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Leveraging RadTox™ cfDNA Testing

for Optimized Tumor Response Management

Circulating cell-free DNA (cfDNA) has emerged as a powerful biomarker for monitoring tumor dynamics and treatment responses across various cancers. This white paper explores the clinical utility of DiaCarta's RadTox™ test, a groundbreaking cfDNA quantification assay, in optimizing tumor response assessment and personalized cancer care. As pioneer Dennis Lo stated, "cfDNA analysis represents a powerful yet minimally invasive approach for molecular analyses in cancer."[1] DiaCarta's RadTox™ test capitalizes on this principle by precisely quantifying cfDNA levels using a proprietary isobDNA technology, providing valuable insights into tumor burden and treatment efficacy.

Introduction

Conventional tumor treatment response monitoring methods, such as imaging, often lag in detecting early treatment responses because (1) tumor size may be too small to be detected by imaging; (2) The tumor size measured by imaging may not reflect the true treatment response as target therapy with efficacy may not shrink the tumor, but enhances tumor necrosis [2]. Traditional protein biomarkers, such as CEA or CA markers are less sensitive and may not provide accurate results.

On the other hand, cfDNA, released into the bloodstream by both cancer and normal cells, represents a real-time snapshot of the tumor's molecular profile and dynamics after different cancer treatments. Many publications have indicated that cfDNA quantification can be used as a prognosis and predictive biomarker for cancer treatments.

Here lists what cfDNA quantification biomarker can offer:

Baseline Assessment: Before initiating treatment, measuring the concentration of cfDNA in the bloodstream can provide baseline information about tumor burden. Elevated levels of cfDNA may indicate a higher tumor burden and suggest a more aggressive disease.

Dynamic Monitoring: Throughout the course of treatment, changes in cfDNA concentration can be monitored to assess treatment response. A decrease in cfDNA concentration over time may indicate a positive response to treatment, reflecting tumor shrinkage or reduced tumor burden. Conversely, an increase or stabilization of cfDNA levels may suggest resistance to treatment or disease progression.

Early Detection of Response or Progression: Changes in cfDNA concentration can often be detected earlier than changes in traditional imaging modalities such as computed tomography (CT) scans. Therefore, monitoring cfDNA levels may enable earlier detection of treatment response or disease progression, allowing for timely adjustments to treatment strategies.

Non-Invasive and Serial Monitoring: Unlike tissue biopsies, which are invasive and can only provide a snapshot of the tumor at a single time point, cfDNA analysis allows for non-invasive and serial monitoring of tumor dynamics. Serial monitoring of cfDNA levels can provide valuable insights into the temporal evolution of tumor response or progression and help guide treatment decisions accordingly.

Prognostic Value: In addition to monitoring treatment response, baseline cfDNA concentration and changes in cfDNA levels during treatment have prognostic implications. Higher baseline cfDNA levels and failure to achieve a decrease in cfDNA concentration during treatment have been associated with poorer outcomes and shorter progression-free survival in various cancer types.

Potential for Personalized Medicine: Integration of cfDNA monitoring into clinical practice may facilitate the development of personalized treatment strategies by enabling real-time assessment of treatment response and the identification of patients who are likely to benefit from specific therapies. RadTox™, in conjunction with other cfDNA monitoring tests, offers a comprehensive approach to monitoring treatment responses. Zhou et al. demonstrated the predictive value of plasma cfDNA kinetics in non-small cell lung cancer patients, highlighting the potential for utilizing cfDNA dynamics in predicting clinical responses [11].

RadTox™ Technology

RadTox™ leverages DiaCarta's proprietary isobDNA™ technology to directly quantify total cfDNA without DNA extraction. This isothermal amplification method targets highly repeated human Alu sequences, enabling sensitive cfDNA detection from low plasma volumes (10-20µL). The isobDNA assay workflow involves the following steps: 1) Plasma sample is combined with the isobDNA reaction mix containing primers specific to the Alu repeats. 2) Isothermal strand-displacement amplification occurs, generating millions of copies of the Alu sequences present in the cfDNA. 3) A fluorescent dye binds to the amplified products, enabling real-time quantification of the cfDNA level based on the fluorescence signal intensity.

Tumor Response Monitoring with RadTox™

Tumor monitoring only by imaging normally has 3 to 4 months of intervals. Patients are left with anxiety while waiting for the imaging results. Combining the RadTox™ test with imaging benefit both patients and physicians in monitoring the whole process of cancer management. Here is how RadTox™ can be used clinically:

1. Tumor Burden Monitoring

   When RadTox™ test is used to measure the cfDNA level before the treatment, this baseline level of cfDNA indicates the tumor burden. The higher cfDNA indicates higher cancer burden. This information can also provide prognostic value for untreated conditions [3,4,5].

2. Early Detection of Treatment Response

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RadTox™ enables early detection of treatment responses by measuring cfDNA level changes during or within days of initiating therapy. This rapid feedback allows timely adaptation of treatment regimens, maximizing efficacy and minimizing unnecessary toxicities. According to Victor Velculescu, "Increases in cfDNA levels can precede clinical progression by months, allowing anticipation of progression."[6]

Studies have shown that progressive disease, stable disease and partial response are correlated with the levels of cfDNA. The level of cfDNA turn to be higher for patients with disease progression, lower for patients with partial response, and constant for patients with stable disease [7].

Our clinical studies with more than 50 patients indicate that when the median cfDNA decreases by 2.45-fold when patients enter from disease progression to stable disease state (Figure 1).

A landmark study by Diehl et al. found that "cfDNA levels were profoundly prognostic, with higher levels associating with worse overall survival independent of clinical parameters."[8]

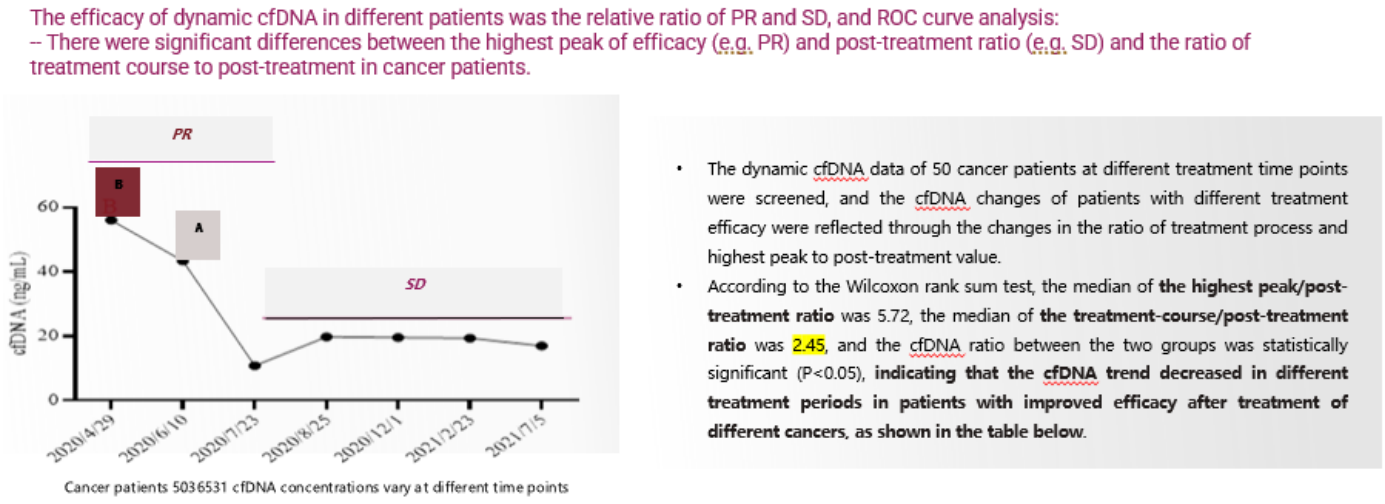

Figure 1. Our clinical studies with over 50 cancer patients showing the median ratio of cfDNA levels at PD and SD is 2.45, transitioning from PD to SD.

3. Monitoring Tumor Recurrence

When cfDNA levels stays constantly low level after treatment, the patients may stay at stable disease conditions. However, when the cfDNA rises significantly, it may indicate tumor recurrence. Our clinical studies with more than 200 patients of 22 different cancer types indicate that when the median cfDNA increases by 2.29-fold, tumor recurrence occurs (Figure 2).

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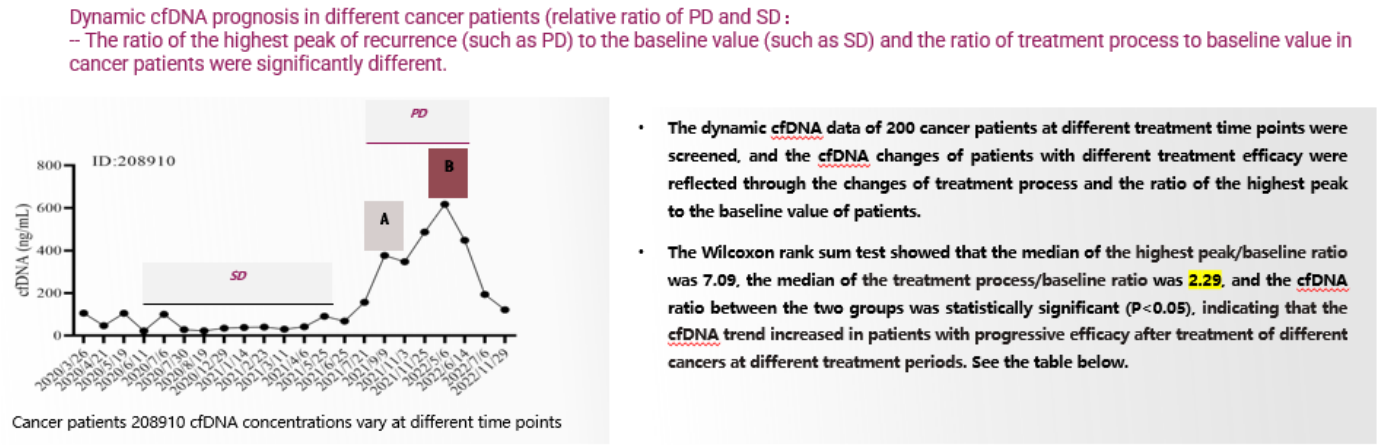

Figure 2. Our clinical study showing the median ratio of cfDNA level at PD and SD is 2.29 (transitioning from SD to PD).

4. Combination of RadTox™ Test and ctDNA Monitoring

The biggest resistance in using cfDNA level as a biomarker in clinical setting is the doubt on the cfDNA's specificity compared to ctDNA because cfDNA comes from both the normal and tumor cells while the ctDNA is totally tumor cells. Because currently it is not technically feasible to separate ctDNA from the rest of cfDNA, ctDNA is often analyzed through mutation or methylation status. Although cfDNA quantification does not represent ctDNA quantification, studies have shown good correlations between cfDNA and ctDNA quantifications [9] (Figure 3) and their correlation with prognosis [10] (Figure 4). The ctDNA increases when cancer progresses and the proportion of ctDNA in cfDNA can increase from 0.1% to 10%, or even 95%.

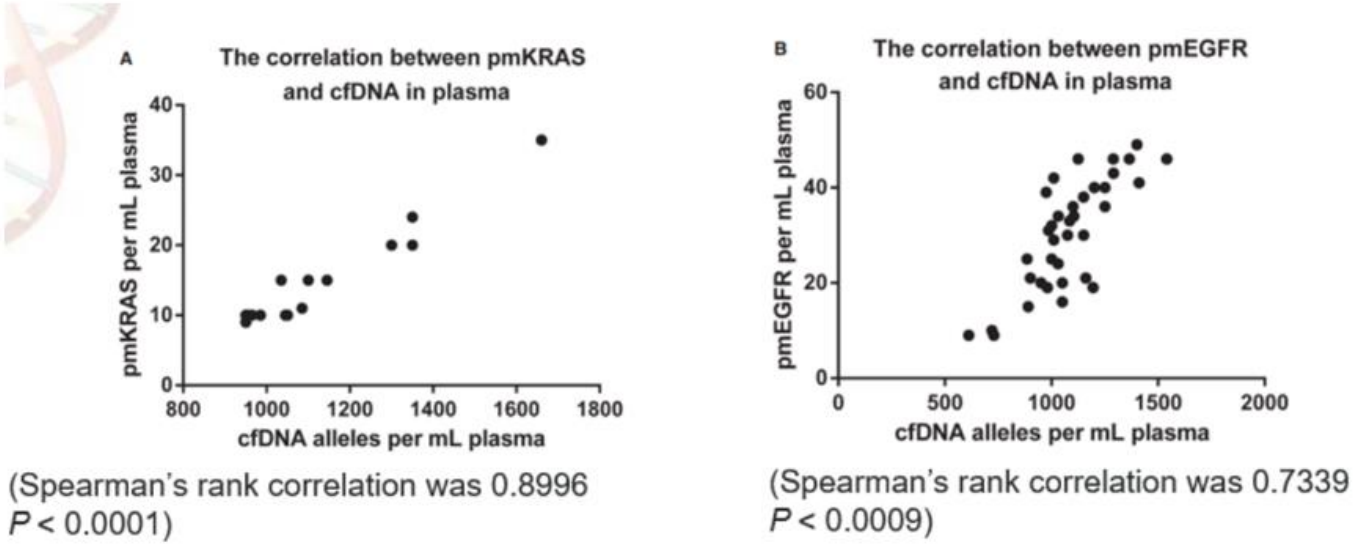

Figure 3. Adapted from Hu et al. 2017 showing the cfDNA and ctDNA levels are correlated with each other in non-small cell lung cancer.

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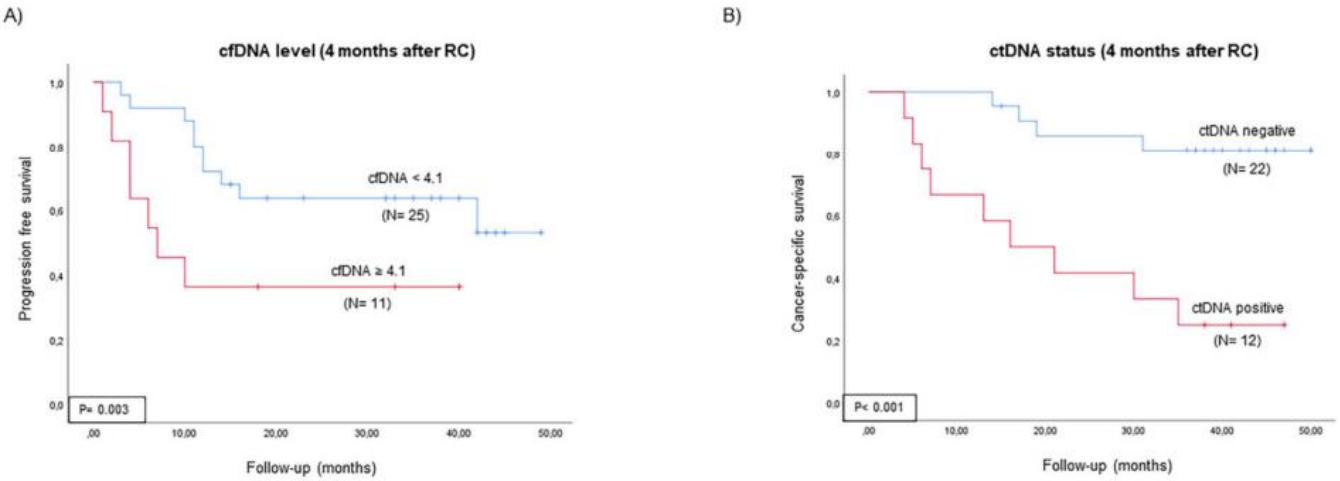

Figure 4. Adapted from Carrasco et al. 2022 showing bpth cfDNA and ctDNA from breast cancer study are correlated to prognosis.

Currently, ctDNA sequencing panels and MRD (molecular residual disease) are the two major classes of ctDNA tests that gradually gained clinical acceptance. ctDNA sequencing panels sequence the gene mutations including actionable mutations and can be used to guide selection therapy and monitoring treatment responses. The MRD monitoring tests, on the other hand, are super-sensitive tests that can detect mutations at 0.01 to 0.1% variant allele frequencies. These tests are often used to monitor if the cancer cells are coming back. Although ctDNA monitoring is a great tool for treatment monitoring, they need sequencing and bioinformatics expertise and has two to three weeks of turn-around time.

Despite the coverage of the high expenses by Medicare and commercial insurances, it is hardly done as a routine monitoring tool as RadTox does. Combination of RadTox™ test with other ctDNA monitoring tests can provide more comprehensive monitoring information to the whole treatment and recovery process. Together with the traditional imaging tool, the liquid biopsy tests provide great value in understanding the treatment response.

Major Advantages of RadTox™ cfDNA Testing

1. Minimally Invasive and Frequent Monitoring

Abstract: Requiring only 20 µL of plasma, RadTox™ enables frequent, minimally invasive monitoring, providing a comprehensive temporal overview of tumor dynamics without the risks and limitations of tissue biopsies.

2. Pan-Cancer Applicability

Abstract: Unlike tumor-specific mutation detection assays, RadTox™ is a versatile pan-cancer test applicable across various tumor types and stages, eliminating the need for prior tumor profiling. This broad applicability simplifies clinical workflows and enables rapid deployment in diverse oncology settings, supporting

personalized cancer care.

3. Cost-Effective and Rapid Turnaround

Abstract: RadTox™ offers a cost-effective solution for frequent monitoring compared to complex genomic assays. Its simple workflow and rapid turnaround time of 5-7 days facilitate timely clinical decision-making.

Conclusion

DiaCarta's RadTox™ cfDNA test represents a paradigm shift in tumor response management, empowering physicians with real-time, actionable insights into treatment efficacy and disease dynamics. By integrating RadTox™ into clinical practice, oncologists can optimize treatment strategies, predict outcomes, and ultimately improve patient care through personalized, data-driven decision-making. Adopting cfDNA testing with RadTox™ can enhance clinical workflows, support value-based care initiatives, and contribute to improved patient outcomes and quality of life.

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