FRIENDS of CANCER RESEARCH

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Assessing the Use of ctDNA as an Early Endpoint in Early-Stage Disease

Friends of Cancer Research Annual Meeting 2021

Introduction

Circulating tumor DNA (ctDNA) is a dynamic biomarker with potentially broad clinical and regulatory applicability in oncology. To date, the use of ctDNA has been studied to the greatest extent in the metastatic solid tumor setting for molecular profiling at diagnosis, targeted therapy selection, treatment response monitoring, and long-term post-treatment tumor surveillance.[1] However, there is great opportunity and potential value to patients to further explore the use of ctDNA in early-stage solid tumors including:

- Determining the need for adjuvant therapy after definitive surgery, radiation, or chemoradiation by indicating the presence of minimal (or molecular) residual disease (MRD) or optimizing neoadjuvant therapy regimens,
- Monitoring for disease recurrence in a simpler and less invasive way compared to existing tools (e.g., clinical imaging, biopsies),
- Enabling the identification of patients at the highest risk of recurrence for enrollment in clinical studies (prognostic enrichment strategies), reducing patient numbers as well as the time and cost of studies, and
- Serving as a potential predictive biomarker for a patient's response to therapy as an early endpoint to predict long-term survival outcomes, allowing for faster identification of drugs that may be most efficacious and support regulatory decision-making.[2]

Objectives

Detail the opportunities and challenges in using ctDNA in the early-stage disease setting.

Identify and prioritize clinical questions supporting its use as an early endpoint to support regulatory approval.

Define data elements where alignment is needed across datasets for easier contextualization and analysis to answer these questions.

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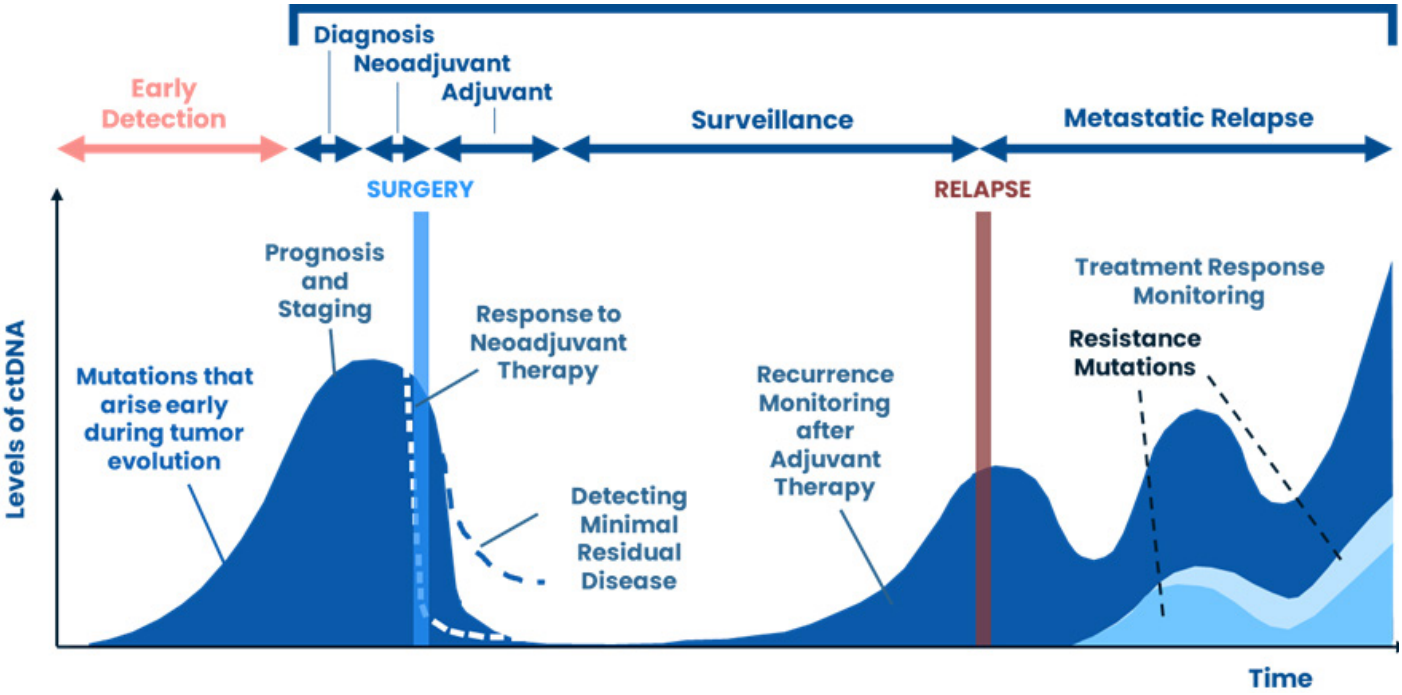
Ultimately, the hope is that use of ctDNA in early-stage disease will improve the approach to drug development in this setting, enabling effective therapies to get to patients faster.
To explore the opportunities and unique challenges for use of ctDNA in early-stage solid tumors, Friends of Cancer Research (*Friends*) convened a multi-stakeholder group of experts in ctDNA and early-stage disease including the U.S. Food and Drug Administration (FDA), drug sponsors, ctDNA assay developers, and academic clinicians. The working group focused on the use of ctDNA as an early endpoint to predict long-term survival outcomes to support regulatory approval, noting the need for collaboration across sponsors. The working group strongly endorsed this collaboration for multiple reasons. There is recognition that validating the use of ctDNA as an early endpoint in early-stage disease will require large amounts of data from multiple prospective clinical trials. These data will need to represent robust clinical outcomes and come from multiple sources.[3] Also, a more coordinated and collaborative approach will help to accelerate the understanding of ctDNA in this setting and to establish ctDNA as a potential early endpoint earlier. Lastly, previous collaborative efforts in this space have laid the foundation for this effort. Friends established a multi-phased collaborative research initiative to harmonize the use of ctDNA to monitor treatment response (ctMoniTR) to determine if changes in ctDNA levels accurately reflect the therapeutic effect of immunotherapies in advanced lung cancer.[4,5] The ctMoniTR Project affirmed that multiple sponsors can work collaboratively to effectively combine data from multiple clinical trials to demonstrate a correlation between ctDNA and response, and has expanded efforts to a second phase that includes additional cancer types and treatments. With this foundation for a collaborative framework, the working group discussed the investigation of ctDNA as an early endpoint in early-stage disease to support its use in regulatory decision-making. Through these findings and leveraging previous ctMoniTR work, we propose a collaborative effort to align data from multiple trials for the investigation of the use of ctDNA as an early endpoint in early-stage disease.

Opportunities for Use of ctDNA in Early-Stage Disease

There are numerous opportunities to utilize ctDNA in early-stage disease that rely on the potential to detect disease burden, such as MRD or molecular relapse, earlier and in a less invasive manner than standard of care imaging technologies or tissue biopsy. Opportunities exist at various stages of established use and validity in oncology, summarized in **Figure 1**. Within the use cases of ctDNA in early-stage disease, one large category pertains to informing and assessing efficacy of therapies. We detail these use cases below.

Figure 1: Potential Use Cases of ctDNA in Oncology. Depicted is a time course through a patient's cancer treatment journey and the opportunities for use of ctDNA to guide treatment. (Adapted from Natera)



ctDNA for Risk Stratification and Treatment Selection

Evidence is emerging on the potential to detect MRD by ctDNA assessment post-surgery to guide decisions on adjuvant therapy. The prognostic value has been demonstrated across multiple tumor types, demonstrating that the detection of ctDNA post-definitive intervention could be utilized to direct patients to appropriate adjuvant therapy in early-stage disease or potentially spare them of unneeded treatment. A study of patients with operable urothelial cancer found that the presence of ctDNA after surgery was significantly associated with poor prognosis and those with detectable ctDNA appeared to derive the most relative benefit with adjuvant immunotherapy.[6] Additionally, multiple studies in early-stage colorectal cancer found the presence of ctDNA after surgery strongly correlated with recurrence[7,8] and inferior disease-free survival (DFS), after adjusting for clinicopathological risk factors.[9]

ctDNA for Patient Selection

Utilizing the prognostic value of ctDNA in the early-disease setting, MRD-selected adjuvant trials can help define a more homogenous patient population with higher relapse-event rates, leading to smaller, higher-risk patient populations and reduced time to reach endpoints. In the early-stage disease setting, adjuvant trial patient populations are heterogeneous with low relapse-event rates leading to the need for large numbers of patients to adequately power studies to analyze outcomes and reach their endpoints, which can also take a significant amount of time and expose some patients to treatments which they ultimately may not need. The potential value of ctDNA in this setting is highlighted by recently launched Phase III trials, including the MERMAID-1 and MERMAID-2 trials to assess adjuvant treatment in patients with

resected stage II and III NSCLC with MRD by ctDNA measurement[10] and the IMvigor011 trial to assess adjuvant treatment in patients with muscle-invasive bladder cancer who are ctDNA positive after cystectomy.[11]

ctDNA to Monitor and Predict Treatment Response in the Neoadjuvant and Adjuvant Settings

Measuring serial ctDNA prior to and throughout treatment may be useful to monitor response to treatment as an early endpoint to potentially predict long-term outcomes. This has been illustrated in a retrospective analysis of prospectively collected samples in early-stage breast cancer where clearance of ctDNA was a predictor of pathological complete response (pCR) to neoadjuvant treatment and was associated with a lower risk of recurrence.[12] In the adjuvant setting, ctDNA can also potentially be used to monitor and predict treatment response as an early endpoint. Early work by a pair of small prospectively designed studies at academic institutions investigating serial ctDNA collection during adjuvant chemotherapy treatment from patients with locally resected colon cancer found that increases in ctDNA levels during treatment was an early indicator of radiologic recurrence[7] and could be an early predictor of relapse.[9] Evidence from these early phase studies supports the association of ctDNA changes as an early predictor of treatment outcome and suggests there is an opportunity in both the neoadjuvant and adjuvant settings to further generate robust evidence.

Defining a Specific Use Case: ctDNA Changes as an Early Endpoint

While there are many possible use cases for ctDNA in early-stage disease, for the purposes of this white paper, the group decided to focus on ctDNA changes (e.g., clearance, reductions, kinetics) in response to therapy as an early endpoint to predict long-term survival outcomes to support regulatory approval. We use the term "early endpoint" for the purposes of this white paper to distinguish the potential to measure ctDNA changes earlier than other endpoints (e.g., disease-free survival, event-free survival, and overall survival) rather than defining the timeframe of when the endpoint is measured (i.e., not insinuating ctDNA measurement occurs early in a clinical trial, as this may vary based on the context of different cancer types or treatment settings). In order for ctDNA to support an Accelerated Approval as a primary efficacy endpoint, ctDNA changes would need to be proven to be reasonably likely to predict clinical benefit. This utility may have therapeutic class specific (e.g., chemotherapy, immunotherapy, targeted therapy, etc.) and tumor type specific considerations, however more data and evidence are needed to delineate these factors. Many clinical and technical questions exist regarding use of ctDNA as an early endpoint and robust evidence generation will be necessary to support its use for regulatory decision-making.

Challenges and Variability in ctDNA Detection

In early-stage disease, there are low amounts of ctDNA due to the small, localized nature of these tumors, and detecting the levels may be limited by current technologies. Furthermore, ctDNA levels vary due to differences in tumor growth rate (e.g., indolent vs. fast-progressing), tumor ctDNA shedding rates, and other biological factors, which vary significantly between different tumor types and metastatic sites (e.g., intracranial metastases).[3] Additionally, both personalized (tumor informed) and non-personalized (plasma only) approaches integrating

varying single-omic or multi-omic approaches (e.g., sequence mutations, structural alterations, methylation, fragmentomics, etc.) and platforms are currently being utilized with many others in development. These approaches, coupled with clinical variables and trial methodology, result in significant sources of variability in early-stage disease ctDNA clinical studies **(Table 1)**.

Table 1: Sources of Variability in Early-Stage Disease ctDNA Clinical Studies

Clinical Variables	Tumor type, histology, stage of disease
	Definitive therapy type (e.g., surgery, radiation, chemoradiation)
	Therapeutic setting (neoadjuvant, adjuvant)
	Current treatment regimens (dosing/timing) and prior regimens
	Therapeutic class (e.g., targeted, IO, cytotoxic, hormonal, etc.)
ctDNA Collection and Methodology	Sample collection timepoints
	Whole blood collection (i.e., tube type, storage)
	Plasma sample processing (i.e., centrifugation)
Captured Endpoints	Endpoints for clinical and radiographic associations, including methodology and definitions of endpoints
	Timing of radiographic surveillance
	Statistical plan (e.g., interim analysis timing, etc.)
Diagnostic Assay and Analysis	Performance parameters (e.g., reference range/interval, LOB, LOD, accuracy, repeatability, reproducibility, clinical cut-off for molecular residual disease)
	Biomarker features assessed (e.g., sequence mutations, structural alterations, methylation, fragmentation, etc.)
	Tumor informed or plasma only platform
	Algorithm design for ctDNA detection and status reporting
	Algorithm design for ctDNA quantification

Key Questions for the Use of ctDNA in Early-Stage Disease

Amidst this variability, there are many questions regarding the ability to use ctDNA in early-stage disease as an early endpoint that will be critical to address. These include both technical and clinical questions.

Key Technical Questions to Be Addressed to Enable the Use of ctDNA in Early-Stage Disease

Due to the multiple approaches and platforms for ctDNA detection in the early-stage disease setting, there are several technical questions regarding the feasibility and best approach for aligning the various methodologies to generate meaningful data on the use of ctDNA as an early endpoint. Questions fall into two categories:

Multi-Use Case Considerations

- Are there different minimum analytical performance requirements for different early-stage disease applications (e. g., neoadjuvant vs. adjuvant, tumor type, stage of disease, etc.)?
- Assuming there are minimum diagnostic analytical performance requirements, are there mechanisms to baseline/compare analytical performance (e.g., LOD, LOB, etc.) across different platforms from both a qualitative (ctDNA detection) and quantitative (ctDNA levels) perspective, and is there a common unit of measurement across assays?

Early Endpoint Considerations

- Given similar analytical performance, are different ctDNA features equally informative to reflect long-term outcomes after surgical or therapeutic intervention (neoadjuvant or adjuvant)?
- Do differences in sample collection (e.g., timing) and pre-analytical processing (e.g., whole blood collection and plasma preparation) affect the ability of ctDNA changes to reflect long-term outcomes?
- Given various lower LOD for different platforms, how can data be pooled and stratified based on the absence or presence of ctDNA to correlate with long-term outcomes?

Future work is needed by multi-stakeholder groups to prioritize the questions and further expand on the necessary evidence to answer these technical questions. There are few harmonized definitions across assays suggesting a need to define common assay metrics and standards to align across datasets.

Key Clinical Questions to be Addressed to Support ctDNA as an Early Endpoint

There are multiple clinical questions regarding the use of ctDNA changes as an early endpoint in early-stage disease. The prioritized questions center around whether changes in ctDNA following treatment reflect long-term outcomes (DFS/EFS and/or OS) at the patient and trial level, as well as whether the ability to use ctDNA as an early endpoint varies by the therapy setting, therapeutic class, or tumor type. Additional considerations explore the nuances of these questions, focusing on the appropriate timing of ctDNA measurement to predict long-term outcomes, including further delineating the predictive value of a drug on reduction, increase, or clearance of ctDNA as reflected in long-term outcomes. These questions will also likely have

different answers depending on the therapeutic setting and tumor type. In the adjuvant setting, it is important to look at ctDNA clearance, while percent change of ctDNA levels may be more relevant in the neoadjuvant setting where the tumor has not been removed. Key questions include:

Do ctDNA changes in response to a drug reflect long-term outcomes (DFS/EFS and/or OS)?

- For example, are certain categorical changes (reduction or rise) in ctDNA more predictive of long-term survival outcomes?

Does the predictive value of ctDNA vary by:

- early-stage disease therapy setting (e.g., neoadjuvant vs. adjuvant)?
- therapeutic class (e.g., immunotherapy, chemotherapy, targeted therapy)?
- tumor type?

When should ctDNA be measured (i.e., should there be set time points for measurement throughout treatment for all trials)?

What is the optimal threshold, in terms of percent change in ctDNA levels (or clearance), that should be used to define ctDNA response?

At what time point does ctDNA response (e.g., early response from pre-treatment to on-treatment, maintaining ctDNA response at a landmark on-treatment timepoint) correlate with long-term survival benefit?

Aligning on a Core Set of Data Elements for Assessing the Use of ctDNA in Early-Stage Disease

There are multiple pragmatic challenges with early-stage disease ctDNA studies including the size and time needed to reach clinical endpoints. Therefore, proactive planning of data elements and analysis methodology is important. To generate sufficient evidence to begin to answer these key clinical questions, collaboration across groups and clinical trials to aggregate data is necessary. If alignment on a core set of data elements occurs before prospective clinical trials are designed and executed, validating ctDNA as an early endpoint can be achieved more efficiently.

Technical Considerations

Due to the significant variability in the analytical approaches and platforms used to measure ctDNA levels, an important first step will be to align on key definitions and metrics for measuring ctDNA to begin to address the technical questions. Studies of ctDNA changes should include sufficient detail regarding the specific approach and measurements of the assay **(Table 2)**, such that the data can be optimally understood and appropriately analyzed across multiple studies to answer the key technical questions.

Table 2: Examples of Analytical Data Elements to Align Across Clinical Trials

Assay Data Element Type	Data Element	Description
Approach	Assay Approach	Tumor informed, plasma only; Personalized or non-personalized
	Genomic Features Assessed	Sequence mutations, structural alterations, methylation, fragmentation, etc.
	Assay Performance Metrics	Reference range/interval, LOB, LOD, accuracy, repeatability, reproducibility
	Platform for ctDNA Assessment	NGS, ddPCR, etc.
	ctDNA Positive Definition	Threshold for calling samples positive
	ctDNA Detection and Quantification Approach	Requirement for ctDNA+ result, how ctDNA levels are calculated, and unit of measurement (e.g., mean tumor molecules (MTM)/ml plasma, and/or Variant Allele Fraction (VAF))
	Non-Genomics Features Assessed	Protein biomarkers, lipid biomarkers, etc.
Measurements	cfDNA Assay Input and Method of Measurement	
	ctDNA Level at Baseline	
	ctDNA Level at Detection	
	ctDNA Fraction at Detection	VAF
	Plasma Volume	
	Per Sample LOD	Estimated LOD for an individual sample

Clinical Considerations

To generate large datasets with robust clinical outcomes and ctDNA data, it is important to align on a core set of data elements that should be captured in randomized controlled clinical trials to allow for better data contextualization and to optimally assess the use of ctDNA as an early endpoint. In the previous ctMoniTR efforts, challenges arose when harmonizing the data across data sources to answer key clinical questions due to the fact that this was a retrospective analysis and key data elements varied, making it challenging to answer some clinical questions of interest. For example, in order to evaluate how early changes in ctDNA can predict response, there must be appropriate timepoint measurements of ctDNA levels at baseline and prior to the first imaging assessment. However, these timepoints were not routinely collected in all clinical trials, making it challenging to effectively answer the clinical question of how early changes in ctDNA levels can predict response. Therefore, pre-specifying the necessary elements to embed in clinical trial protocols can help maximize the types of clinical questions that can be answered and prevent later analysis issues due to discordant clinical trial methodology.

Table 3: Examples of Clinical Data Elements to Align Across Clinical Trials

Data Elements	Considerations for Alignment
Baseline Disease Characteristics and History	Timing of adjuvant therapy after definitive therapy (adjuvant) History and timing of prior therapy (neoadjuvant and adjuvant)
Timing and Frequency of Plasma Collection	Time of day sampling
	Timing of collection relative to definitive therapy (adjuvant)
	Timing of collection with therapy (e.g., cycle of administration)
	Timing of collection at baseline measurement
Frequency of Radiographic Tumor Surveillance and Imaging Modality	Frequency of imaging
	Timing of imaging with plasma collection

For each of these core data elements there are multiple considerations, each with the opportunity to align on a standard methodology for the data elements to maximize learnings later. For example, there is varying methodology for the timing and frequency of plasma collection, and further work is needed to define the frequency so that analysis of ctDNA changes over time can be more effectively analyzed across datasets. The frequency of collection may depend on the therapeutic class, cycle of administration, treatment setting (neoadjuvant or adjuvant), or tumor type. These clinical variables will also inform the clinical endpoints that are measured in a clinical trial (e.g., DFS, EFS, OS, and pCR), and will therefore affect the conclusions that can be drawn regarding the use of ctDNA as an early endpoint. Future work is needed to align on these core data components and set forth recommendations to be followed in future clinical trials to allow for effective assessment of ctDNA.

Conclusions

There is great opportunity in early-stage solid tumors to assess the use of ctDNA changes as a potential early endpoint to predict long-term patient outcomes for regulatory approval. The focus on establishing ctDNA as an early endpoint has the potential to expedite and improve confidence in the efficacy of novel therapies, bringing beneficial treatments to patients sooner. To rigorously evaluate the use of ctDNA as an early endpoint for regulatory decision-making, aggregating data across studies will be necessary. There are many critical clinical and technical questions to address to establish ctDNA as an early endpoint, and alignment on key data elements in clinical studies will help to accelerate the answers to these questions. As the group continues to develop a roadmap for assessing the use of ctDNA as an early endpoint in early-stage disease, ongoing conversations and collaboration between stakeholders is crucial.

As a first step, a landscape assessment of the current data available from previously conducted randomized controlled trials in early-stage disease is needed. The group aims to establish an inventory of data availability, categorizing the data available by clinical variables such as tumor type, treatment setting, and therapeutic class. An analysis will need to be conducted to understand the methodology for obtaining the core clinical data elements, such as the frequency of plasma collection in the studies. This insight into current practice, with an understanding and justification of the clinical context supporting the practice, will inform future recommendations for data capture in clinical studies. Prospectively designed studies, following the specified recommendations for data capture, will then lessen the variability seen in retrospective datasets allowing for more effective analysis.

There are additional significant technical and analytical questions about the assays that measure ctDNA to be addressed. Activities must be coordinated with other relevant stakeholders, as efforts to set pre-analytical and analytical standards for assays measuring ctDNA will be important. Further, the technical and statistical considerations for effectively conducting pooled meta-analyses from multiple trials, given the variability, will need to be discussed to determine the optimal statistical approaches and potential limitations of meta-analyses.

As demonstrated by the previous ctMoniTR work in late-stage disease, collaboration across sponsors for data analysis from multiple clinical trials is possible. Collaboration will be necessary to generate large datasets with robust and aligned clinical data to evaluate the use of ctDNA as an early endpoint in early-stage disease. Initial considerations are presented in this white paper, and work will continue to build a roadmap for assessment of this early endpoint that has the potential to transform drug development and benefit patients.

Abbreviations

cfDNA – Cell Free DNA

cfRNA – Cell Free RNA

ctDNA – Circulating Tumor DNA

ctMoniTR – ctDNA to Monitor Treatment Response (*Friends*' collaboration)

ddPCR – Digital Droplet Polymerase Chain Reaction

DFS – Disease-Free Survival

EFS – Event-Free Survival

IO – Immuno-Oncology

LOB – Limit of Blank

LOD – Limit of Detection

MRD – Minimal (or Molecular) Residual Disease

MTM - Mean Tumor Molecules

NSCLC – Non-Small Cell Lung Cancer

NGS – Next Generation Sequencing

OS – Overall Survival

pCR – Pathological Complete Response

VAF – Variant Allele Fraction

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