Perspective

Practical recommendations for using ctDNA in clinical decision making

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The continuous improvement in cancer care over the past decade has led to a gradual decrease in cancer-related deaths. This is largely attributed to improved treatment and disease management strategies. Early detection of recurrence using blood-based biomarkers such as circulating tumour DNA (ctDNA) is being increasingly used in clinical practice. Emerging real-world data shows the utility of ctDNA in detecting molecular residual disease and in treatment-response monitoring, helping clinicians to optimize treatment and surveillance strategies. Many studies have indicated ctDNA to be a sensitive and specific biomarker for recurrence. However, most of these studies are largely observational or anecdotal in nature, and peer-reviewed data regarding the use of ctDNA are mainly indication-specific. Here we provide general recommendations on the clinical utility of ctDNA and how to interpret ctDNA analysis in different treatment settings, especially in patients with solid tumours. Specifically, we provide an understanding around the implications, strengths and limitations of this novel biomarker and how to best apply the results in clinical practice.

Over the past 20 years, there has been an incremental and consistent improvement in cancer survival rates[1], largely attributed to more effective treatments and improved patient management strategies[2]. Refining the identification of patients who may benefit from adjuvant therapy following definitive management is critical to optimizing patient care. As appropriate interventions may improve outcomes, the judicious use of additional therapy can spare patients at low risk of recurrence from adverse treatment effects and unnecessary costs. The current paradigm of disease management centres around tumour-specific, stage-based recommendations, primarily relying on pathology and imaging results to optimize treatment plans for patients[3]. Although imaging is the accepted standard method to monitor disease progression or relapse and measure response to treatment, radiological findings are sometimes difficult to interpret correctly, leading to high rates of false positivity and negativity[4]. Blood-based metabolic tumour markers (for example, carcinoembryonic antigen (CEA), cancer antigen (CA)-125, CA19-9 and lactate dehydrogenase (LDH)) represent a non-invasive approach to evaluate the status of disease. However, many of these established biomarkers are considered unreliable, as they can be elevated due to conditions unrelated to cancer, leading to low sensitivity and specificity[5–9].

ctDNA has emerged as a non-invasive, blood-based biomarker that broadly reflects somatic variants found in the tumour tissue[10]. Several approaches to measure ctDNA have been developed, such as panel-based assays, next-generation sequencing and droplet digital polymerase chain reaction, and are discussed in depth elsewhere[11,12]. Beyond the inherent biologic differences for each tumour type, the variation in the underlying ctDNA detection methodologies (such as sequencing depth[12]) and analytical validation measures (such as sensitivity and specificity) for each assay affect the subsequent interpretations of results[11,13,14]. Regardless of the assay, quantitatively measuring circulating tumour burden, either through variant allele fraction (as a percentage) or tumour fraction (as the mean number of tumour molecules per millilitre), should also be considered while interpreting ctDNA results clinically[13]. ctDNA has several applications, including early cancer detection, comprehensive genomic profiling for treatment selection, detection of molecular residual disease (MRD), surveillance of recurrence and monitoring of treatment response[14]. The appropriate assay for each of these clinical applications may be different depending on what must be evaluated in that specific clinical scenario.

Investigations in the clinical setting have established associations between ctDNA detection and its concentration with tumour burden, response to therapy and prognosis[15,16]. Numerous studies using different assay technologies indicate that ctDNA is a sensitive and specific biomarker for MRD detection. It may precede radiological imaging and other standard-of-care (SOC) methods by months but is optimally applied in conjunction with standard surveillance diagnostics[17–20]. However, the current knowledge of the clinical utility of ctDNA is limited to studies that were largely observational or anecdotal in nature and were narrowly defined to specific indications. There is a need to examine the clinical utility of ctDNA-based MRD testing in interventional trials across indications. The current landscape of findings of such clinical trials has been reviewed previously[12,16,21,22]. Finally, only a few ctDNA-based MRD assays are currently available commercially to clinicians, and each has a different degree of predictive and prognostic value[23–25]. Thus, although existing data on the use of ctDNA in cancer patients is promising, it is crucial that ordering providers understand the implications, strengths and limitations of this novel biomarker in order to optimally apply the results to their clinical practice.

In this Perspective, we discuss our view on optimizing the use of ctDNA testing specifically for MRD detection for solid tumours. We also highlight how ctDNA can help guide clinical management of cancer patients during the course of their disease (Tables 1 and 2). The application and utility of ctDNA testing for haematological malignancies

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Table 1 | General recommendations for utilizing ctDNA in clinical practice

Setting	Clinical position
Early stage	
Prior to treatment	ctDNA-positive patients to be treated with SOC.
After surgery	ctDNA could be evaluated for prognostication, ideally starting two weeks after definitive therapy; adjuvant therapy should be given per standard guidelines.
After completion of best SOC including systemic therapy	Persistent ctDNA-positive patients could be considered for clinical trials that accept ctDNA-positive patients. More intense imaging surveillance should be considered.
Treatment-response monitoring	
Neoadjuvant treatment monitoring	In cases where clinical complete response to neoadjuvant therapy permits consideration of non-operative management, persistent detection of ctDNA positivity may deter a non-operative approach.
Unresectable or advanced disease	Early assessment of response to systemic therapy with potential to switch treatment regimens if ctDNA does not decrease.
Immunotherapy setting	
True progression	Consideration should be given to altering the treatment regimen for patients who may have radiological and molecular (ctDNA) evidence of progression.
Pseudoprogression	Consideration should be given to not cease immunotherapy regimen prematurely for patients who have unconfirmed radiological progression accompanied by a ctDNA decrease.
Hyperprogression	Understanding whether large fluctuations in DNA could potentially identify hyperprogression requires additional research.
Exceptional responders	Treatment discontinuation could be considered for ctDNA-negative patients with continued surveillance monitoring with ctDNA monitoring added to SOC approaches.
IRAEs	Treatment could be discontinued for ctDNA-negative patients with continued monitoring using ctDNA and SOC approaches.

IRAEs, immune-related adverse events; ctDNA, circulating tumor DNA; SoC, standard of care; IO, immunotherapy.

have been discussed previously[26,27]. Screening methods for early cancer detection also examine cell-free DNA (cfDNA), although other features (for example, methylation) are also assayed. Preliminary data suggest the clinical utility of such screens[28,29], but they are not designed to monitor disease progression, and are thus not included in this discussion. Here we begin by addressing the interpretation of ctDNA results prior and subsequent to surgery or definitive treatment. We then discuss technical considerations when ctDNA detection is indicated for MRD. We provide recommendations regarding the management of patients with ctDNA-positive and negative results after the completion of SOC therapy. Last, we discuss the use of ctDNA testing for treatment-response monitoring in the neoadjuvant setting, specifically in the context of immunotherapy. In all cases, results from evaluating ctDNA should be taken in the context of the larger comprehensive assessment of the patient, thereby refining standard clinical staging and risk stratification.

Baseline ctDNA detection prior to surgery

Tumour fraction has been observed to vary both between tumour types and between patients with the same type of cancer[30]. The release of ctDNA from the primary tumour can be influenced by a number of factors, including tumour size, location, metabolic activity, histological subtype and grade and lymph node status[31]. In certain cases, clinical limitations may result in subclinical ctDNA levels, where patients appear to be ctDNA-negative (false negative). For example, attempted MRD detection while a patient is concurrently receiving adjuvant chemotherapy may produce a false-negative result because of the systemic treatment.

For tumour-informed ctDNA testing, which selects the blood-based biomarkers from previously acquired tumour tissue (either from a biopsy or resection specimen), the source and quality of tumour tissue has a significant role in the success of plasma-based ctDNA testing. If the patient has undergone radiation or chemotherapy treatment prior to obtaining the tissue specimen, it is likely that non-clonal tumour-specific variants may disappear in response to the selective pressure of treatment. Thus, ctDNA testing based on detecting these non-clonal variants may fall below the limit of detection in subsequent plasma samples.

In another scenario, as demonstrated in Fig. 1a, small primary tumour size and certain histologies (including sarcoma, renal cancer, lung adenocarcinoma, hormone receptor-positive breast cancer and brain cancer[32,33]) may result in lower tumour shedding and consequently, undetectable ctDNA at the time of diagnosis and/or progression. Thus, ctDNA detection varies depending on tumour biology. As ctDNA-positive patients undergo cancer-directed therapy, a significant reduction in tumour burden may lead to ctDNA negativity. Of note, in contrast to post-treatment timepoints, the correlation between baseline and/or pre-operative ctDNA status and long-term outcomes is not fully understood. Some studies have shown that there is no correlation between baseline or pre-operative ctDNA status and outcome[34], whereas others have shown a strong correlation between pre-operative ctDNA positivity and survival outcomes[35,36]. Thus, it remains unclear whether and how these pre-operative ctDNA levels should influence clinical decision making, and how the variability may be related to assay design in addition to clinical features.

Below we present data demonstrating the prognostic value of tumour-informed ctDNA status prior to surgery in lung, breast, kidney and bladder cancers, wherein patients who test ctDNA-negative prior to surgery (at baseline) are observed to have better outcomes compared with ctDNA-positive patients (Fig. 1b). It should be noted that these observations are based on personalized and tumour-informed ctDNA testing (Signatera™ multiplex polymerase chain reaction–next-generation sequencing-based test). These observations may or may not be applicable to other ctDNA testing methodologies as the underlying technologies and interpretation of results may differ[16].

ctDNA detection subsequent to surgery

When ctDNA is detected (that is, detection of MRD) following definitive surgery, patients have a risk of relapse approaching 100%, varying on the basis of the cancer type, ctDNA assay and whether repeated (longitudinal) testing is performed[20,24,37]. The frequency at which ctDNA becomes detectable after surgery or treatment with curative intent is dependent on the tumour biology[38] and the aggressiveness of the residual disease. For example, patients whose ctDNA is detectable at the MRD timepoint but is undetectable following adjuvant therapy may have improved long-term outcomes compared with those with persistent positivity. This latter scenario suggests resistance to treatment, possibly owing to tumour heterogeneity and clonal evolution, contributing to an eventual relapse[39].

Table 2 | Representative clinical application of ctDNA in solid tumours in ongoing clinical trials across different treatment settings

Cancer type	Neoadjuvant setting	Adjuvant setting	Surveillance
Colorectal cancer	Rectal: post-total neoadjuvant treatment to inform interventions (surgery versus watch and wait) in combination with other traditional methods.	Inform risk-based adjuvant treatment decisions (escalate or de-escalate adjuvant treatment). Clinical trials: • BESPOKE (NCT04264702) • CIRCULATE Japan (comprising GALAXY (UMIN000039205), ALTAIR (NCT04457297) and VEGA (jRCT1031200006)) • CIRCULATE USA (NRG-GI008) • CIRCULATE Germany (AIO-KRK-0217) • DYNAMIC II (ACTRN12615000381583) • COBRA (NCT04068103) • DYNAMIC III (ACTRN12617001566325) • PEGASUS (NCT04259944) • TRACC Part C (NIHR128529)	Monitor for early recurrence detection. Clinical trial: • BESPOKE (NCT04264702) Treating on molecular recurrence Clinical trial: • CIRCULATE Japan (comprising GALAXY (UMIN000039205), ALTAIR (NCT04457297), and VEGA (jRCT1031200006)) • NCT03803553
Oesophagogastric cancers	Assess response to neoadjuvant therapy. Clinical trial: • CURE (NCT04576858)	Inform risk-based adjuvant treatment decisions.	Monitor for early recurrence detection. Clinical trial: • CURE (NCT04576858)
Breast cancer	Identify non-responders, with possible changes in treatment prior to surgery. Clinical trial: • I-SPY 2.2 TRIAL (NCT01042379)	Inform risk-based adjuvant treatment decisions, in conjunction with other clinical, pathological and genomic risk factors. Clinical trials: • PERSEVERE (NCT04849364) • ASPRIA (NCT04434040)	Identify recurrence earlier than traditional tools, before the patient becomes symptomatic. Treating on molecular recurrence. Clinical trials: • LEADER (NCT03285412) • DARE (NCT04567420) • c-TRAK-TN (NCT03145961)
Bladder cancer	Assess response to neoadjuvant therapy. May guide treatment strategy for exceptional responders and non-responders.	Inform risk-based adjuvant treatment decisions and identify patients likely to benefit from immunotherapy. Clinical trials: • IMvigor010 trial (NCT02450331) • TOMBOLA (NCT04138628) • IMvigor011 (NCT04660344)	Monitor for disease recurrence. Clinical trial: • IMvigor011 (NCT04660344)
Gynaecologic malignancies	Not applicable	Inform risk-based adjuvant treatment decisions. Clinical trial: NCT05212779	Monitor for recurrence. Clinical trial: NCT05212779
Lung cancer	Identify non-responders, with possible changes in treatment prior to surgery.	Inform risk-based adjuvant treatment decisions, including chemotherapy and sequential immunotherapy, or targeted therapy. Clinical trials: • IMpower010 (NCT02486718) • MERMAID (NCT04385368) • LUCID (NCT04153526) • ADAURA (NCT02511106) • MELROSE (NCT03865511) • NCT04367311 • NCT04585477 • NCT02759853	Monitor for disease recurrence. Clinical trial: • LUCID (NCT04153526)
Skin cancer	Not applicable	Inform risk-based adjuvant treatment decisions and whether combination immunotherapy is needed for patients with advanced-stage disease. Clinical trials: • DETECTION (NCT04901988) • CheckMate 76K (NCT04099251) • Keynote 716 (NCT03553836) • INTERIM (NCT03352947) • CAcTUS (NCT03808441) • SECOMBIT (NCT02631447) • EBIN (NCT03235245) • AVAST-M (ISRCTN 81261306)	Monitor for disease recurrence.

Technical aspects of ctDNA detection

MRD is a subclinical disease that is associated with a high risk for recurrence, which cannot be detected by standard imaging techniques. Evaluating MRD using ctDNA enables the detection of micrometastatic disease. It should be noted that a negative ctDNA result suggests a decreased risk of recurrence, rather than a guaranteed lack of recurrence[18–20,24,34,36,37]. SOC post-surgical surveillance is limited to imaging and/or blood-based biomarkers (that is, CEA, CA-125, CA 15-3 and LDH) that are a proxy for ongoing disease, but these have demonstrated poor sensitivity and specificity for assessing MRD[6–9]. The addition of ctDNA to standard surveillance can complement the current paradigms and may improve the time to detection of a cancer recurrence.

Figure 2a depicts a hypothetical clinical scenario regarding the probability of ctDNA-based MRD detection post-surgery. Post-surgical ctDNA levels (MRD timepoint) may fall below the assay's limit of detection, exhibiting a low probability of MRD detection (that is, a false negative). At this level, it would be ideal to interpret results in a binary fashion (that is, positive or negative). The likelihood of detecting MRD when present improves as the ctDNA levels increase. However, at concentrations

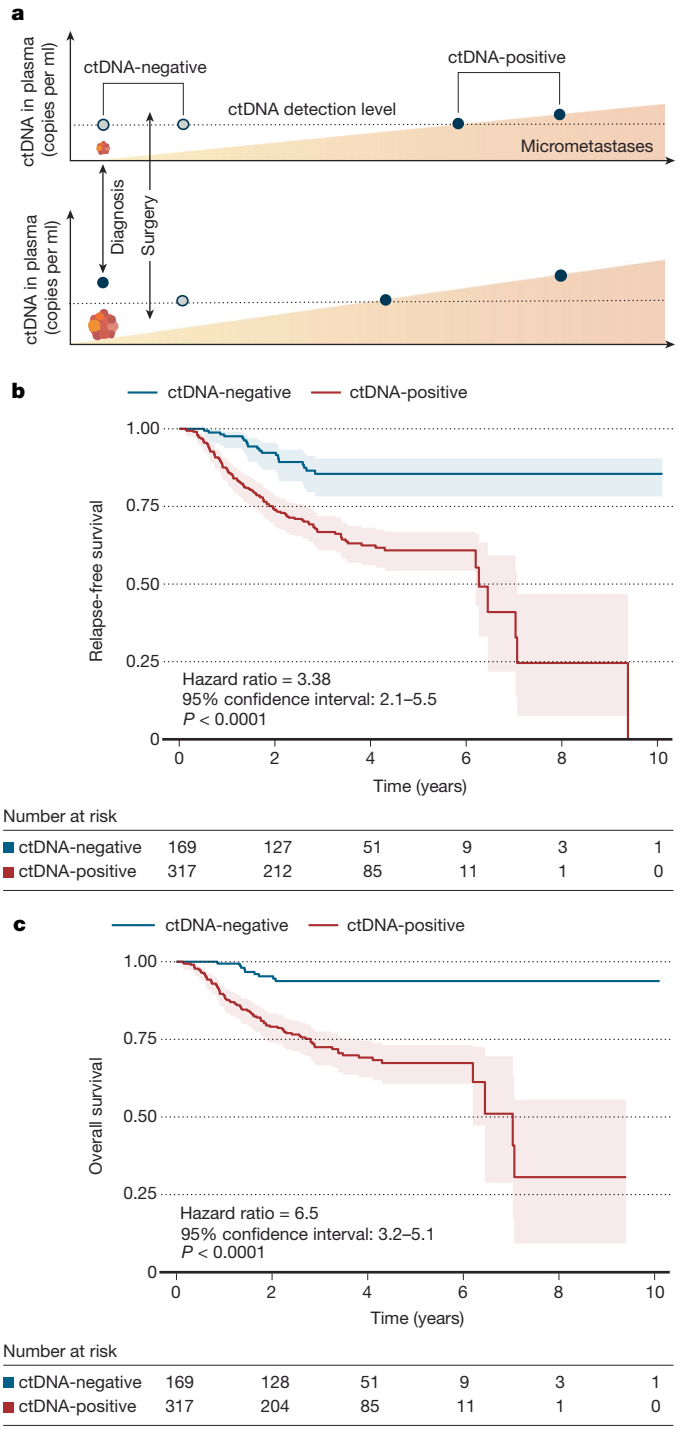

Fig. 1 | Interpretation of ctDNA results in the peri-operative setting and prognostic value of ctDNA status prior to surgery for predicting survival outcomes. a, Hypothetical schema demonstrating that tumour subtype and size can influence tumour shedding and consequently ctDNA status at baseline prior to surgery. In the top example, the tumour subtype and size result in ctDNA that is not detected prior to surgery. Following surgery, the patient remains ctDNA-negative, but with serial sampling over time, the tumour progresses and can be detected through ctDNA. In the bottom example, ctDNA is detected in the patient prior to surgery. Immediately after surgery, the ctDNA status can return to negative, but this does not preclude the possibility of MRD, as ctDNA levels can fall below the limit of detection. Repeat testing over time may identify micrometastatic disease in advance of radiological relapse. Tumour size and cancer subtype can also influence the rate by which micrometastatic disease can become detectable using ctDNA testing. **b**, Association of ctDNA status prior to surgery with recurrence-free survival in patients with early-stage cancer. **c**, Association of ctDNA status prior to surgery with overall survival in patients with early-stage cancer. These data are based on the Signatera™ multiplex polymerase chain reaction–next-generation sequencing-based test (average sequencing depth >105,000). Cancer types included: non-small cell lung cancer (NSCLC) ($n = 93$), breast cancer ($n = 296$), renal cell carcinoma ($n = 36$) and muscle-invasive bladder cancer (MIBC) ($n = 61$). Hazard ratio (HR) values were adjusted by cancer type.

below 0.1 copies per millilitre, ctDNA detection still remains dependent on probabilistic sampling; thus, a patient who tests ctDNA-positive may falsely test negative if subsequent sampling is performed very close to the previous timepoint. ctDNA data can be interpreted more quantitatively at levels above 1.0 copies per millilitre, making it more desirable for treatment-response monitoring.

Another factor to consider is the timing of blood draw relative to the time of surgery. Previous studies have suggested that cfDNA levels can increase in response to surgical trauma, along with many cancer-related and other factors[40]. Detection of ctDNA in instances of elevated baseline cfDNA may be more difficult, leading to more false negatives. This can be overcome by longitudinal monitoring. On the basis of existing evidence[41], we suggest a waiting period of two weeks post-surgery, before an initial blood sample is drawn for ctDNA-based MRD testing[42]. It is also recommended to consider a short interval follow-up draw (for example, one month later) to confirm a negative result. This testing interval should allow for timely initiation of adjuvant treatment if indicated.

Several retrospective studies have shown improved performance of MRD detection with serial ctDNA (Fig. 2b), where a high sensitivity and specificity have been reported for detection of recurrence (sensitivity 79–100%, specificity 88–100%) across a range of solid tumours[16]. Furthermore, in initial studies in which ctDNA was obtained in conjunction with standard surveillance, testing agnostic to other clinical parameters provides a substantial average lead time of 3 to 18 months between ctDNA-based MRD detection and recurrence detected by radiological imaging for these cancers[18–20,43–48]. Now that ctDNA is being used more widely in clinical practice with rapid result turnaround times, we anticipate a trend in shorter lead times to be reported, as the detection of positive ctDNA logically prompts radiographic evaluation[49]. However, the historical data is still impactful in establishing a role for ctDNA in conjunction with current SOC surveillance strategies. The exceptional performance of ctDNA was recently acknowledged in a task force consensus statement from the US National Cancer Institute, concluding that the presence of ctDNA was strongly associated with high risk of disease recurrence in patients with colorectal cancer, and that current results suggested that ctDNA is a robust marker for MRD[50,51].

Although an appreciation of differences in the performance of various ctDNA-based assays awaits further research, there is an overall strong indication that ctDNA-based MRD detection identifies a subgroup of patients at high risk for recurrence within multiple cancers[52], with a growing body of literature for specific types, including colorectal[20,53,54], breast[19], bladder[18], lung[55] and pancreatic[56] cancers, and multiple myeloma[37]. Adapted management of disease based on ctDNA positivity can now be envisioned in the clinical setting.

Management of ctDNA-positive patients

Many studies have outlined the role of ctDNA testing in disease management as an early indicator of cancer recurrence[18–20,23,24,44,46,48,57]. However, recent studies have shown that the poor prognosis associated with ctDNA positivity can be modified by effective adjuvant systemic therapy[58–60]. Although on-treatment ctDNA clearance is correlated with a favourable prognosis[61–63], the current evidence does not suggest that ctDNA clearance alone is sufficient for prediction of long-term survival benefit (disease free survival or recurrence-free survival). Thus,

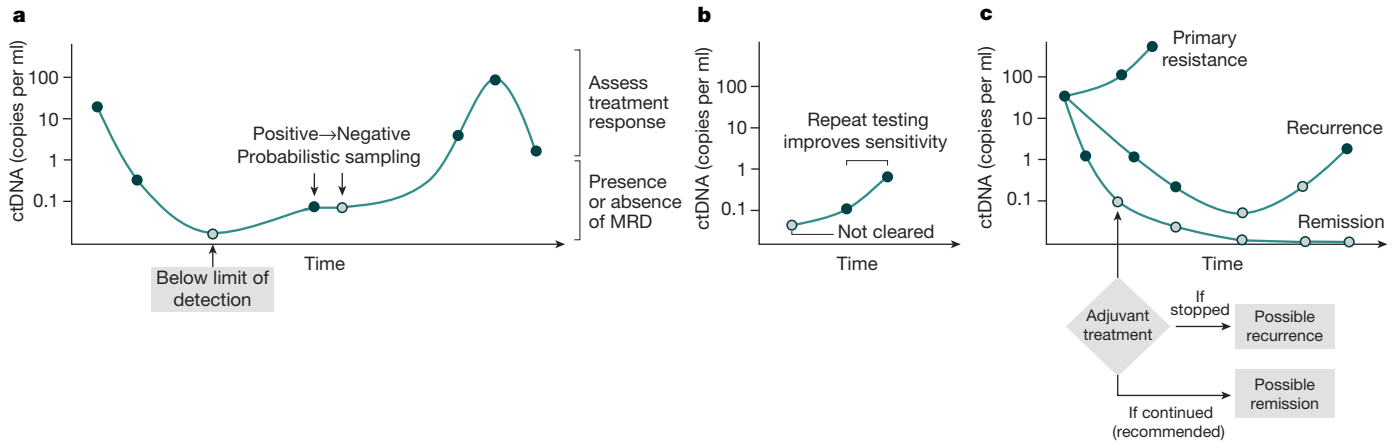

Fig. 2 | ctDNA detection in different clinical scenarios. Representative models of longitudinal ctDNA testing results are depicted for critical clinical settings. **a**, The probability of residual disease detection and eventual treatment-response monitoring. A patient who is identified as ctDNA-positive at diagnosis undergoes surgery with curative intent. The probability of detecting a residual disease (MRD) may be affected by tumour biology and increased cfDNA levels immediately after surgery—among other factors—and thus the result is subject to probabilistic sampling. As ctDNA levels increase, the probability of ctDNA detection increases and becomes suitable for treatment-response monitoring. **b**, Serial testing improves the sensitivity of the ctDNA test. Serial testing for ctDNA is recommended to avoid any false-negative or false-positive results, and thus improves the overall sensitivity of the assay in detecting disease recurrence. **c**, Dynamic changes in ctDNA following treatment. A patient who tests positive for ctDNA is recommended to be on adjuvant chemotherapy. Continued elevation in ctDNA is suggestive of primary resistance to therapy. A decline in ctDNA level followed by clearance is indicative of a successful therapeutic response. At this point, it is recommended that the patient complete the course of treatment to achieve remission, as cessation of therapy may lead to disease recurrence.

although on-treatment ctDNA clearance is an indicator of treatment response, completion of the full planned course of treatment based on established clinical practice irrespective of on-treatment ctDNA results is still recommended to minimize the chance of a future relapse (Fig. 2c).

Below, we discuss in depth four representative cancer types of different disease biology—colorectal, breast, lung and bladder cancer—that demonstrate the clinical utility of ctDNA testing in disease management. For each of these cancers, we present the data supporting the use of ctDNA testing for MRD detection. We describe the clinical accuracy of ctDNA tests in these settings, outline current management strategies and highlight ctDNA-based disease management options. Ongoing investigation of the utility of ctDNA testing in these and other cancer types are outlined in Table 2.

Colorectal cancer

Although the vast majority of patients with stage I and II colon cancer experience good outcomes after surgery alone, a minority of patients relapse. A small number of clinical prognostic factors exist that can aid in the identification of early-stage patients who can effectively benefit from adjuvant systemic therapy (that is, stage III or high-risk stage II), potentially leading to a reduction in recurrence risk[64]. It is also recognized that tumours with microsatellite instability (MSI-high tumours) do not benefit from standard adjuvant chemotherapy[65]. In the absence of these factors, a positive ctDNA test, found either postoperatively or during follow-up, may be used to identify patients at high risk of recurrence who would benefit from adjuvant chemotherapy. Although prospective data remains limited, recent results from DYNAMIC II found that ctDNA-guided adjuvant chemotherapy effectively reduced the number of stage II patients receiving chemotherapy compared with standard management, without compromising survival outcomes, even among patients with high-risk histologies[66]. More recently, results from the GALAXY cohort of the CIRCULATE study demonstrated that high-risk stage II patients with colorectal cancer who were ctDNA-positive at four weeks post-surgery could benefit from chemotherapy[60]. In addition, two studies aimed to further evaluate this concept prospectively—COBRA (NCT04068103) and CIRCULATE Germany (AIO-KRK-0217)—are underway[67,68].

Patients with stage III colon cancer are recommended to receive adjuvant chemotherapy, although more than 50% may be cured by surgery alone[69]. Patients in whom ctDNA is detected post-surgery (MRD timepoint) are likely to have recurrence, and evidence demonstrates that these patients should receive adjuvant chemotherapy. Although 20% of stage III patients treated with adjuvant chemotherapy have a recurrence of their cancer[69], it is currently unknown whether the detection of ctDNA positivity after surgery should influence the chemotherapy regimen that is selected or if patients should receive subsequent therapy after the completion of SOC adjuvant treatment. Of note, a large number of ongoing clinical studies in colorectal cancer, including COBRA, CIRCULATE Japan (comprising GALAXY (UMIN000039205), ALTAIR (NCT04457297) and VEGA (jRCT1031200006)), CIRCULATE-US, DYNAMIC II (ACTRN12617001566325), DYNAMIC III (ACTRN12617001566325), PEGASUS (NCT04259944), TRACC Part C (NIHR128529) and NCT03803553 are designed to use MRD detection by ctDNA to guide adjuvant treatment decisions[16,70–72]. Data from the GALAXY cohort from CIRCULATE Japan has provided validation for this hypothesis among patients with stage III colorectal cancer[60]. These studies use a variety of different assays, each of which has observational data suggesting that these markers are prognostic, however, these ongoing studies will be key in determining how ctDNA fares as a predictive biomarker[22].

Breast cancer

ctDNA-based MRD detection can have an important role in disease management of breast cancer, as up to 30% of women with breast cancer relapse and die after treatment with curative intent, despite presenting with imaging indicating no evidence of disease[73]. For early-stage breast cancer, the current SOC is surgery, often followed by adjuvant therapy[74]. Post-surgical ctDNA positivity is prognostic of relapse[19,43,45]. Longitudinal monitoring of ctDNA subsequent to surgery may therefore inform choices regarding escalation of treatment (for example, adding chemotherapy to hormone-based therapy) by providing an early indication of active micrometastatic disease relative to SOC monitoring[19,75,76] (that is, imaging, monitoring with tumour markers and multigene assays). We therefore recommended that patients with detected ctDNA be classified as clinically high risk, and providers

should consider giving treatments that are accordingly indicated by the US Food and Drug Administration and clinical practice guidelines. For patients who test ctDNA-negative, especially serially, we recommend classification as lower risk with consideration for less intensive treatments, and in certain cases, observation and monitoring alone[77,78]. Ongoing clinical studies to assess MRD to guide treatment decisions[16] include studies focused on early-stage oestrogen receptor (ER)-positive breast cancer (LEADER (NCT03285412)), ER-positive/HER2-negative stage II/III breast cancer (DARE (NCT04567420)), early-stage triple-negative breast cancer (c-TRAK-TN (NCT03145961)) and metastatic breast cancer (PERSEVERE (NCT04849364)).

Lung cancer

Although patients most commonly present with advanced disease, patients with early-stage disease can be treated with curative intent and subsequently monitored for recurrence. In these cases, ctDNA results could facilitate the selection of adjuvant or targeted therapy, or could be used for ongoing surveillance following treatment[24,55]. The presence of ctDNA has been shown to be prognostic in non-metastatic NSCLC, which was treated with curative intent (LUCID[24] (NCT04153526), IMpower010[79] (NCT02486718) and NCT02759835[80]). There are currently ongoing clinical trials assessing whether ctDNA is prognostic of outcomes in EGFR tyrosine kinase inhibitor therapies (MELROSE[81] (NCT03865511)).

Bladder cancer

Patients with early-stage non-muscle invasive bladder cancer may be simply treated with transurethral resection with fulguration[82,83]. A positive ctDNA test following transurethral resection may identify patients who require more aggressive treatment along the lines of recommendations for MIBC, including neoadjuvant chemotherapy (NAC) followed by radical cystectomy. Similarly, a positive ctDNA test following NAC and radical cystectomy in patients with MIBC may indicate the need for additional therapy. Data from the IMvigor010 trial (NCT02450331) suggest that patients who test positive for ctDNA post-surgery may benefit from adjuvant atezolizumab[84]. Currently, there are some clinical studies exploring the utility of ctDNA at the MRD timepoint in guiding adjuvant therapy, in metastatic bladder cancer (TOMBOLA (NCT04138628)) and MIBC[16] (IMvigor011 (NCT04660344)).

Management of ctDNA-negative patients

In the adjuvant setting, patients who remain or become ctDNA-negative while on treatment, have been observed to have a significantly improved prognosis[60]. However, ctDNA negativity does not preclude recurrence. How risk of relapse is estimated depends on the cancer type. Longitudinal testing with serial negative ctDNA results suggests a lower risk of recurrence than single-timepoint testing. However, the MRD timepoint appears to be a key indicator of disease outcome, but patients with longitudinal assessment who remain serially ctDNA-negative appear to have the best outcomes. Detecting recurrent disease in areas with low shedding and less communication with the bloodstream, such as the central nervous system[85], peritoneal cavity[86,87] and the lung[87,88] can be challenging. Measuring ctDNA in samples other than blood, such as urine, saliva or cerebrospinal fluid, have demonstrated promising results as a prognostic biomarker for monitoring disease progression[33]. Particularly for brain cancers, where biopsies represent a very invasive and high-risk procedure and there is heavy reliance on imaging for surveillance, ctDNA from cerebrospinal fluid represents a promising, less invasive approach.

Recent studies have shown a reduced absolute benefit of adjuvant therapy for ctDNA-negative patients, mainly as a result of their reduced risk of recurrence. However, whether the relative risk of adjuvant therapy is also reduced or absent compared with ctDNA-negative patients treated with surgery alone is currently being studied. Early data for MIBC (IMvigor010) and colorectal cancer (IDEA-FRANCE) suggest that patients who test ctDNA-negative may not derive as much benefit from adjuvant therapy as those who are found to be ctDNA-positive[84,89]. A similar trend was observed in patients with resected NSCLC receiving NAC[90]. However, an investigation in a larger cohort is needed to validate these findings.

Application of ctDNA negativity could potentially guide de-escalation and/or omission of therapy in patients who are borderline candidates for systemic therapy owing to other moderate risk factors. In such cases, it would be reasonable to consider the ctDNA-negative status among other patient factors in determining the use and duration of adjuvant systemic therapy.

As described previously for non-metastatic colorectal cancer, several clinical studies are using ctDNA-guided approaches as rationale for de-escalation of adjuvant therapy for ctDNA-negative patients. When and how this may influence the administration of adjuvant therapy is of great interest and is currently being studied in the VEGA trial, enroling ctDNA-negative patients with stage I–IV colorectal cancer, with the goal of comparing surveillance alone to SOC adjuvant therapy[70] (3 months of CAPOX).

Treatment-response monitoring

ctDNA testing is a powerful tool in the treatment-response monitoring setting. Below, we describe what is currently known about ctDNA monitoring in the neoadjuvant and adjuvant settings, specifically in the context of immunotherapy.

Neoadjuvant treatment monitoring

NAC is used for many neoplastic diseases, including breast cancer, rectal cancer and MIBC. Clinical and pathological response to NAC provides important prognostic information.

In breast cancer and MIBC, the prognostic role of NAC is to downstage the tumour, ideally achieving a pathological complete response[91–93] (pCR). A growing number of studies have demonstrated the ability of ctDNA to assist in early response assessment following NAC. In breast cancer, several studies have concluded that ctDNA testing during or after NAC is predictive of pCR and/or patient survival outcomes, including for early-stage disease[94,95] and stage II/III disease[96], as well as in triple-negative breast cancer[97,98]. Several studies have also demonstrated the prognostic value of ctDNA testing during NAC in MIBC[84,99]. In patients enroled in ABACUS, a prospective phase 2 study examining the benefit of neoadjuvant atezolizumab before cystectomy found that longitudinal ctDNA testing results accurately predicted response to the therapy, including pCR and major pathological response[84]. Ongoing clinical studies aimed at exploring the clinical utility of ctDNA in the neoadjuvant setting include I-SPY-2 (NCT01042379) for breast cancer, and the PRE-PREVENCYS trial (NL8678) for MIBC[100].

Given that ctDNA dynamics provide an early indication of response to NAC, it is recommended that providers consider this information to optimize patient outcomes. For example, in cases where patients are treated with NAC that can have long-term and cumulative effect on survivorship, such as anthracyclines in breast cancer[101], ctDNA clearance can provide a rationale for early cessation of therapy. Conversely, early identification of non-responders may enable a timely switch to more effective therapies. This is a novel extrapolation from what is done with interval imaging restaging. In addition, the prognosis of cases with residual disease can further be refined by differentiating cases in which ctDNA persists from those in which clearance is achieved. Furthermore, in MIBC, excellent response to NAC can provide a rationale for avoidance of cystectomy and urinary diversion[102].

Similar strategies are currently under investigation in the neoadjuvant setting for locally advanced rectal cancer. The SOC paradigm has largely shifted in support of total NAC with chemoradiotherapy and systemic chemotherapy prior to surgical resection. If NAC is found to be

effective, consideration can be given to non-operative management[103]. ctDNA monitoring in the neoadjuvant setting can help in prediction of complete clinical response prior to surgery and be prognostic of survival outcomes. This may enable ctDNA to guide the need for subsequent therapy, wherein ctDNA negativity may suggest watchful waiting, and persistent ctDNA may indicate a need for surgery[103–106]. However, additional prospective studies and clinical trials are needed to better define the utility of ctDNA in this space, especially given the limitations of ctDNA in defining the status of local disease.

Immunotherapy

Immune checkpoint blockade (ICB) therapy designed to target PD-1, PD-L1 and CTLA-4 have shown to improve survival in multiple cancers including NSCLC, melanoma, head and neck squamous cell carcinoma, renal cell carcinoma and urothelial carcinoma[107]. Although only a minority of cancer patients (less than 20%) respond to ICB, durable clinical benefit has been observed in patients who do respond[17]. Atypical responses such as pseudoprogression and hyperprogression can also occur, which can make it difficult to achieve or confirm therapeutic efficacy[107–109].

There is a growing body of evidence indicating that ctDNA measurement may help in the interpretation of clinical response for patients receiving ICB therapy. Bratman et al. demonstrated that changes in ctDNA level measured at baseline and shortly after commencement of treatment are predictive of response to treatment in advanced-stage patients receiving ICB therapy. In the same study, ctDNA clearance was also associated with outcome, with 12 patients with metastatic disease who cleared ctDNA during treatment being alive at the end of the study, with a median follow-up time of 25 months. By contrast, patients whose ctDNA increased or remained stable had median overall survivals of 13 and 23 months, respectively[17].

Pseudoprogression

Pseudoprogression refers to an initial radiographic increase in size of the primary tumour, followed by radiographically apparent tumour regression[107]. The phenomenon is defined as tumour's response to treatment after initial increase in volume, due to the infiltration of tumoral tissue by immune cells. It has been observed to occur in approximately 10% of solid tumours treated with ICB[107]. Pseudoprogression is problematic for clinicians to determine whether a change in treatment is warranted or whether the patient is responding and needs additional therapy with the same regimen.

Currently, the distinction between pseudo- and true progression is defined by immunotherapy RECIST (iRECIST) guidelines[110], where immune unconfirmed progressive disease of >20% in the sum of the diameter of the lesions is followed up at least 4 weeks later by imaging, to confirm progressive disease[107]. Importantly, ctDNA has been shown to identify pseudoprogression accurately and in real time at the molecular level, without the need for a 4-to-8-week follow-up period. Wherein, an unconfirmed radiological progression may be accompanied by a decrease in ctDNA level, resulting in eventual ctDNA clearance. However, current data on the clinical utility of ctDNA in this setting has been limited to a handful of smaller retrospective cohorts[17,111].

Timely distinction of pseudoprogression from true progression may help avoid both premature discontinuation of an effective therapy (for pseudoprogressors) and avoid exposing patients to prolonged, ineffective or costly treatments (for true progressors). Furthermore, in cases of true progression, ctDNA status can provide rationale for switching to an alternative therapy more quickly.

Hyperprogression

Recent studies have reported hyperprogressive disease in 4–29% of patients with solid tumours who receive ICB therapy, which may be associated with a shorter overall survival following progressive disease[112]. Key criteria for hyperprogression include time to treatment failure of less than two months, with a two-fold or greater increase in disease progression and at least a doubling of the patient's tumour burden compared with pre-baseline imaging[113]. It is anticipated that large, rapid increases in ctDNA could potentially identify hyperprogression. There are, however, little data relating ctDNA dynamics to hyperprogression in the immunotherapy setting[112]. Future studies that include adequate numbers of patients who experience hyperprogression are needed to establish whether ctDNA can effectively distinguish hyperprogession from other forms of progression and the potential utility for patient management in the immunotherapy setting.

Exceptional responders

Patients with unusually favourable responses to a specific treatment protocol are defined as exceptional responders[114]. Rapid clearance of ctDNA is known to be associated with exceptional treatment response[17]. Identification of exceptional responders may aid in determining treatment duration, allowing for earlier discontinuation, and sparing patients from treatment-associated toxicities and costs. Prospective studies and clinical trials evaluating the implications of longitudinal changes of ctDNA are needed to validate the benefits of discontinuing treatment in exceptional responders defined by ctDNA.

Immune-related adverse events

While ICBs are designed to activate immune responses against tumour cells, they can also induce immune responses against other tissues, organs, and systems, leading to undesirable symptoms in patients[115]. When IRAEs occur, ctDNA monitoring may assist in determining whether immunotherapy should continue. Cessation of immunotherapy, regardless of disease grade, could potentially spare patients from IRAEs and reduce costs for patients and the healthcare system. We therefore recommend continuation of ctDNA monitoring along with SOC monitoring after discontinuation of immunotherapy, to determine whether and when, alternative therapy should be pursued. Importantly, the role of ctDNA in this space remains to be investigated in detail. One study found that specific ctDNA-detected alterations in *CEBPA*, *FGFR4*, *MET* and *KMT2B* were associated with a greater likelihood of IRAEs[116].

Outlook

Over the past decade, our understanding of the potential clinical utility of ctDNA testing in patients with solid tumours has increased substantially. Although this information applies to a broad variety of cancer types, this Perspective summarizes the current knowledge of how to best utilize ctDNA testing, highlighting specific applications to colorectal, breast, lung and bladder cancers, as well as ICB-treated solid tumours. Numerous assays for ctDNA are available or in development; it is crucial to understand and recognize the strengths and limitations of a particular platform when interpreting the clinical effects of adding ctDNA to the current SOC treatment. We have highlighted some of the key data that are available and described how to interpret ctDNA results and to best proceed according to the current knowledge prior to treatment (baseline measurement), after definitive therapy or surgery with curative intent (the MRD timepoint), during the surveillance period, and during active treatment in the neoadjuvant, adjuvant and ICB settings.

Although evidence of clinical utility is still emerging, early results from largely observational studies demonstrate that ctDNA is a highly significant prognostic factor compared with other established clinicopathological risk factors. Thus, ctDNA testing may add to the overall patient assessments for risk stratification, wherein postoperative ctDNA-positive status indicates a higher risk of recurrence. Implementation of ctDNA testing can inform prognosis and assist in determining the level of treatment that may be needed to clear existing disease, prevent relapse and improve chances of long-term survival.

A potential development from ctDNA-guided decision making is the mitigation of unnecessary treatment and the accompanying side-effects and financial burden to patients, or targeted second-line therapy decisions if first-line therapy does not halt disease progression[117,118]. Although a number of post hoc analyses, clinical experiences and case series have been published to date, prospective studies to define the utility of ctDNA testing in clinical practice are still needed. We recommend that ongoing and future trials aiming to examine novel therapy approaches consider utilizing ctDNA testing in their study design, to enable stratification to identify those patients who are most likely to benefit from the studied therapeutic intervention. Ultimately, as the specific indications, ctDNA platforms, treatment decision points and therapy implications are refined and validated, it is likely that ctDNA will be incorporated into many aspects of clinical practice. Prospective studies with well-established clinical end points will determine whether ctDNA can supplement or even replace current standard clinical metrics.

Data availability

All data generated or analysed during this study are included in this Perspective. Informed consent was obtained as part of the ordering assay. This study was approved by the corresponding Ethical and Independent Review Services (protocol no. 20-049-ALL) and was conducted in accordance with the Declaration of Helsinki. Further enquiries can be directed to the corresponding author.

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Additional information
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