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Review Article

Circulating tumor DNA (ctDNA) as a biomarker for lung cancer: Early detection, monitoring and therapy prediction

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Abstract. Circulating tumor DNA (ctDNA), i.e., DNA shed from tumor cells into the bloodstream, is emerging as one of the most useful plasma biomarkers in patients with multiple types of cancer, including patients with non-small cell lung cancer (NSCLC). Indeed, NSCLC was the first malignancy in which measurement of ctDNA was approved for clinical use, i.e., mutational testing of *EGFR* for predicting response to EGFR tyrosine kinase inhibitors in patients with advanced disease. Although historically the gold standard method for *EGFR* mutational analysis required tumor tissue, the use of ctDNA is more convenient and safer for patients, results in a faster turn-around-time for return of results, provides a more complete representation of genetic alteration in heterogeneous tumors and is less costly to perform. Emerging uses of ctDNA in patients with lung or suspected lung cancer include screening for early disease, surveillance following initial treatment and monitoring response to therapy in metastatic disease. For evaluating therapy response, ctDNA appears to be especially useful in patients receiving targeted therapies against driver oncogenes or immunotherapy. Further work should not only validate these emerging findings but also aim to optimize and standardize ctDNA assays.

Keywords: Lung, cancer, ctDNA, liquid biopsy, tumor marker, biomarker

1. Introduction

Biomarkers play an important and sometime vital role in the management of patients with cancer [1, 2]. However, until relatively recently, circulating biomarkers were not widely used in patients with lung cancer. In the last decade this situation has changed, especially in the use of predictive biomarker for targeted therapies and immunotherapy in patients with the most common form of lung cancer, i.e., non-small cell lung cancer (NSCLC) [3–5]. Indeed, NSCLC is one of the cancers pioneering the use of predictive biomarkers for selecting molecularly targeted therapies [3–5].

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Of the various new blood-based biomarkers proposed for lung cancer, one of the most promising is the measurement of DNA shed form tumors into the systemic circulating, i.e., circulating tumor DNA or ctDNA [3–6]. NSCLC was the cancer in which ctDNA was first approved for clinical purposes, i.e., mutation testing of *EGFR* for predicting response to anti-EGFR therapies when tissue was unavailable. The primary aim of this article is to discuss the use of ctDNA as a predictive biomarker for anti-EGFR as well other therapies in patients with NSCLC. In addition, I discuss emerging data on the use of ctDNA in screening for lung cancer, follow-up after curative surgery and monitoring therapy effectiveness. As mutational analysis of *EGFR* is one of the most widely investigated NSCLC biomarkers and one of the most widely used in clinical practice, the article will predominantly focus on it.

2. *EGFR* mutations for predicting response to EGFR tyrosine kinase inhibitor: The prototype ctDNA therapy predictive biomarker

As mentioned above, the first ctDNA tests to enter clinical application was the use of *EGFR* mutations for predicting benefit from EGFR tyrosine kinase inhibitor (TKIs) in patients with advanced NSCLC. EGFR (also known as HER1) is a member of the HER/ErbB family of transmembrane proteins that also includes the structurally related molecules, HER2 (c-erbB2), HER3 and HER4 [7]. These 4 proteins contain an extracellular ligand-binding region, a transmembrane region and an intracellular domain that contains tyrosine kinase (TK) activity. Activation of EGFR which is normally mediated by specific ligand binding to the extracellular region leads to intracellular signalling, altered gene transcription and ultimately increased cell proliferation. Aberrant EGFR signalling resulting from mutations in exon 18–24 of the tyrosine kinase domain leads to uncontrolled proliferation which can eventually culminate in malignancy.

3. Prevalence and types of *EGFR* mutations in non-small cell lung cancer

One of the most frequent genetic alterations in NSCLC are mutations in *EGFR* [8]. The prevalence of such mutations in NSCLC patients varies, depending on race or global location. Thus, in a comprehensive review of 136,533 *EGFR* mutation tests performed worldwide, Graham et al. [9] found a mutation prevalence of 46% in southern Asia, 30% in northern Asia, 16% in Africa and the Middle East, 13% in Europe, and 9% in North/South America.

The most frequently occurring mutations in *EGFR* are in exon 19 (exon19del) (44%) and exon 21 (L858R) (40%) [10]. The 3rd most frequent type of mutation involves insertions in exon 20 (ex20ins) [9, 11]. Although ex20ins mutations are present in approximately 10% of mutant *EGFR* NSCLC cases, they are found in only 1-2% of all NSCLC patients It is unclear however, if the prevalence of this mutation varies by race. Other rare mutations in *EGFR* include S768I, G719X and L861Q [12].

All the above mutations are referred to as activating mutations as they lead to increased and constitutive downstream signaling from EGFR, potentially resulting in cancer formation and progression. Exon19del and L858R are also known as sensitizing mutations as they confer sensitivity to several clinically approved EGFR TKIs including the first-generation TKIs, erlotinib and gefitinib, the second-generation TKIs, afatinib and dacomitinib and the third generation TKI, osimertinib.

4. Mutant *EGFR* in tissue as a predictive biomarker for EGFR TKIs

Although early studies found no relationship between EGFR protein levels determined in tissue by immunohistochemistry and response to EGFR TKIs, subsequent reports showed that the presence of

EGFR exon19del or L858R point mutation were strongly associated with response [13–15]. In the first proof concept showing this relationship, Lynch et al. [13] reported that 8/9 patients with EGFR TKI-responsive NSCLC contained somatic mutations in the *EGFR* gene compared to 0/7 mutation-negative patients who showed no response. Subsequently, large trials confirmed these finding, showing that 60–80% of *EGFR*-mutation-positive patients exhibited an objective response with median progression-free survival periods of 9 to 12 months with the first generation of TKIs such as gefitinib and erlotinib. In contrast, *EGFR* mutation-negative patients almost never benefited from these inhibitors. Subsequently, several randomized prospective trials reported superior response rate and progression-free survival with erlotinib or gefitinib versus standard platinum-based chemotherapy in patients with the *EGFR*-sensitizing mutations [14, 15].

Unlike the findings with exon19del or exon 21 L858R mutations, most of the ex20ins mutations do not confer sensitivity to the standard TKIs. More recent work however, found that the presence of these mutations was associated with benefit from a newly introduced TKI known as mobocertinib [16] or a bispecific antibody that targets both EGFR and MET, known as amivantamab-vmjw [17].

5. Use of circulating tumor DNA to detect *EGFR* mutations

All the early studies relating *EGFR* mutational status with response to TKIs used tumor tissue to detect the relevant mutations. However, obtaining lung cancer tissue is an invasive procedure that may lead to patient discomfort or possible harm. For example, the reported rate of pneumothorax following lung biopsy was 17 to 38% of patients undergoing the procedure [18]. A further problem with biopsy is that for 20–30% of patients with advanced NSCLC, adequate amounts of suitable tissue for biomarker analysis cannot be obtained [18]. Even if suitable and adequate amounts of tissue are procured, a biopsy taken from a single location within a tumor may not provide comprehensive molecular information, especially in molecularly heterogeneous samples or when multiple metastases are present.

Many of these problems can be circumvented by using ctDNA, rather than tissue, for mutational analysis. In addition to the relatively non-invasive procurement of plasma, use of this fluid enables a more rapid turn-around time for results as well as being less costly to assay (for review, see ref. 4). Furthermore, all tumor sites (e.g., multiple metastatic sites) might be expected to shed mutant DNA, enabling ctDNA to potentially provide a more comprehensive overview of all the genetic alterations present, than might be obtained with a single site biopsy. Interestingly, in one study, therapeutically actionable mutations were found in ctDNA in 10–20% of NSCLC patients who had insufficient tissue or failed tissue analysis [19].

Overall, good concordance has been found between the mutational status of tissue and corresponding ctDNA, especially for the exon19del and L858R point mutations [4]. Thus, in a meta-analysis of 32 studies involving a total of 4527 patient with advanced NSCLC, the pooled sensitivity of ctDNAs for detecting tissue mutations was 0.70 [95% confidence interval (CI), 0.63–0.75], the pooled specificity was 0.98 (95% CI, 0.96–0.99), the pooled positive predictive values (PPV) was 0.97 (95% CI, 0.95–0.99) and the pooled negative predictive values (NPV) was 0.75 (95% CI, 0.74–0.76)tumour [20]. Most of the individual studies investigated in the meta-analysis were small and retrospective in design. However, in a single center prospective trial involving 180 patients with advanced non-squamous NSCLC, ctDNA was found to have a specificity of 100% for both *EGFR* 19 del and L858R mutations [21]. The sensitivity of ctDNA however was less good, i.e., was 82% for *EGFR* 19 del and 74% for L858R mutations.

Based on these and other reports [3, 4, 14], ctDNA appears to have excellent specificity for detecting *EGFR* mutations present in NSCLC tissue. Thus, if a ctDNA result is positive for *EGFR* mutations, patients should be considered for treatment with an EGFR TKI. On the other hand, a negative finding

may not reliably reflect the tissue status. In this situation, tissue analysis should be performed where possible. Despite the lower sensitivity for ctDNA, both types of assays when applied to the measurement of exon19del and L858R mutations, were shown to have similar predictive value for response to EGFR TKIs in patients with advanced NSCLC [22, 23].

Because of its convenience, relative non-invasive sampling, faster turn-around-times and good agreement with tissue finding, several international expert panels state that ctDNA (at least in some situations) may be used to assess the mutational status of *EGFR* for predicting benefit from EGFR TKIs. Indeed, as far back as 2018, jointly published guidelines by the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) stated that "in some clinical settings in which tissue is limited and/or insufficient for mutation testing, physicians may use a ctDNA assay to identify *EGFR* mutations" [24]. The guidelines, however, cautioned that because the sensitivity of ctDNA assay may be inadequate, a negative result using ctDNA cannot provide reliable evidence that the corresponding tumor is also *EGFR* mutation-negative.

In 2021, updated guidelines published by the International Association for the Study of Lung Cancer (IASLC) stated that "in patients with oncogene addicted NSCLC, liquid biopsy is emerging as not only complimentary to tissue analysis but is also acceptable as the initial approach (plasma first)" [25]. In contrast, the most recent National Comprehensive Cancer Network (NCCN) guidelines state that ctDNA should not be used instead of a histological tissue diagnosis. However, the organization also added that a ctDNA test could be considered if a patient was unfit for tissue sampling or if there was insufficient tissue for analysis [26]. Finally, the 2022 European Society of Medical Oncology (ESMO) states that ctDNA testing can be used "in treatment-naive patients and is especially recommended when a significant delay is expected in obtaining tumour tissue for genotyping, when invasive procedures may be risky or contraindicated, or bone is the only site that could be biopsied". ESMO however, cautions that small-volume tumors such as intrathoracic tumours, or predominantly intracranial disease, can result in high false-negative findings [27].

It was mentioned above that patients with ex20ins mutations tend to be resistant to the first generation of EGFR TKI but may respond to amivantamab-vmjw or mobocertinib. The US Food and Drug Administration have approved the Guardant360 CDx ctDNA test for predicting response to amivantamab-vmjw.

6. Use of circulating tumor DNA to detect *EGFR* Mutations conferring resistance to first or second generation TKIs

Although EGFR TKI such a gefitinib or erlotinib are initially effective in inducing tumor regression in most patients with *EGFR*-sensitizing mutation, resistance inevitable develops in effectively all treated patients. In approximately 50% of cases, this acquired resistance is caused by a secondary mutation in *EGFR*, i.e., T790M. Patients developing this specific mutation, however, can be treated with the 3rd generation EGFR TKI, osimertinib [28].

Similar with the *EGFR* sensitizing mutations discussed above, T790M mutations can also be detected using ctDNA [4]. Concordance between the *EGFR* T790M mutational status in plasma and tumor tissue appears to be less strong than that found with the sensitizing mutations. Thus, following a systematic review and meta-analysis, the pooled sensitivity of ctDNA for tissue mutations was 0.67 (95% CI: 0.64–0.70), the pooled specificity was 0.80 (95% CI: 0.77–0.83), the pooled positive PPV was 0.85 (95% CI: 0.82–0.87) and the pooled NPV was 0.60 (95% CI: 0.56–0.63) [29]. As with all analytes, the sensitivity of ctDNA *EGFR* T790M mutations depend on the type of assay used. Indeed, using certain PCR assays, the sensitivity for detecting T790M mutation in plasma reached 93% [3].

Table 1
Biomarkers for predicting response to targeted therapies in the treatment of advanced non-small cell lung cancer

Drug target/ biomarker	Genetic alteration	Prevalence (%)	Therapy
EGFR	Mutation (del19, L858R)	19	Gefitinib, erlotinib, afatinib, dacominitib, osimertinib
EGFR	Mutation (T790M)		Osimertinib
EGFR	Mutation (ins20)	1-2	Amivanttamab-vmjw, mobocertinib
ALK	Translocation	6	Alectinib, brigatinib, ceratinib, lorlatinib
MET	Mutation (exon 14 skipping)	5	Capmatinib, tepotinib
ROS	Translocation	1	Cerotinib, crizotinib
BRAF	Mutation (V600E)	5	Dabrafenib + trametinib
RET	Translocation	1	Selpercatinib, pralsetinib
KRAS	Mutation (G12C)	23	Sotorasib

Data relating to the prevalence of the different genetic alterations taken from ref. 8. Data relating to therapy as recommended by the National Comprehensive Cancer Center (NCCN) [26] and The European Society of Medical Oncology (ESMO) [27].

According to the 2017 joint guidelines published by CAP, IASL and AMP, ctDNA may be used to detect *EGFR* T790M mutations in lung adenocarcinoma patients following progression or acquired resistance to first/second generation of EGFR TKIs. Again, reflexing to tumor tissue was recommended if the plasma result was negative [24]. In 2021, the IASL stated that when acquired resistance to EGFR TKIs develop, initial use of ctDNA is the preferable way to identify the T790M resistance mutation [25].

Thus, in patients developing resistance to a first/second generation TKIs, an emerging practice is to first test for the T790M mutation in plasma and if positive, administer osimertinib. On the other hand, if the T790M mutation is undetectable in plasma, tissue analysis for the mutation should be carried out if feasible.

7. Use of ctDNA to detect other therapy predictive biomarkers in NSCLC patients

While *EGFR* mutation analysis was the first therapy predictive biomarker used in patients with NSCLC, several others were recently recommended for determining response to their specific matching therapy (Table 1). Similar with *EGFR* mutations, most of these genetic alterations can also be detected using ctDNA. Although less extensively investigated than *EGFR* mutations, good agreement between tissue and ctDNA has also been obtained especially for *ALK* translocations, *ROS* translocations, *BRAF* mutations, *MET* exon-14 skipping mutations and *RET* translocations [30]. Depending on the assay used however, detecting translocation such as those of *ALK* or *ROS* may be difficult using ctDNA. Indeed, these translocations may be best measuring using RNA-seq. Despite possible difficulties with ctDNA, the 2021 IASL guidelines state that this fluid may be used to measure all of the above biomarkers [25].

Finally, ctDNA is undergoing investigation for predicting response to immunotherapy, especially immune checkpoint inhibitors (ICI). For predicting response to ICI, the mutations detected in ctDNA are usually converted to what is referred to as the tumor mutational burden (TMB). Tissue TMB

(tTMB) is one of a small number of recommended biomarkers for identifying patients likely to benefit from immune checkpoint inhibitors [5]. Although only a moderate correlation has been found between tTMB and plasma TMB (pTMB) [31–33], several emerging studies suggest that high pTMB levels are also associated with benefit from immune checkpoint inhibitors in patients with advanced NSCLC [34–37]. However, due to lack of standardization, lack of a universally accepted cut-off value and lack of adequate clinical validation, measurement of TMB using ctDNA is not currently recommended for clinical use.

8. Emerging clinical uses of ctDNA in patients with NSCLC

8.1. Screening for early disease

Screening for lung cancer in high-risk patients using low dose computed tomography (CT) is currently performed in some countries. Compared to CT, blood-based biomarkers has several advantages including convenience, high-throughput, lower costs and lack of exposure for participants to potentially dangerous ionizing radiation. Conventional serum protein biomarkers for lung cancer such as CYFRA 21-1, GRP, NSE and CEA however, lack specificity and sensitivity for the early detection of lung cancer [38]. Thus, research for the identification of new lung cancer screening biomarkers in recent years has shifted from protein to non-protein molecules. Of the various non-protein biomarkers evaluated to-date, one of the most promising is the measurement of ctDNA.

Many of the studies investigating ctDNA as a screening test for lung cancer have used "pan-cancer" assays in which lung cancer was one of several different cancer types included in the analysis. In one of these early studies on this topic, Phallen et al. [39] compared the ctDNA mutational profile of 58 cancer-associated genes from 200 patients with a previous diagnosis of several different types of cancer and 44 apparently healthy controls. In this study 71 patients had lung cancer (histology type not specified) and of these 44 (62%) were positive for ctDNA. Similar to the situation with standard biomarkers, sensitivity increased with increasing disease stage, i.e., 45% in stage I, 72% in stage II, 75% in stage III and 83% in stage IV. Importantly, all the 44 healthy controls investigated were negative for the driver gene mutations analyzed. However, 16% of healthy controls had mutations in genes associated with a benign condition known as clonal haematopoiesis of indeterminate potential (CHIP) (see below).

Rather than detecting mutations, Klein et al. [40] investigated targeted methylation of ctDNA in combination with artificial intelligence as a pan-cancer screening test. Specificity was investigated in 2069 "non-cancer" controls and set at 99.5%. At this specificity, 302/404 patients with lung cancer were deemed positive, i.e., a sensitivity of 75%. Positivity was 20.9% in stage I disease, 79.5% in stage II, 90.7% in stage III and 95.2% in stage IV. As in the study of Phallen et al. [39] mentioned above, the histology type of the lung cancers investigated in this study was not specified.

In a study that focused specifically on patients with NSCLC, Chabon et al. [41] developed and prospectively validated a machine-learning ctDNA test which they dubbed 'lung cancer likelihood in plasma' (Lung-CLiP). The authors initially trained Lung-CLiP using samples from a discovery group of 104 patients with early-stage NSCLC and 98 non-cancer controls (56 of which were risk-matched controls undergoing CT screening for suspected lung cancer). At 98% specificity, sensitivity was 41% in patients with stage I disease, 54% in patients with stage II disease and 67% in patients with stage III disease. Using the 98% specificity threshold obtained in the training set, Lung CLiP was validated using a different population of early stage NSCLC patients ($n = 46$) and risk-matched controls ($n = 48$). According to the authors, performance in the validation cohort was statistically similar with that observed in the training set as evaluated using area under the curve (AUC) analysis and sensitivity metrics.

In another study specifically on lung cancer (all histological types), Mathios et al. [42] used a machine learning model for detecting genome-wide ctDNA fragmentation patterns, known as DELFI (DNA evaluation of fragments for early interception). Validation of DELFI was performed in a prospective study of 385 non-cancer individuals and 46 lung cancer patients. At 80% specificity, the sensitivity for cancer by combining fragmentation profiles, clinical risk factors, CEA and CT imaging, was 94% across all disease stages. At 80% specificity, the sensitivity was 91% for stages I/II and 96% for stages III/IV disease.

A limitation of all of the above screening studies is that they were retrospectively performed in patients with an established diagnosis of lung cancer. Sensitivity might thus be lower in an unscreened asymptomatic population. Furthermore, in the studies that used apparently healthy subjects as controls, specificity might be less if patients with benign lung disease or other benign diseases were included. In summary, although ctDNA assays appear to have reasonably high specificity for lung cancer, its sensitivity, especially for stage 1 disease is limited. However, because of its relative high specificity, it could complement low dose CT in screening for lung cancer.

Because of the less than ideal sensitivity of ctDNA for lung cancer detection, Nair et al. [43] measured mutations present in bronchoalveolar lavage (BAL). Using samples from 38 cases and 21 high-risk control individuals without detectable cancer, mutations were detected more frequently in BAL than in plasma cfDNA in all disease stages ($P < 0.001$). Using a Random Forest model with leave-one-out cross-validation, preliminary results suggested that the BAL DNA assay identified lung cancer with 69% sensitivity and 100% specificity and importantly detected more cancers than BAL cytology. Clearly, these promising preliminary findings require validation in studies with larger numbers of samples.

8.2. ctDNA in surveillance following treatment with curative-intent surgery

For some cancers such as those of the prostate, colon-rectum and ovary, biomarkers are widely used in surveillance following initial curative-intent treatment. The main aim of measuring biomarkers in this setting is the early detection of emerging recurrences/metastases, the assumption being that the early detection of recurrent disease, followed with an early salvage course of treatment, improves outcome. At least 3 studies have investigated the potential value of serial ctDNA determinations in NSCLC patients treated with curative-intent [45–47]. Across the studies, the sensitivity of the first post-treatment ctDNA value for predicting early recurrence varied between 36% and 100%, while the specificity varied from 71% to 100%. However, when using all the post-treatment follow-up time-point values, the sensitivity for detecting relapse increased to >80% while the specificity remained unchanged.

The median lead-time provided by serial ctDNA measurements over clinical and/or radiological finding in this setting was found to vary from approximately 2 to 7 months [45–47]. Whether initiating early therapy based on this lead-time versus waiting for clinical/radiological evidence of recurrence, improves outcome is currently unclear. However, a preliminary report involving 65 patients with locally advanced NSCLC showed that early treatment with consolidation immunotherapy improved outcome in those with ctDNA-positive disease but had no significant benefit in those with ctDNA-negative findings [48]. Although this study included a relatively small number of patients with a short follow-up period and involved retrospective analysis, the results are sufficiently encouraging to merit validation in a larger prospective randomized trial.

In summary, the measurement of ctDNA following initial treatment may identify patients at high risk of relapse who may benefit from additional courses of adjuvant therapy. Similarly, it may be able to identify patients at low risk of relapse for whom it might be possible to administer less intensive or possibly no systemic therapy. Trials addressing these questions such as the MERMAID-2 trial (NCT04642469) and the MERMAID phase III (NCT04385368) are ongoing [3].

8.3. ctDNA in monitoring response to systemic therapy in advanced disease

For evaluating therapy effectiveness in patients with advanced NSCLC, ctDNA has been best investigated in patients receiving anti-EGFR inhibitors or immunotherapy. One of the most consistent findings in this setting is that an early decline in ctDNA following the commencement of therapy correlates with good outcome [48–53]. Conversely, a failure to clear ctDNA is generally associated with a poor outcome.

While T790M mutations are amongst the most frequent mechanisms of acquired resistance to first/second generation TKI, see above, the secondary resistance mutations are more variable for the third generation, TKI, osimertinib. Amongst the mechanisms causing acquired resistance to osimertinib in the advanced disease setting are the emergence of the *EGFR* C797S mutation, amplification of *MET* and histological transformation to small cell lung cancer [54]. While the latter cannot be detected using ctDNA, amplified *MET* has been determined using ctDNA [55, 56]. Several trials are currently investigating novel drugs for targeting MET in this setting, including savolitinib, tepotinib and capmatinib [57].

In addition to targeted therapies, ctDNA has also been investigated for evaluating response to immunotherapy with immune checkpoint inhibitors in patients with metastatic NSCLC. Indeed, for monitoring response to immunotherapy in patients with advanced NSCLC, ctDNA is one of the most widely investigated circulating biomarkers [51, 52]. In this setting, patients with high pre-therapy levels (VAFs) of ctDNA tend to a have a poor outcome, but as with anti-EGFR therapy, an early decline in levels is also generally associated with a favourable response [52, 53]. Emerging findings suggest that the use of ctDNA in monitoring response to immunotherapy may be particularly useful in patients with radiologically-stable disease [52, 53] and in those exhibiting pseudoprogression [58], i.e., where transient enlargement of tumors based on radiology is found that is not accompanied by clinical evidence of progression.

Finally, in the context of using ctDNA to monitor therapy efficacy, it is important to bear in mind that as with the classical protein biomarkers, spikes or transient increases in ctDNA can occur following the initiation of therapy in patients with advanced NSCLC [59].

9. Limitations of ctDNA as a biomarker for lung cancer

Although ctDNA has multiple attractive features and indeed has potential to be a transformative biomarker for lung cancer, it also has limitations. These limitations include lack of specificity for cancer, i.e., when genetically altered DNA shed from white blood cells (WBC) is detected. This can occur when CHIP is present [60]. The prevalence of CHIP depends on age of patient (increasing frequency with increasing age), cut off value selected for the variant allele frequency (VAF) value and sensitivity of assay used. Indeed, according to some publications, CHIP may be universal in aging subjects when highly sensitive assays are employed [61, 62]. The shedding of DNA from WBC may contribute to an incorrect interpretation of ctDNA mutations, especially when the (VAF) is low. The potential problem of interference by CHIP can however, be overcome by performing paired sequencing of DNA from peripheral blood mononuclear cells. Alternatively, employment of specific bioinformatic algorithms that filter out potential CHIP mutations may be used, although this procedure may miss genetic alterations that are infrequently associated with CHIP.

A further disadvantage of ctDNA is limited sensitivity when tumor volume/burden is low. This is especially the situation when only intrathoracic tumors or brain metastasis are present [27]. Furthermore, the detection of gene fusions and alteration in gene copy numbers is more difficult with ctDNA than with tissue [27]. Thus, the reported values using ctDNA may have been underestimated. The

limited sensitivity in the presence of low volume disease however, may be negated/reduced by simultaneously measuring epigenetic alterations, by performing combined measurements at both the ctDNA and the mRNA level [4, 63] or combined measured with standard protein biomarkers. Yet another potential strategy for increasing sensitivity is to measure mutations in fluids close to metastatic sites rather than in plasma, see above.

Finally, it should be stated that compared with the measurement of standard biomarkers, detection of ctDNA requires relative labour-intensive assays, has relative slow turn-around times for results and are expensive to measure (compared to standard biomarkers).

10. Conclusion

NSCLC is the tumor type leading the way with the clinical use of ctDNA assays. As mentioned above, mutational analysis of *EGFR* using ctDNA can be currently used to predict response or resistance to specific EGFR TKIs, especially when suitable tissue is not available. Considerably further work is required before ctDNA can be used in screening for lung cancer, surveillance following initial treatment or monitoring therapy effectiveness. This additional work requires standardization of the pre-analytical steps as well as standardization of ctDNA assays. In addition, it will be necessary to demonstrate clinical utility for ctDNA in surveillance following initial treatment and in monitoring response to systemic therapy in metastatic disease. Achieving success in these areas should result in ctDNA being a widely used biomarker for patients with NSCLC.

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Conflict of interest

Michael J. Duffy is an editorial board member of Tumor Biology, but had no participation in the peer review process of this paper.

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