Journal for ImmunoTherapy of Cancer

Challenges in the implementation of ultrasensitive liquid biopsy approaches in precision oncology

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To cite: Desai A, Lovly CM. Challenges in the implementation of ultrasensitive liquid biopsy approaches in precision oncology. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e006793. doi:10.1136/jitc-2023-006793

This article is published simultaneously in Journal for ImmunoTherapy of Cancer.

Accepted 30 March 2023

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INTRODUCTION

Cell-free DNA (cfDNA) is DNA present in the cell-free component of blood (plasma and serum) or other human bodily fluids.[1] In patients diagnosed with cancer, a component of this cfDNA includes tumor-derived DNA arising from primary or metastatic cancer sites, also known as circulating tumor DNA (ctDNA).[2] Since ctDNA may harbor the same somatic genomic alterations as the tumor,[3] ctDNA detection may have utility in screening and diagnosis, assessing therapeutic response, monitoring for therapeutic resistance, and quantitatively assessing molecularly measurable/minimal residual disease (MRD).[4]

In the article highlighted by this viewpoint, Semenkovich *et al* provide a comprehensive review of the various sources, laboratory techniques, clinical applications, and challenges with utilization of ctDNA. Currently, ctDNA testing is increasingly used in clinical practice with sufficient evidence of utility for genotyping advanced cancer when there is insufficient tissue available for analysis. For example, ctDNA blood based testing is approved for detection of *EGFR* mutations to identify patients eligible for EGFR-directed targeted therapies. Food and Drug Administration subsequently granted approval for two ctDNA platforms (Guardant360 CDx and FoundationOne Liquid CDx) to detect genomic alterations in multiple solid tumor types to identify targetable tumor mutations which now include *KRAS*, *BRAF*, *EGFR* exon 20, *MET* exon 14, and *KRAS* G12C in non-small cell lung cancer and *BRCA*1/2 in ovarian cancer among others. However, much remains to be done prior to clinical applicability in cancer screening, MRD assessment and monitoring, and therapeutic response monitoring. Here, we outline the clinical challenges and next steps for these technologies to improve patient outcomes across the cancer continuum (figure 1).

The ctDNA can provide a complimentary approach to tissue-based testing for advanced cancer genotyping.[5] However, several limitations exist and require attention in this setting. Given ctDNA fractions may vary based on tumor shedding, there is potential for false-negative results. It is important to consider this as 'non-informative' rather than true-negative when ctDNA is used in lieu of tissue-based genotyping, which may still be able to identify targetable alterations. Also, identification of somatic copy number variants and tumor mutational burden can vary based on preanalytical variables, such as amount of cfDNA and tumor stage, among others, and thus should be interpreted with caution for clinical decision-making. It is imperative to take into account the disparities in the bioinformatic filtering of suspected germline variants and the limitations posed by varying signal-to-noise ratios across different vendor platforms when interpreting ctDNA test results.[6] To enhance clinical decision-making, it is recommended that the reporting of ctDNA results include transparent information regarding the limitations of the test that may lead to false-negative reports. Additionally, the report should include information on the ctDNA fraction and the specific genetic alterations that are evaluated for within the test. The usage of longitudinal ctDNA evaluations, which monitor changes in ctDNA levels over time, holds the potential to augment the clinical sensitivity of the tests and bolster the confidence of healthcare providers in relying on ctDNA test results.[7]

Identifying mechanisms of resistance to targeted agents is crucial for tailoring appropriate treatment to overcome drug resistance. The ctDNA may enable such evaluation of mechanisms of resistance, especially when tissue biopsies cannot be obtained post progression. The ctDNA dynamics may provide early assessment of treatment

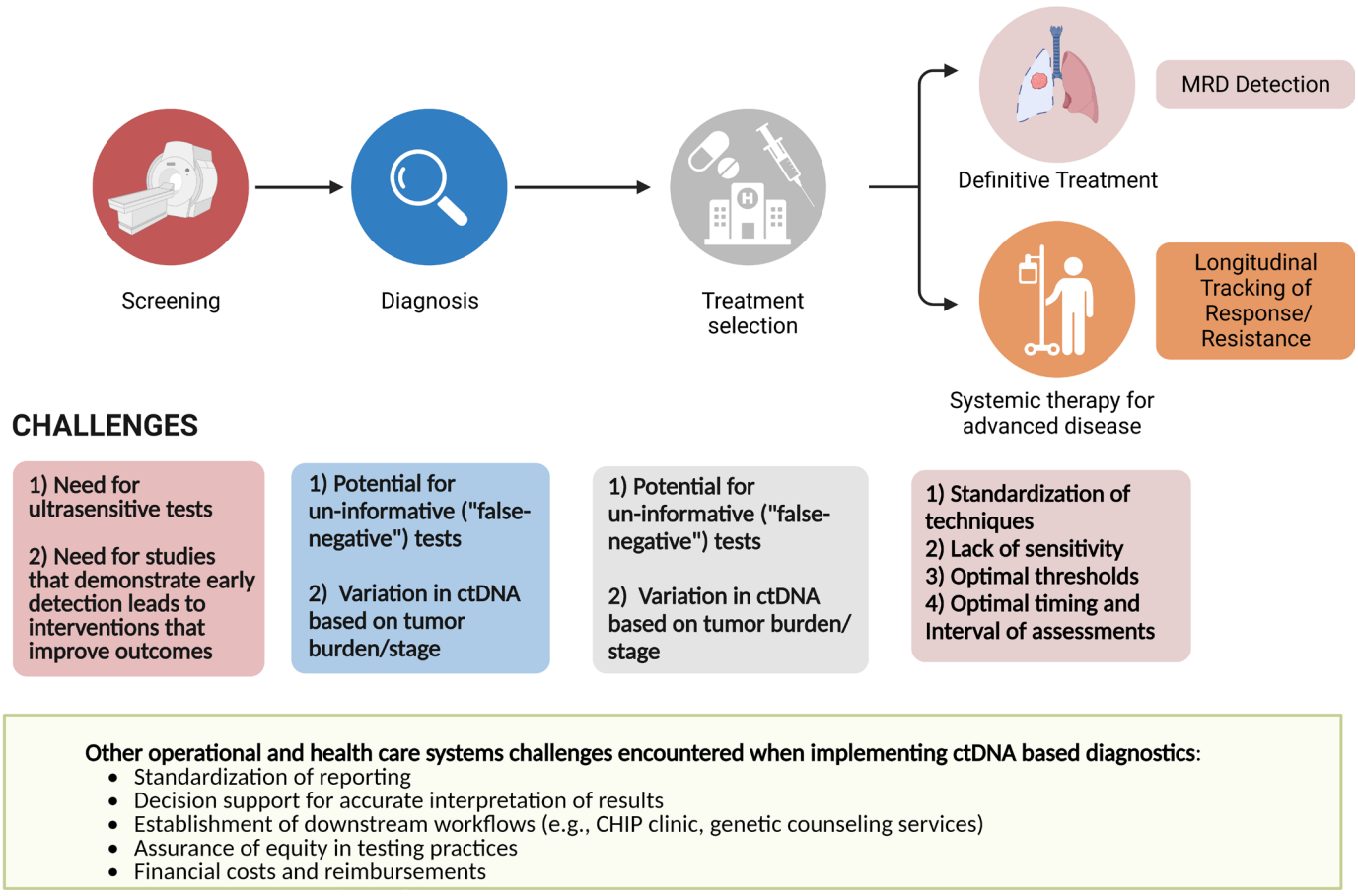

Figure 1 Applications and challenges of ctDNA assessments across the cancer continuum. ctDNA, circulating tumor DNA; MRD, minimal residual disease; CHIP: Clonal hematopoiesis of indeterminate potential

efficacy, differentiation of true progression from pseudoprogression, and opportunity to alter or escalate treatment prior to clinical progression.[8] Standardization and identification of optimal strategies (tumor informed vs tumor agnostic tests) and thresholds for monitoring and timing for ctDNA-based response prediction are necessary to determine best practices for clinical application. This requires well-designed randomized interventional clinical trials to provide high-level evidence for the value of ctDNA monitoring, response prediction, and clinical actionability of ctDNA dynamic assessment.

As we implement novel therapies to early-stage cancers, detection of MRD and identification of molecular relapse during adjuvant treatment or surveillance using ctDNA may provide a window of opportunity to improve outcomes. Most currently available ctDNA assays have adequate sensitivity for advanced cancer genotyping but lack sensitivity for MRD detection, where the tumor fraction is low.

Numerous studies across different cancer types have shown that the presence of ctDNA post-definitive therapy portends worse outcomes.[9] One of the most exciting and evolving utilities of ctDNA is for the detection of MRD. The detection of MRD through ctDNA holds the promise of capturing disease recurrence sooner than radiographic progression, clarifying ambiguous radiographic studies, and affording opportunities to intervene on disease progression sooner to potentially alter the natural history of the disease. Despite the tremendous enthusiasm for ctDNA MRD detection, there are technical and clinical obstacles that currently limit its use. In their review, Semenkovich *et al* elegantly describe the various technical challenges in its application. Detection of MRD can be achieved through either tumor-informed or tumor-agnostic methods. Tumor-informed MRD detection is based on genotyping of both tissue and plasma samples, resulting in higher sensitivity. Currently available tumor-agnostic approaches, which rely on plasma-only next generation sequencing, are likely not sufficiently sensitive for broad implementation of MRD detection.[10]

From a clinical perspective, the timing and impact of ctDNA to tailor adjuvant or consolidation therapies, as well as its value as a surrogate endpoint for early-stage cancer studies, remains to be defined. The ctDNA MRD may provide prognostic information with increased levels of ctDNA indicative of higher cancer burden, associated with worse prognosis.[11 12] Whether ctDNA MRD will help predict the need and response to adjuvant therapies, remains to be elucidated with well-designed studies. Prospective randomized trials using ultrasensitive ctDNA technologies are currently underway to understand the potential utility of ctDNA MRD detection and its role in

personalizing adjuvant or consolidation systemic therapies. For example, the CORRECT study (NCT05210283) is enrolling patients with Stage II or III colorectal cancer who have undergone surgical resection in order to prospectively monitor the association of ctDNA with recurrence-free interval.

Coincident with the development of ultrasensitive ctDNA-based approaches for MRD detection, orthogonal approaches for increasing sensitivity are being explored. For example, analysis of alternative sources of ctDNA (such as other bodily fluids proximal to the primary cancer site) and evaluation of other analytes in the blood (DNA methylation signatures, RNA, exosomes, and circulating tumor cells), both of which may provide complementary approaches to increase sensitivity and optimize liquid biopsy technology for clinical utility. Furthermore, several studies are evaluating the utility of ctDNA technologies for cancer screening and early detection. As ctDNA use increases, standardization of reporting, provider education and establishment of downstream genetic counseling (for when germline mutations are detected) and hematology referrals (for when clonal hematopoiesis of indeterminate potential is detected) must be prioritized. Furthermore, we must ensure that we develop parallel strategies to mitigate any possible barriers (including cost, financial toxicity, implementation, and disparities in testing) in implementation.

In conclusion, ctDNA has the potential to truly enable precision oncology where early screening, diagnostic, response prediction, monitoring and surveillance can be performed to tailor the right treatments to our patients at the right time.

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Contributors Both authors contributed to the content, design and writing of the manuscript.

Funding CML was supported in part by NIH NCI R01CA227833, UG1CA233259, 5P01CA129243-12, U54CA217450-01, U01CA224276-01, LUNGevity Foundation with funds raised by EGFR Resisters for the 2021 EGFR Resisters/LUNGevity Foundation Lung Cancer Research Award Program, and by the ALK Positive patient advocacy organization through the GO2 Lung Cancer Research organization. Figure was created with BioRender.com

Competing interests Both authors have completed the ICMJE uniform disclosure form. AD is a consultant/advisory board member for Amgen and Sanofi. Over the past 2 years, CML has been a consultant/advisory board member for Amgen, Arrivent, AstraZeneca, Blueprints Medicine, Cepheid, D2G Oncology, Daiichi Sankyo, Eli Lilly, EMD Serono, Foundation Medicine, Genentech, Indupro, Janssen, Medscape, Novartis, Pfizer, Puma, Roche, and Takeda.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES

1 Mandel P. Les acides nucleiques du plasma sanguin chez 1 homme. *CR Seances Soc Biol Fil* 1948;142:241–3.
2 Anker P, Mulcahy H, Chen XQ, *et al*. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. *Cancer Metastasis Rev* 1999;18:65–73.
3 Stroun M, Anker P, Maurice P, *et al*. Neoplastic characteristics of the DNA found in the plasma of cancer patients. *Oncology* 1989;46:318–22.
4 Bettegowda C, Sausen M, Leary RJ, *et al*. Detection of circulating tumor DNA in early-and late-stage human malignancies. *Sci Transl Med* 2014;6:224.
5 Rolfo C, Mack P, Scagliotti GV, *et al*. Liquid biopsy for advanced NSCLC: a consensus statement from the international association for the study of lung cancer. *J Thorac Oncol* 2021;16:1647–62.
6 Stetson D, Ahmed A, Xu X, *et al*. Orthogonal comparison of four plasma NGS tests with tumor suggests technical factors are a major source of assay discordance. *JCO Precis Oncol* 2019;3:1–9.
7 Openshaw MR, Mohamed AA, Ottolini B, *et al*. Longitudinal monitoring of circulating tumour DNA improves prognostication and relapse detection in gastroesophageal adenocarcinoma. *Br J Cancer* 2020;123:1271–9.
8 Shaw AT, Martini J-F, Besse B, *et al*. Early circulating tumor (CT) DNA dynamics and efficacy of lorlatinib in patients (PTS) with advanced ALK-positive non-small cell lung cancer (NSCLC). *JCO* 2019;37(15_suppl):9019.
9 Peng Y, Mei W, Ma K, *et al*. Circulating tumor DNA and minimal residual disease (MRD) in solid tumors: current horizons and future perspectives. *Front Oncol* 2021;11:763790.
10 Kasi PM. Tumor-informed versus plasma-only liquid biopsy assay in a patient with multiple primary malignancies. *JCO Precis Oncol* 2022;6:e2100298.
11 Kotaka M, Shirasu H, Watanabe J, *et al*. Association of circulating tumor DNA dynamics with clinical outcomes in the adjuvant setting for patients with colorectal cancer from an observational galaxy study in circulate-japan. *JCO* 2022;40(4_suppl):9.
12 Verzè M, Pluchino M, Leonetti A, *et al*. Role of ctDNA for the detection of minimal residual disease in resected non-small cell lung cancer: a systematic review. *Transl Lung Cancer Res* 2022;11:2588–600.