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Clinical Practice of ctDNA in Immunotherapy for Solid Tumours: Molecular Residual Disease Detection, Prognostic Assessment and Treatment Stratification

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Abstract. Immune checkpoint inhibitors have improved clinical outcomes in selected patients with solid tumours, while differences in tumour biology, recurrence risk and treatment-related toxicity limit equal benefit across patients. Conventional imaging and tissue biopsy cannot reliably identify molecular residual disease or support frequent longitudinal monitoring. Circulating tumour DNA (ctDNA) may address this clinical need because it can be assessed minimally invasively and can reflect residual disease and tumour dynamics over time. In this article, the clinical utility of ctDNA in molecular residual disease detection, prognostic assessment and treatment stratification is evaluated across four solid tumour types. Among all these clinical settings, which include postoperative, neoadjuvant, consolidation and advanced-disease settings, there is a common relationship between the detected result of ctDNA and disease progression: persistent ctDNA can generally indicate a high recurrence potential, while its clearance is positively related to a better treatment response and outcome. Longitudinal assessment often provided information beyond a single measurement and, in some studies, preceded radiological recurrence. However, the evidence for prognostic utility is considerably stronger than that for treatment-guiding utility. The c-TRAK TN study demonstrated the feasibility of ctDNA-triggered intervention but also showed that molecular relapse may be detected after metastatic disease has already become radiologically visible. Overall, ctDNA can complement imaging, pathology and clinicopathological factors, although it should not currently determine immunotherapy escalation, de-escalation or duration outside prospective trials. Before ctDNA-guided immunotherapy can be translated reliably into real practice, standardised assays, earlier and more frequent sampling, and randomised interventional validation are required.

Keywords: circulating tumour DNA, solid tumours, immune checkpoint inhibitors, molecular residual disease

1. Introduction

Immunotherapies, such as immune checkpoint inhibitors, have recently demonstrated efficacy in selected patients and can reduce the risk of disease recurrence. However, they cannot provide equal

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benefit in all patients because of differences in their tumour biology and the potential for recurrence. Moreover, some adverse effects, such as immune-related toxicity, can occur in those patients who derive limited benefit from the immunotherapy. Reliable biomarkers should be assessed precisely. In this way, patients who have a higher likelihood of benefiting from immunotherapies can be identified, and more precise treatment stratification can then be supported [1].

However, conventional approaches are limited in identifying molecular residual disease (MRD), which leaves this clinical need unmet. Standard imaging techniques can only assess the radiological disease status, whereas molecular remission cannot be reliably confirmed when radiological remission is shown. That is to say, tumour cells are not necessarily absent when no obvious focus is shown in imaging. Additionally, there are some drawbacks to tissue biopsy, including the invasive operation and the limitation in representing the spatial and temporal heterogeneity of the tumour. Because of these limitations, tissue biopsy is challenging and not suitable for high-frequency longitudinal monitoring; it is more like a snapshot of tumour biology instead. As a result, a dynamic biomarker that can be detected in a minimally invasive way and is capable of reflecting MRD is required [1].

Among the numerous liquid biopsy biomarkers, circulating tumour DNA (ctDNA) is considered one of the tools that are most likely to address this unmet need because it can reflect tumour dynamics in real time and has the convenience of collection. Circulating tumour DNA (ctDNA) is defined as fragments of DNA that are derived from the tumour and enter the blood during apoptosis, necrosis or other processes. Being minimally invasive is one of the advantages of using ctDNA as a dynamic biomarker, as ctDNA is present in blood and the blood samples can be collected repeatedly over time. As a result, assessment of ctDNA can support longitudinal monitoring of tumour dynamics. Additionally, ctDNA can facilitate MRD detection that may remain undetectable by conventional imaging, because tumour-derived DNA with extremely low concentration is still assessable. Furthermore, the information offered by ctDNA is useful, as there is a positive correlation between ctDNA-detectable MRD and higher recurrence risk, and ctDNA can sometimes detect recurrence even earlier than conventional imaging. In this way, treatment can be stratified according to ctDNA detection. The positive result of ctDNA reflects the high risk of recurrence or even potential treatment escalation, while ctDNA clearance gives evidence of potential treatment de-escalation [1].

Overall, ctDNA has already shown unique potential in several aspects, such as addressing the limitations of conventional imaging and biopsy testing and identifying MRD, owing to its merits of minimally invasive sampling, dynamic monitoring and high sensitivity, with abundant retrospective studies showing that positive ctDNA test results can reflect the probability of disease recurrence after receiving immunotherapy. Nevertheless, prospective interventional trials are still required to give more solid proof of the reliability of using ctDNA to predict individual patient benefit from undergoing immunotherapy and the method of safely translating test results into practice to guide treatment escalation and de-escalation. This article is going to systematically review the existing evidence, analyse the key technical aspects of testing and clinical considerations across different application scenarios, and discuss challenges such as insufficient standardisation, variability in assay sensitivity, and barriers to clinical translation, focusing on the three core application domains, molecular residual disease detection, prognostic assessment and treatment stratification, in order to offer a reference for the clinical decision making of precise immunotherapy and future study directions (Table 1) [1].

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2. Clinical practice of ctDNA in non-small-cell lung cancer

Non-small-cell lung cancer (NSCLC) is a common category of lung cancer, which represents one of the most extensively investigated solid-tumour settings with ctDNA applied in its treatment. The treatment strategies for NSCLC show a wide difference according to its stage and resectability: the key point for patients with resectable tumours is lowering the recurrence by adjuvant immunotherapy, while the individual difference in relapse risk is wide and it is hard to reflect the dynamic molecular residual condition by conventional biopsy; as for those with unresectable stage III lung cancer, chemoradiotherapy combined with consolidation immunotherapy afterwards is given, whereas a fixed duration of treatment which cannot take the degree of tumour remission into account may lead to unnecessary toxicity or missing the timing of consolidation. The unmet need for both of them is a high-sensitivity biomarker that can be dynamically traced and provide early warning of recurrence. Because minimally invasive sampling allows high-frequency testing and can detect changes earlier than imaging, ctDNA has already been used in those settings for identifying residual disease and precise prognostic stratification, and its feasibility of personalised immunotherapy strategies is primarily tested. This section is going to organise the existing evidence of ctDNA in molecular residual disease detection, prognostic assessment and treatment decision support and discuss the technology and biological considerations in clinical translation, focusing on resectable and unresectable NSCLC.

Table 1. Summary of the clinical evidence for ctDNA across tumour types

Cancer and clinical setting	Core studies	Main ctDNA utility and findings	Principal limitation
Non-small-cell lung cancer (NSCLC): perioperative, neoadjuvant and consolidation settings	Jun et al.; Provencio et al.; Xia et al.; Zhang et al. [2-5].	Postoperative or persistent positivity indicated MRD and higher recurrence or progression risk; clearance or sustained negativity was associated with better outcomes.	Analyses were mainly observational or correlative, and treatment was not assigned according to ctDNA status.
Triple-negative breast cancer (TNBC): neoadjuvant and postoperative adjuvant settings	Pusztai et al.; Lynce et al.; Turner et al. [6-8].	ctDNA further stratified risk among patients with residual disease; c-TRAK TN prospectively used ctDNA detection to trigger pembrolizumab.	Small exploratory cohorts and late molecular detection limited evidence for predictive treatment benefit.
Melanoma: postoperative adjuvant and advanced disease	Marchisio et al.; Martines et al. [9, 10].	Baseline or persistent ctDNA was associated with relapse or shorter progression-free survival (PFS), while clearance may reflect treatment response.	Small cohorts, mutation-restricted assays and no ctDNA-guided treatment assignment.
Oesophageal squamous cell carcinoma (ESCC): neoadjuvant, postoperative and definitive-treatment settings	Morimoto et al.; Wang et al. [11, 12].	Serial ctDNA showed potential for response assessment, postoperative MRD detection and earlier recurrence warning.	The outcome study evaluated chemotherapy, while ECMRD-001 is a protocol without reported clinical outcomes.

2.1. Postoperative ctDNA-MRD for recurrence risk stratification in resectable NSCLC

Evidence of the effectiveness of ctDNA in MRD assessment in NSCLC was established in patients receiving curative-intent surgery. The visible solid tumour is removed during surgery, after which detected ctDNA may indicate residual disease. For this reason, patients undergoing surgery represent an informative clinical model for testing the efficacy of ctDNA-MRD [4, 5].

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In LUNGCA-1, a prospective multicentre cohort study of 330 resectable stage I–III NSCLC patients, perioperative ctDNA was assessed before and shortly after surgery. It illustrates that postoperative ctDNA positivity, defined as positive ctDNA at three days and/or one month after surgery, is related to recurrence (hazard ratio [HR]. 11.1) and may reflect relapse prognostically beyond what conventional clinicopathological factors do. These findings can support the meaning of postoperative ctDNA as an indicator of residual disease and recurrence risk, although the study is an observational design which cannot establish its ability to guide adjuvant treatment [4].

Zhang et al. further demonstrated the value of longitudinal monitoring in 261 patients with resected stage I–III NSCLC. In this study, only a small portion of patients with persistently undetectable MRD experienced recurrence, corresponding to a negative predictive value (NPV) of 96.8%, whereas patients with longitudinally detectable MRD had a positive predictive value (PPV) of 89.1%. Furthermore, it was shown that ctDNA detection preceded clinical recurrence by a median of 3.4 months. The results gained suggest that sustained MRD negativity may indicate lower recurrence risk more reliably than a single postoperative measurement, despite the limited sensitivity remaining for brain-only recurrence [5].

Generally, LUNGCA-1 primarily establishes the prognostic utility of perioperative ctDNA assessment, while Zhang et al. further illustrated the importance of dynamic monitoring, which can distinguish sustained negativity from transient or later-emerging MRD [4, 5].

2.2. ctDNA dynamics and prognosis during neoadjuvant chemoimmunotherapy in resectable NSCLC

In the NADIM study, ctDNA concentration before surgery showed potential value for prognostic stratification in patients with resectable stage IIIA NSCLC receiving neoadjuvant chemoimmunotherapy. Patients were classified using a maximum mutant allele fraction threshold of 1%. Compared with the high-ctDNA group, those below this threshold experienced more favourable progression-free and overall survival, with corresponding hazard ratios of 0.20 and 0.07. These findings suggest that ctDNA measurement during neoadjuvant treatment may help distinguish patients with different prognostic profiles [3].

Crucially, among patients with undetectable ctDNA who underwent three cycles of neoadjuvant therapy prior to surgery, both progression-free survival (PFS) and overall survival (OS) were significantly improved (hazard ratios of 0.26 and 0.04), suggesting that molecular assessment provides insights earlier than radiological evaluation and is independent of it. The study further compared the discriminative ability of the two assessment methods; the concordance index (C-index) for predicting OS based on ctDNA status reached 0.82, compared to 0.72 for imaging, indicating that molecular response captures more comprehensive biological information [3].

Although the single-arm trial cannot definitively confirm the utility of ctDNA in guiding treatment adjustments in a single-arm trial, the clinical significance of ctDNA was extended to dynamic monitoring during adjuvant therapy before surgery, where it can offer an assessment of the depth of tumour regression at the molecular level through this study. In the future, its clinical value requires further validation through randomised controlled trials [3].

2.3. ctDNA kinetics for risk assessment during consolidation immunotherapy in unresectable stage III NSCLC

BTCRC LUN 16-081 investigated serial ctDNA changes in 38 patients with unresectable stage III NSCLC undergoing a short course of consolidation immunotherapy. Following chemoradiotherapy

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(CRT), participants received either nivolumab or nivolumab plus ipilimumab for up to six months. CtDNA measurements were performed at three treatment points: following CRT, after the first cycle of immune checkpoint inhibition, and upon completion of consolidation therapy. This design allowed post-CRT ctDNA status to indicate possible residual disease, while subsequent measurements were used to monitor molecular changes during immunotherapy [2].

CtDNA status separated patients into groups with different outcomes at each measurement point. Following CRT, patients with detectable ctDNA had a 24-month PFS rate of 29%, whereas the rate reached 65% among those with no detectable ctDNA. A larger difference was seen after the first checkpoint inhibitor cycle, with rates of 0% and 72% in the ctDNA-positive and ctDNA-negative groups, respectively. By the end of consolidation therapy, 24-month PFS was 15% in patients with detectable ctDNA and 67% in those without it. In addition, every patient whose ctDNA level increased after one treatment cycle developed disease progression within the following 12 months. Therefore, an early rise in ctDNA may indicate an unfavourable response before the full course of immunotherapy has been completed [2].

These findings suggest that ctDNA may have a role beyond the detection of MRD after CRT, as repeated measurements can also provide information during consolidation immunotherapy. In clinical practice, such monitoring might help recognise patients who could complete treatment earlier, as well as those who need more intensive therapy or follow-up. Nevertheless, the analysis was based on a small cohort and only examined the association between ctDNA and outcomes. Treatment duration was not determined by ctDNA results. It therefore remains uncertain whether changing treatment according to ctDNA status can improve patient outcomes, and this question should be addressed in prospective randomised trials [2].

Across these four studies, the clinical meaning of ctDNA varies with the disease settings and the treatment strategies, while the utility of identifying residual disease and recurrence risk remains unchanged. LUNGCA-1 demonstrates that ctDNA can reflect MRD and high risk of postoperative relapse, and Zhang et al. focus more on dynamic monitoring. To aid immunotherapies, ctDNA shows its ability to assess risk at the molecular level. Across these clinical settings, the evidence for the prognostic meaning of ctDNA is considerably stronger than the evidence for its treatment-guiding utility. There are some potential strategies for treatment stratification according to the ctDNA level, guiding the escalation or de-escalation of adjuvant immunotherapy, duration of treatment and risk assessment. Nevertheless, these ctDNA analyses were primarily observational or correlative. Because treatment was not assigned according to ctDNA status, they could not provide predictive treatment benefit. Overall, ctDNA can support MRD detection and prognostic assessment, although it cannot guide or determine the escalation, de-escalation or duration of treatment [2–5].

3. Clinical application of ctDNA in triple-negative breast cancer

Triple-negative breast cancer (TNBC) provides a useful context for evaluating ctDNA, since residual invasive disease after neoadjuvant therapy is linked to a greater likelihood of relapse. However, patients do not benefit equally from additional immunotherapy. In KEYNOTE-522, pembrolizumab was administered with neoadjuvant chemotherapy and then continued as adjuvant treatment, resulting in improved pathological response and event-free survival (EFS) in patients with high-risk early-stage TNBC. An exploratory residual cancer burden (RCB) analysis found that pembrolizumab increased the proportion of patients in lower residual cancer burden categories. The greatest reduction in EFS events was observed in the RCB-2 subgroup (HR 0.52; 95% confidence interval (CI), 0.32–0.82). Nevertheless, all patients continued adjuvant pembrolizumab regardless of their pathological response, while RCB was available only after surgery. Therefore, an additional

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molecular marker may be needed to monitor residual disease over time and to separate patients who may need intensified therapy from those with a relatively low risk of relapse [6].

The OXEL randomised phase II trial explored this issue in 45 patients who had residual TNBC following neoadjuvant chemotherapy and surgery. Participants received adjuvant nivolumab, capecitabine or both, and ctDNA was included as an exploratory secondary biomarker. Testing was successful in 38 patients, of whom 13 (34%) had detectable ctDNA at enrolment. After a median follow-up period of 20.4 months, distant relapse occurred in 14 patients; 10 of them had been ctDNA-positive at baseline. Median invasive disease-free survival was 4.52 months in the baseline-positive group but was not reached in patients without detectable ctDNA. This association remained significant after multivariable adjustment (HR 50.70; 95% CI, 6.52–393.98). The six-week results also showed a clear difference: all nine patients with persistent ctDNA later developed distant recurrence, whereas three of the four patients who achieved ctDNA clearance remained recurrence-free at the data cut-off [7].

These results illustrate that ctDNA can provide prognostic information beyond the presence of residual invasive disease. That is to say, pathological residual disease identifies a clinically high-risk population, whereas ctDNA may further identify the patients with molecularly active residual disease. Nevertheless, ctDNA was not used to assign treatment in OXEL; the distribution of ctDNA-positive patients was unbalanced across treatment arms, and the small sample size made the ctDNA analysis exploratory. Therefore, the study supports ctDNA as a prognostic and dynamic monitoring biomarker, but cannot establish whether nivolumab, capecitabine or their combination should be selected according to ctDNA status [7].

Unlike previous observational studies, c-TRAK TN prospectively examined whether ctDNA-detected molecular relapse could be used to initiate immunotherapy. This multicentre phase II trial enrolled 161 patients in a surveillance programme, with blood collected at three-month intervals. During the first year, ctDNA became detectable in 44 patients (27.3%). Of the 32 patients who entered the intervention group, staging imaging showed that 23 already had metastatic disease when ctDNA was first identified. Consequently, pembrolizumab was started in only five patients, and sustained ctDNA clearance was not observed in any of them [8]. The trial demonstrated that prospective ctDNA monitoring and molecularly triggered intervention were possible. However, it also suggested that the opportunity for early treatment may be missed if molecular relapse is detected only after the disease has become radiologically visible. More sensitive assays or shorter sampling intervals may therefore be required for a genuinely pre-emptive strategy.

Generally, the evidence in TNBC demonstrates a progression from pathological risk stratification to molecular risk assessment and finally to ctDNA-triggered intervention. KEYNOTE-522 provides the therapeutic context in which residual disease remains clinically meaningful; OXEL shows that baseline positivity and persistent ctDNA are strongly associated with recurrence, and c-TRAK TN tests whether this information can be translated into treatment. Across these studies, ctDNA has a clear prognostic meaning, but the predictive value for individual benefit from immunotherapy remains uncertain. Future interventional trials may need earlier postoperative sampling, more sensitive assays and shorter testing intervals before ctDNA can reliably guide the escalation or de-escalation of adjuvant immunotherapy [6-8].

4. Clinical application of ctDNA in melanoma

In melanoma, ctDNA can be applied in both the postoperative adjuvant setting and advanced disease treated with immune checkpoint inhibitors. The ability to quantify a known B-Raf proto-oncogene, serine/threonine kinase (BRAF) or Neuroblastoma rat sarcoma viral oncogene homolog (NRAS)

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mutation repeatedly makes melanoma particularly suitable for longitudinal monitoring, while the biological and clinical meaning of ctDNA still differs according to whether the patient has radiologically undetectable disease after surgery or measurable metastatic disease [9, 10].

In a prospective study by Marchisio et al., 32 patients with resected stage III BRAF-mutant cutaneous melanoma were monitored during adjuvant treatment. Dabrafenib combined with trametinib was administered to most patients, while nivolumab was given to three patients. CtDNA was detected at baseline in 11 patients, and its presence was related to an increased likelihood of relapse (HR 3.79; 95% CI, 1.20–12.00). A marked difference in three-year OS was also observed according to baseline ctDNA status, with rates of 54.6% in the ctDNA-positive group and 95% in the ctDNA-negative group. The corresponding HR for death was 7.92 (95% CI, 1.56–40.36). Serial monitoring provided further information, because ctDNA clearance by the end of treatment occurred only in baseline-positive patients who remained relapse-free, while continued ctDNA detection appeared to indicate recurrence at an earlier stage [9].

This study supports postoperative ctDNA as a biomarker which can reflect molecular residual disease and demonstrates the additional value of serial testing. Nevertheless, its relevance specifically to immunotherapy is limited, because only a small proportion of patients received nivolumab and most received targeted therapy. In addition, the mutation-specific droplet digital PCR (ddPCR) approach can only assess patients with a trackable BRAF mutation. Therefore, the study establishes prognostic utility more strongly than the ability to guide adjuvant immunotherapy [9].

The LIQUID-MEL pilot study prospectively included 23 patients with advanced melanoma harbouring BRAF or NRAS mutations. Participants were treated with either nivolumab alone or nivolumab combined with ipilimumab. Plasma samples were obtained at baseline, before cycle two, during the first imaging evaluation and when disease progression occurred. CtDNA was present at baseline in five patients. Median PFS was 3.88 months in patients without initial ctDNA shedding, compared with 0.69 months in those with detectable ctDNA ($p=0.012$). Although a difference in OS was observed between the two groups, it was not statistically significant. Baseline ctDNA shedding also lost its prognostic and predictive significance after adjustment in the multivariable analysis. Because only a small number of patients were included, findings from serial ctDNA monitoring remained descriptive [10].

Across these two melanoma studies, ctDNA positivity indicates higher tumour burden or residual disease, while ctDNA clearance may reflect treatment response. The postoperative study demonstrates a strong association with relapse and survival, and LIQUID-MEL extends dynamic monitoring to advanced disease during immune checkpoint inhibition. Nevertheless, there are limitations in both studies due to the small number of patients included, restricted mutation coverage and the absence of ctDNA-guided treatment assignment. As a result, ctDNA can support prognostic assessment and complement radiological monitoring in melanoma, although it cannot currently determine the choice, duration or discontinuation of immunotherapy [9, 10].

5. Clinical utility of ctDNA in gastro-oesophageal tumours

Oesophageal squamous cell carcinoma (ESCC) frequently involves alterations in genes such as epidermal growth factor receptor (EGFR), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and Neurogenic locus notch homolog protein 1 (NOTCH1), together with abnormal activity in signalling pathways including mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog 1 (AKT). These molecular changes contribute to tumour development and progression. Meanwhile, DNA fragments released from tumour cells can enter the circulation and provide the biological basis for ctDNA

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detection and monitoring. The treatment paradigm for this disease encompasses multiple modalities —including neoadjuvant therapy, surgery, chemoradiotherapy, and immunotherapy—and given the high degree of tumour heterogeneity and the inability of conventional tissue biopsies to capture dynamic clonal evolution in real time, ctDNA can provide molecular-level information at various treatment milestones [11, 12]. Therefore, ctDNA may offer molecular information at several time points across treatment [11, 12].

Morimoto et al. conducted serial plasma ctDNA testing in patients with locally advanced ESCC who underwent neoadjuvant chemotherapy and subsequent oesophagectomy. Before treatment, mutations detected in plasma were consistent with those in primary tumour tissues in 11 of 13 patients. Following neoadjuvant chemotherapy, ctDNA remained detectable in all pathological non-responders, whereas it was present in only 25% of responders ($p=0.007$), indicating that changes in ctDNA status may help assess treatment response before surgery. A clearer association was observed after surgery. Among the 16 patients included in the postoperative analysis, the one-year recurrence-free survival rate was 90% for those without detectable ctDNA, compared with 0% for patients who remained ctDNA-positive ($p=0.0008$). Furthermore, in two patients who subsequently developed recurrence, ctDNA became detectable approximately 5.5 months before the disease was identified by imaging [11].

These findings demonstrate the probability that ctDNA can be used to assess neoadjuvant response, identify postoperative MRD and provide earlier warning of recurrence. However, this study evaluated neoadjuvant chemotherapy rather than immunotherapy and included a small number of patients. Consequently, it provides a biological and clinical basis for ctDNA monitoring in ESCC, but does not prove that changing immunotherapy according to ctDNA can improve outcomes [11].

ECMRD-001 prospectively evaluates the potential role of MRD in supporting treatment decisions for patients with inoperable stage II–III ESCC. The treatment pathway includes neoadjuvant immunochemotherapy, radical chemoradiotherapy given with immunotherapy, and subsequent adjuvant immunotherapy. MRD is monitored at different treatment stages to examine molecular changes and their relationship with clinical response. The protocol plans ctDNA detection at multiple stages, including before and after neoadjuvant treatment, during radical chemoradiotherapy and during adjuvant immunotherapy [12]. In this way, the study may clarify whether ctDNA clearance can identify molecular response and whether persistent positivity indicates a population requiring intensified treatment or closer monitoring.

Nevertheless, ECMRD-001 is a study protocol and no clinical outcome results were available in the cited publication. It cannot currently support ctDNA-guided escalation or de-escalation as an established strategy. Generally, the evidence in ESCC illustrates the transition from observational detection of molecular response and recurrence risk to prospective integration with immunotherapy. The prognostic utility is promising, while treatment-guiding utility still requires completed prospective studies and comparison between ctDNA-informed and standard clinical management [11, 12].

6. Conclusion

Across NSCLC, TNBC, melanoma and ESCC, ctDNA has shown consistent potential in three core application domains: MRD detection, prognostic assessment and treatment stratification. After curative-intent treatment, detectable ctDNA can identify residual disease that remains invisible on conventional imaging. During neoadjuvant, adjuvant or advanced treatment, longitudinal changes can provide a dynamic assessment of tumour response. Persistent positivity or re-emergence is

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generally associated with recurrence or progression, while sustained negativity and clearance indicate a more favourable prognosis.

However, the strength of evidence is not equal across these applications. The prognostic meaning of ctDNA is supported by multiple prospective cohorts and correlative analyses, but most studies did not assign treatment according to ctDNA status. Even in c-TRAK TN, where ctDNA detection triggered pembrolizumab, many patients already had radiologically detectable metastatic disease and very few received the planned intervention. This illustrates that demonstrating an association with recurrence is different from proving predictive treatment benefit.

There are also several technical and clinical barriers. Assay sensitivity varies according to tumour type, disease burden, shedding condition, mutation coverage and sampling schedule. Residual disease cannot always be excluded when a negative result is shown, especially in low-shedding tumours or sanctuary sites such as the brain. Moreover, thresholds for positivity, definitions of clearance and optimal testing intervals remain insufficiently standardised. False-negative results may lead to inappropriate de-escalation, whereas a positive result without an evidence-based intervention may cause anxiety, additional imaging and treatment-related toxicity.

Overall, ctDNA can already support risk assessment and complement imaging, pathology and clinicopathological factors, but limitations still exist, and it should be combined with other clinical evidence instead of being used in decision-making of immunotherapy escalation, de-escalation or duration outside prospective studies without other support. Future trials need to demonstrate that ctDNA-guided decisions improve patient outcomes rather than only predict them. With standardised assays, earlier and more frequent sampling and randomised interventional validation, ctDNA may become an important tool for selecting patients who need additional immunotherapy and sparing low-risk patients from unnecessary toxicity and cost.

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