Use of Circulating Tumor DNA for Curative-Intent Solid Tumor Drug Development

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Oncology Center of Excellence (OCE)
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
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Contains Nonbinding Recommendations

Use of Circulating Tumor DNA for Curative-Intent Solid Tumor Drug Development Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance is intended to help sponsors planning to use circulating cell-free plasma derived tumor DNA (ctDNA) as a biomarker in cancer clinical trials conducted under an investigational new drug application (IND) and/or to support marketing approval of drugs and biological products[2] for treating solid tumor malignancies in the early-stage (curative-intent) setting. This guidance reflects FDA's current thinking regarding drug[2] development and clinical trial design issues related to the use of ctDNA as a biomarker in clinical trials for solid tumor malignancies in the curative-intent setting. Standardization and harmonization of ctDNA assays and methodologies will also be discussed, with a particular focus on assay considerations to assess for molecular residual disease (MRD). Manufacturers interested in developing a specific MRD assay for solid tumors for clinical use should consult the Office of In Vitro Diagnostics in the Center for Devices and Radiological Health (CDRH).

This guidance does not address the use of ctDNA for the early detection of cancer or cancer screening (e.g., situations where cancer has not yet been diagnosed). This guidance also does not address the use of ctDNA in the metastatic cancer setting, although some principles may be applicable across multiple disease settings (e.g., meta-analytical approaches for clinical validation of early endpoints). As the focus of this guidance is on use of ctDNA for drug development, this guidance does not detail development of ctDNA assays solely as in vitro diagnostics to monitor for disease recurrence. Additional information on the related topic on use of minimal residual disease in hematologic malignancies can be found in guidance for industry *Hematologic Malignancies: Regulatory Considerations for Use of Minimal Residual Disease in Development of Drug and Biological Products for Treatment* (December 2020).[3]

[1] This guidance has been prepared by the Oncology Center of Excellence in collaboration with the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER), and the Center for Devices and Radiological Health (CDRH) at the Food and Drug Administration.

[2] For the purposes of this guidance, all references to *drugs* include both human drugs and therapeutic biological products unless otherwise specified.

[3] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Drug development for solid tumors in the early stage, non-metastatic setting, typically involves large trials and multiple years of conduct and follow-up with time-to-event endpoints. Certain patients with early-stage solid tumors can be cured with local therapy alone (e.g., surgery, radiation or chemoradiation), other patients require (neo)adjuvant systemic therapy, and others may progress to metastatic disease despite definitive local therapy and/or systemic therapy. Patients may receive neoadjuvant therapy to improve outcomes of curative-intent local therapies and to prevent metastatic spread of cancer. In the adjuvant treatment setting, patients typically receive curative local therapy followed by systemic treatment to prevent disease recurrence. Better approaches are needed to determine which patients may benefit from (neo)adjuvant and escalated therapy, and which patients are unlikely to benefit and may be spared from unnecessary toxicity.

ctDNA is tumor-derived fragmented DNA shed into a patient's bloodstream that is not associated with cells. Measurement of ctDNA from blood draws offers a minimally invasive approach to obtain information about a patient's cancer. ctDNA as a biomarker has a number of potential regulatory and clinical uses in the early stage setting that may assist and expedite drug development. In the early-stage cancer setting, ctDNA may be used to detect a certain targetable alteration, to enrich a high- or low-risk population for study in a trial, to reflect a patient's response to treatment, or potentially as an early biomarker of efficacy. We will discuss each of these potential uses below.

Multiple small studies have suggested that residual ctDNA detecting molecular residual disease (MRD) after surgery or completion of standard systemic therapy confers a poor prognosis and selects a population at high risk of relapse.[4] However, the evidence to support the clinical validity or clinical utility of ctDNA varies across solid tumor malignancies, patient populations,

[4] Powles, T. et al. ctDNA Guiding Adjuvant Immunotherapy in Urothelial Carcinoma. Nature, 85(2): 114-122; 2021; Tie J, et al. Circulating Tumor DNA Analysis Detects Minimal Residual Disease and Predicts Recurrence in Patients with Stage II Colon Cancer, Sci Transl Med 8(346); 2016; Garcia-Murillas et al. Assessment of Molecular Relapse Detection in Early-Stage Breast Cancer. JAMA Oncol.; 5(10): 1473-1478; 2019; Chaudhuri et al. Early Detectio of Molecular Residual Diseases in Localized Lung Cancer by Circulating Tumor DNA Profiling. Cancer Discovery, 2017; 7: 1394-1403; Christensen et al. Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients with Urothelial Bladder Carcinoma. J Clin Oncol. 2019 Jun 20;37(18):1547-1557; Reinert, H. et al., Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients with Stages 1 to III Colorectal Cancer, JAMA Oncol. 5(8): 1124-1131; 2019; Coombes, P. et al., Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin Cancer Res . Jul 15; 25(14): 4255-4263; 2019; Abbosh B. et al., Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature, 545(7655), 446-451. 2017.

and testing modalities. ctDNA quantity can vary among individuals and depends on the type of tumor, location, stage, tumor burden, and response to therapy. Incorporating ctDNA endpoints into prospective randomized clinical trials is important for further evidence generation to support regulatory and clinical use of ctDNA.

ctDNA assessments can vary among laboratories and technologies used to detect ctDNA which can result in discrepant results. Many clinical laboratories develop their own protocols that can impact ctDNA measurements and detection. Further standardization and harmonization of assays will allow for better use of ctDNA in a regulatory setting and will allow for analyses across studies to validate the use of ctDNA, as described in Sections IV and V of this guidance.

III. DEVELOPMENT OF CTDNA AS A BIOMARKER FOR REGULATORY USE IN EARLY-STAGE SOLID TUMOR CLINICAL TRIALS

Sponsors should consult the FDA if they plan to incorporate ctDNA for patient selection or as an endpoint in early-stage solid tumor clinical trials. The level of evidence to support the use of ctDNA as a biomarker varies depending on the specific use of ctDNA in a clinical trial. The following are potential uses for ctDNA:

A. ctDNA for Patient Selection based on Molecular Alteration:

Sampling a patient's plasma can allow for detection of ctDNA and for potential selection of a patient population harboring genetic or epigenetic alterations that could be targetable by a given drug under study. This may be helpful in the neoadjuvant setting if tumor tissue is unavailable or inadequate for testing. ctDNA may also be used in the adjuvant setting if tissue is unavailable, such as after curative-intent treatment with chemoradiation rather than surgery.

- ctDNA can be used for patient selection for detection of genomic alterations (e.g., EGFR exon 19 deletion) for eligibility criteria for a clinical trial.
- ctDNA identifying specific molecular alterations can also be used as a stratification factor if a trial enrolls both a biomarker-positive and biomarker-negative population. Hierarchical testing procedures with the control of Type-I error rate may allow testing of multiple ordered endpoints in both the intent-to-treat population and biomarker-selected (ctDNA biomarker-positive) subgroup.
- As ctDNA testing may include false negative results, the sensitivity of the ctDNA assay for detecting variants of clinical interest should be evaluated using appropriate Limits of Detection studies. Tumor testing may need to be performed, if feasible, to confirm a negative ctDNA result.

B. ctDNA Molecular Residual Disease for Patient Enrichment:

In the early-stage setting, residual ctDNA after definitive local therapy and/or after (neo)adjuvant therapy is indicative of MRD. ctDNA MRD can be used as a

biomarker to enrich a trial for patients with higher risk disease and increased events of disease recurrence or death.

- ctDNA MRD testing after surgery or (neo)adjuvant therapy could determine study eligibility of a biomarker positive population.
- ctDNA MRD status at baseline could alternatively be used as a stratification factor in a study enrolling both patients who are ctDNA MRD negative and positive. Hierarchical testing procedures could be performed to test both the intent-to-treat population (including both the ctDNA MRD positive and negative group) as well as just the ctDNA MRD positive group.
- ctDNA MRD could be used for treatment optimization, to add on therapy for patients who are at higher risk of disease recurrence (i.e., ctDNA MRD positive) or to de-escalate therapy for patients with lower risk of disease recurrence (i.e., ctDNA MRD negative). Design options could include an escalation design of adding an experimental therapy to standard of care treatment compared to standard of care treatment alone for patients who are ctDNA MRD positive or a de-escalation design for patients who are ctDNA MRD negative. A de-escalation trial might evaluate a novel therapy that has the potential to be less toxic than current standard of care therapy, reduce the length of treatment or dose of standard of care therapy, or eliminate use of standard of care therapy altogether. The clinical trial should be randomized.
- The MRD assay should have high sensitivity and negative predictive value (NPV) for supporting de-escalation of treatment and high specificity and positive predictive value (PPV) for supporting escalation of treatment.
- The primary endpoint should be disease-free survival (DFS) if only adjuvant therapy is given or event-free survival (EFS) if neoadjuvant therapy is given (with or without adjuvant therapy), or overall survival (OS).[5] If DFS or EFS is the primary endpoint, there should be no evidence of detriment to OS based on a prespecified analysis plan.
- There should not be any early interim efficacy analyses of the primary endpoints due to limited events. Later interim analyses may be considered however these should be prespecified near the start of the trial, adjusted for the multiple testing and set at a reasonable point with robust data maturity. For example, it would be expected that most patients should have completed treatment prior to any interim analyses being conducted.

[5] See guidance for industry *Clinical Trials Endpoints for the Approval of Cancer Drugs and Biologics* (December 2018).

C. ctDNA as a Measure of Response for Drug Development:

Changes in ctDNA levels could be used in early phase clinical trials to aid in signal finding of drug activity and to potentially aid sponsors in their drug development plans. Preliminary signals correlating a change in levels or clearance of ctDNA may inform the design of future randomized trials which incorporate both ctDNA endpoints along with time-to-event efficacy outcome measures.

- Early in clinical drug development, changes in ctDNA levels could be used to estimate antitumor activity of an investigational therapy either alone or in addition to information from imaging assessments.
- Monitoring changes in ctDNA levels may provide relevant clinical data that may be used in conjunction with other clinical data, relevant nonclinical data, and dose- or exposure-response relationships to select an optimized dosage(s) for subsequent clinical trials.[6]
- FDA encourages sponsors to develop evidence regarding the usefulness of ctDNA response in addition to or supporting pathologic complete response information after neoadjuvant therapy.

D. ctDNA as an Early Endpoint in Clinical Trials:

Although not currently validated for use, changes in ctDNA in response to a drug may have the potential to be used as an early endpoint to support drug approval. Changes in ctDNA or ctDNA clearance may be particularly useful as an early endpoint in the early-stage, curative-intent cancer setting. Unlike the metastatic disease setting, clinical trials in the (neo)adjuvant disease setting after definitive local therapy cannot use imaging-based, tumor outcomes such as overall response rate to measure response to an investigational therapy.

- Further data from prospective randomized clinical trials incorporating ctDNA endpoints are required to support the use of ctDNA as an endpoint reasonably likely to predict long term outcomes (DFS/EFS/OS).
- Trials that collect ctDNA data before and after drug treatment should also collect long term outcome data to characterize the association between the reduction or clearance of ctDNA and long term outcome.
- Quantitative ctDNA analysis and multiple ctDNA time points are generally recommended and can enable more robust readouts.
- Various statistical criteria have been proposed for validating an endpoint and often meta-analytical approaches have been used. [7] An appropriate meta-analysis to validate ctDNA at a trial level association

[6] For additional information on dose optimization in oncology clinical trials, see the draft guidance for industry *Optimizing the Dosage of Human Prescription Drugs and Biological Products for the Treatment of Oncologic Diseases* (January 2023). When final, this guidance will represent FDA's current thinking on this topic.

[7] For additional information on meta-analyses, see the draft guidance for industry *Meta-analyses of Randomized Controlled Clinical Trials to Evaluate the Safety of Human Drugs or Biological Products* (November 2018). When final, this guidance will represent FDA's current thinking on this topic.

should include only randomized trials. Sponsors should discuss and provide details of any proposed meta-analysis plan to validate use of ctDNA in a particular context of use with the FDA.

- The plan should include details of trial designs, inclusion and exclusion criteria, ctDNA assessment methods, and disease setting. A justification for the suitability of pooling the studies should be provided and should consider tumor histology, stage of disease, drug class (e.g., chemotherapy, immunotherapy, targeted therapy), and other important variables.
- Trials should include a patient population representative of the population in which the endpoint ultimately will be used.
- An adequate number of randomized trials with sufficient follow-up time should be included and justified.
- Analysis based on individual patient-level data should allow an assessment of individual-level association.
- Prespecified criteria for concluding association based on both trial-level and individual-level association measures, including prespecified timing and window of ctDNA assessment should be provided.
- Long term clinical endpoints, such as EFS/DFS and OS that have been clearly and consistently defined across studies should be included.
- Sponsors should explore the effects of missing data on trial results.

IV. ASSAY CONSIDERATIONS

ctDNA assays should be analytically validated and have sufficient performance characteristics prior to using in clinical trials. An essential part of developing ctDNA for regulatory use in oncology clinical trials is standardization and harmonization of assays and methodologies. Although the guidance discusses various ctDNA applications in early-stage solid tumor drug development, this section focuses on assay considerations for MRD applications.

A. Types of Molecular Residual Disease Assays

ctDNA MRD assays can utilize tumor-informed methods, tumor-naïve methods, or a smaller panel of candidate genes and/or multi-omics biomarkers, each with its own strengths and limitations. As MRD applications and technologies used are novel, the choice of the method selected should consider the intended use and user needs. These assays need to have adequate performance to achieve the necessary sensitivity and specificity to support the intended use. Section IV of this guidance provides examples of MRD assays, but does not represent an exhaustive list of technologies or approaches, or their strengths and limitations.

- Tumor-informed approaches are constructed by sequencing the tumor and then selecting a set of variants to follow. Tumor-informed panels

may not detect emerging mutations after tumor sampling or reversions to a wild-type state.
    - An advantage of this approach is that it may lead to higher specificity.
    - Limitations of this approach include lag time between tumor testing and ctDNA panel creation, and sensitivity and specificity may depend on clinical cutoffs and analytical sensitivity of the device as well as the number of tumor-informed targets assayed.
    - It is important to consider the reliability of the variants selected by a tumor-informed panel. Tumor-informed panels may be more reliable from tumors with higher tumor mutation burden and with highly characterized variants (i.e., mutation hotspots) compared to panels from tumors with lower mutation burden and with variants that are not well characterized.
- Tumor-naïve or "tumor-agnostic" panels are generic panels that are not informed by sequencing or by specific mutations known to be present in the primary tumor. This approach uses panel-based next generation Sequencing (NGS) to ascertain MRD.
    - An advantage of this approach is faster turn-around time.
    - Limitations include tumor biomarkers not covered by the ctDNA panel. Additional characterization of panels would be needed to understand what percentage of patients are trackable with such techniques.
    - Whole genome sequencing (WGS) could potentially be used in a tumor-naïve fashion. This would allow the use of various biomarkers including but not limited to mutations, epigenetic alterations (e.g., methylation) or fragmentomic analysis of ctDNA to capture tumor derived ctDNA signals.

Multiple biomarkers on a candidate gene panel could help assure that the MRD assay will serve its function, even with the development of additional cytogenetic changes.

B. Sampling Considerations

There are several sampling considerations related to the clinical trial design and the intended use patient population that should be taken into account.

- The shedding of ctDNA is affected by histology, grade, stage, and size of the tumor thus timing of ctDNA testing should be discussed with the FDA and should be supported by performance characteristics of the test, disease characteristics and tumor biology.
- A set time point(s) should be chosen for enrollment into the study and prespecified.
- If a sponsor wishes to use multiple ctDNA time points to determine eligibility (e.g., screening paradigm evaluating if intervention at early detection of recurrence would influence outcome) this should be

supported by scientific data/rationale. Sensitivity analyses based on different time windows could be explored (but should be predetermined and discussed in advance).

- The timing of ctDNA sample collection should be the same across study arms.
- A baseline pre-treatment blood sample should be collected to allow for consideration of the impact of variation in tumor shedding rates on assay performance. In addition, this sample will allow for interpretation of the post-treatment sample for study enrollment.
- All sites in the study should follow standardized protocols for sample collection, storage, and processing and handling. Pre-analytical factors that could impact background DNA levels as well as ctDNA levels should be controlled as they can impact the sensitivity and specificity of the test.

C. Assay analytical validation considerations for marketing applications

Analytical validation ensures that the assay measures the analyte or analytes that are intended to be measured in the intended tumor type. Analytical validation should be conducted to establish the performance characteristics of the assay. Validation studies should be acceptable in terms of the assay's sensitivity, specificity, accuracy, precision, and other relevant performance characteristics using a specified technical protocol, which may include specimen collection, handling, and storage procedures.[8] The acceptance criteria for the validation studies should be adequately justified to support clinical use.

- MRD assay validation should encompass the entire assay system from sample collection (e.g., blood collection in the specific collection tube that will be used with the final market ready assay) to the output of the assay including the detection threshold (cut-off) that determines positive versus negative patients. The cut-off should be prespecified and optimized to minimize misdiagnosis based on the intended use clinical setting. Additionally, the distribution of results observed from testing specimens from patients should be considered to determine the potential for misdiagnosis based on the imprecision of the assay. For tests that do not use a cut-off, positive result reporting should be demonstrated to be above the noise of the assay and evidence that a positive result reported below the detection limit is accurate should be provided.
- The predefined assay cutoff and the predefined specific time windows should be established to optimize assay sensitivity and specificity for the clinical trial use. Analytical performance should be robust to detect MRD positivity accurately and reproducibly.

[8] For example, see the Summary of Safety and Effectiveness Data (SSED) for the Guardant360 CDx PMA P200010: https://www.accessdata.fda.gov/cdrh_docs/pdf20/P200010B.pdf

- The validation approach of an MRD test will depend on the type of MRD testing modality. As noted in section IV A., there are different types of MRD testing approaches that are currently under development. For tumor-naïve NGS-based MRD panels, panel-based validation of fixed panel content will be needed; however, for tumor-informed NGS-based personalized panels, the panel content will vary for each patient and therefore the assay validation will be based on each personalized assay. We recommend that the validation of a representative set of personalized assays should include different variant types, classes, numbers, and distributions seen in the intended use population. The validation strategy to support the device marketing application should be discussed with CDRH/FDA.
- Clinical specimens are recommended to be used for key assay validation studies such as confirmation of the assay limit of detection (LoD), assay precision, analytical accuracy, and assay input studies. For analytical accuracy, samples from pivotal clinical trials should be included. In some analytical validation studies since a large volume of sample will be needed, clinical samples may be supplemented by contrived samples. In general, when using contrived samples in assay validation studies, the functional equivalency between the contrived and clinical samples should be demonstrated and rationale should be provided. The detectability of variants across the assay range should be shown to be equivalent between contrived and clinical specimens.
- For fixed panels, cell lines carrying the specific alterations (i.e., cell line DNA spiked into an appropriate matrix), representing fragmented tumor DNA, may be used as contrived samples. For personalized assays, cell lines that represent a distribution of the number and type of variants based on early clinical study data should be developed.
- Assay precision should be demonstrated using samples across the detection range of the assay including samples at the assay cutoff and samples with the minimum analyte requirements.
- An appropriate set of reference materials should be developed to allow for comparability across multiple MRD assays. Collaborative efforts with standards organizations (e.g., National Institute of Standards and Technology, World Health Organization) and multiple stakeholders may be needed.
- Sponsors should discuss assay analytical validation plans with CDRH through a Q-Submission.[9]

[9] See guidance for industry and FDA staff *Requests for Feedback and Meetings for Medical Device Submissions: The Q-Submission Program* (January 2021).

V. INVESTIGATIONAL DEVICE CONSIDERATIONS

- An investigational ctDNA device used for enrollment or for treatment decision-making in a trial is subject to FDA's investigational device exemption (IDE) regulations as well as 21 CFR parts 50 and 56.[10]
- Whether a sponsor needs to submit an IDE application is dependent on whether the device used in the trial is considered significant risk (SR), non-significant risk (NSR), or exempt.[11]
- Sponsors can submit a Study Risk Determination pre-submission through CDRH's Q-submission program.
- Sponsors may also seek a risk determination through the optional streamlined submission process for investigational devices in oncology trials for new INDs.[12]

[10] See 21 CFR 812.
[11] See guidance for industry *Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors. Significant Risk and Nonsignificant Risk Medical Device Studies* (January 2006).
[12] See guidance for industry *Investigational In Vitro Diagnostics in Oncology Trials: Streamlined Submission Process for Study Risk Determination* (October 2019).