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Regulatory implications of ctDNA in immuno-oncology for solid tumors

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ABSTRACT

In the era of precision oncology, use of circulating tumor DNA (ctDNA) is emerging as a minimally invasive approach for the diagnosis and management of patients with cancer and as an enrichment tool in clinical trials. In recent years, the US Food and Drug Administration has approved multiple ctDNA-based companion diagnostic assays for the safe and effective use of targeted therapies and ctDNA-based assays are also being developed for use with immuno-oncology-based therapies. For early-stage solid tumor cancers, ctDNA may be particularly important to detect molecular residual disease (MRD) to support early implementation of adjuvant or escalated therapy to prevent development of metastatic disease. Clinical trials are also increasingly using ctDNA MRD for patient selection and stratification, with an ultimate goal of improving trial efficiency through use of an enriched patient population. Standardization and harmonization of ctDNA assays and methodologies, along with further clinical validation of ctDNA as a prognostic and predictive biomarker, are necessary before ctDNA may be considered as an efficacy-response biomarker to support regulatory decision making.

INTRODUCTION

Circulating tumor DNA (ctDNA) is an increasingly important biomarker being developed for the diagnosis and management of patients with cancer and is emerging as a regulatory tool to support efficient clinical trials and drug development. Measurement of ctDNA offers a minimally invasive approach to obtain valuable information about a tumor without having to rely on tissue biopsies.[1] There are a number of clinical uses of ctDNA in development including for the diagnosis of cancer, to assess prognosis, and guide treatment decisions.[2 3] In clinical trials, ctDNA may be used for patient selection based on detection of alterations for eligibility in a clinical trial, to identify high-risk and low- risk study populations, and to assess response to anticancer therapies.[4] Although not currently validated for use, ctDNA also has potential as an early endpoint in clinical trials to support drug approvals.

To date, several ctDNA-based companion diagnostic (CDx) assays have been approved by the US Food and Drug Administration (FDA) as essential for the safe and effective use of a number of targeted therapies, primarily for the treatment of patients with metastatic cancers (table 1).

The earliest approvals were for PCR-based devices, such as Cobas EGFR Mutation Test V.2, for *EGFR* exon 19 deletions or exon 21 L858R mutations, for selection of patients with non-small cell lung cancer (NSCLC) for treatment with specific *EGFR* tyrosine kinase inhibitors. This was followed by approval of another PCR-based device, the *therascreen PIK3CA* RGQ PCR Kit, for identification of 11 *PIK3CA* mutations in ctDNA for the treatment of patients with breast cancer with alpesilib. Following the approval of these targeted assays, next-generation sequencing (NGS)-based liquid biopsy panels such as the Guardant360 CDx and F1 Liquid CDx tests were approved as CDx as well as for tumor profiling for certain indications listed in table 1. With major breakthroughs in the metastatic setting, targeted therapies along with ctDNA tests are increasingly being developed for earlier stage cancers as well. And while the initial approvals of ctDNA tests have been for targeted therapies directed at oncogenic driver mutations, ctDNA also holds promise to guide treatment decision-making for immuno-oncology (IO)-based therapies and may lead to future FDA approvals of CDx tests for immunotherapies. There are several potential uses of ctDNA for IO that may be used for patient management in the clinical setting but have yet to be validated. Future FDA approvals of ctDNA-based CDx tests for immunotherapies for specific intended uses would require thorough analytical and clinical validation.

Over the past decade, immune checkpoint inhibitors have led to a paradigm shift for the treatment of numerous cancers with over 85 FDA-approvals for antibodies directed against programmed death 1 (PD-1) or programmed

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Table 1 FDA approvals of circulating tumor DNA-based companion diagnostics

Device	Cancer type	CDx biomarker(s)	Drug(s)
Cobas EGFR Mutation Test v2 PCR test	NSCLC	EGFR exon 19 deletions or exon 21 L858R mutations	Group labeling claim for FDA approved TKIs Tarceva (erlotinib), Tagrisso (osimertinib, Iressa (gefitinib)
		Exon 20 T790M substitution mutation	Tagrisso (osimertinib)
therascreen PIK3CA RGQ PCR Kit PCR test	Breast Cancer	11 mutations in PIK3CA	Piqray (alpelisib)
Guardant360 CDx Test NGS test	NSCLC	EGFR exon 19 deletions, L858R, and T790M	Tagrisso (osimertinib)
		EGFR exon 20 insertions	Rybrevant (amivantamab-vmjw)
		KRAS G12C	Lumakras (sotorasib)
F1 Liquid CDx Test NGS test	NSCLC	EGFR exon 19 deletions, L858R	Iressa (gefitinib), Tagrisso (osimertinib), Tarceva (erlotinib)
		MET exon 14 SNVs and indels lead to skipping	Tabrecta (capmatinib)
		ALK	Alecensa (alectinib)
	Prostate Cancer	BRCA1, BRCA2	Rubraca (rucaparib)
		BRCA1. BRCA2, ATM	Lynparza (olaparib)
	Ovarian Cancer	BRCA1, BRCA2	Rubraca (rucaparib)
	Breast Cancer	11 mutations in PIK3CA	Piqray (alpelisib)

CDx, companion diagnostic; FDA, Food and Drug Administration; indel, insertion-deletion; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; SNV, single-nucleotide variant; TKI, tyrosine kinase inhibitor.

death ligand (PD-L1).[5] Similar to targeted therapies, anti-PD-1 and anti-PD-L1 (anti-PD-(L)1) antibodies were initially developed for the treatment of advanced or metastatic disease but more recently are also gaining FDA approvals for the treatment of earlier stage disease.[6–14] Although immune checkpoint inhibitors are often well tolerated, some patients will experience serious and life-threatening toxicities from this class of drugs. Furthermore, some patients with non-metastatic tumors may be cured after local therapy (eg, surgery, radiation, or chemoradiation) with or without neoadjuvant or adjuvant ((neo)adjuvant) chemotherapy, and additional treatment with anti-PD-(L)1 therapy may only increase risk of toxicity without improving long-term clinical outcomes. Thus, more data are needed to determine which patients are most likely to gain benefit from (neo)adjuvant anti-PD-(L)1-based therapy and how long they should receive such therapy.

In the early-stage setting, residual ctDNA after definitive local therapy is indicative of molecular residual disease (MRD) and can be used to identify patients at highest risk of recurrent or metastatic disease. Across numerous studies and tumor types, the presence of ctDNA after curative intent therapy is associated with worse disease-free survival (DFS).[15–25] While residual ctDNA is associated with a poor prognosis, MRD may also be harnessed to identify those most likely to benefit from additional therapy, including immunotherapy, or for those without residual ctDNA to select patients appropriate for de-escalation. In these early studies, the positive predictive value (PPV) of MRD after definitive treatment has been uniformly high and the negative predictive value (NPV) has been reasonable. Repeat testing at multiple time points, can serve to increase the aggregate NPV of the composite MRD negative call. ctDNA as a biomarker of MRD is also being used to select for patients with a high risk of recurrence in multiple ongoing, prospective clinical trials. As patients with MRD have increased events of disease recurrence, this enrichment strategy may allow for more efficient clinical trials in a more homogeneous patient population with smaller sample sizes and shorter study periods.

There are also important clinical uses of ctDNA in the metastatic setting, which include monitoring tumor evolution, evaluating for mechanisms of treatment resistance, and deciding when to switch anticancer therapies. Longitudinal monitoring of ctDNA has also demonstrated potential to differentiate pseudoprogression from true progression for patients with metastatic melanoma being treated with anti-PD-1 antibody therapy alone or in combination with ipilimumab, an anti-CTLA-4 antibody.[26]

In this review, we will discuss uses of ctDNA for solid tumors in the early-stage and advanced disease settings, with particular emphasis on IO-based therapies. We will review the evidence supporting ctDNA as a prognostic biomarker and enrichment tool for clinical trials. While evidence is currently lacking, we will also review FDA guidance documents explaining what data must be generated

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to validate ctDNA as a marker of MRD for potential use as an early endpoint for regulatory decision making. We will also review emerging ctDNA-based biomarkers relevant to IO, including tumor mutation burden (TMB), and examine concordance with tissue-based assays. Importantly, we will highlight the strengths and limitations of different diagnostic assays and platforms for ctDNA tests, and discuss efforts to standardize ctDNA collection, processing, and analysis. Using ctDNA for the early detection of cancer or for cancer screening is not within the scope of this article but are reviewed elsewhere and are important areas of potential future use.[27]

CLINICAL APPLICATIONS OF CTDNA FOR PATIENTS WITH EARLY-STAGE DISEASE

ctDNA as a prognostic biomarker

In multiple small studies, the presence of ctDNA after curative intent therapy (eg, surgical resection or chemoradiotherapy) has been associated with worse DFS for early-stage cancers in numerous tumor types.[15–25] A single ctDNA test as early as 2 to 4 weeks after surgery, may detect MRD and select for a high-risk population of patients.[15] Further, assessment at serial time points every few months may improve the sensitivity of detection of MRD.[15 16] Across solid tumors, the sensitivity of ctDNA-based detection of MRD in the adjuvant setting has been reported at 80% to 100% with specificity ranging from 88% to 100% with serial testing.[15 20 22 28–33] Detection of MRD with ctDNA is also associated with median lead times ahead of radiographic or clinical disease recurrence of 1.7 to 18.9 months.[15 16 18 20 22 24 25 28–36]

Clearance of ctDNA after radiotherapy for treatment of virally mediated head and neck cancers has also been identified as a good prognostic biomarker. For patients with Epstein-Barr virus (EBV)-associated advanced nasopharyngeal carcinoma, clearance of EBV ctDNA 1 week after completion of radiotherapy has been associated with improved relapse-free survival and overall survival (OS) compared with patients with persistently detectable plasma EBV DNA.[37] More recently, rapid clearance of circulating plasma human papilloma virus (HPV) Type 16 DNA by week four of definitive chemoradiotherapy has also been associated with improved relapse-free survival for patients with HPV-associated oropharyngeal squamous cell carcinoma.[38]

While collectively these data demonstrate the prognostic potential of ctDNA in solid tumors after definitive surgery or chemoradiation, ctDNA as a prognostic biomarker has not been formally validated. There are also notable limitations to these small studies and standardization of testing is needed. Timing of sampling, length of follow-up, propensity of tumors for ctDNA shedding, and assay characteristics and performance (discussed below) impact the sensitivity of ctDNA for MRD detection. In addition, for cancers with metastatic relapse restricted to the brain, ctDNA may not be detectable prior to or at the time of radiographic disease recurrence due to poor entry of ctDNA across the blood brain barrier into circulation.[15 23] The specificity of ctDNA for radiographic disease recurrence is also impacted by length of follow-up, and lead time measurements similarly depend on timing of ctDNA collection in relationship to imaging assessments and intervals between scans. Furthermore, the kinetics of ctDNA clearance likely varies between tumor type, stage and other baseline characteristics of disease, and extent and modality of curative-intent therapy (ie, surgery vs chemoradiotherapy). Nonetheless, these data overall support the incorporation of ctDNA for certain tumor types into prospective, interventional clinical trials including in IO, with multiple potential regulatory uses for ctDNA as a biomarker.

ctDNA for patient selection

In the (neo)adjuvant treatment setting, detection of genetic and epigenetic alterations in ctDNA can select for a patient population for clinical trial enrolment.[4] One advantage for enrolment based on ctDNA-based assays is if available tissue is limited for testing and would otherwise require an additional, invasive biopsy. However, it is important to consider potential discordance between tissue-based and plasma-based assays, which may effectively select for different patient populations. In fact, a key limitation of currently FDA-approved ctDNA-based assays is that a substantial proportion of patients whose tumors are mutation-positive in tissue-based testing are not detected. This low sensitivity has led to the addition of limitation language in ctDNA CDx approvals to 'reflex' ctDNA test negative results to tissue biopsy. The cause for these false negative results from plasma-based assays may be linked to the amount of ctDNA shedding from the tumor or limitations of device performance.

For IO-based therapies, identification of MRD is a major potential use of ctDNA-based assays for the selection of patients for clinical trial enrolment (discussed in the next section). Other possibilities for patient selection for IO-based trials include an assessment of TMB-status with adequate analytical and clinical validation (ie, TMB-high vs TMB-low ctDNA), and identification of mutations that may predict for primary or acquired resistance to immune-checkpoint blockade (eg, mutations in *STK11*). While these biomarkers may be used for early-stage cancers, they may also be used in the metastatic setting and are broadly discussed across tumor stages later in the article.

ctDNA MRD for patient enrichment

Given the natural history of early-stage cancers and longer life expectancy of patients, barriers to drug development in the non-metastatic setting include the requirement for large trials conducted over an extended amount of time to obtain long-term survival data. However, ctDNA after definitive therapy can be used as a marker of MRD to enrich a trial for patients with higher risk disease and increased events of disease recurrence or death.[4] A potential trial design is obtaining a baseline ctDNA sample

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prior to any neoadjuvant therapy or surgery followed by another assessment for ctDNA after surgery. ctDNA status after surgery could be used as a stratification factor in a study enrolling patients both negative and positive for ctDNA. Patients would then be randomized to receive standard of care (eg, adjuvant therapy or observation) with or without an investigational therapy (figure 1A). Hierarchical testing with control of the type I error rate may allow multiple ordered endpoints in the intention-to-treat (ITT) population (all comers), including both the ctDNA positive and negative subgroups, as well as just the ctDNA-positive subgroup. Another alternative using this design, would be to treat the ctDNA-positive and ctDNA-negative cohorts as separate trials. If there is no intention to analyze data across ctDNA-positive and ctDNA-negative cohorts, this traditionally would not require type I control across both studies.

An enrichment strategy that builds off this approach would be to study an investigational therapy only in the group of patients who are biomarker positive (figure 1B). As patients who are ctDNA-positive are expected to be a higher risk population, increased event rates are anticipated which would allow for smaller patient sample sizes and shorter trials in a more homogeneous patient population. An adaptive enrichment strategy is another variation of this design, which initially includes a ctDNA-negative arm, which could be closed at an interim analysis for futility (figure 1C). Yet another potential trial design is to obtain a ctDNA sample after curative-intent therapy and at the completion of standard adjuvant therapy. Patients with ctDNA-positive disease after adjuvant therapy could then be randomized to an investigational therapy or observation (figure 1D). Assessment for genetic alterations may also be incorporated to inform the selection of the investigational therapy. Another potential use of ctDNA is for enrolment into a de-escalation trial by identifying patients who are negative for ctDNA and have potentially lower risk disease, with an ultimate goal of reducing unnecessary and potentially toxic adjuvant therapies. Patients who are negative for ctDNA after curative intent therapy may be randomized to standard-of-care therapy versus a de-escalated regimen (figure 1E). As the NPV for ctDNA improves with repeat testing, serial testing for ctDNA may be performed to allow patients receiving de-escalated therapy to crossover to receive standard-of-care therapy if patients become ctDNA positive.

For enrichment trials based on ctDNA MRD, the preferred primary endpoints are DFS for trials in the adjuvant setting, event-free survival (EFS) if neoadjuvant therapy is given (with or without adjuvant therapy), or OS.[4] Due to limited events and as the treatment effect may not be robust, early interim analyses may not provide an accurate estimate of the treatment effect size. However, later interim analyses which are pre-specified, adjusted for multiple testing, and planned to occur after most patients are expected to have completed treatment may provide more reasonable estimates of the treatment effect.

The IMvigor010 (NCT02450331) clinical trial was a randomized study of adjuvant atezolizumab versus observation after complete resection in 809 patients with urothelial carcinoma. The trial did not reach its primary efficacy endpoint of DFS nor show a significant benefit in OS in the ITT population.[39] However, in a post hoc exploratory analysis of 214 patients who were positive for ctDNA, DFS and OS were improved in the atezolizumab arm versus the observation arm (DFS HR 0.58, 95% CI 0.43 to 0.79; OS HR 0.59, 95% CI 0.41 to 0.86).[40] Based on these results, IMvigor011 (NCT04660344) was designed as a follow-up, prospective study of adjuvant atezolizumab versus observation enriched for only patients who are ctDNA positive after cystectomy.[41 42] In addition to these studies in urothelial carcinoma, there are ongoing, prospective studies across multiple tumor types of IO-based therapies using ctDNA MRD status for patient selection[42–47] (table 2). For some studies, eligibility is limited to patients who are ctDNA-positive after curative-intent therapy[42 44 45] while other trials use postoperative ctDNA MRD status to assign patients to adjuvant therapy.[43 46 47]

One example of this paradigm, although not an IO study, is the DYNAMIC (ACTRN12615000381583) clinical trial in which ctDNA MRD was used to refine patient selection for adjuvant treatment in stage II colon cancer.[48] Adjuvant chemotherapy after resection of stage II colon cancer is not routinely recommended for patients who are not in a high-risk subgroup as the potential benefit is low.[49] Although the presence of ctDNA after surgery for stage II colon cancer has predicted for high risk of disease recurrence,[16] expert consensus guidelines have thus far not recommended use of ctDNA when making treatment decisions regarding adjuvant chemotherapy.[49] To prospectively evaluate the use of ctDNA, the DYNAMIC trial randomized 455 patients with stage II colon cancer to have adjuvant chemotherapy treatment decisions guided by either ctDNA results or standard clinicopathological features. In the ctDNA-guided group, patients with a positive ctDNA result at 4 or 7 weeks after surgery were treated with oxaliplatin-based or fluoropyrimidine chemotherapy while patients who were negative for ctDNA did not receive any chemotherapy. In total, 15% of patients in the ctDNA-guided group received adjuvant chemotherapy compared with 28% of patients in the standard-management group (relative risk 1.82; 95% CI 1.25 to 2.65). Despite lower rates of adjuvant chemotherapy in the ctDNA-guided group, the primary endpoint of 2-year recurrence-free survival was not compromised in the ctDNA-guided group compared with the standard management group (93.5% vs 92.4%, respectively; absolute difference 1.1%; 95% CI −4.1% to 6.2% (non-inferiority margin: −8.5%)).

ctDNA as an efficacy-response biomarker

ctDNA may be used in early phase clinical trials to assist in evaluating antitumor response for investigational agents, but also has future potential as an early endpoint to support drug approval. In early phase trials, ctDNA can

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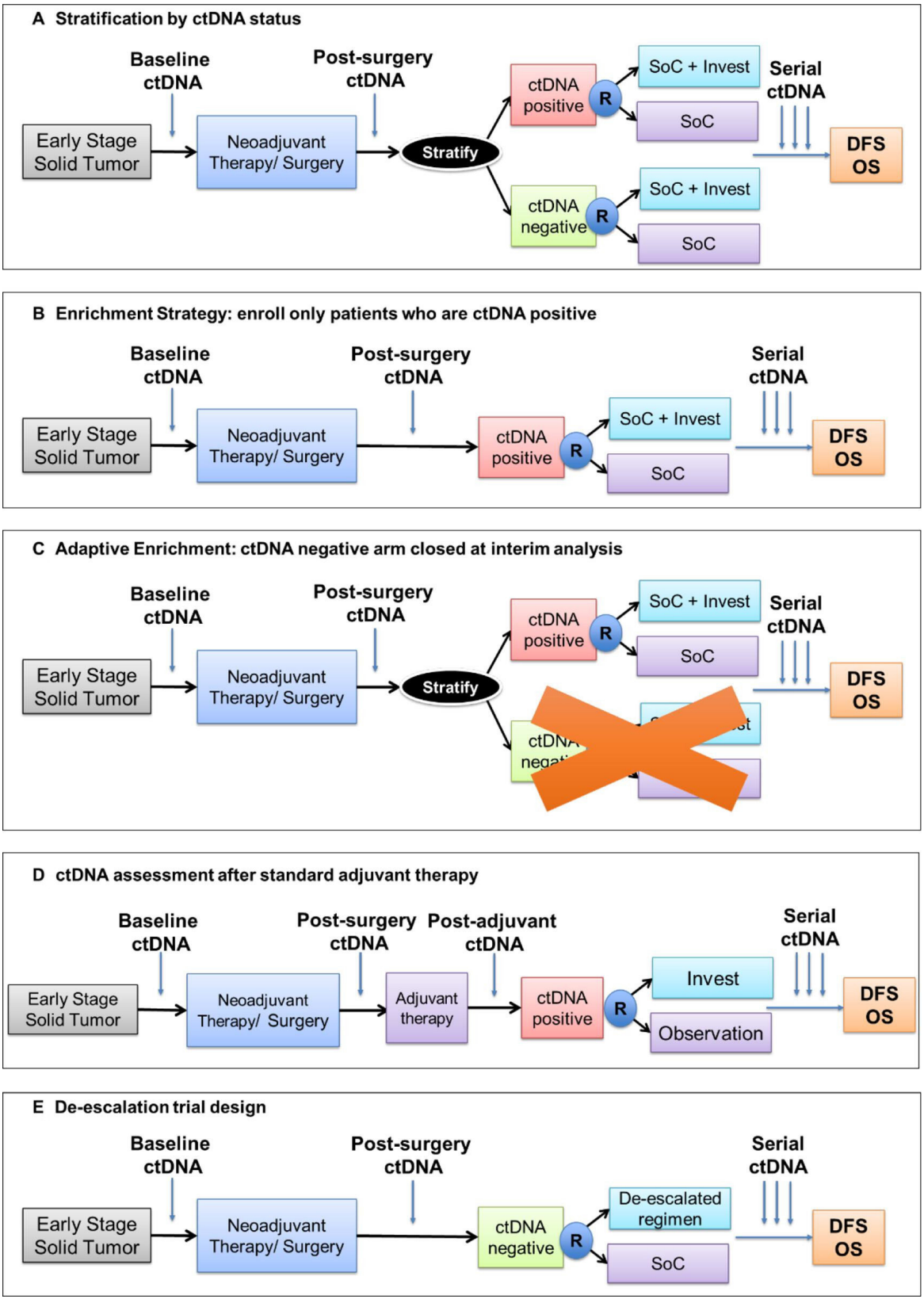

Figure 1 Use of ctDNA molecular residual disease as an enrichment tool in clinical trials. (A) ctDNA status after surgery can be used as a stratification factor, enrolling patients both negative and positive for ctDNA. (B) An enrichment strategy is to only enroll patients who are positive for ctDNA and may have higher risk disease. (C) An adaptive enrichment strategy is to include a ctDNA-negative arm that may be closed at an interim analysis for futility. (D) Patients with ctDNA-positive disease after curative-intent surgery and standard adjuvant systemic therapy may be randomized to an investigational therapy or observation. (E) Patients who are negative for ctDNA after curative-intent surgery may be randomized to standard-of-care (SoC) adjuvant therapy versus a de-escalated regimen. ctDNA, circulating tumor DNA; DFS, disease-free survival; OS, overall survival.

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Table 2 IO-based randomized trials using ctDNA MRD for patient selection, treatment decisions, or as an early endpoint

Trial and disease	Study arms	Primary endpoint(s)	Use of ctDNA
c-TRAK TN NCT03145961 Moderate or high-risk early-stage TNBC	Adjuvant pembrolizumab versus observation	ctDNA by 12 months; ctDNA by 24 months; absence of ctDNA/ DFS at 6 months	Assign treatment: ctDNA-positive within 12 months randomized to pembrolizumab or observation; ctDNA-negative observed
MERMAID-1 NCT04385368 NSCLC stage II, III	Adjuvant durvalumab+SOC chemotherapy versus placebo+SOC chemotherapy	DFS	Inclusion criteria: ctDNA-positive after surgery
MERMAID-2 NCT04642469 NSCLC stage II, III	Adjuvant durvalumab versus placebo	DFS	Inclusion criteria: ctDNA-positive during 96-week surveillance period
IMvigor011 NCT04660344 Muscle-invasive bladder cancer	Adjuvant Atezolizumab versus chemotherapy	DFS	Inclusion criteria: ctDNA-positive within 20 weeks after cystectomy
DETECTION NCT04901988 Melanoma stage IIB/C	Adjuvant nivolumab versus observation	OS	Assign treatment: ctDNA-positive treated with nivolumab
SCION NCT04944173 NSCLC stage I	Durvalumab+SABR (ctDNA-negative) versus durvalumab+SABR (ctDNA-positive) versus Durvalumab+SABR followed by eight additional cycles of Durvalumab (ctDNA-positive)	DFS	Assign treatment: ctDNA-positive randomized to either no further therapy or eight additional cycles of durvalumab

ctDNA, circulating tumor DNA; DFS, disease-free survival; IO, immuno-oncology; MRD, molecular residual disease; NSCLC, non-small cell lung cancer; SABR, stereotactic ablative radiotherapy; SOC, standard of care; TNBC, triple negative breast cancer.

aid in signal finding and estimating antitumor activity for an investigational therapy, and to help researchers refine drug development strategies. For example, a preliminary signal correlating a change in levels or clearance of ctDNA with treatment response such as overall response rate (ORR) may inform the design of future randomized trials to use ctDNA endpoints along with long-term efficacy outcome measures. For neoadjuvant therapies, ctDNA response can also be correlated with pathologic complete response (pCR) information and response per radiographic imaging assessments.[50] Serial measurements of ctDNA at baseline, during neoadjuvant therapy (if applicable), after curative intent therapy, and during and after adjuvant therapy (if applicable) would allow for exploration of the utility of ctDNA as a measure of treatment response. Such ctDNA measurements can help assess the prognostic value of ctDNA, correlate ctDNA with treatment response (eg, ORR, pCR), and identify patients who may benefit from additional adjuvant therapy.

An ultimate goal is to validate ctDNA as an early endpoint that may support a drug approval. However, ctDNA endpoints alone are not currently validated or sufficient to support approval of a drug marketing application. Data generation is essential for potential future use of ctDNA endpoints as the basis of a drug approval. Although many trials have incorporated ctDNA measurements as exploratory endpoints, fewer ongoing trials for IO-based therapies have included formal testing of ctDNA endpoints with type I error control. Inclusion of alpha-controlled ctDNA endpoints in randomized trials, along with time-to-event efficacy endpoints, are important to generate data required to support its use more broadly as an early endpoint reasonably likely to predict long term outcomes (eg, DFS, EFS, and OS).

For validation, a single trial correlating ctDNA endpoints with long-term clinical outcomes is insufficient to establish ctDNA as an endpoint that can be used as the sole basis for future drug approvals even in the same disease setting (ie, for the same tumor type, stage of disease, type of therapy, patient population) However, such data from a single trial could provide evidence for use of ctDNA in future trials, and multiple trials correlating ctDNA with long-term outcomes would further strengthen its use as supportive evidence for a marketing application.

Meta-analyses of multiple large, randomized trials can help establish ctDNA as an early endpoint that can be used as the basis for a drug approval. As there may be important differences in the relationship between ctDNA endpoints and long-term clinical outcomes depending on the tumor type, stage of disease, type of therapy, and other variables, well-designed meta-analyses should include clinical trial data from patients that define a distinct population with similar baseline characteristics. Based on current available data, validation of ctDNA endpoints in one tumor type and disease setting generally could not be extrapolated for use of ctDNA for regulatory approval in another disease setting.

To validate ctDNA as an early clinical endpoint, an association should be correlated with long-term clinical outcomes at both the patient and trial level in a meta-analysis.[51 52] Patient-level correlation may involve conducting a responder analysis of patients based on ctDNA status using the Kaplan-Meier method to estimate survival (ie, EFS, DFS and OS). At the patient level,

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Box 1 Meta-analyses to validate circulating tumor DNA (ctDNA) as an early endpoint

Considerations for using meta-analyses to validate ctDNA as an early endpoint

- ⇒ Discuss planned meta-analyses with the Food and Drug Administration prior to their initiation.
- ⇒ For validation at the trial level, meta-analyses should only include randomized trials with sufficient follow-up time.
- ⇒ The meta-analysis plan should clearly outline the particular context of use of ctDNA, and include details regarding the trial designs and ctDNA assessment methods.
- ⇒ Justification for pooling studies and patient populations should be provided, and should consider tumor biology and natural history of the disease, disease setting, eligibility criteria, and drug class of the investigational therapy.
- ⇒ The patient population in pooled trials should be representative of the population in which the endpoint is planned for use.
- ⇒ The ctDNA devices across pooled trials should be harmonized in terms of molecular residual disease cut-off, analytical sensitivity, and timing of measurements.
- ⇒ Studies should include long-term clinical endpoints such as event-free survival, disease-free survival, and overall survival for correlation with ctDNA.
- ⇒ The timing and window of ctDNA assessment should be prespecified, for concluding an association between ctDNA and long-term clinical outcomes.
- ⇒ There should be a plan to evaluate the impact of missing data on trial results.

association of ctDNA with long-term survival outcomes is important to provide insight about an individual's prognosis and can be used to counsel patients. Trial-level correlation would involve observing a consistent relationship between the effect size of ctDNA endpoints (eg, ctDNA clearance) and the effect size of survival endpoints across multiple randomized trials. With a trial-level association, ctDNA may predict for treatment benefit within an entire study population. Important considerations for sponsors regarding meta-analyses to validate ctDNA as an early endpoint are outlined in box 1 and the FDA Draft Guidance to Industry: Use of ctDNA for Early-Stage Solid Tumor Drug Development.[4]

The Friends of Cancer Research initiative, 'ctDNA for Monitoring Treatment Response (ctMoniTR) project' is an example of a pooled analysis demonstrating patient-level correlation between changes in ctDNA and long-term clinical outcomes, and is discussed further in the next section of this review.[53] However, to date, there have been no trial-level meta-analyses evaluating the association between ctDNA and long-term survival outcomes.

A prior meta-analysis of patients with breast cancer serves as an example to assess patient-level and trial-level correlations between an early endpoint, in this case pCR, and long-term clinical benefit.[54] While a strong association was identified between pCR and both EFS and OS at the patient level, there was little association at the trial level and thus pCR could not be formally validated as an early endpoint for improved EFS and OS. The inclusion of clinical trials with heterogeneous patient populations, with different breast cancer subtypes, and use of heterogeneous therapies, with some patients receiving targeted therapy in addition to chemotherapy, may have contributed to the lack of trial-level association between the endpoints. These results suggest that demonstration of trial-level associations between early-endpoints and long-term clinical outcomes may rely on pooling clinical trials with well-defined and relatively homogeneous patient populations. However, due to the individual patient correlation between pCR and outcome, the standardization of pCR across trials, and a reliance on totality of evidence to support drug approval, pCR in breast cancer has been used as an approval endpoint and use of pCR in this setting is detailed in FDA guidance.[55 56] In addition, pCR in breast cancer can be used as an enrichment biomarker to support drug approval for higher-risk populations similar to the potential use of ctDNA in MRD.[57]

CLINICAL APPLICATIONS OF CTDNA FOR PATIENTS WITH METASTATIC DISEASE

Thus far, FDA approvals of ctDNA-based CDx assays have been limited to targeted therapies with no approvals for use with IO-based therapies (table 1). The use of ctDNA to predict outcomes with immunotherapy compared with targeted therapies is less established and is associated with several challenges, including inability to assess the tumor microenvironment, intratumoral heterogeneity and divergent clonal evolution, and costs associated with sequencing. Despite these challenges, several ctDNA-based assays have the potential to identify patients most likely to benefit from immunotherapy, including in the metastatic disease setting.[58] Monitoring early ctDNA kinetics after initiation of immunotherapy may predict clinical response in patients with lung cancer[59–61] and melanoma[62] and further exploration and validation are warranted. For example, in a small study of patients with metastatic NSCLC, early clearance of ctDNA within approximately 9 weeks of initiation of an anti-PD-1-based therapy was associated with improved PFS and OS compared with patients with no evidence of ctDNA elimination.[59] In addition, Friends of Cancer Research initiated a public-private partnership entitled 'ctDNA for Monitoring Treatment Response (ctMoniTR) project'.[53] This project aligned methodologies to analyze ctDNA from smaller lung cancer immune checkpoint inhibitor clinical trials to assess whether decreases in the variant allele frequencies of ctDNA would predict longer term outcomes in the larger pooled dataset. Disparate clinical trials with different ctDNA time points, collection sampling schedules, and methods were successfully pooled, and associations were still observed between reduction in ctDNA and OS, PFS, and ORR.

ctDNA may also be used to assess for genetic determinants of response to IO. In targeted sequencing of ctDNA, *TP53* and *KRAS* mutations were associated with improved PFS in patients with advanced NSCLC while

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mutations in *PTEN* or *STK11* were correlated with early disease progression.[63] Similarly, in gastric cancer, the mutation status of *TGFBR2*, *RHOA*, and *PREX2* detected in ctDNA have been associated with worse PFS while mutations in *CEBPA*, *FGFR4*, *MET*, or *KMT2Bb* have been associated with increased rates of immune related adverse events.[64] Across multiple tumor types, mutations in DNA polymerase epsilon (*POLE*) and DNA polymerase delta 1 (*POLD1*) identified in tumor tissue have been associated with a DNA hypermutated molecular phenotype and response to immune checkpoint inhibitors; subsequent investigations have also assessed for these mutations in ctDNA.[65 66]

Immune checkpoint blockade is effective in tumors that are DNA mismatch repair deficient (dMMR) or have a microsatellite instability-high (MSI-H) phenotype. In 2017, FDA granted accelerated approval to pembrolizumab for the first tissue agnostic indication for the treatment of adult and pediatric patients with previously treated unresectable or metastatic MSI-H or dMMR solid tumors based on an ORR of 40% with durable responses.[67] Using ctDNA assays to assess MSI-H status is appealing particularly when tissue testing is unavailable or infeasible. Several PCR-based and NGS-based assays have been developed to assess MSI-H status from ctDNA, with favorable concordance between plasma-based and tissue-based analyses.[68 69] However, limitations of these assays include the limited sensitivity and number of microsatellites screened in PCR-based assays and costly high-depth sequencing needed for NGS-based assays.[70]

TMB as a proxy for neoantigen load, detected in either tissue or blood, may also predict for response to immune checkpoint inhibitors.[71–75] Based on an ORR of 29% and durable responses as demonstrated in the KEYNOTE-158 trial, the FDA granted accelerated approval to pembrolizumab for the treatment of adult and pediatric patients with refractory unresectable or metastatic solid tumors with high TMB (≥10 mutations/megabase (mut/Mb)) as detected in tissue.[76] However, further validation of TMB cut-offs across tumor types, standardization of TMB measurements and practices, and understanding of the relationship between TMB assessed from tissue (tTMB) and blood (bTMB) are needed. Significant discordance has been reported between bTMB and tTMB levels and multiple factors may contribute to this including differences between assays with respect to gene panel size, depth of coverage, the allele frequency cut-off for a variant positive call and additional issues with the bioinformatics pipeline, and what type of variants are counted towards the numerator of TMB.[77 78] A limitation of tTMB is that the prognostic value from a single tumor biopsy might be negatively impacted by intratumor heterogeneity and a change in TMB over time with treatment. Alternatively, potential advantages for bTMB are that a single assessment may better reflect the composite of subclones in a tumor and multiple assessments are much more feasible with repeat blood draws compared with repeat tissue biopsies.[58] Importantly, the association between high bTMB and long-term clinical outcomes including OS has varied depending on the study[74 75 79 80] and further prospective studies are needed to better validate the prognostic and predictive value of bTMB.

While there are a number of potential blood-based biomarkers which may predict for response to IO-based therapies for metastatic cancers, the clinical and regulatory utility are currently limited without further evaluation in prospective clinical trials. As a marker of disease progression, it's unclear these ctDNA-based assays represent a meaningful improvement over, or in addition to, current, standard radiographic assessments. One possibility is to use ctDNA-based assays to monitor tumor evolution and treatment resistance. Although serial ctDNA evaluations may be costly and impractical for routine clinical care if large gene panels or whole exome sequencing are needed for each assessment, ctDNA may be useful to differentiate between pseudoprogression versus true progression in the setting of anti-PD-(L)1 therapy. For example, evaluation of ctDNA in patients with metastatic melanoma receiving treatment with anti-PD-1 therapy was able to accurately distinguish between pseudoprogression and true progression.[26]

ASSAY CONSIDERATIONS

MRD test considerations

As several IO-based trials are evaluating the role of MRD, here we include key considerations for MRD assays. Since the presence of MRD predicts for worse clinical outcomes, detection of MRD may be an important mechanism for early intervention over the course of cancer therapy. Different methods including tumor-informed methods, tumor-naïve methods, or a smaller panel of candidate genes can be utilized for MRD detection.[81] For a tumor-informed approach, the primary tumor of each patient is sequenced to select a set of personalized variants to follow. For a tumor-naïve or 'tumor-agnostic' approach, prespecified gene panels are used across patients, without following unique mutations for individual patients. Whole genome sequencing may also be used in a tumor-naïve fashion allowing the use of other biomarkers besides mutations, including epigenetic alterations (eg, methylation) or fragmentomic analysis of ctDNA to capture tumor derived ctDNA signals.[82–85]

Sampling considerations for MRD testing

It is important that all sites in a clinical study follow standardized protocols for sample collection (eg, blood collection in the specific collection tube that will be used with the final market ready assay), storage, timing of sample collection, and processing and handling, for consistent measurement of ctDNA across patients.[3] Since the shedding of ctDNA is affected by histology, grade, stage, and size of the tumor, a baseline pretreatment sample allows for consideration of the impact of variation in tumor shedding rates on assay performance and interpretation of results from the post-treatment sample for study

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enrolment. As noted earlier, repeat ctDNA testing may be needed to evaluate the true NPV of an MRD assay, which generally improves with serial testing. BloodPAC consortium, which is a not-for-profit consortium consisting of members from industry, academia, not-for-profits, and US government agencies, worked together collaboratively to develop consensus recommendations on data elements for the liquid biopsy field as a whole and has published minimum technical data elements (preanalytical variables) that are needed for liquid biopsy sample collection.[86]

Assay analytical validation considerations for marketing applications

Analytical validation establishes the analytical performance characteristics of a test.[87] Analytical validation studies generally specify procedures for specimen collection, handling, and storage of samples to ascertain the sensitivity, specificity, accuracy, precision, and other relevant performance characteristics of an assay. Justification of the acceptance criteria for the validation studies is important to support clinical use of a ctDNA MRD assay. Studies have shown that clonal hematopoiesis of indeterminate potential, or CHIP, may lead to the detection of mutations in healthy, aging individuals, and result in a high false positive rate in liquid biopsy tests. Therefore, in order to obtain reliable and accurate ctDNA-based liquid biopsy test results, it is best practice to have a CHIP filter or to sequence paired white blood cells, to subtract CHIP mutations. BloodPAC consortium has developed a document describing a set of generic analytical validation protocols and standard methods customized explicitly for NGS-based ctDNA assays.[88]

In addition, to potentially achieve harmonization between different assays, an appropriate set of reference materials could be developed to allow for comparability across multiple assays. Reference materials may be used to understand the differences in analytical sensitivity between different assays. Analytical sensitivity would likely be different between assays, resulting in different clinical cut-offs and identification of different subgroups of patients. This can directly impact the clinical validity of the proposed intended uses (ie, the clinical claims for the assays). Therefore, each clinical claim may be for a specific ctDNA-based CDx assay.

Validation of an MRD assay encompasses the entire assay system from sample collection to the output of the assay. Optimizing assay sensitivity and specificity for clinical use also involves establishing an appropriate assay cut-off. While MRD assays with high sensitivity and NPV are important to support de-escalation of treatment, MRD assays with high specificity and PPV are better to support escalation of treatment. For key assay analytical validation studies such as confirmation of the assay limit of detection, assay precision, analytical accuracy, assay input studies, and specimen stability studies, use of clinical samples is important to assess the analytical performance of the test. For other analytical studies, both clinical and contrived samples with equivalent performance characteristics may be adequate.

CONCLUSION

ctDNA holds significant promise to assist in the diagnosis, management, and prognostication of cancers. Particularly for earlier-stage solid tumors, ctDNA is also emerging as an important enrichment tool for clinical trials, which may help expedite drug development including for IO-based therapies. However, further work is needed for validation of ctDNA as an efficacy-response biomarker to support regulatory decision making and drug approvals. Numerous ongoing trials are exploring the relationship between ctDNA and long-term clinical outcomes which may strengthen the evidence for ctDNA MRD as an early endpoint. Standardizing guidelines related to the methods and timing of ctDNA collection, performance characteristics of assays, and assay cut-offs will also be essential to fully realize the regulatory use of ctDNA.

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