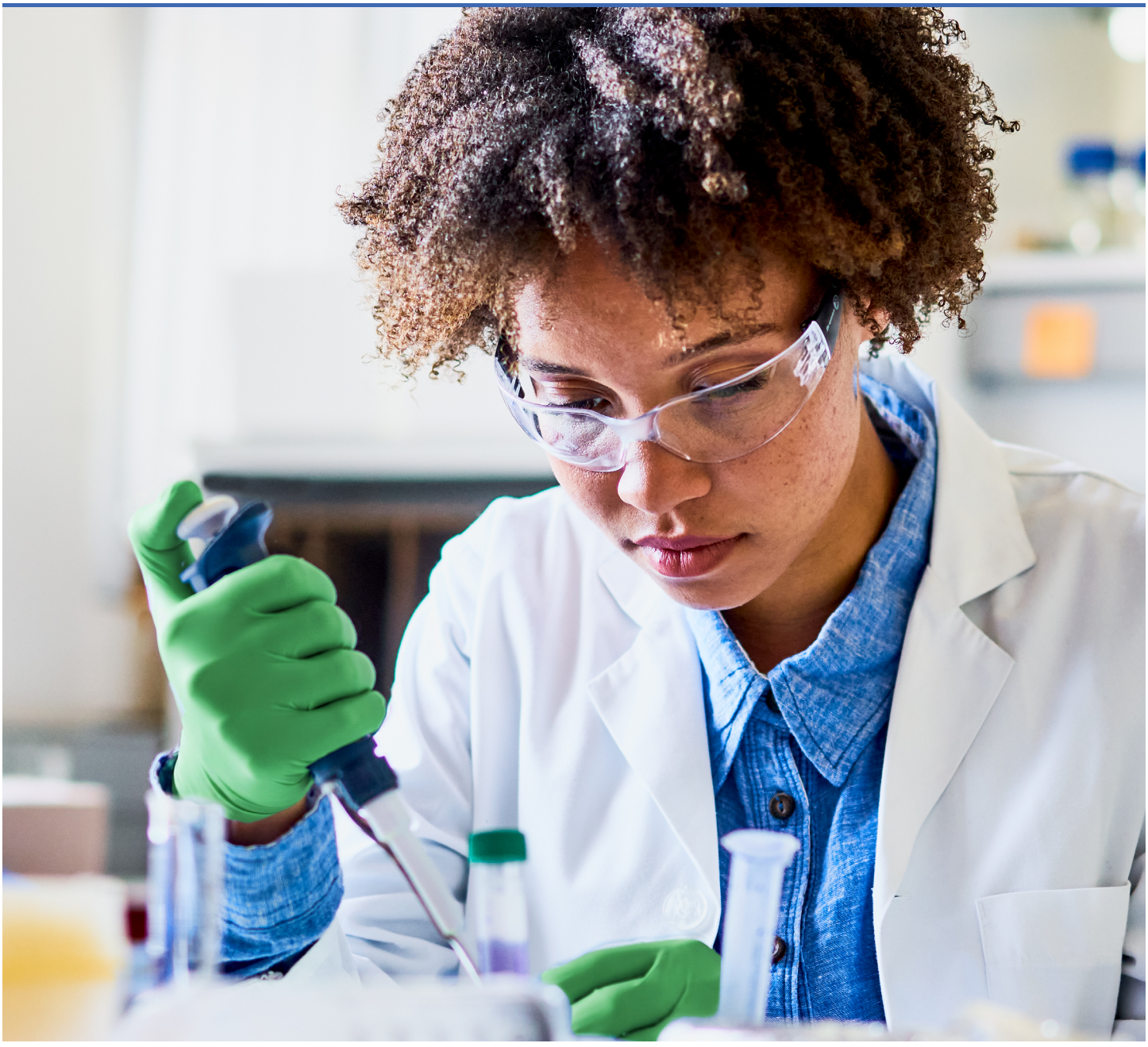
ONCOLOGY RESEARCH
EBOOK

ctDNA Analysis Across the Cancer Research Continuum

Advancing Research for a New Era of Personalized Care



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Bulletin 3947

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ctDNA and the Modern Clinical Research Landscape

Circulating tumor DNA (ctDNA) analysis has transformed cancer research by offering a non-invasive method for sampling solid tumors. This approach offers a powerful alternative to traditional, intrusive tissue biopsies, enabling researchers to continuously monitor tumor-derived genomic biomarkers, tumor evolution, tumor progression, treatment response, and recurrence.

Unlike invasive biopsies—which require hospitalization, surgical procedures, specialized equipment, and come with risks and complications—ctDNA analysis can be performed using standard clinical laboratory techniques, such as a blood draw. In addition, several different biofluid samples can be collected, including blood, urine, plasma, and saliva, and used for ctDNA analysis, offering a safer, easier, and more efficient sample collection procedure.

Moreover, ctDNA analysis holds significant prognostic value in cancer research. Ideally, this research can lead to better-informed decisions about therapeutic strategies and assess the risk of relapse by tracking molecular responses and detecting molecular residual disease (MRD). Real-time monitoring of tumor dynamics could greatly improve the probability of efficacious treatment selection.

Importantly, the minimally invasive aspect of ctDNA sample collection means that analyses can more easily be repeated sequentially to support longitudinal analyses. Such longitudinal studies can provide clinical researchers the ability to monitor evolving tumor biology over time. With a more comprehensive view of tumor clonal evolution over time, researchers can monitor for emergent resistant mutations that feed into tumor drug response analysis. Further, data sets from longitudinal studies will help determine the predictive accuracy of these assays.

For example, a recent study by Aggarwal et al. focusing on advanced, metastatic non–small cell lung cancer (NSCLC) demonstrated that mutation detection was significantly better in plasma analysis alone compared to tissue alone, obviating the need for invasive biopsies.[1] Similarly, Sacher et al. demonstrated that Droplet Digital™ PCR (ddPCR™) analysis of plasmadetected common driver mutations in *EGFR and KRAS* with high specificity, in a matter of days.[2] They suggested that ddPCR technology can assist in therapy selection, reduce the need for additional biopsies, and may help detect *EGFR T790M* drug-resistant variant, usually missed in tissue biopsy samples due to high tumor heterogeneity.

PLACEHOLDER

What are ctDNA and cfDNA?

Cell-free DNA (cfDNA) is released in healthy individuals in the bloodstream (or other biofluids), as part of cellular homeostasis.

However, in patients with cancer, the concentration of cfDNA in the blood and plasma significantly increases, scaling with the cancer stage.[3] The increased concentration of cfDNA in biofluid samples is attributed to the presence of tumor-derived cfDNA, also called circulating tumor DNA (ctDNA), which can make up 0.01% to 90% of the total cfDNA concentration.[4] ctDNA carries the genetic mutations from solid tumors, making it an invaluable biomolecule for driver and passenger mutation analysis.[5,6]

One notable characteristic of ctDNA is its short half-life, typically less than two hours.[7] This short half-life allows researchers to analyze tumor dynamics in near–real time, providing valuable insights into the evolving genetic landscape of the tumor. By monitoring changes in ctDNA levels over time, clinical researchers can assess treatment response, disease progression, and the emergence of treatment resistance.

ctDNA Detection Methods and Utility

In ctDNA analysis, various molecular methods are available for detecting and analyzing genetic biomarkers. Each method has advantages, limitations, and optimal R&D applications, allowing clinical researchers to tailor their approach based on specific requirements.

Next-Generation Sequencing (NGS)

One widely used technique is NGS, which offers an unbiased and high-throughput solution for identifying genetic biomarkers in ctDNA.

Whole genome sequencing and whole exome sequencing provide a comprehensive view of the genetic landscape of a tumor sample. This approach can identify known and unknown mutations, including copy number variations and structural rearrangements.[8] However, while NGS provides a holistic view, it can be expensive and time-consuming. For researchers without computational experience, a prebuilt and validated bioinformatics workflow, or an in-house bioinformatics team, data analysis can be complicated, impeding progress.

As an alternative, amplicon-based NGS focuses on specific known mutations or loci, offering a lower-cost and higher-depth option. While data analysis is much simpler for target NGS, error rates can make identifying rare variants difficult. Thus, using NGS may necessitate additional rework or re-analysis by other complementary assays to improve confidence in the genetic biomarkers present in ctDNA.

PCR-Based Methods

PCR-based methods are another commonly employed approach for ctDNA analysis. These methods are generally low-cost, easy to set up, and provide rapid turnaround times.[9]

Droplet Digital™ PCR (ddPCR™) technology stands out in this category, enabling multiplexed workflows for analyzing multiple loci simultaneously. IIt offers high sensitivity compared to NGS and quantitative PCR (qPCR; also called real-time PCR) and provides absolute quantification of mutations present in ctDNA. The simplicity and sensitivity of ddPCR technology make it an attractive option for various applications within clinical cancer research.

While ddPCR system analysis offers many advantages over both sequencing and other PCR-based assays, mutation detection requires prior sequence information about the locus. Therefore, it's not optimal for biomarker discovery-focused research.

	NGS	ddPCR Technology
		
Absolute quantification	No	Yes
Workflow difficulty	Complex	Simple
Bioinformatics burden	High	Low
Turnaround time	Days	Hours
Sensitivity	Good	Best
Cost	High	Cost-effective

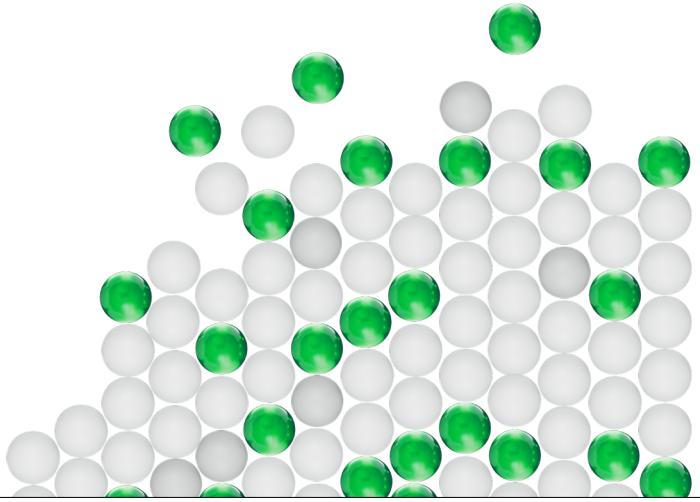
Integrating Complementary Technologies for Optimized ctDNA Analysis

Given the above advantages and challenges of NGS- and PCR-based methods, these techniques can play distinct yet complementary roles to provide a more complete analysis of ctDNA biomarkers. The best fit ctDNA analysis method will depend on where in the cancer timeline a subject is and what area of clinical research is being investigated.

For instance, NGS is vital in screening, where identifying relevant driver and passenger mutations or other genetic biomarkers is crucial. The comprehensive nature of whole genome or exome NGS allows researchers to explore the entire genetic landscape and identify potential targets for early intervention.

On the other hand, ddPCR technology has a central role in follow-up validation of NGS results, therapy response tracking, and relapse monitoring. It provides a rapid and reliable method for confirming the presence of specific genetic mutations detected by NGS, tracking changes in ctDNA levels during treatment, and monitoring for signs of disease recurrence.

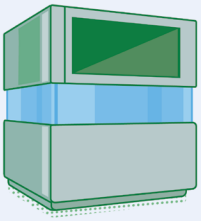
By integrating these complementary approaches, researchers can leverage the strengths of each technique throughout R&D, enhancing the accuracy, efficiency, and clinical utility of ctDNA analysis.



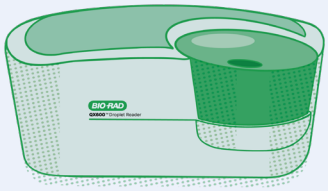
PLACEHOLDER

What's the Right Method?

Best Fit ctDNA Analysis Method



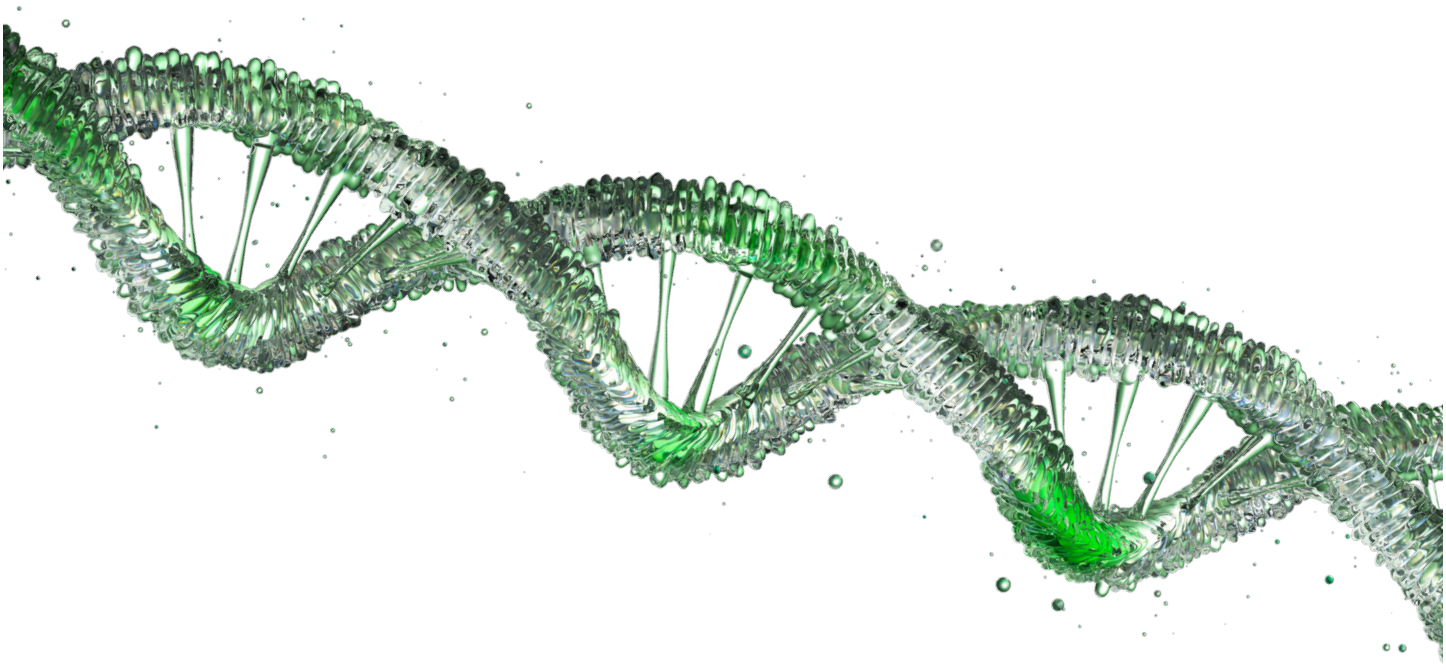
Next Generation Sequencing (NGS)



ddPCR Technology

Area of Clinical Research

Diagnosis and Treatment	MRD Assessment	Post-Treatment Monitoring	Recurrence and New Treatment	Post-Treatment Monitoring	Recurrence Monitoring



ddPCR Applications in ctDNA Detection and Recent Clinical Research

The use of ddPCR technology for ctDNA analysis has emerged as a valuable tool across various cancer types, aiding in developing personalized treatment strategies and monitoring disease relapse. There has also been significant focus on using ddPCR technology-based analysis ctDNA analysis to monitor MRD, which could be used as a surrogate end point for clinical trials. Ultimately, this would mean that trials could be completed more rapidly, at lower cost and with fewer participants. There have been successful applications in monitoring progression and/or relapse in leukemia and the studies referenced here suggest that similar implementation for solid tumors may be possible.[11,12] The application of ddPCR technology in these areas has provided important validation points, advancing its adoption for deeper understanding of genomic biomarkers and their potential for clinical utility across many different cancer types.

Breast Cancer

Identifying high-risk breast cancer and monitoring for relapse are crucial aspects of disease management. Several studies have demonstrated the prognostic value of ddPCR technology-based analysis in this context. Garcia-Murillas et al. have shown that ddPCR technology predicts relapse in early-stage breast cancer, providing valuable insights into disease recurrence.[13] By analyzing ctDNA, the group also found that ctDNA sequencing could uncover the genetic mutations underlying MRD more accurately than sequencing the original tumor could. Their findings enable the identification of high-risk patients and the development of personalized treatments.

Additionally, ctDNA analysis has been utilized to predict responses to neoadjuvant chemotherapy, particularly in early triple-negative breast cancers (TNBCs). Cavallone et al. looked at the concentrations of ctDNA using ddPCR technology before and after the treatment of TNBCs.[14] They found that the absence of ctDNA after treatment was associated with a lower chance of relapse and improved overall survival. The predictive value of ddPCR technology was similar to other factors commonly used to assess treatment response

Furthermore, Berger et al. employed ddPCR technology-based assays in a phase III trial study to evaluate whether monitoring ctDNA levels and making treatment changes based on those levels can improve the prognosis for patients with metastatic ER+ HER2- breast cancer.[15] Though the study has not read out yet (trial results will be completed in 2025), this demonstrates the utility of ctDNA analysis for continuous monitoring.

NSCLC

In non-small cell lung cancer (NSCLC), clinical monitoring often relies on imaging methods that primarily detect gross changes. However, a recent study highlighted the utility of single-gene ddPCR analysis in predicting early disease progression and durable treatment responses to immune checkpoint inhibitors.[16] They demonstrated that ddPCR analysis of ctDNA is a cost-effective monitoring tool that offers a precise and dynamic approach to evaluating treatment outcomes and disease progression in patients with NSCLC.

Melanoma

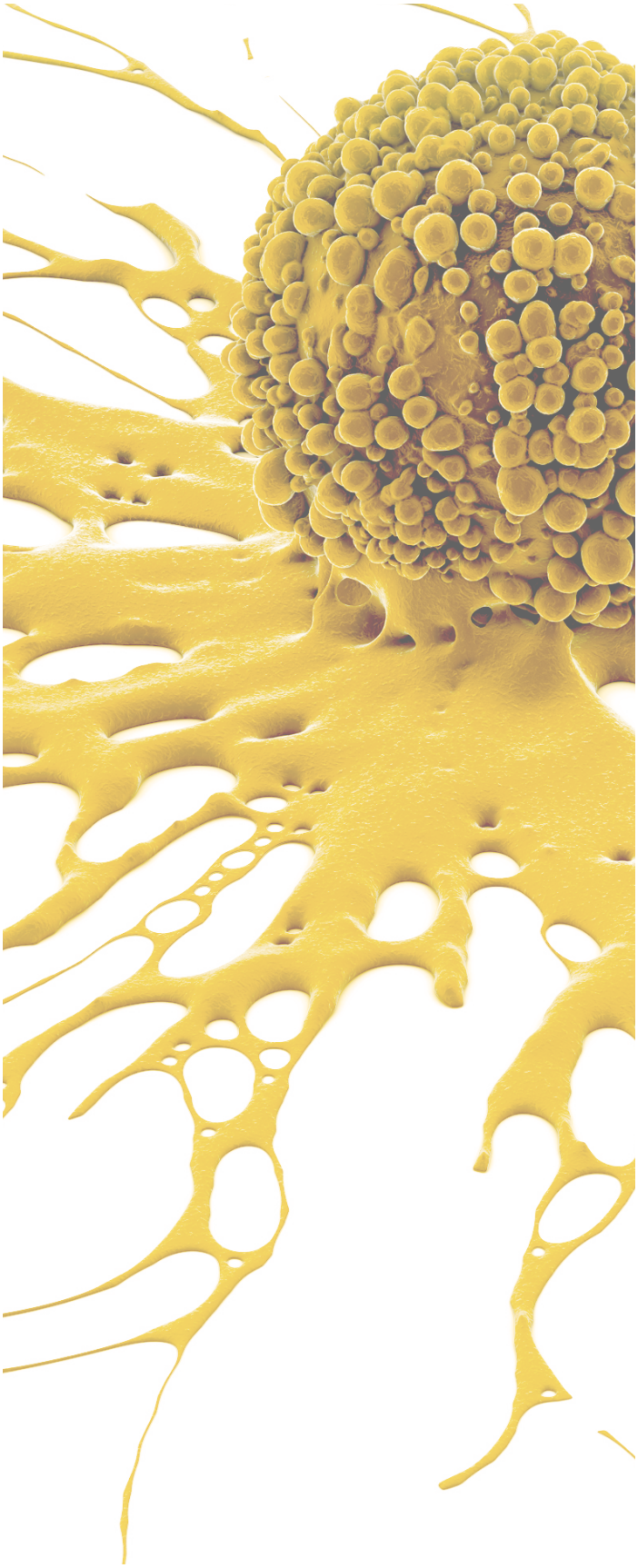
Melanoma, another cancer type that can be challenging to manage, has seen significant advancements in understanding of disease course by applying ddPCR technology. Traditionally, melanoma progression and relapse detection have relied on computer tomography, a costly and time-consuming imaging technique.

However, recent studies have demonstrated the clinical value of ddPCR technology-based methods. Forthun et al. used ddPCR-based methods to measure ctDNA in melanoma.[17] They found that ddPCR assays had higher sensitivity than NGS in predicting treatment response and providing faster results at lower costs. Lee et al. used ddPCR to differentiate between pseudoprogression and true progression in response to immune checkpoint inhibitor treatment.[18] Similarly, a 2021 study, published in The Lancet Oncology, used ddPCR in melanoma to predict survival and treatment outcomes.[19] This information aids clinical researchers in developing a better understanding of the treatment of melanoma.

Colorectal Cancer

Colorectal cancer poses a significant challenge in terms of early detection of relapse using current imaging and laboratory methods. In a 2020 study, Holm et al. demonstrated the potential of ddPCR technology-based ctDNA analysis in the early detection of disease progression with metastatic colorectal cancer undergoing treatment.[20] By monitoring ctDNA levels, ddPCR technology identified disease progression at an early stage, enabling prompt intervention and potential improvement of patient outcomes.

These diverse applications across breast cancer, NSCLC, melanoma, and colorectal cancer highlight the versatility and clinical significance of ddPCR technology-based ctDNA analysis. These published findings show that ddPCR technology enables researchers to identify valuable predictive and prognostic ctDNA biomarkers, facilitating the future improvement of personalized treatment strategies and patient outcomes.

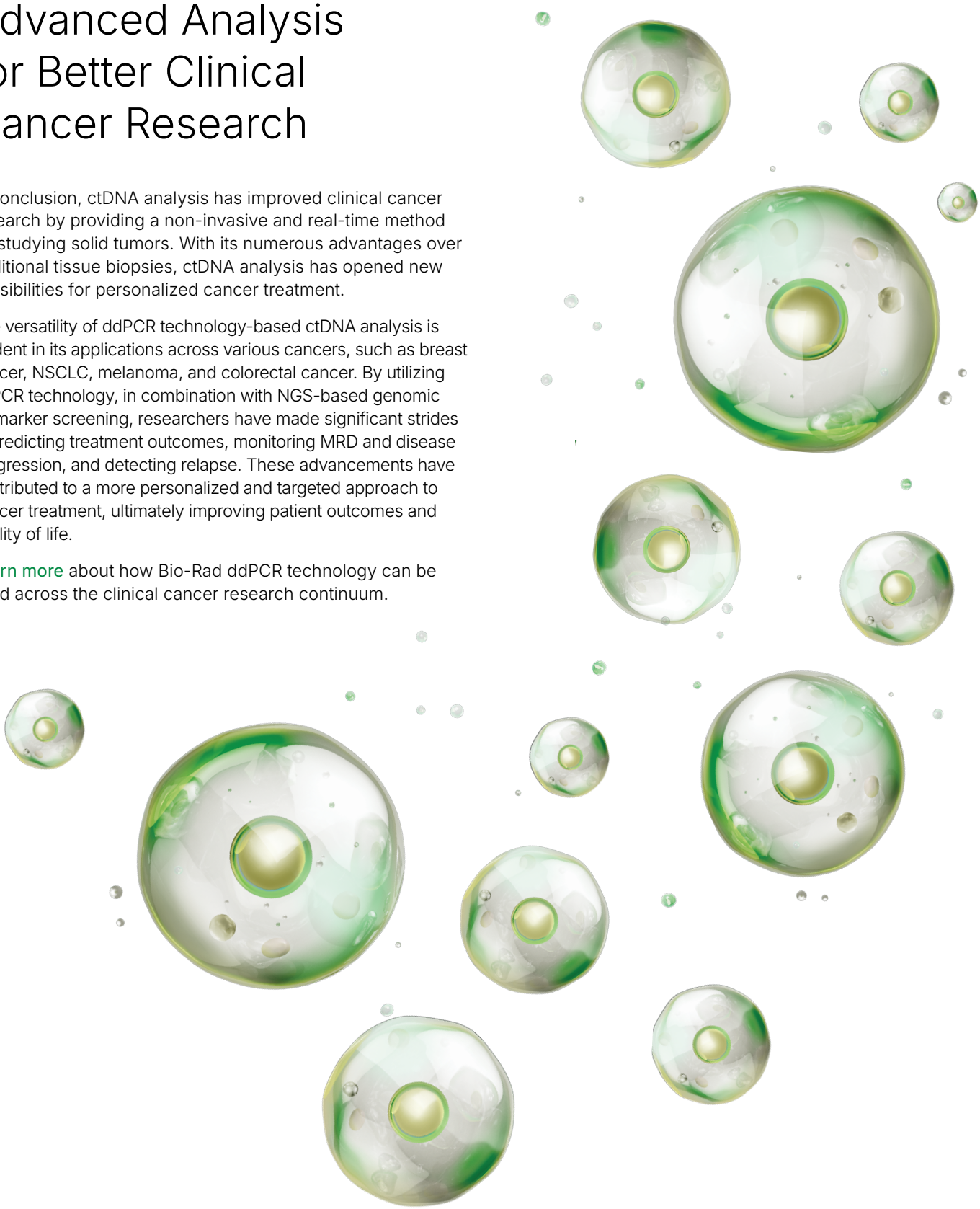


Advanced Analysis for Better Clinical Cancer Research

In conclusion, ctDNA analysis has improved clinical cancer research by providing a non-invasive and real-time method for studying solid tumors. With its numerous advantages over traditional tissue biopsies, ctDNA analysis has opened new possibilities for personalized cancer treatment.

The versatility of ddPCR technology-based ctDNA analysis is evident in its applications across various cancers, such as breast cancer, NSCLC, melanoma, and colorectal cancer. By utilizing ddPCR technology, in combination with NGS-based genomic biomarker screening, researchers have made significant strides in predicting treatment outcomes, monitoring MRD and disease progression, and detecting relapse. These advancements have contributed to a more personalized and targeted approach to cancer treatment, ultimately improving patient outcomes and quality of life.

Learn more about how Bio-Rad ddPCR technology can be used across the clinical cancer research continuum.



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