous monitoring the response of CRC patients receiving therapy

References

1. Vallania, F. et al. Plasma-derived cfDNA to reveal potential biomarkers of response prediction and monitoring in non-small cell lung cancer patients on immunotherapy. *J. Clin. Orthod.* 38, 9588-9588 (2020).
2. Lin, J. et al. Evaluation of a sensitive blood test for the detection of colorectal advanced adenomas in a prospective cohort using a multiomics approach. *J. Clin. Orthod.* 39, 43 (2021).
3. Darwiche, N. Epigenetic mechanisms and the hallmarks of cancer: an intimate affair. *Am. J. Cancer Res.* 10, 1954–1978 (2020).

Acknowledgements

This study was sponsored by Freenome Holdings, Inc.

Disclosures

All authors are employees of Freenome Holdings, Inc.

Presented at the American Association for Cancer Research Annual Meeting, San Diego, CA. April 5-10, 2024.