Circulating Tumor DNA in Development of Therapies for Cancer: An Evidentiary Roadmap to an Early Endpoint for Regulatory Decision-Making

Approach

Friends of Cancer Research (*Friends*) assembled a multi-stakeholder working group comprised of pharmaceutical companies, diagnostic labs, government health officials, patient advocates, and academic researchers to develop an aligned strategy for generating data and evidence to support using the measurement of *circulating tumor DNA (ctDNA)*[1] levels in patients with solid tumors as a drug development tool in regulatory decision-making. This objective includes using ctDNA as an early endpoint to predict long-term outcomes in patients being treated for early-stage cancer. We thank the numerous stakeholders for their thoughtful input and expertise in the development of this evidentiary roadmap.

Background

Recent technological innovations allow for the detection of ctDNA in the blood. ctDNA is a biomarker with potentially broad clinical and regulatory applicability in oncology:[2]

- **ctDNA for Patient Selection based on Molecular Alteration**
  - Using molecular features identified in ctDNA to select for patients with alterations targetable by therapy, allowing for the development and evaluation of targeted therapeutic approaches that bestow the most benefit to the patient.
- **ctDNA Molecular Residual Disease for Patient Enrichment**
  - Determining the need for adjuvant therapy after definitive surgery, radiation, or chemoradiation by indicating the presence of *molecular (or minimal) residual disease (MRD)*.
  - Enabling the identification of patients with elevated risk of recurrence for enrollment in clinical studies. This may optimize clinical studies by reducing the overall

number of trial participants needed, and in turn the time and cost of studies.

- **ctDNA as a Measure of Response**
  - Detecting a change in the degree or extent of disease burden by serial measurements while on treatment.
  - Supporting early response/resistance identification (signal finding) in early phase clinical trials to support decision-making in drug development.
- **ctDNA as an Early Endpoint in Clinical Trials**
  - Detecting a change in the degree or extent of disease burden by serial measurements while on treatment.
  - Evaluating treatment efficacy and support regulatory decision-making as an early endpoint capable of predicting long-term survival outcomes.

This document outlines considerations and evidentiary needs to support the use of changes in ctDNA levels while on treatment as an early endpoint in clinical trials that predicts long-term clinical outcomes. The term "early endpoint" signifies measuring *ctDNA changes* earlier than other longer-term endpoints (i.e., progression-free survival (PFS), event-free survival, and overall survival (OS)) 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). This roadmap outlines evidentiary needs to support the use of this early endpoint in cancer, including an endpoint to support accelerated approval. To support accelerated approval, the *surrogate endpoint* must be reasonably likely to predict a clinical benefit. The long-term goal of using ctDNA as a validated surrogate endpoint to replace a clinical outcome is a much higher bar of evidence. As we work towards accomplishing this long-term goal, useful information will also be generated to help inform its use as an early endpoint.

The introduction of novel therapeutics, especially targeted therapies, has changed the paradigm for treating solid tumors. While the availability of these new therapies provides increased clinical benefit for patients, the concomitant increase in survival time creates a unique challenge in the development of new therapies. With these novel therapeutics, traditional clinical trial approaches using long-term clinical outcome endpoints such as PFS or OS may not allow for an efficacy determination in a timely manner. Early endpoints that are "reasonably likely to predict a clinical benefit," are becoming increasingly important in oncology drug development. However, it is critical to obtain adequate data to fully qualify and validate ctDNA as an early endpoint for solid tumors. The use of ctDNA levels (e.g., presence, changes, or clearance) represents an emerging early endpoint that holds great promise.

Aligned methodologies are needed to support robust data generation and enable evaluation of data across trials throughout academia and industry. A recent meta-analysis by *Friends* in non-small cell lung cancer (NSCLC) suggests decreases in ctDNA levels due to therapeutic intervention are associated with improved outcomes.[3] Individual trials have also demonstrated this trend. However, evaluating findings from across individual trials can be challenging due to potential impacts of differences in therapeutic modalities investigated, inconsistencies in how ctDNA is collected, measured, and reported, and the variability in the performance of the tests measuring ctDNA (**Table 1**). This can make it difficult to generalize findings and may not meet the necessary evidentiary threshold for use of ctDNA levels as an endpoint in regulatory decision-making.

Table 1: Sources of Variability in ctDNA Clinical Studies

Clinical Variables	Tumor type, histology, stage of disease
	Definitive therapy type (e.g., surgery, radiation, chemoradiation)
	Therapeutic setting (advanced/recurrent/metastatic, 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, time in transport)
	Plasma sample processing (i.e., centrifugation speeds, double spins, long-term stability)
Captured Endpoints	Endpoints for clinical and radiographic associations, including methodology and definitions of endpoints (e.g., 50% decrease)
	Timing of radiographic surveillance
	Statistical plan (e.g., interim analysis timing, etc.)
Diagnostic Assay and Analysis	Performance parameters (e.g., reference range/interval, Limit of Blank (LOB), Limit of Detection (LOD), accuracy, repeatability, reproducibility, clinical cut-off for molecular residual disease, unit of measurement for ctDNA)
	Biomarker features assessed (e.g., somatic variant mutations, structural variant alterations, methylation, fragmentation, etc.)
	Tumor informed or liquid only platform
	Sequencing platform
	Algorithm design for ctDNA detection and status reporting
	Algorithm design for ctDNA quantification

Source: Adapted from Friends of Cancer Research White Paper. Assessing the Use of ctDNA as an Early Endpoint in Early-Stage Disease.[4]

Clinical Questions that Support the Use of ctDNA Measurement as an Early Endpoint

Generating evidence to support the use of changes in ctDNA levels as an endpoint requires careful consideration of several critical clinical questions.

The primary question is:

- Do changes in ctDNA levels while on treatment predict long-term outcomes (i.e., disease free survival/event free survival (DFS/EFS), overall response rate (ORR), PFS, and/or OS at the individual- and trial-level?

Secondary questions should also be explored to investigate additional nuances:

- Does the predictive value of ctDNA levels vary by:
  - stage of disease (e.g., early stage, advanced stage)
  - disease therapy setting (e.g., neoadjuvant, adjuvant)?
  - therapeutic class (e.g., immunotherapy, chemotherapy, targeted therapy)?
  - tumor type?

- How does timing of ctDNA measurement impact the predictive value, i.e., should there be set time points for measurement before and throughout treatment for all trials? How do different treatment regimens and cancer types influence timing?

- What is the optimal threshold, in terms of percent change in ctDNA levels (or clearance), that should be used to define ctDNA response? Does this threshold used to define ctDNA response depend on the disease setting (e.g., advanced disease, neoadjuvant) and tumor type?

- How does the depth and durability of ctDNA response (i.e., early response from pre-treatment to on-treatment, maintaining ctDNA response at a landmark on-treatment timepoint) correlate with long-term survival benefit?

Key Considerations for Validating the Use of ctDNA as an Early Endpoint

Understanding the application of ctDNA levels as a biomarker is important when designing studies to validate its use for ascertaining therapeutic efficacy. There are multiple technical and clinical characteristics contributing to variability that should be adequately accounted for, such as assay type, underlying disease, patient heterogeneity, therapeutic context, target of therapy, or a combination of disease parameters, when conducting validation studies.

Technology Considerations

There are a variety of assays that measure ctDNA with differing approaches, which can impact the interpretation of assay results. Assays analyze different molecules, use different technology platforms, and have different methodologies for measuring ctDNA. The variability these differences introduce to ctDNA measurement should be considered when developing evidence that supports the use of ctDNA as an endpoint.

1. *Molecular Alterations Analyzed*

Assays measure different molecules, including genetic alterations and epigenetic modifications. For genetic alterations, there is a tendency to evaluate single nucleotide variants (SNVs), while some assay developers assess insertions, deletions, and/or classic gene fusions. Most ctDNA assays do not account for large structural events or gene copy number variation (CNV), however, some may account for these alterations through low depth whole genome sequencing (WGS) or other more targeted approaches. For assays measuring epigenetic modifications, most focus on measuring methylation or fragment size distribution/DNA fragment patterns. In some cases, assays use a multimodal approach that incorporate genetic alterations and epigenetic modifications.

2. *Platforms*

Different platforms analyze different variants/signatures at varying sensitivities and specificities. **Table 2** highlights platforms that measure ctDNA and the opportunities and challenges for each.

3. *Methodological Approaches*

There are two main methodological approaches for identifying ctDNA variants to monitor:

- *Tumor Informed Assays* use individualized sequencing information from a patient's tumor tissue to determine which genes should be monitored in the patient's blood. ddPCR typically focuses on a single genetic mutation that is often the target of the patient's therapeutic treatment. Monitoring a single tumor marker, however, may result in a false negative due to other subclones that can emerge while on treatment. Other assays use a proprietary algorithm to select the optimal variants from the tumor to include in the bespoke panel. Logistical challenges, including the time to develop patient-specific marker panels, require careful consideration when selecting this approach in certain disease settings (e.g., advanced disease).

- *Liquid Only or Tumor-Naïve/Tumor Agnostic Assay* does not require tumor tissue or prior knowledge of a tumor's mutation profile. This approach uses either a pre-determined gene-panel to identify ctDNA variants or a WES/WGS assay. The former approach depends on the panel of genes/methylation loci, which are sometimes selected based on the tumor of origin (e.g., a lung cancer panel). Genes can be analyzed individually or as a signature. WGS and WES can also be used in a tumor-agnostic manner to generate significant coverage across genes, increasing the assay's sensitivity.

4. *Assay Performance*

Assay performance depends on a variety of factors and alignment on metrics will be critical for harmonizing the use of ctDNA as an early endpoint. The BloodPAC Consortium developed recommendations[5] for 11 required preanalytical attributes to support standards development and robust ctDNA assay development. There are also a series of measurements used to

Table 2: Characteristics of ctDNA Assay Platforms

Platform	Description	Opportunities	Challenges	Sensitivity	Coverage
Droplet Digital PCR (ddPCR)	• Used for targeted somatic variants	• Targeted nature focuses on known variants • Smaller volume necessary to measure ctDNA	• Not suitable for most fusions because of complexity in junctions or numbers of isoforms generated in splicing • Less suited for capturing novel emerging alterations over time	Highest	Lowest
Fixed size next generation sequencing (NGS) panels	• Generally, includes targeted NGS somatic nucleotide variant panels, and "methylation only" approaches, or both • May also include CNVs and fusions	• Can cover a broader range of genomic alterations (~30-650 genes) while controlling size and cost	• Sensitivity may vary based on the number of analytes included, GC-rich bias and depth of sequencing	Moderate	Moderate
WGS/ Whole Exome Sequencing (WES)	• Algorithms identify ctDNA from whole genome/exome	• Coverage advantage, whole genome/exome are covered with a single test	• Lower sensitivity and variable specificity for targets	Lowest	Highest

determine assay performance that need to be harmonized:

- Sensitivity: In general, sensitivity is variable and mostly depends on shedding. There are circumstances where the gene alteration is present in the tumor but no, or very low levels of, ctDNA are detected in the blood.

- Specificity: Specificity depends on the variant targeted. Tumor sequences are unique/ specific to the presence of a tumor; however *clonal hematopoiesis of indeterminate potential (CHIP)* can be mislabeled as a somatic variant and complicate the results if not controlled for. In addition, if germline mutations are not adequately filtered, bioinformatically, or by normal tissue sequencing, the presence of germline alterations may affect the specificity of these assays.

- Accuracy: Greater than 95% accuracy is desirable, but this may be difficult depending on the technologies, panels, target variant frequency, and availability of clinical specimens.

- Precision: Precision should be greater than 95% inter-day, intra-day and inter-instrument, inter-operator.

- *Limit of Quantitation (LOQ)/Limit of Detection (LOD):* LOQ/LOD is the lowest concentration of analyte that can be consistently detected 95% of the time in a defined type of specimen. LOQ/LOD will be driven by the input level, molecular conversion rate, noise reduction method (e.g., Unique Molecular Identifier), ctDNA input/blood volume, and depth of sequencing and will vary at the specific allele level. Minimum performance characteristics will differ for various platform technologies as well as various providers.

There may be opportunities to use contrived samples to align on approaches for determining assay performance across different assay platforms. However, limitations in certain factors such as number of mutations, chromosome copies, and tissue *tumor fractions* impact how contrived samples reflect clinical sample performance.

5. Sampling Considerations

Plasma is the default choice for nearly all liquid biopsy applications; however, a few diagnostics developers use serum. Early data shows that serum is more likely to be contaminated by leukocyte DNA and this can impact analyses. Given the need for standardization in this space, we recommend adopting a ubiquitous matrix such as plasma.

6. Standardization Needs

A few additional standardization needs include:

- Measurement outputs may vary across assays including outputs such as *variant allele frequency (VAF)* and *mean tumor molecules per milliliter (mtm/mL)*.
- A common language to describe epigenetic modifications.

- Statistical considerations, including *baseline* measurement versus change from baseline. Different statistical questions require different data.

Clinical Considerations

The data that will support the use of ctDNA as an early endpoint will depend on the clinical context of use including the cancer type, disease stage, treatment setting, and treatment regimen as these may impact ctDNA kinetics. These components should be considered when developing a framework for evidence generation and designing clinical trials supporting the use of ctDNA as an endpoint.

Likely, validating the use of ctDNA levels as an early endpoint will be a stepwise process, initially validating its use in one tumor type, treatment setting, and drug class where there are strong and existing data and evidence for changes in ctDNA levels anchored to a standard measure of response to treatment in that indication. This approach was seen in FDA's pathological complete response (pCR) guidance[6], with use of pCR as an endpoint specific to high-risk early-stage breast cancer in the neoadjuvant setting. From a single indication, there may be opportunities to use lessons learned across treatments or tumor types to support use in other settings. For ctDNA levels, it will be important to understand how and when it is feasible to extrapolate findings from one indication to other indications.

1. Current Data Availability

While the long-term goal of the evidentiary roadmap is to support the use of ctDNA levels in early-stage disease, there are lessons to be learned from data that are currently available. Trials are underway to collect data and evidence that support the use of ctDNA levels as a biomarker for treatment response and long-term outcomes in the metastatic setting in multiple tumor types including NSCLC, bladder cancer, colorectal cancer, renal cell carcinoma, and breast cancer. Additionally, trials focused on the use of ctDNA levels to determine MRD may also support an understanding of ctDNA dynamics in different cancer types, especially in earlier stages of disease. This is another active area of research focused in the colorectal and NSCLC settings.

2. Variability Based on the Disease and Therapeutic Characteristics

Identifying clinical characteristics that influence monitoring ctDNA levels in a specific disease setting may support a rationale for indications that could be categorized together and/or prioritized for evidence generation. As noted above, and through *Friends'* previous work, the value of the use of ctDNA levels to monitor treatment response has been demonstrated in the metastatic NSCLC setting, and the indication serves as a use case to apply the characteristics of the indication to other indications (**Table 3**).

Table 3: Characteristics of Therapeutic Indication Impacting Use of ctDNA as an Endpoint

Characteristic	Considerations	Use Case: Metastatic NSCLC
Biology of Cancer Type	The biology of the specific tumor type, histology, size, vascularity, and location may result in variable ctDNA shed rates impacting the relevance and feasibility of using ctDNA levels as an endpoint.	Shown to have high shed rates in the metastatic setting
Tumor Stage	The stage of the tumor may impact the ctDNA shed rates and early-stage tumors may shed less, impacting detection of ctDNA levels for use as an early endpoint.	
Drug Class	ctDNA kinetics in response to treatment may vary depending on the mechanism of action of the treatment, which may impact the use of ctDNA to compare outcomes across treatment arms.	Ongoing work by ctMoniTR in different drug classes (IO, TKIs)
Treatment Schedule	The treatment schedule may impact the ctDNA shed rates and ctDNA kinetics as well as the timing of ctDNA collection and measurement, therefore a fixed time to sample may not be optimal for each study.	Some drugs for NSCLC are given weekly or every 3-4 weeks in most instances
Definitive Treatment (Early-Stage Setting)	The type of definitive treatment (e.g., surgery, radiation, chemoradiation, radiofrequency ablation) and success of the resection or therapy will alter ctDNA levels and should be considered for establishing baseline ctDNA levels. Additionally, surgery and radiation may impact ctDNA levels differently with and without treatment.	Use of ctDNA is mostly in the advanced setting, where definitive treatment does not occur
Medical Necessity	The utility of other established surrogate endpoints for certain cancers or patients (e.g., patients with unmeasurable disease) may vary by indication necessitating other types of endpoints that can potentially readout sooner or be relevant in a specific patient population.	Safety/risk concerns with repeated tissue biopsies, and toxicities associated with therapies demand a need for a less invasive monitoring tool, such as liquid biopsies

Sampling Considerations

The timing of when to measure ctDNA levels, both initially (baseline) and during follow-up, is not currently standardized, and understanding ctDNA dynamics will require a level of standardization for use as an endpoint. Differences in timing decisions are driven by disease characteristics, treatment regimen and schedule, and assay technology. Additionally, the time to therapeutic response and response durations may vary by types of treatment regimens. Alignment across trials for similar drug classes and cancer types should be considered. For clinical trials that use ctDNA levels as an early endpoint to measure treatment efficacy, sponsors should:

- Ensure impacts on the patient are considered when making decisions about timing for blood draws. Focus on aligning with other clinical activities such as treatment administration and scans.

- Collect a pre-treatment (including pre-surgery) baseline sample. A surgical sample may also be valuable, especially for tumor informed assays.

- Consider timing of imaging, including collecting on-treatment ctDNA samples in parallel with CT/MRI imaging scans to facilitate the exploration of how ctDNA response correlates with accepted measures of clinical response, such as RECIST. However, additional timepoints should be considered, especially before imaging scans, since changes in ctDNA levels may be detected much earlier than disease progression assessed by an imaging scan in early-stage disease.

- Measure ctDNA levels at the end-of-treatment response assessment to fully capture the treatment effect and consider collecting ctDNA during the DFS or OS follow-up period.

Evidence is needed to understand how timepoints impact our understanding of ctDNA dynamics. Key questions to answer include:

- How frequently should samples be collected?

- Does frequency differ based on use case (e.g., tumor type, therapy)?

- How does the frequency of therapy administration impact ctDNA kinetics, and therefore collection?

- How do you define a baseline sample within a specific clinical trial and therapy setting (e.g., neoadjuvant versus adjuvant), and how soon should the sample be drawn (e.g., early-disease setting after definitive surgery)?

Knowledge Gaps and Approaches to Support Evidence Generation

Current data provide a limited understanding of the variability in ctDNA dynamics across different tumor types, tumor stages, treatment regimens, and treatment settings. Meta-analytic approaches will help evaluate and support the use of ctDNA levels as an early endpoint to monitor treatment across various disease settings and provide an opportunity for better alignment in data collection and evidence generation.

Baseline ctDNA Levels Associated with Different Cancer Types

- **Challenge:** Current data in the metastatic setting have shown variable baseline ctDNA shed rates across different cancer types.[7,8] However, there is not a wealth of data on baseline ctDNA shed rates in the early-stage setting, and data continues to be from disparate ctDNA technologies, making pooled analyses across studies challenging. A better understanding of pre-treatment ctDNA levels across tumor types and stages, assayed with multiple ctDNA assay technologies, would be informative to begin to understand how the biology of the cancer type and stage impacts ctDNA levels.

- **Potential Solution:** Establish evidence regarding baseline ctDNA levels for cancer type and stage across assays through a collaborative effort involving multiple diagnostic developers. These data could support efforts to develop guidance on the use of ctDNA as an early endpoint that can encompass multiple cancers that have shared characteristics, such as shed rates, rather than focusing on individual cancers. Not only would baseline levels support an understanding of the range of ctDNA levels in different stages and tumor types to inform ongoing strategies, comparing different assay outputs will build a foundation and identify key questions to support the harmonization of the endpoint across ctDNA assays. This evidence could also support harmonized metrics for quantifying ctDNA levels, including in early-stage disease.

Association Between Changes in ctDNA Levels and Response to Treatment in Early- and Late-Stage Disease

- **Challenge:** The majority of available data assessing associations between ctDNA changes and patient outcomes to date is in the *late-stage disease* setting. Data is limited in early-stage disease and determining whether associations observed in the advanced setting are generalizable to early-stage disease is needed to help inform its use as an early endpoint in the early-stage setting.

- **Potential Solution:** The ctMoniTR Step 2 Project asks the question: Do changes in ctDNA reflect treatment response in metastatic disease? This project serves as a foundation for an aligned methodology to generate evidence for use of ctDNA to track treatment response in the metastatic setting, but also provides a framework to ask a similar question in early-stage disease to determine whether associations between ctDNA level and patient outcomes varies between these two settings and types of endpoints (e.g., ORR, PFS, OS).

Harmonizing Assays that Measure ctDNA

- **Challenge:** There are different methodologies, technological approaches, and metrics for quantifying and reporting ctDNA levels (e.g., VAF, mtm/mL) across assays, which also impacts the LOD of a given assay. These differences can impact the ability to conduct meta-analyses and could lead to differences in how data are interpreted.

- **Potential Solution:** Evaluating the ability of ctDNA to assess response to treatment using a meta-analytical approach across multiple clinical trials and ctDNA assays, such as in the ongoing ctMoniTR project, can help inform methodological approaches for analyzing data across different assay technologies. This data can also help identify opportunities to support greater alignment across assay quantification, determination of ctDNA "positivity", and reporting. Comparing associations between ctDNA and other established endpoints, such as RECIST, will help evaluate differences in timing and duration of response and inform what constitutes "meaningful change." Once established, this could serve as a performance benchmark for determining whether an assay is optimal for detecting clinically meaningful changes in ctDNA levels.

Tumor Specific ctDNA Dynamics

- **Challenge:** The extent to which changes in tumor clonality, and the tracking of specific clones, can have an impact on the interpretation of associations between measured ctDNA levels and clinical outcomes is not fully known. In studies investigating targeted therapies, assays may be tracking molecular alterations of the therapeutic target. An important consideration is whether only tracking those specific mutations impacts the understanding of tumor dynamics as new subclones may emerge during the course of treatment. Comparing results with assays that are not tumor informed may help understand the impacts of subclones.

- **Potential Solution:** For these studies, sponsors should identify the mutations known to be sensitive to the therapy and those that are known to be resistant to the therapy. If using only the target of the therapy as the ctDNA marker, it is important to understand the impact clonality changes may have on interpreting the results of the ctDNA measurements. Tumor samples from patients with late-stage/metastatic disease could be used to assess both tumor-informed and tumor-naïve approaches, where a high disease burden increases the tumor ctDNA and tissue amount for study. Alternatively, a similar analysis could be conducted in specimens collected in the adjuvant setting (i.e., following post-surgery with curative intent), with blood specimens collected pre- and post surgery.

Standards

- **Challenge:** Patient samples, especially from clinical trials, are limited. Some evidence suggests that contrived samples may be challenging to use across assays due to differences in technologies of the assays and that it may be difficult to use contrived samples for evidence development with tumor-informed methodologies.

- **Potential Solution:** A review and discussion of the various contrived samples available would most likely show that some enable more assays to be evaluated than others. There may be an opportunity to test contrived samples across multiple assays to identify the best approach to creating them, if possible. Additionally, assay developers should consider creating a shared resource of retained blinded remnants for ongoing quality control assessments for assay performance.

Regulatory Considerations for Use of ctDNA in Oncology Drug Development

Use of ctDNA as an Early Endpoint for an Accelerated Approval

Validated surrogate endpoints that predict clinical benefit can be used as the primary efficacy endpoint in some clinical trials to replace traditional clinical outcome measures like OS. In oncology drug development, this has helped spur innovation and bring life-saving therapies to patients quickly and safely. When the surrogate endpoint is intended to replace a clinical outcome for the purposes of regular approval, the validity of the surrogate endpoint to predict a clinical benefit in a specific context of use must be established using robust evidence from clinical trials and meta-analytical analyses. However, in the absence of a validated surrogate endpoint, an early endpoint supported by less extensive evidence can support an accelerated approval when the early endpoint is "reasonably likely to predict a clinical benefit." This would be limited to drugs for serious conditions that fulfill an unmet medical need and would require confirmatory trials to verify benefit using a traditional clinical outcome measure. The use of ctDNA as an early endpoint could be a plausible approach for use in oncology drug development, as more extensive data continues to be generated, which may ultimately result in ctDNA becoming a validated surrogate endpoint to support regular approval. When using ctDNA as an endpoint in a clinical trial, it is necessary to define what is a clinically meaningful change. Use of ctDNA as an early endpoint could be defined in many ways, including as a categorical change from baseline to an on-treatment measurement (e.g., a 50% decrease from baseline), based on the absence of ctDNA (e.g., ctDNA clearance), or including an assessment of duration of the observed changes in ctDNA at a landmark timepoint. How clinical benefit is being defined based on changes in ctDNA levels and what constitutes a clinically meaningful change should be defined a priori. This will likely be dependent on the clinical setting and will also likely vary by treatment, tumor, and assay type will impact use of ctDNA as an endpoint.

Meta-Analytical Approach for Analyzing Data to Validate Use of ctDNA as an Early Endpoint

Meta-analytical approaches merge findings from independent studies to measure an overall effect and have been used to validate other novel early endpoints. Pooled analyses can also increase confidence in observed associations between ctDNA levels and patient outcomes. The terminology and definitions below provide further detail about statistical principles relevant to the validation of an early endpoint:

- *Individual-level association* is the strength of the association between the early endpoint and the true clinical endpoint.

- *Trial-level association* is the strength of the association between the effects of treatment on the early endpoint and the true clinical endpoint.

As outlined in recent FDA guidance documents,[9] to maximize the interpretability of data aimed at supporting the use of ctDNA as an early endpoint, meta-analytic approaches should include:

- Details of trial designs, inclusion and exclusion criteria, ctDNA assessment, and disease setting as well as justification for the suitability of pooling the studies.
- Trials that include a patient population representative of the population in which the endpoint will be used.
- An adequate number of randomized trials with sufficient follow-up time. The number of trials to be included in the meta-analysis should be justified.
- An analysis based on individual patient level data to allow an assessment of individual level surrogacy.
- Prespecified criteria for concluding association based on both trial-level and individual-level association measurements, including prespecified timing and window of ctDNA assessment. Should explore sensitivity analyses based on different time windows.
- Long-term clinical endpoints, such as DFS/EFS, PFS, and OS, that have been clearly and consistently defined across studies.
- Missing ctDNA assessments and reasons for missing data.
- Sensitivity analyses to demonstrate robustness of the early endpoint and subgroup analyses.
- Statistical handling of unevaluable samples.
- Description of the rate of technical failure (e.g., no ctDNA detectable in sample, especially relevant at baseline).
- Potential confounding factors.
- Comparison of trials using different ctDNA assays and level cutoffs, capturing the assay performance metrics (sensitivity, number of alterations examined, etc.)

Evidence Needed to Support Regulatory Use of ctDNA

While the number of studies demonstrating an association between decreased levels of ctDNA and improved patient outcomes continues to grow, these studies on their own likely do not meet the necessary threshold needed to support the use of ctDNA as a validated surrogate endpoint. Evidence needed to justify use of ctDNA as an early endpoint will depend on the regulatory context, whether it is being used as a supportive or primary endpoint, and other criteria. One approach to accumulate evidence to support the use of ctDNA as an early endpoint is through meta-analytic approaches. In some instances, these meta-analyses could potentially justify the use of ctDNA as an early endpoint in the context of an accelerated approval. Meta-analyses

can be challenging given the variability across trials incorporating ctDNA, including differences in technologies used, disease and therapeutic types, and the schedule of assessment. Collaborative research partnerships, such as the consortium that supported pCR as a surrogate endpoint in breast cancer and the ctMoniTR project for use of ctDNA, are helping elucidate strategies for bringing together disparate datasets and identifying opportunities for alignment to support future meta-analyses across clinical trials.

Glossary of Key Terms

Baseline: The time before a treatment intervention for cancer, to assess ctDNA levels prior to therapy.

Clonal Hematopoiesis of Indeterminate Potential (CHIP): The alteration or mutation of bone marrow stem cells that gives rise to an outgrowth of affected cells.

Circulating tumor DNA (ctDNA): Tumor-derived fragmented DNA shed into a patient's bloodstream that is not associated with cells.[10]

ctDNA Changes: A variation or fluctuation in the levels of measured ctDNA over time, most likely a change from pre-treatment levels to on-treatment levels. This may also be a categorical change, such as presence or absence of ctDNA.

Early Endpoint: An endpoint that is reasonably likely to predict long-term clinical outcomes and may be used to support an accelerated approval.

Early-Stage Disease: Specific TNM staging will vary depending on the cancer type but is generally a localized cancer amenable to local intervention with curative intent.

Fixed size next generation sequencing (NGS) panels: A next generation sequencing (NGS) assay that evaluates a pre-specified number and type of alterations in ctDNA.

Late-Stage Disease: Specific TNM staging will vary depending on the cancer type, but is generally a cancer that has metastasized, and is not amenable to local intervention.

Limit of Quantitation (LOQ)/Limit of Detection (LOD): The lowest concentration of analyte that can be consistently detected 95% of the time in a defined type of specimen.

Liquid Only or Tumor-Naïve/Tumor Agnostic Assay: An assay that does not require tumor tissue for creation and use of the assay, and relies on a pre-determined gene panel or whole genome/whole exome sequencing to identify ctDNA variants.

Molecular Residual Disease (MRD): The persistence of a small number of malignant cells, which may be undetectable by conventional screening methods, that is measurable by next generation sequencing of the plasma.

Mean Tumor Molecules per milliliter (mtm/mL): The average number of tumor molecules detected in the ctDNA per milliliter of the patient's plasma.

Tumor Fraction: The proportion of cell-free DNA derived from tumor cells in a blood sample.

Tumor Informed Assay: An assay that uses a patient's tumor tissue to determine which genes and alterations should be monitored in the blood.

Surrogate Endpoint: An endpoint used in clinical trials that does not directly measure clinical outcomes as the clinical outcomes may take a very long time to study. The surrogate endpoint predicts, or correlates with, clinical benefit and is accepted by the FDA as evidence of benefit and can support a regular approval.

Variant Allele Fraction (VAF): The frequency at which the variant of interest at a specific locus is detected in sequencing reads from a specimen.

References

1. Throughout the document, words that are defined in the appendix are italicized and underlined at first use.
2. FDA. Use of Circulating Tumor DNA for Early Stage Solid Tumor Drug Development Guidance for Industry. May 2022. Accessed on May 20, 2022 from: https://www.fda.gov/media/158072/download
3. Vega et al. JCO PO 2022 [In Press].
4. Friends of Cancer Research White Paper: Assessing the Use of ctDNA as an Early Endpoint in Early-Stage Disease. https://friendsofcancerresearch.org/wp-content/uploads/Assessing_Use_of_ctDNA_Early_Endpoint_Early-Stage_Disease-1.pdf
5. Febbo et al. Minimum Technical Data Elements for Liquid Biopsy Data Submitted to Public Databases. CPT 2020.
6. FDA. Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval, Guidance for Industry. July 2020. from: https://www.fda.gov/media/83507/download
7. Zill OA, Banks KC, Fairclough SR, et al. The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clinical Cancer Research 2018; 24(15): 3528-3538.
8. Zhang Y, Yao Y, Xu Y, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. Nature Communications 2021; 12(11): 1-14.
9. FDA. Use of Circulating Tumor DNA for Early Stage Solid Tumor Drug Development Guidance for Industry. May 2022. Accessed on May 20, 2022 from: https://www.fda.gov/media/158072/download
10. FDA. Use of Circulating Tumor DNA for Early Stage Solid Tumor Drug Development Guidance for Industry. May 2022. Accessed on May 20, 2022 from: https://www.fda.gov/media/158072/download