DiaCarta, Inc.
4385 HOPYARD RD, SUITE 100
PLEASANTON, CA 94588, USA

OFFICE 510 878 6662
FAX 510 735 8636
INFORMATION@DIACARTA.COM

DiaCarta's cfDNA RadTox Test vs ctDNA Analysis Tests

Introduction

Value of both cfDNA and ctDNA biomarkers

As liquid biopsy technologies continue to advance, oncologists are increasingly relying on circulating cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) biomarkers to inform patient treatment and care management [1, 2]. These innovative approaches offer valuable insights into cancer diagnosis and treatment response.

cfDNA quantification provides a broad perspective on tumor burden, treatment efficacy, and disease progression. This method offers oncologists a comprehensive understanding of the patient's overall condition, aiding in decision-making regarding treatment adjustments and disease monitoring.

In contrast, ctDNA analysis, which includes quantification, mutation detection, and methylation profiling, offers a more detailed assessment. It plays a crucial role in various aspects of cancer management, such as early detection, tumor-specific genomic profiling, guidance for targeted therapies, monitoring treatment responses, and predicting prognosis. Additionally, ctDNA analysis facilitates the monitoring of disease recurrence through molecular residual disease (MRD) surveillance.

By integrating both cfDNA quantification and ctDNA analysis, oncologists can tailor treatment strategies more precisely, optimizing patient outcomes while minimizing unnecessary interventions. These liquid biopsy technologies represent a promising avenue in personalized cancer care, offering non-invasive and dynamic insights into tumor biology and treatment responses.

Integration of liquid biopsy biomarkers to standard care tools

The integration of circulating cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) biomarkers offers a complementary approach to traditional cancer care tools, enhancing the precision and effectiveness of cancer management alongside standard methods such as imaging and biomarker analysis (e.g., CEA or CA19-9).

Studies have demonstrated that liquid biopsy assays can detect cancer recurrence at earlier stages compared to conventional imaging techniques, which are currently considered the gold standard in cancer care alongside biomarker analysis like CEA [1,3,4]. Furthermore, liquid biopsy utilizing cfDNA exhibits superior sensitivity and specificity compared to biomarkers such as CEA and CA19-9 [5].

The inclusion of liquid biopsy tools supplements imaging modalities, particularly in scenarios where imaging results are inconclusive for monitoring treatment response, such as distinguishing between disease progression and pseudo-progression [3,6,7]. By providing dynamic insights into tumor biology and treatment response, liquid biopsy enhances the clinician's ability to make informed decisions, ultimately optimizing patient care outcomes.

In summary, the integration of cfDNA and ctDNA biomarkers into cancer care protocols complements standard tools like imaging and biomarker analysis, offering a more comprehensive approach to cancer management. These innovative techniques not only detect cancer recurrence earlier but also provide valuable insights when conventional methods yield ambiguous results, thus enhancing the precision and efficacy of cancer treatment.

This comprehensive overview explores the roles of cfDNA and ctDNA testing, highlighting the advantages of DiaCarta's RadTox™ test for total cfDNA analysis and its complementary clinical utility alongside ctDNA tests from companies such as Natera (Signatara) and Guardant Health (Guardant 360 Response and Guardant Reveal).

Technical Background: cfDNA vs. ctDNA

What is cfDNA?

Cell-free DNA (cfDNA) refers to all free-circulating DNA fragments (average size at 166 bp in length) present in the bloodstream, originating from both tumor and non-tumor cells undergoing secretion, apoptosis or necrosis [8]. Although there are reports indicating the length differences between the DNA from circulating tumor cells (ctDNA, see below) and the rest of cfDNA, there is no reported physical separation method used in clinical studies.

Since cfDNA is less specific (other reasons or diseases may change the cfDNA level) and there is only a very small fraction of ctDNA (see below) in the total cfDNA at early stage of cancer, total cfDNA does not serve well as a diagnostic biomarker, rather than a predictive biomarker for cancer prognosis and treatment response. Numerous publications have indicated the elevated total cfDNA levels often correlate with increased tumor burden and treatment response and can be used as a prognosis biomarker [2]:

- Baseline cfDNA level before treatment is correlated with prognosis [9,10].
- cfDNA level after treatment (e.g. chemotherapy) compared to baseline correlates with the disease conditions and treatment responses:
    - Higher cfDNA levels in patients with disease progression (PD) [11,12, 13].
    - Consistent cfDNA levels in patients with stable disease conditions [11, 14].
    - Lower cfDNA levels in patients with partial responses to treatments [11,12, 13].
- Higher cfDNA ratio (post-treatment/before treatment) is correlated with therapy responses and prognosis [15].
- Transition monitoring from disease progression to stable disease to cancer recurrence (DiaCarta, unpublished).

A research study has indicated that the rise in cfDNA levels among patients with poorer prognoses stems not from an increase in ctDNA, but rather from heightened DNA release from granulocytes in such patients [16]. Nevertheless, the significant correlation between increased total cfDNA and poorer prognosis remains unchanged. Total cfDNA continues to demonstrate promise as a reliable biomarker for prognosis across various solid tumors, including high-grade brain tumors like glioblastoma, where the presence of ctDNA within cfDNA is constrained proven by somatic mutation sequencing.

While the values of cfDNA quantification are notable, it's important to acknowledge its limitations:

- Effective diagnostic biomarker: Despite suggestions from some studies, cfDNA quantification does not currently serve as a reliable diagnostic biomarker.
- Identification of cancer mutations or methylation information: cfDNA quantification cannot provide detailed information regarding specific cancer mutations or methylation patterns.
- Guidance for targeted therapies: It does not offer guidance for targeted therapy selection.
- Tracking tumor clone evolution and resistance: cfDNA quantification does not facilitate the monitoring of tumor clone evolution or the emergence of resistance. cfDNA quantification detects tumor recurrence by significant cfDNA increase, not by monitoring the return of ctDNA.

What is ctDNA?

ctDNA represents a specific fraction of cfDNA originating from tumor cells, carrying somatic alterations such as mutations, copy number variations, and methylation patterns [17]. In the early stages of cancer, ctDNA levels typically remain low, comprising only 0.01 to 1% of the total cfDNA [18]. However, as the tumor progresses, the proportion of ctDNA escalates, reaching up to 90% in late-stage metastatic cancer [19, 20]. This increase in ctDNA can be attributed to several factors, including the rapid proliferation of tumor cells, particularly those clinically significant, heightened turnover of tumor cells, and increased shedding or release of ctDNA into the bloodstream due to the formation of new blood vessels around tumors. Metastasis further exacerbates this phenomenon by spreading tumors to multiple sites, resulting in a greater number of tumor cells and consequently more release of ctDNA. These insights help elucidate why cfDNA can serve as both a prognostic marker and an indicator of treatment response, despite not exclusively originating from tumor cells.

While physically separating ctDNA from the remainder of cfDNA presents challenges, distinguishing ctDNA from normal cell-derived cfDNA is achievable through the identification of mutations or methylation patterns. Modern next-generation sequencing (NGS) has emerged as a potent and indispensable tool for quantifying and analyzing ctDNA, enabling the detection of tumor-specific genomic and epigenomic aberrations, as well as facilitating the tracking of tumor clone evolution.

Undoubtedly, ctDNA analysis, including ctDNA quantification, furnishes oncologists with more comprehensive insights into tumor cells compared to total cfDNA quantification. Beyond its role as a prognostic, treatment response, and disease progression biomarker, ctDNA analysis contributes significantly

to early detection, diagnosis, and therapeutic guidance, capabilities not afforded by cfDNA quantification alone. However, ctDNA analysis is not without its unresolved issues:

- Limited mutation detection**:** Despite the inclusion of a limited number of actionable mutations in targeted NGS panels, not all tumor mutation information is unveiled due to the panels' inherent limitations.
- Discrepancies between tissue and blood NGS results**:** Gold-standard tissue NGS and blood NGS results often diverge due to tumor heterogeneity and the different sample types utilized, potentially leading to conflicting therapy guidance.
- Accuracy of ctDNA quantification**:** ctDNA quantification may lack precision as it often relies on the highest mutant allele fraction (MAF) for quantification. This approach may overlook crucial low-MAF but fast-growing mutant allele clones, particularly in the context of tumor heterogeneity.
- Challenges in detecting dynamic clone evolution**:** Mutations arising from tumor dynamic clone evolution may go undetected or may not be promptly detected, given the rapid mutation rate under drug pressures. This necessitates multiple rounds of complex yet costly and time-consuming ctDNA analyses.
- Limitations in clinical application**:** Constrained by the availability of centralized Laboratory Developed Tests (LDTs) and prolonged turnaround times for complex and sensitive assays, the scalability of ctDNA analysis for frequent and serial monitoring in clinical settings remains challenging.

These unresolved issues underscore the need for further refinement and innovation in ctDNA analysis to enhance its utility and reliability in clinical practice.

The RadTox™ Test and Advantages

RadTox leverages DiaCarta's proprietary isobDNA™ technology to directly quantify total cfDNA without DNA extraction. This testing method targets highly repeated human Alu sequences, enabling sensitive and accurate cfDNA detection from low plasma volumes (as little as 10-20µL) [21].

Advantages of the RadTox™ test:

- Pan-cancer applicability: The RadTox™ test stands out for its pan-cancer applicability. Unlike ctDNA tests, which rely on mutation or methylation markers specific to a patient or cancer type, the RadTox™ test quantifies total cfDNA independently of tumor-specific biomarkers. This feature enables cancer-agnostic monitoring and disease management [22].
- No prior mutation knowledge required**:** Another advantage of the RadTox™ test is its ability to monitor high-level tumor dynamics without prior knowledge of cancer mutation information. This

simplicity sets it apart from ctDNA analysis, making the RadTox™ test more accessible and straightforward [22].

- Early response monitoring**:** The RadTox™ test offers the capability to detect changes in cfDNA levels before radiographic evidence becomes apparent. This early response monitoring feature allows for the early detection of treatment efficacy [23, 24].

- Frequent, serial, minimally invasive monitoring: The RadTox™ test boasts a practical advantage in terms of sample volume requirements. Its low plasma volume needs make frequent and serial monitoring more clinically feasible compared to ctDNA analysis, which often requires 20 ml or more of blood [11, 15, 25].

- Cost-effectiveness: In terms of cost-effectiveness, the RadTox™ test offers a viable solution for cfDNA monitoring. Its streamlined approach reduces financial burdens on patients and healthcare systems compared to complex genomic assays [26].

- Complementary to ctDNA analysis: While ctDNA provides valuable genomics profiling information, cfDNA analysis through the RadTox™ test offers a different perspective on tumor dynamics. These two approaches complement each other, providing a more comprehensive understanding of the disease [27].

- Ease of validation: The RadTox™ test's simplicity also extends to its ease of validation in other clinical labs if necessary. This feature facilitates scalability to meet the demand for upscale, frequent, and serial real-time monitoring requirements.

These features collectively position the RadTox™ test as a promising tool for cancer monitoring and management, offering benefits in terms of accessibility, effectiveness, and cost-efficiency.

ctDNA Analysis Monitoring

ctDNA analysis serves various purposes in cancer management, including screening and early detection, as evidenced by initiatives such as Grail's Galleri and Guardant Health's Guardant Shield. However, in this discussion, our focus is on the utility of ctDNA analysis for treatment monitoring, along with the features and benefits of DiaCarta's RadTox test. We'll also compare RadTox with ctDNA analysis tests offered by Natera and Guardant Health.

MRD Monitoring:

- These tests can track the trace presence of tumor-derived ctDNA after curative treatment to monitor tumor recurrence. Detecting MRD can guide the next-step therapy decisions.

Residual Disease and Resistance Tracking:

- After treatment, ctDNA analysis allows monitoring of residual active disease by quantifying existing tumor mutations/signatures and new resistance mutations appeared due to treatment pressure. This provides a real-time assessment of the response.

Therapy Selection:

- By identifying the tumor's specific genomic alterations from ctDNA, these tests can stratify patients to targeted therapies and newer treatments based on their molecular profiles.

The key enablers are advanced technologies like digital sequencing, targeted capture, and methylation analysis to detect ctDNA tumor biomarkers with high sensitivity and specificity. However, these tests require either prior knowledge of the tumor's mutations for customization or specific gene panels that are known to cover the patient-specific alterations for utility.

Here, we compare the RadTox™ tests and the other well-known MRD or treatment response monitoringtests:

Features	**DiaCarta RadTox**	**Natera Signatera**	**Guardant Health Guardant360 Response**	**Guardant Health Guardant Reveal**
Test Type	Total cfDNA quantification	Customized ctDNA MRD monitoring for recurrence	Assessment of ctDNA changes and treatment response monitoring	Fixed panel ctDNA MRD monitoring for recurrence
Primary Use	Disease progression and treatment response monitoring	Detecting MRD post-treatment	treatment response monitoring after 4 weeks of treatment	4 weeks after post-surgery or/and surveillance monitoring
Technology	IsobDNA™ assay	Deep sequencing	Digital sequencing	Digital sequencing and Methylation analysis
Sample Required	10-20µL plasma	2x10 mL tubes of whole blood or more	2x10 mL tubes of whole blood or more	2x10 mL tubes of whole blood or more
Turnaround Time	~5-7 days	~28 days	~14 days	~14 days
Pre-treatment testing	The same test before treatment	Tissue NGS before treatment	Guardant 360 test before treatment	Not required
Cancer Types	Pan-cancer	Tumor-informed (based on tissue biopsy)	Tumor-naive	Colorectal cancer, breast cancer and lung cancer
Sensitivity	High (Limit of detection at 0.09 ng/ml)	High (>100k sequencing depth)	High (Limit of detection at 0.25% mutant allele frequency)	High (Limit of detection at 0.01% mutant allele frequency)
Cost per Test	Low	High	High	High

Features	DiaCarta RadTox	Natera Signatera	Guardant Health Guardant360 Response	Guardant Health Guardant Reveal
Frequency	Multiple times, even weekly	Multiple times depending on the treatment plan	1 to 3 times between treatment week 4 to 10	Multiple times depending on the treatment plan
Advantages	Frequent monitoring, rapid results, simple assay, cost-effective	Biopsy then blood MRD detection/monitoring	Genomic profiling, therapy guidance	Blood samples only Residual disease and recurrence monitoring

While the RadTox™ test monitors total cfDNA as a general biomarker, the tests from Natera, Guardant Health can provide tumor-specific genomic insights complementary to the RadTox™ test, such as MRD detection, residual disease tracking, genomic profiling for therapy selection.

Conclusion

As the field of liquid biopsy advances, the quantitative cfDNA analysis offered by the RadTox™ test emerges as a valuable complement to ctDNA genomic testing. By offering a versatile and cost-effective method to monitor tumor burden and treatment response across various cancer types, the RadTox™ test equips physicians with practical, real-time insights, facilitating more informed and tailored treatment approaches. Integrating both cfDNA quantification and ctDNA genomic analysis can significantly enhance clinical decision-making in oncology, augmenting traditional standard care by providing additional insights beyond imaging tools. These complementary approaches not only offer greater sensitivity but also address the limitations of imaging tools, which can be less frequently utilized, more expensive, and occasionally inconclusive.

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