RadTox™ – A Complementary Diagnostic Test with Radiotherapy and Chemotherapy for a Variety of Cancers

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Abstract

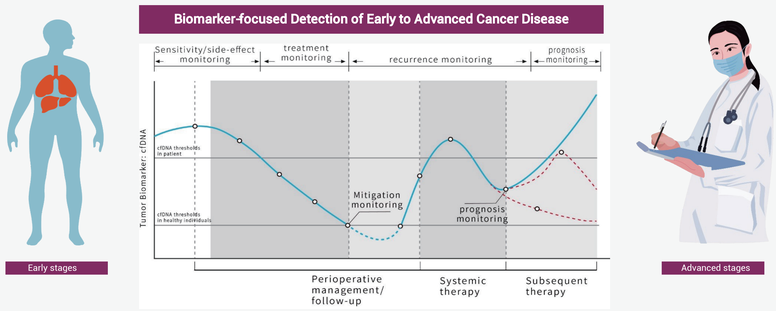
Biomarker detection using liquid biopsy for cancer diagnosis and monitoring treatment response has gained attraction for development of modern personalized, precision diagnostics. This methodology overcomes invasive and difficult to get traditional biopsy, and provides routine, non-invasive tests beneficial to patients. Development of rapid, near real-time liquid biopsy lab tests allows easier and more precise cancer care management for individual patients from diagnosis to treatment. This will ultimately shed light on how to better treat cancer when the right therapy tools are applied in time.

We have developed the RadTox™ test based on our isobDNA™ and Luminex xMAP™ Technology. The test accurately measures cell-free DNA (cfDNA) in plasma before, during, and after treatment. With high sensitivity and specificity, the test provides oncologists invaluable information with treatment efficacy, prognosis, and progression of disease. Here, we have shown our results indicate that RadTox test on the Luminex platform is an easy and accurate assay for cfDNA measurement, with advantages over qPCR assays that often need DNA extraction. We also demonstrated how the RadTox™ test has been used in our clinical studies to monitor patients' responses to therapies including radiotherapy and chemotherapy, regardless of cancer types.

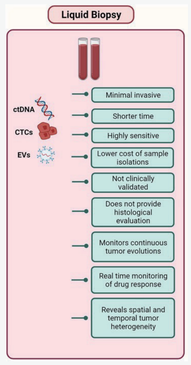
Introduction

Traditional standard cancer care heavily relies on imaging for cancer detection and monitoring. However, this may delay cancer diagnosis as imaging may not be able to detect cancer until the tumor grows to a certain size. Imaging may also delay proper treatment procedure as it takes 3 to 6 months to check the patient's response to the therapy and start a new therapy if necessary. However, modern liquid biopsy can bring great benefit to patients. While a key liquid biopsy biomarker can help detect the presence of the cancer at early stage, another one can help monitor the whole treatment procedure, so physicians know what happens during and after treatment. What the patient really needs is a high-precision and personalized test that provides real-time monitoring during the whole process of cancer care. Biomarker detection using liquid biopsy provides just that. Based on the market needs, we have developed the RadTox ™ test that directly measures cfDNA in near real-time at different stages of treatment. The test provides meaningful information for physicians and patients regarding their therapy responses at different periods.

Doctor's Needs: A Biomarker to Monitor the Whole Cycle of Cancer Treatment



Patient Needs: Real Time Assessment of Treatment Response



LIQUID BIOPSY:

- **Non-invasive**
- **Simulated histopathology:** degree of carcinogenesis, drug sensitivity, and molecular markers.
- **Quantitative:** large-scale dynamic quantitative analysis.
- **Rapid, accurate and cost-saving:** Treatment response can be assessed in real time, enabling low-cost rapid testing.
- **Assessing normal tissue tolerance:** reducing and avoiding normal tissue damage is both efficient and safe.
- **Reflect the tumor microenvironment and molecular metabolic regulation:** anti-tumor,auto immune response, tumor metabolism and molecular response under hypoxia.

Comparison of Cancer Therapy Monitoring Tools

Traditional CEA biomarkers

- Widely recognized and used in medical community
- Not always reliable due to lower sensitivity
- Cost covered by insurance

Modern MRD biomarker

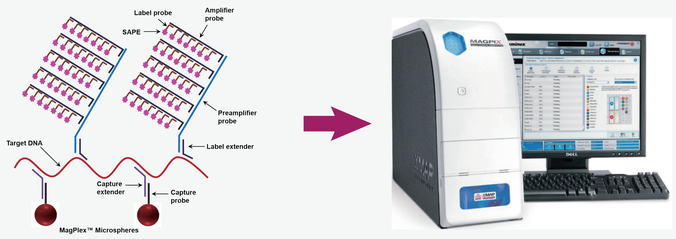
- Most advanced ultradeep NGS technology with 20 ml or more blood
- High sensitivity, but needs multiple target tracking for accuracy
- Costly and only a few tests have insurance reimbursement
- Technology expertise required for testing and analysis
- Customized tests necessary

RadTox cfDNA Test

- Simpler technology platform: branched DNA on Luminex xMAP platform with only 10 µl plasma needed
- High sensitivity and specificity based on our clinical studies
- Much less cost and insurance re-imbursement approval in process
- No technology experts required for testing
- No customized test is required

RadTox™ is Based on IsobDNA™ and Luminex xMAP™ Technology

Figure 1: Illustration of RadTox™ test on Luminex MAGPIX

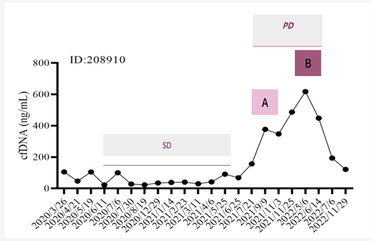


RadTox™ - Monitor Cancer Recurrence Based on Statistical Data

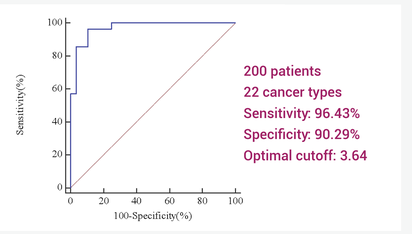
Dynamic cfDNA levels in different cancer patients (relative ratio of PD-progression of disease and SD-stable disease study indicates that the ratio of the highest peak of recurrence (such as PD) to the baseline value (such as SD) and the ratio of treatment process to baseline value in cancer patients were significantly different.

Figure 2. RadTox™ is a sensitive marker for cancer recurrence monitoring

Cancer patient 208910 cfDNA concentrations onitoring for 2 years 8 months



ROC curves with different chemotherapy time ratios to assist in the diagnosis of different efficacy



200 patients
22 cancer types
Sensitivity: 96.43%
Specificity: 90.29%
Optimal cutoff: 3.64

Table 1. Comparison of cfDNA ratios before and after chemotherapy in cancer patients with different therapeutic effects

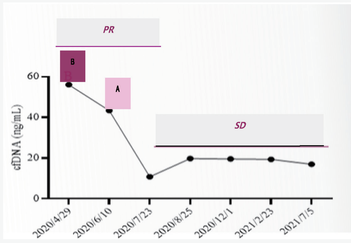
Indicators	Course of treatment /baseline values SD	Highest peak /baseline values SD	Z	P
Different time ratio, M(P25.P75)	2.29(1.85,3.89)	7.09(5.06,11.16)	-3.291	0.001

RadTox™ - Monitor Cancer Prognosis Based on Statistical Data

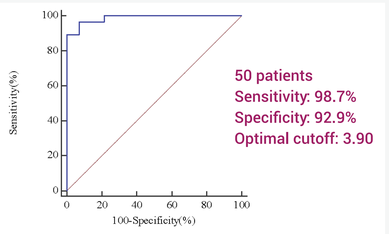
The efficacy of dynamic cfDNA in different patients was the relative ratio of PR and SD, and ROC curve analysis: There were significant differences between the highest peak of efficacy (e.g. PR) and post-treatment ratio (e.g. SD) and the ratio of treatment course to post-treatment in cancer patients.

Figure 3. RadTox™ is a sensitive marker for cancer prognosis monitoring

Cancer patients 5036531 cfDNA concentrations vary at different time points



ROC curves with different chemotherapy time ratios to assist in the diagnosis of different efficacy



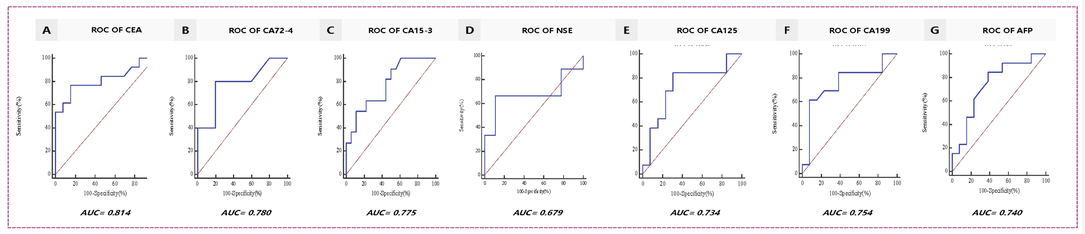
50 patients
Sensitivity: 98.7%
Specificity: 92.9%
Optimal cutoff: 3.90

Table 2. Comparison of cfDNA ratios before and after chemotherapy in cancer patients with different therapeutic effects

Indicators	Course of treatment /baseline values SD	Highest peak /baseline values SD	Z	P
Different time ratio, M(P25.P75)	2.45(1.74,3.37)	5.72(3.30,9.68)	-3.296	0.001

RadTox™ - Higher Sensitivity Compared to Traditional Cancer Biomarkers in Monitoring Treatment Efficacy

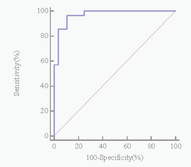
Figure 6. Based on ROC curve, cfDNA biomarker is better than any of the traditional biomarkers shown



Thirty patients with lung cancer, brain cancer, pancreatic cancer and blood cancer were screened, and the ROC curve analysis showed that the efficacy of auxiliary diagnosis of cancer patients was the ROC curve analysis of the relative ratio of PD and SD.

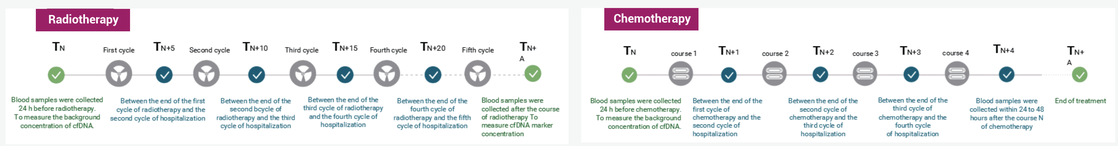
- **CEA** the area **AUC under the ROC curve is 0.814**, sensitivity is 76.92%, specificity is 84.62%;
- **CA72-4** the area **AUC under the ROC curve is 0.780**, sensitivity is 80.00%, specificity is 80.00%;
- **CA125** the area **AUC under the ROC curve is 0.734**, sensitivity is 84.62%, specificity is 69.23%;
- **CA19-9** the area **AUC under the ROC curve is 0.754**, sensitivity is 61.54%, specificity is 92.31%;
- **CA15-3** the area **AUC under the ROC curve is 0.775**, sensitivity is 54.55%, specificity is 88.89%;
- **AFP** the area **AUC under the ROC curve is 0.740**, sensitivity is 84.62%, specificity is 61.54%;
- **NSE** the area **AUC under the ROC curve is 0.679**, sensitivity is 66.67%, specificity is 88.89%.

- **The efficacy of cfDNA in the diagnosis of cancer patients is the area under the ROC curve of the relative ratio of PD to SD.**
- *AUC (95% CI) is 0.969* (0.884-0.997),**sensitivity is 96.43%,specificity is 90.29%.**



RadTox™ - Suggested Blood Sample Collection Schedule

Figure 5. Based on ROC curve, cfDNA biomarker is better than any of the traditional biomarkers shown



Conclusion

We have shown cfDNA is a sensitive and specific biomarker for cancer therapy monitoring based on our RadTox™ Test. Our test has two great advantages:

（1）Does not involve cfDNA extraction, keeping high accuracy.

（2）Only uses 10 ul plasma, making multiple sample collection practical.

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