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Viewpoint

Lightning does strike twice: leveraging phased variants to enhance minimal residual disease detection

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SUMMARY

Liquid biopsy detection of residual cancer after therapy offers to transform oncology care. Nonetheless, in the residual cancer context, signals are sparse and are hindered by technical sequencing noise. Kurtz et al.[1] introduce phased variant enrichment and detection sequencing (phasED-seq) to increase the circulating tumor DNA signal-to-noise ratio and detect minimal residual disease with unprecedented sensitivity.

If successful, cancer treatment will lead to remission. However, the absence of radiographically visible disease can obscure a large number of cancer cells that remain after treatment. These remnants, known as minimal residual disease (MRD), may go on to cause lethal disease relapse. Sensitive MRD detection may thus empower clinical care to provide additional therapy, enhancing the likelihood of cure.

As tumor DNA may enter biofluids as circulating tumor DNA (ctDNA), identification of ctDNA via tumor-specific mutations has shown promise in the identification of MRD in many cancer types.[2–5] Deep-targeted sequencing techniques, such as cancer-personalized profiling by deep sequencing (CAPP-seq[6]), have been developed to deal with the scarcity of mutated fragments but are restricted by technical noise, limiting the ability to distinguish single nucleotide variants (SNVs) signals from sequencing errors[7]. To reduce technical background noise, error suppression methods such as duplex sequencing (duplex-seq) have been developed.[7,8] Duplex-seq relies on identifying an SNV on both strands of a ctDNA molecule, thereby reducing the probability of sequencing errors. However, duplex-seq suffers from lower sequencing yields given that unpaired top and bottom DNA strands are discarded.[8] In an elegant twist, Kurtz et al. reasoned that another avenue to curb the impact of sequencing errors would be to detect SNVs that are located in close proximity in the genome such that they may be identified on the same sequencing read (termed phased variants) (Figure 1).[1] The key guiding intuition is that such occurrences can leverage conditional probabilities, whereby the likelihood of observing a rare event twice is the product of multiplying their separate probabilities. Thus, if the chance of observing a random sequencing error is $1{:}10^3$, the chance of observing two such events on the same sequencing read will be the product of multiplying the probabilities, or $1{:}10^6$. This principle allows reducing the impact of sequencing errors, without the decrease in yield inherent to duplex-seq.

To identify SNVs that could be present on the same ctDNA molecule, the authors mined whole genome sequences of 24 cancer types for SNVs at most 170 base pairs apart (the modal length of circulating DNA fragments). While most cancer histologies contained potential PVs, they were particularly enriched in B cell lymphomas. The authors found that PVs in B cell lymphoma occurred in signatures typically associated with AID/AICDA-induced clustered mutations. Importantly, clustered AID/AICDA-induced mutations tend to affect specific areas in the genome, and therefore many of these PVs are conserved throughout different B cell lymphoma histologies.[9] This allowed the authors to design a one-size-fits-all phased variant enrichment and detection sequencing platform (phasED-seq) to target multiple PVs specific for B cell lymphoma.

Kurtz et al. performed an *in vitro* mixing study to evaluate the lower limit of detection of phasED-seq and compared it to previous assays (CAPP-seq and duplex-seq).[1] To demonstrate the strength of their assay, they mixed minute fractions of cell-free DNA from lymphoma patients to cell-free DNA of healthy individuals for estimated tumor fractions ranging from 0.5 to 1000 parts per million (ppm). CAPP-seq allowed detection in ctDNA concentrations as low as 100 ppm, while duplex-seq was accurate down to ~10 ppm. phasED-seq radically increased ctDNA detection compared to CAPP-seq and duplex-seq, accurately estimating tumor burdens as low as 0.5 ppm. To demonstrate the clinical value of highly sensitive MRD evaluation, the authors studied plasma samples from a patient with diffuse large B cell lymphoma undergoing therapy. While ctDNA was undetectable by CAPP-seq after one

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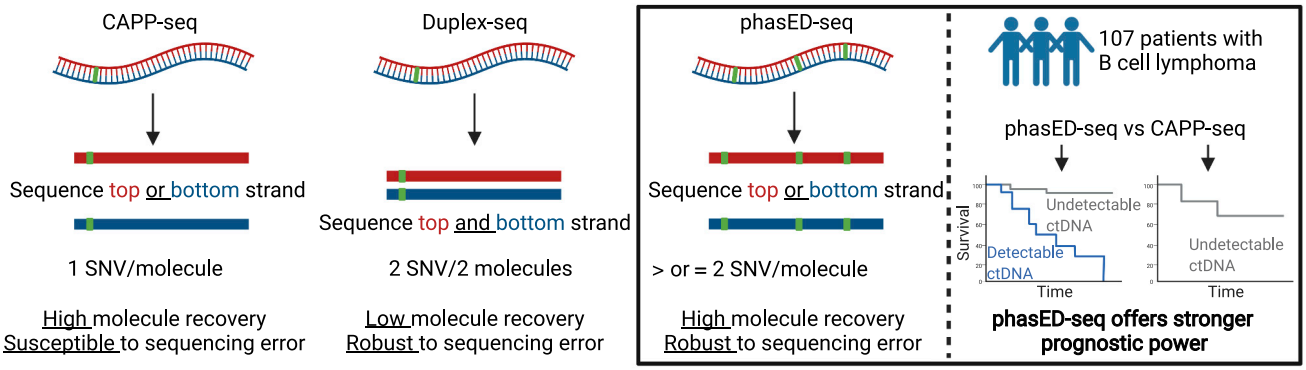

Figure 1. Next generation ctDNA sequencing assays

(Left) CAPP-seq profiles SNVs present on circulating DNA fragments.

(Middle) Duplex-seq identifies original top and bottom strands via duplex adapters. Identification of the SNV on both DNA strands decreases the potential for sequencing errors.

(Right) phasED-seq targets circulating DNA containing two or more SNVs for robust sequencing error suppression and high molecule recovery. phasED-seq was applied to plasma samples of 107 patients with B cell lymphoma and demonstrated higher prognostic power compared to CAPP-seq. Figure created using biorender.com

round of treatment, phasED-seq consistently detected ctDNA, and indeed the malignancy recurred 13 months later. These results highlight the potential of phasED-seq to detect MRD in curative settings.

To further validate the clinical utility of phasED-seq, the authors investigated 277 samples from 107 patients with B cell lymphomas. They found that phasED-seq identified ctDNA in 25% of cases undetected through CAPP-seq after two cycles of treatment. Importantly, the detection of ctDNA via phasED-seq was prognostic of event-free survival. In a post-treatment cohort of 19 patients, the five patients who ultimately experienced disease recurrence were identified through phasED, compared to only two through CAPP-seq. Together, these results suggest that phasED-seq offers greater sensitivity for residual disease detection than previous state-of-the-art ctDNA assays.

Solid organ tumors also contain putative PVs. However, unlike in lymphomas, they are distributed more broadly in the genome, limiting the ability to create a generally applicable targeted panel. Notably, as each location in the genome is represented by only thousands of physical fragments, a single detection is likely insufficient to robustly detect ctDNA at the parts per million range[10]. To overcome this challenge and harness phasED-seq to MRD measurement in solid tumors, Kurtz et al. developed patient-specific panels to target tens to hundreds of tumor-informed PVs.[1] These panels were applied to 24 plasma isolates from 6 patients (5 lung cancer and 1 breast cancer). CAPP-seq assays detected tumor burden in 9/24 samples, while phasED-seq recovered ctDNA from an additional 6 samples. In a patient with phase III lung adenocarcinoma, CAPP-seq failed to detect ctDNA after the initiation of treatment, despite measurable tumor volume by computed tomography scans. In all of these samples, ctDNA was detectable through phasED-seq. These results suggest that the relatively high limit of detection of CAPP-seq, determined by its SNV false positivity rate, leads to false negatives that are recovered through phasED-seq.

ctDNA promises to transform clinical oncology through the ability to monitor response to therapy in real time, allowing closed-loop treatment optimization. Nonetheless, minimal residual disease detection is limited by the sparsity of tumor derived DNA in the plasma, making it hard to distinguish true mutations from technical background sequencing error. This study by Kurtz et al. is an important step in overcoming technical noise and improving the sensitivity of MRD detection.[1] The unprecedented sensitivity of phasED-seq to detect cancer during and after treatment may find use in guiding treatment decisions, where physicians may de-escalate therapy when ctDNA levels become undetectable via phasED-seq, sparing patients toxic and costly therapies. Similarly, the increased sensitivity of phasED-seq could allow for earlier detection of treatment failure, allowing to offer patients alternative therapies while the tumor burden is low and the disease is still within the curative window.[2,11] Although further validation is needed, this study by Kurtz et al. highlights the potential of ctDNA-based assays to accurately monitor residual disease and to inform clinical decision-making in low tumor burden settings.

DECLARATION OF INTEREST

D.L. is a co-founder of C2i Genomics and a member of its scientific advisory board.

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