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FORESIGHT DIAGNOSTICS

Closing the gap on minimal residual disease detection in cancer

Foresight Diagnostics has developed a platform that detects circulating tumor DNA at levels below one part-per-million, allowing accurate identification of minimal residual disease well below current levels of detection. With testing available for B cell lymphomas and solid tumors, Foresight offers partnering opportunities ranging from investigational through prospective clinical studies and companion diagnostic development.

Current liquid biopsy assays are able to detect circulating tumor DNA (ctDNA) levels when tumor burden is high, but are not sensitive enough to detect minimal residual disease (MRD) when ctDNA levels are low. A significant portion of lymphoma patients eventually experience disease progression, and existing ctDNA assays fail to detect MRD in the majority of patients who will later relapse. Foresight Diagnostics, a privately-held cancer diagnostics company based in Aurora, Colorado, USA, has developed a novel liquid biopsy testing platform, PhasED-Seq, that allows detection of MRD with up to a 100-fold improvement in sensitivity over existing assays. Phased Variant Enrichment & Detection Sequencing (PhasED-Seq) detects ctDNA at levels below one part-per-million (<0.0001%) (ppm), a level of sensitivity that allows, for the first time, robust detection of ctDNA during and immediately after curative intent treatments across a variety of clinical settings (Fig. 1). One such application is the earlier detection of disease progression following curative intent treatment in patients with B cell lymphomas. PhasED-Seq provides an average gain of 200 days of lead time over existing disease surveillance platforms, which represents a fundamental shift in the ability to personalize treatment approaches for cancer patients and improve their overall survival prospects. The company is developing additional MRD assays based on PhasED-Seq technology for other tumor types, including a variety of solid tumors.

Potential applications of PhasED-Seq include the efficient development of new oncologic drugs, the design of new registrational pathways with MRD as an endpoint, and eventually the enhancement of clinical practice through MRD-guided treatment decisions.

"Despite the success of standard therapies in curing a large fraction of patients with B cell lymphomas, there is still a high unmet need for tools that can accurately and rapidly identify patients at risk of progression," said Jake Chabon, co-founder and CEO of Foresight. "Our ctDNA-based MRD detection platform, PhasED-Seq, provides a powerful new diagnostic tool for monitoring disease status during periods of low and previously undetectable tumor burden so that alternative treatments that could impact patient lives in very meaningful ways can be initiated as soon as possible."

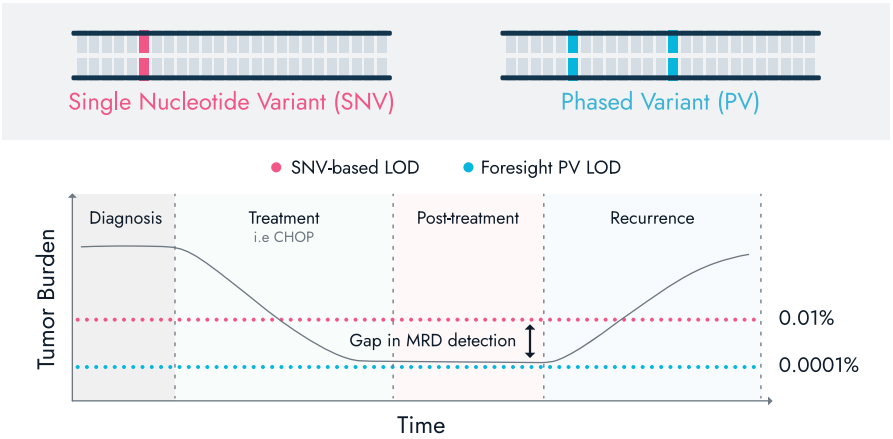

Fig. 1 | PhasED-Seq enables measurement of MRD at levels lower than SNV-based ctDNA detection. PhasED-Seq detects phased variants, ctDNA molecules harboring two or more SNVs, resulting in a 100-fold improvement in sensitivity over traditional SNV-based MRD detection platforms. This improvement in the limit of detection affords the possibility for continuous tumor detection both during and following treatment, thereby closing a critical gap in cancer management. ctDNA, circulating tumor DNA; LOD, limit of detection; MRD, minimal residual disease; SNV, single nucleotide variant.

Foresight is seeking collaborations with clinical and pharmaceutical partners interested in exploring and jointly developing novel diagnostic and therapeutic strategies based on the enhanced MRD detection capabilities afforded by Foresight's platform.

Zeroing in on MRD

A successful cancer treatment is defined by remission of the disease to undetectable levels. Unfortunately, the ability to determine whether remission is total and permanent is dictated by the ability to detect any traces of remaining disease, or MRD, that might seed a relapse. Over the past few decades the ability to detect MRD has rapidly evolved from radiographic determinations and histological analysis of solid biopsies to more sensitive and less invasive, liquid biopsy-based methodologies. Liquid biopsy refers to the analysis of bodily fluids, most commonly blood, to detect tumor-derived analytes such as circulating tumor cells (CTCs) or ctDNA.

The detection of ctDNA has shown great promise in helping identify MRD in several cancers, but the detection limits of the existing technologies still impose a 'treatment blind spot' of many months during which patients and their doctors must wait before making any decisions on potential follow-up treatment decisions.

Many existing ctDNA-based MRD approaches rely on detecting the minuscule fraction of tumor-specific single nucleotide variants (SNVs) present among the mostly non-cancerous freely circulating DNA contained in a liquid biopsy. The limit of detection (LOD) of a liquid biopsy platform thus rests on the ability to differentiate true, cancer-derived SNVs from non-tumor derived SNVs and potential sequencing errors in a large pool of background DNA.

Targeted sequencing techniques that track SNVs, such as cancer-personalized profiling by deep sequencing (CAPP-Seq) have improved the LOD of liquid biopsy approaches to approximately 100 ppm, depending on the approach. While these improved LODs have helped reduce the 'blind spot' following therapy when it is unclear whether a patient is cured, these approaches fail to detect MRD at the end of therapy in the majority of patients destined to relapse, delaying treatment decisions that could be lifesaving if implemented earlier.

"There are multiple milestones, including during therapy, at the end of therapy, and during the surveillance period after therapy, when physicians and patients could be making critical treatment decisions, but our inability to detect the exceedingly low MRD levels characteristic of those time points

prevents this from happening," said Foresight co-founder and scientific advisor Ash Alizadeh, MD. "There is a need for new ways to track disease and therapy progression and improve individualized predictions and treatment recommendations."

Improving the odds on ctDNA detection

Foresight has developed a new platform for MRD detection that decreases the LOD to tumor burden levels as low as 0.5 ppm, potentially eliminating treatment blind spots by enabling continuous MRD surveillance during and following therapy. This improved sensitivity for ctDNA detection is possible because of the implementation of PhasED-Seq, a new methodology recently developed by the cofounders of Foresight[1].

PhasED-Seq substantially improves the LOD for MRD detection by detecting two or more SNVs on individual DNA molecules. This approach overcomes the limitations of CAPP-Seq, which detects single SNVs on single DNA strands. The exponentially reduced probability of two or more SNVs being present on the same DNA strand as a result of co-occurring sequencing errors rather than true tumor-derived events results in a strong improvement in the signal-to-noise ratio and the resultant improved accuracy and performance of PhasED-Seq.

The key advance in Foresight's approach is taking advantage of the fact that many SNVs are located within less than 170 base pairs from other SNVs. Given that the average size of a ctDNA fragment is ~170 base pairs, it's possible to simultaneously identify two or more SNVs on the same DNA molecule. Such phased variants (PVs) are ubiquitous in cancers—some types of tumors such as B cell lymphomas are particularly enriched for PVs.

For the first implementation of its PhasED-Seq platform, the company focused on detecting MRD in liquid biopsy specimens from patients with B cell lymphomas. Specifically, the assay detects PVs in regions of the genome enriched for mutations arising due to the activation-induced cytidine deaminase (AID/AICDA) mutational process. These PVs are located in stereotypical areas of the genome and conserved across B cell malignancies, allowing for an off-the-shelf MRD solution that does not require patient-specific customization. In addition to PV-based MRD detection, Foresight's MRD testing platform also provides comprehensive SNV and copy number variation genotyping that can be used for genetic subclassification and cell of origin determination in patients with diffuse large B cell lymphoma (DLBCL). The company has validated the clinical utility of the Foresight Lymphoma Assay in longitudinal clinical cohorts consisting of 678 specimens from 213 patients with DLBCL. In a recent publication[1], MRD detection using Foresight's PhasED-Seq platform was directly compared to SNV-based ctDNA detection both during and following two cycles of immunochemotherapy. During treatment, the assay correctly detected 88% of patients in need of additional therapy versus only a 46% correct detection rate with the SNV-based ctDNA approach. Post-treatment, the success rate for the assay went up to 100% compared to 40% for SNV-based ctDNA detection (Fig. 2).

A PhasED approach to solid tumors

Foresight's research has revealed that while solid tumors also contain PVs, these tend to be more broadly distributed across the genome. To take advantage of the improved detection sensitivity and specificity of PhasED-Seq with solid tumors, Foresight generates panels of patient-specific PVs.

In a pilot study of five patients with lung cancer and one with breast cancer, PhasED-Seq was 62.5% successful in detecting potential relapse versus only 37.5% with an SNV-based ctDNA assay. Importantly, the sensitivity achieved with PhasED-Seq in solid tumors—down to 1 ppm—is equivalent to those achieved with B cell lymphomas. While the implementation of PhasED-Seq for MRD detection in solid tumors requires obtaining biopsy samples from individual patients, the potential for making treatment decisions significantly earlier due to the much lower LOD afforded by PhasED-Seq provides the Foresight platform with a clear advantage for patients. Foresight projects that its MRD technology will be applicable to the majority of solid tumors including breast, lung, colorectal, liver, kidney, uterus, skin, and pancreatic cancers.

"Foresight's MRD detection platform has the potential to impact disease management in unprecedented ways, both for blood cancers and solid tumors," said Chabon. "A reliable negative test would help with making a decision about withholding potentially toxic and expensive additional treatment, and conversely, a highly sensitive positive test would provide actionable data to plan follow-up treatment while the disease burden is still low and patients are more likely to respond."

MRD detection with real-time Foresight—a continuum of opportunities

The high sensitivity of Foresight's MRD assay provides, for the first time, the possibility to monitor MRD and the potential for relapse detection in real-time and with high accuracy, opening the door to a bevy of opportunities both in drug development and clinical settings. These encompass efficient development of new oncologic drugs, including new registrational pathways with MRD as an endpoint, and potentially changing clinical practice through MRD-guided treatment decisions. Specifically, a number of immediate clinical trial scenarios in which implementation of the PhasED-Seq-based MRD detection platform would be highly beneficial can be envisioned.

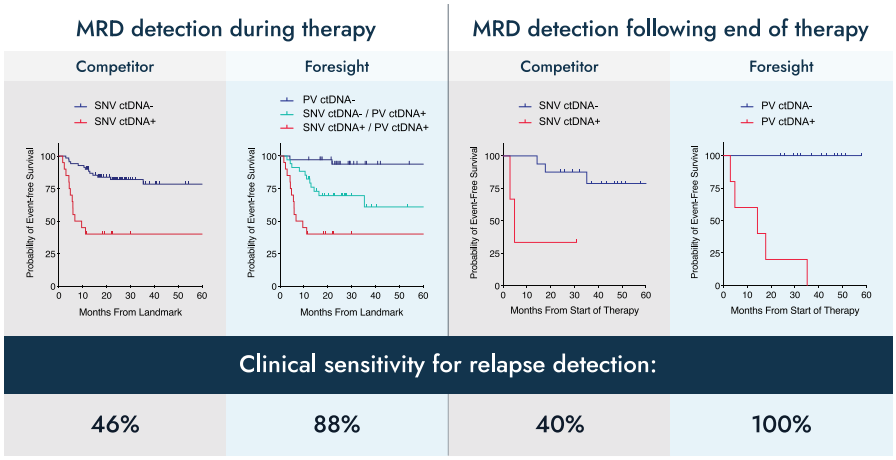

Fig. 2 | PhasED-Seq MRD testing improves clinical outcome stratification. In clinical studies, PhasED-Seq improves MRD detection both during treatment and immediately following treatment compared to SNV-based approaches[1]. During treatment, PhasED-Seq improved recurrence prediction by approximately two-fold; following treatment (left); PhasED-Seq improved recurrence prediction from 40% to 100% (right). MRD, minimal residual disease; SNV, single nucleotide variant.

- ***Risk-adapted randomized trial design.*** Assessment of MRD after only one to two cycles of R-CHOP treatment (rituximab-cyclophosphamide, doxorubicin hydrochloride, vincristine [Oncovin], prednisone) in B cell non-Hodgkin lymphoma would allow for early identification of patients who are not responding to therapy and are in need of additional treatment.
- ***Increased trial size.*** The improved clinical sensitivity of the Foresight MRD assay could allow for higher patient enrollment rates due to better screening capability.
- ***Reduced trial costs.*** Improved screening sensitivity would reduce time and costs to identify the necessary number of registrants for a trial.
- ***MRD as surrogate trial endpoint.*** Higher sensitivity of the MRD test could provide a clinically appropriate endpoint for treatment, allowing trial completion sooner than with existing endpoints such as progression-free survival at 24 months.

To cover the range of possibilities from investigational studies to clinical trials and companion diagnostic development, Foresight is actively building out its operational infrastructure and testing capabilities at the company's US Clinical Laboratory Improvement Amendments (CLIA)-registered laboratories, where it offers research-use-only and CLIA-certified testing options for researchers and clinicians.

"Foresight's MRD detection platform can, for the first time, provide actionable real-time information to physicians and biopharmaceutical companies to enable more personalized treatment approaches for patients with cancer," said Mukul Agarwal, CFO and CBO of Foresight. "We believe our platform will become the future standard of care for MRD detection, and we are looking to partner with others on turning this potential into a reality for cancer patients worldwide."

1. Kurtz, D.M. et al. *Nat. Biotechnol.* **39**, 1537–1547 (2021).

CONTACT
Mukul Agarwal
CFO and Chief Business Officer
Foresight Diagnostics
Aurora, CO, USA
Tel: +1-720-443-3658
Email: press@foresight-dx.com