Commentary

Reporting on circulating tumor DNA monitoring in metastatic cancer—From clinical validity to clinical utility

Caroline Brenner Thomsen, MD, PhD [1,2]; Amanda Dandanell Juul, MD[3]; Anna Cecilie Lefèvre, MD[4,5]; Christina Glismand Truelsen, MD[4,5]; Edina Dizdarevic, MD[1,2]; Heidi Ryssel, MD[6]; Ina Mathilde Kjær, MD[2,7]; Karen Lycke Wind, MD[4,5]; Louise Bach Callesen, MD[4,5]; Louise Faaborg Larsen, MD[2]; Malene Støchkel Frank, MD[8,9]; Rikke Fredslund Andersen, MSc, PhD[1]; Karen-Lise Garm Spindler, MD, PhD, DMSc[4,5]; and Anders Jakobsen, MD, PhD, DMSc[1,2]

INTRODUCTION

Circulating tumor-specific DNA (ctDNA) is a group of biomarkers with rapidly increasing interest and the potential to change clinical practice. The diversity of reported ctDNA results, however, is remarkable and complicates the transition of ctDNA from being clinically valid to becoming a biomarker with proven clinical utility.

From a theoretical point of view, ctDNA detected in blood holds several characteristics of an ideal tumor marker, being timely measured, minimally invasive, and easily accessible for repeated measurement with little discomfort to the patient. Hence, ctDNA can become a critical determinant in driving the care of cancer patients.[1,2] The results of consecutive ctDNA analyses might facilitate personalized follow-up programs, add information to inconclusive scans, and spare patients of comprehensive interventions such as repeated tumor biopsies. Ineffective treatments and the pertaining toxicity may be reduced with a more precise monitoring of treatment efficacy.

Despite the advantages, ctDNA monitoring has not been implemented in the daily clinic for several reasons. Current knowledge is based on retrospective analyses and few prospective studies, and the possible clinical value has not been proven in randomized trials. Application in the diagnostic and adjuvant settings may often involve only a binominal scale with positive and negative values, which are easy to interpret and transfer to clinical use. The situation becomes more complicated in the metastatic setting, in which serial measurement with increasing and decreasing values can pose a challenge. A continuous scale may seem more appropriate, although it also has limitations as discussed in this article. A review by Merker et al[3] discussed the preferred analytical considerations when reporting on ctDNA. They emphasized the rapid pace of research and the necessity of guidance for clinical validity to achieve clinical utility.

The aim of the present study was to perform a critical evaluation of the reporting of ctDNA results in the current literature focusing on monitoring of treatment effect in the metastatic setting.

Analytical Validity

Analytical validity refers to the accuracy with which ctDNA can be identified and quantified in a given laboratory test. A ctDNA fraction of 0.1% is equivalent to approximately 4 ng of input DNA. The detection limit may be set by the total amount of cell free DNA (cfDNA) regardless of method sensitivity.

Subsampling is a problem that cannot be overcome, but it can be reduced by increasing the volume of plasma used for analysis. Partitioning represents another in-born source of variation. Different techniques for sampling preservation, DNA isolation, and ctDNA analysis are beyond the scope of the present article, but it should be underlined that validated control materials are of utmost importance.

The Foundation for the National Institutes of Health ctDNA Quality Control Project[4] is investigating the quality in ctDNA analyses. This work is warranted and required before application in the clinic as recently described by Hayes,[5]

Corresponding Author: Caroline Brenner Thomsen, MD, PhD, Danish Colorectal Cancer Center South, Vejle Hospital, Beriderbakken 4, DK-7100 Vejle, Denmark (caroline.emilie.brenner.thomsen@rsyd.dk)

[1]Danish Colorectal Cancer Center South, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark; [2]Department of Oncology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark; [3]Odense Respiratory Research Unit, Odense University Hospital, Odense, Denmark; [4]Experimental Clinical Oncology, Department of Oncology, Arhus University Hospital, Aarhus, Denmark; [5]Department of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark; [6]Department of Clinical Physiology, PET and Nuclear Medicine, Rigshospitalet, Copenhagen, Denmark; [7]Department of Biochemistry and Immunology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark; [8]Department of Clinical Oncology and Palliative Care, Zealand University Hospital, Roskilde, Denmark; [9]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

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highlighting that analytical "lock down" and hence analytical validity before clinical testing is important. This means that the respective test needs to be defined, validated, and finalized before a biomarker can enter the stage of evaluation for clinical utility.

The literature holds several methods of analyzing ctDNA, which can be assembled in 2 general groups, targeted assays and broad-coverage assays.[3,6] Digital polymerase chain reaction (dPCR) and next-generation sequencing (NGS) are the 2 of the most frequently reported methods.[7-9] They both potentially provide a low limit of detection, which is an important factor. Screening for genetic variations requires NGS, but when the variations are already known, dPCR is the preferred and easiest method of ctDNA analysis with a low turn-around time.

Clinical Validity

The term clinical validity refers to the accuracy of the test in distinguishing between presence and absence of a disease. To achieve clinical validity, it is important to find the right cutoff point and unit of measurement.

Units of measurement

A valid measure of ctDNA is an absolute prerequisite for the establishment of its clinical utility. The measure should be unique and easily accessible, but first of all it should reflect biological characteristics of clinical relevance.

The results can be given in a quantitative measure as ng/mL or copies/mL with great variations in quantity. Shedding of ctDNA is a multifactorial process and tumor size is only 1 of the factors.[10-13] Primarily, the quantitative measure is limited by analytical variation depending on eg, ctDNA purification. The dependence on tumor size and analytical variation can be overcome by using fractional abundance with ctDNA given as a percentage of cfDNA as applied in the majority of the literature.[14,15] Because this assessment can be limited by the fluctuation of cfDNA not necessarily reflecting tumor biology,[13,16] it may represent a problem in the serial analysis of samples during chemotherapy and/or radiotherapy, conceivably affecting cfDNA and ctDNA at different rates.

The current literature favors the use of a relative measure of ctDNA. A reasonable step would be to report a quantitative measure as well to enhance transparency and the possibility of comparing study results.

Assessment of ctDNA Dynamics

The term ctDNA dynamics is used to describe changes in the level of ctDNA during a course of disease. Studies have demonstrated that ctDNA dynamics mirror the disease and that clinically relevant information can be obtained from correlating ctDNA changes with outcome.[17] Several studies have shown the level of ctDNA to increase with the advancement of the disease[18] and changes in ctDNA to correlate with tumor activity.[10,15,19]

Early dynamics

The ability of early dynamics to predict treatment response is of high clinical interest, as it allows for rational discontinuation of ineffective treatment. Early dynamics can be defined as changes in the ctDNA level as measured from the baseline sample to a given early time point during treatment. The baseline measurement (ie, before treatment initiation) is essential and reported in most studies, but the subsequent time points for sampling vary among study groups and cancer subtypes. Intraindividual variation has been shown in relation to the baseline level. Only approximately 40% (10/26) of the patients analyzed with two pretreatment samples had values with less than 20% variation.[20]

A recent consensus statement on colorectal cancer has listed specific recommendations for standardized sample collection at specific time points in several settings, but not in metastatic disease.[21] The current standards could be optimized by collecting the second blood sample before the first status scan.

The majority of studies across cancer types define early dynamics to occur within the first 3 months.[15,19,22] A recent study on metastatic breast cancer analyzing blood sampled at baseline and once during treatment (between 4 and 12 weeks) found that the one ctDNA analysis after treatment initiation held significant prognostic information and correlated with the later radiologic assessment.[23] In advanced melanoma, Syeda et al[9] reported an independent prognostic value of ctDNA. They analyzed ctDNA at baseline and at week 4 from enrollment. Osumi et al[24] investigated the early change in ctDNA in colorectal cancer and found the changed level from baseline to 8 weeks of treatment to correlate with response and survival.

The optimal time point for the second sample in the evaluation of early dynamics has yet to be defined and could be the focus of future studies on ctDNA in metastatic cancer.

Treatment monitoring

Consecutive blood sampling with monitoring of ctDNA levels can potentially monitor disease development during systemic antineoplastic treatment and follow-up as a supplement to radiological assessment as illustrated in Figure 1. Stable or decreasing ctDNA levels indicate

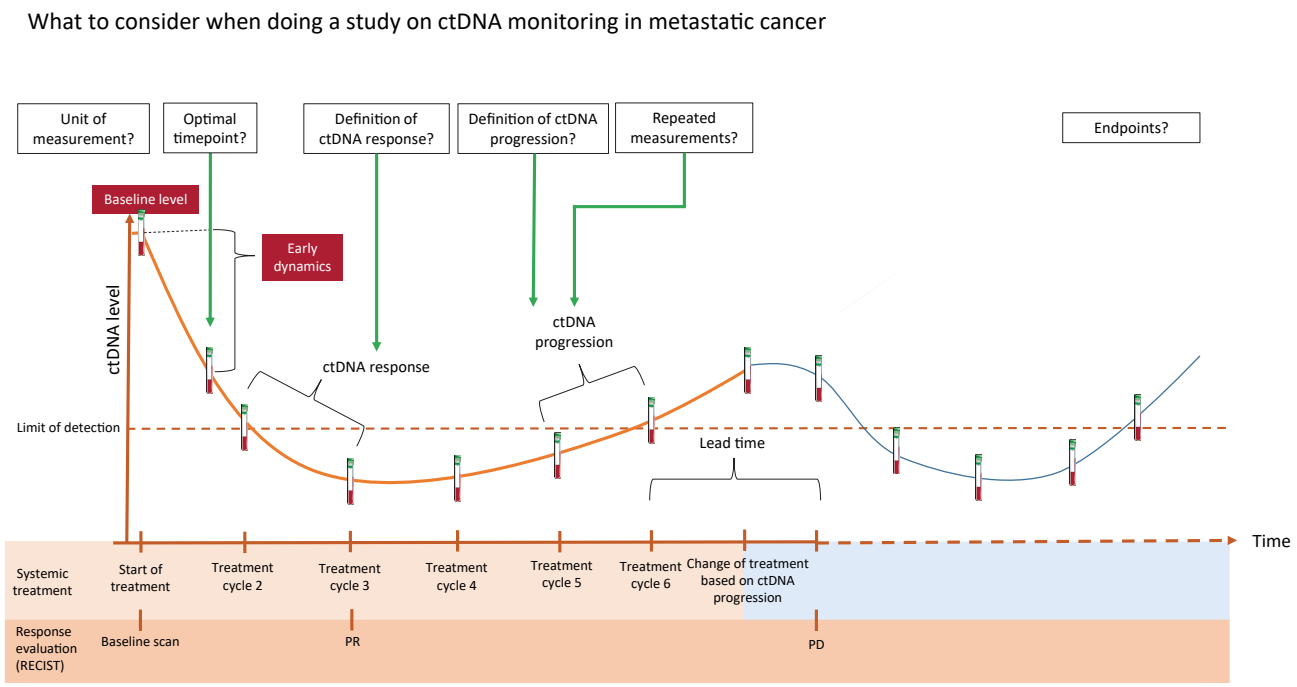

FIGURE 1. Aspects to consider when planning a study on ctDNA monitoring of metastatic cancer. ctDNA indicates circulating tumor DNA; PD, progressive disease; PR, partial response.

TABLE 1. List of Proposed Criteria for Defining a ctDNA Response

Source	Definition
Guibert 2019[30]	Any decrease, and 30% or 50% decrease or increase in allele fraction
Provencio 2021[31]	Cutoff <X% or ≥X% MAF at baseline and over time
Jia 2019[32]	Log2-fold change of ctDNA
Lueong 2020[33]	Above or less than 32 copies/mL of plasma
Thompson 2021[34]	Cutoff of 50%
Anagnostou 2019[19]	Complete reduction in ctDNA
Zou 2021[35]	ctDNA molecules per milliliter levels
Garlan 2017[36]	Decreasing below 0.1 ng/mL
Thomsen 2020[37]	An increase or decrease above or below the 95% confidence interval of the previous value

Abbreviations: ctDNA, circulating tumor DNA; MAF, mutant allele fraction.

disease control whereas an increase reflects progression with a significant lead time to radiological and/or clinical progression.[25,26]

In a study on advanced head and neck cancer, Hanna et al[27] collected blood samples for ctDNA analysis throughout the study period at intervals of 14 to 21 days and found a reflection of treatment response in the ctDNA dynamics. Dawson et al[17] quantified ctDNA in serially collected plasma samples from patients with metastatic breast cancer and showed a correlation between ctDNA level and tumor burden. A recent study in small cell lung cancer evaluated the role of ctDNA by analyzing blood samples at baseline, at every treatment cycle, and at progression and found an association with progression-free survival and overall survival (OS).[28]

The optimal time points for repeated measurement need to be defined. The clinical potential in these observations is substantial, but the heterogeneity of the current literature calls for clear definitions and validation of relevant approaches. To gain insight into treatment efficacy, ctDNA can be analyzed at baseline, before start of every treatment cycle, and at the times of clinical and radiological evaluation. This will provide a detailed overview of the changes in ctDNA and enable correlation with radiological evaluation and treatment effect.

Defining ctDNA progression and response

It is essential to define the clinically relevant increase and decrease of ctDNA to reflect progression and response, respectively. Absolute as well as relative changes are widely used in the literature, and multiple definitions have been reported, eg, "x fold reduction",[29,30] "relative change from baseline,"[31] logarithmic calculations,[32] and absolute changes[33] as shown in Table 1. In a small proof of concept study including patients with non–small cell lung cancer, Cabel et al[15] reported that undetectable ctDNA at week 8 of treatment correlated with long-lasting response and OS (hazard ratio [HR], 10.2). Thompson et al[34] defined

molecular response as a >50% decrease in mean allele fraction at week 9 of treatment in patients with non–small cell lung cancer treated with immunotherapy. The molecular response influenced OS at a significant level (HR, 0.27). Similar results have been published by Anagnostou et al,[19] but the molecular response was defined as complete reduction of ctDNA to undetectable levels. At week 8, the ctDNA response had a significant influence on survival (HR, 5.36). Zou et al[35] reported a quantitative relationship between mutant molecules per mL and survival in patients with non–small cell lung cancer treated with immunotherapy or chemotherapy. Garlan et al[36] found a decrease of ctDNA to normal levels (0.1 ng/mL) to correlate with a better prognosis in patients with metastatic colorectal cancer, and the steepness of the ctDNA decrease held further prognostic information.

The effect of early changes in the level of ctDNA was also found in a study by Thomsen et al.[37] Colorectal cancer patients treated with chemotherapy who had an undetectable level of ctDNA after the first treatment cycle had a median survival of 25.4 months compared to 13.5 months in the group with detectable ctDNA. Another study[22] investigated the correlation between ctDNA level at the first evaluation of objective response and prognosis. An undetectable level or a level with a lower confidence interval (CI) including zero correlated with OS in all 4 cohorts (ie, colorectal, ovarian, and lung cancer). ctDNA response clearly outperformed ORR with respect to prognostic value.

Each definition has advantages and disadvantages, but discussion and comparison of the methods is required to determine which definition is best correlated with outcome. Using relative changes can be a challenge when handling low levels of ctDNA, where a small absolute change represents a high relative change. The contrary applies to high levels. Furthermore, if the ctDNA level is undetectable in one sample it can be difficult to determine the change compared to the immediately preceding or subsequent sample independent of the chosen definition. When using dPCR, an accessible and clinically relevant definition could be an increase or decrease above or below the 95% CI of the previous value.[38] This method overcomes the challenge of an undetectable level of ctDNA. The 95% CI includes the variation of the Poisson distribution but not the analytical and biological variation.

The possible need of confirming a change in the level of ctDNA is still debated. A potential bias rarely discussed is the circadian or biological variation,[39] which can be an argument for repeated measurement. In the development of ctDNA-based monitoring, repeated measurement can be considered as an essential step when evaluating analytical validity before analytical lock down.[5] This could help the initial process of incorporating ctDNA into daily clinical practice to avoid action on potential false-positive results.

A standardization of study methods in the near future could result in a validated definition of a clinically relevant change in ctDNA. The definition of "undetectable" is essential in the settings of screening and minimal residual disease. The level of undetectable ctDNA could be determined based on healthy individuals and the analytical sensitivity.

ctDNA Response as a New Trial End Point

A relevant end point is an integral part of any clinical trial. It should reflect the benefit of the therapeutic measure and be objectively assessable by simple means. In clinical oncology, OS is the gold standard in trials intended to improve the treatment, which often has a perspective of several years. Therefore, end points reflecting OS at an early time are of major interest.

A surrogate end point in a clinical trial is a "substitute for a clinically meaningful end point that measures how a patient feels, functions and survives that is expected to predict the effect of therapy."[40] According to Buyse el al,[41] a new surrogate end point should correlate with the clinical end point and also be associated with treatment effect.

The reduction in tumor size (response) is the first objective measure and overall response rate (ORR) is widely applied. It has been used for approval of several drugs,[42] but correlation with OS is poor.[43] Therefore, new end points with a causative relationship between early treatment response and OS are of high interest. The ctDNA response seems to meet the NIH criteria for a likely early surrogate end point but a generally accepted definition is still lacking. It is conceivable that the clinical efficacy calls for major relative changes in the ctDNA levels. A binary scale (detectable/undetectable) is easy to interpret and scientifically sound if the undetectable level includes values with confidence levels overlapping zero.

The current literature, however, is only suggestive as to the possible utility of ctDNA response as a surrogate marker of OS. The final proof must come from randomized trials comparing treatments with different ctDNA response rates resulting in different rates of OS. Such trials face a number of scientific and ethical challenges but seem reliable when using 2 doses of the same drug.

Recommended Steps Toward Clinical Utility

Clinical utility of ctDNA monitoring in metastatic disease has not yet been proven beyond reasonable doubt

TABLE 2. Summary of Key Topics to Consider in the Planning of Interventional Studies Analyzing ctDNA in the Metastatic Setting of Solid Tumors

Topic	Key Considerations
Analytical method	• Targeted assays using digital PCR or • Broad-coverage assays using NGS[7-9]
Optimal time points	• Baseline • Before every treatment cycle • At radiological and clinical evaluations
Measurements	• A quantitative measure (eg, copies/mL) and • Fractional abundance
Interpretation of ctDNA dynamics	• Increasing ctDNA from one time point to the next with no overlap between the 95% CI is considered significant and should lead to discontinuation of the current treatment • Decreasing or stable ctDNA is considered as response and should allow continuation of the current treatment • Undetectable is a value of 0 or 0 included in the 95% CI
End points	• ctDNA response

Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; NGS, next-generation sequencing; PCR, polymerase chain reaction.

and the issue calls for further dedicated research. Table 2 summarizes the recommendations raised in this article.

The current literature on cancer is marked by an overwhelming number of biomarkers claimed to be of clinical importance. The reality is that very few have survived the steps from the laboratory to clinical application. One reason is the diversity in reporting.

At the moment, ctDNA has interest in the scientific community with convincing results appearing at a rapid rate, but translation into clinical utility is still poor. Full implementation of ctDNA monitoring of metastatic disease depends on well-planned trials showing improved patient outcomes from that approach. The present work clearly indicates that a more uniform reporting of results is an absolute condition for general clinical acceptance. Optimally applied, addition of ctDNA analysis in the monitoring of metastatic disease may represent a major step forward in the treatment of cancer patients.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

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