Cancer Treatment Reviews 78 (2019) 31–41

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Laboratory-Clinic Interface

Opportunities of circulating tumor DNA in lung cancer

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ARTICLE INFO

Keywords:
ctDNA
Lung cancer
Early detection
Targeted therapy
Immune checkpoint blockade

ABSTRACT

Current classification and treatment of lung cancer rely increasingly on molecular and genetic testing. Obtaining tumor tissue is not always feasible and multiple biopsies are undesirable. In response to the demand for non-invasive molecular and genetic testing in cancer care, several liquid biopsy technologies, including circulating DNA (ctDNA), have been developed. ctDNA analysis is now technically feasible to be carried out in large scales and integrated into clinical practice owing to the advances in technology. Despite the challenges in improving test accuracy and cost-effectiveness, there are huge potentials for ctDNA analysis in lung cancer management. This review focuses on the clinical utility of ctDNA analysis in lung cancer, including early detection, monitoring treatment response and detecting residual disease, identification of genetic determinants for targeted therapy, and predicting efficacy of immune checkpoint blockade.

Introduction

Cell-free DNA (cfDNA) refers to non-encapsulated DNA in the bloodstream, which was first discovered in the 1940s [1]. Increased levels of cfDNA were later found to be associated with pathological processes, most commonly with neoplastic diseases [2,3]. In cancer patients, a fraction of their cfDNA is derived from tumor cells and referred to as circulating tumor DNA (ctDNA).

ctDNA could be detected in patients with subclinical and localized cancer, thus potentially serve as a marker for screening and residual disease monitoring. When captured and appropriately analyzed, ctDNA reflects the genome of the tumor(s) and provide similar genetic information to tissue biopsy. Rather than reflecting the genetic composition of a single biopsied or resected tumor, ctDNA is closer to a "snapshot" of the DNA from all locoregional and metastatic tumors in the body. Therefore, ctDNA test can compliment a single biopsy, accounting for tumor heterogeneity when determining targets for treatment, monitoring response, and detecting resistance. Given that ctDNA analyses are blood-based tests and pose minimal risk for cancer patients, it can be easily obtained repeatedly to track tumor dynamics over time. If optimized for specific clinical purposes, ctDNA tests can also have a cost advantage over radiographic examinations and invasive procedures for obtaining tissue. For the reasons mentioned above, there are huge potentials for ctDNA in cancer care.

Today, lung cancer care is at the frontier of personalized medicine; classification of disease subtype, selection of treatment and identification of resistance mechanism all increasingly rely upon molecular and genetic information. It is an area where researchers and clinicians could leverage the advantages of ctDNA analysis to advance patient care rapidly. This review focuses on the clinical utility of ctDNA analysis in lung cancer, including early detection, monitoring treatment response and detecting residual disease, identification of genetic determinants for targeted therapy, and predicting the efficacy of immune checkpoint blockade.

Biological and technical aspects of ctDNA

The mechanism of release of DNA from the tissue into the circulation is not fully understood; necrosis, apoptosis, and secretion are the three presumed mechanisms [4]. Cells that undergo necrosis is considered a source of cfDNA [5]. The high necrotic activity in tumor cells could explain the increased cfDNA levels in cancer patients [2,3]. However, necrosis-derived DNA fragments were not found in some cancer patients. An alternative source of cfDNA is thought to be secretion by viable cells, [6,7]. Some evidence also suggests that the macrophages engulf the DNA fragments from necrotic tissue before they are released into the circulation [8,9]. More recent studies found that cfDNA is enriched in nucleosome-derived DNA fragments, which points

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https://doi.org/10.1016/j.ctrv.2019.07.002
Received 14 May 2019; Received in revised form 2 July 2019; Accepted 6 July 2019

to apoptosis as the primary source of cfDNA [10,11].

Non-tumor and tumor cells release DNA into circulation. The proportion of ctDNA in cfDNA is highly variable, ranging from 0.01% to over 90% [12], and correlates with disease burden [13]. ctDNA can be differentiated from other cfDNA by the presence of mutations. Given the rarity of ctDNA, methods used to detect mutations associated with ctDNA require exquisite sensitivity. False-positive due to the detection of targeted mutations released by non-cancer cells is another concern, particularly when ctDNA analysis is used for diagnostic purposes. One well-recognized cause of genotyping discordance between plasma and tumor samples is clonal hematopoiesis. A portion of cfDNA is derived from white blood cells. As hematopoietic stem cells divide, some random mutations confer an advantage for self-renewal or proliferation while not affecting the function of the white blood cells [14,15]. Some of these random mutations, such as *TP53*, *IDH2*, *GNAS*, and *KRAS,* are also frequently identified in cancer cells and may be falsely picked up by ctDNA assays [15–17]. An ideal cfDNA/ctDNA test would need to overcome the aforementioned sensitivity and specificity issues before being used in cancer care.

Allele-specific polymerase chain reaction (PCR) has been used to detect epidermal growth factor receptor (EGFR) mutations in ctDNA in lung cancer patients since the 2000s [18]. In 2016, the US FDA approved the cobas® *EGFR* Mutation Test v2 kit as a companion diagnostic test for EGFR tyrosine kinase inhibitors (TKIs) using plasma specimens [19]. Digital PCR (dPCR), developed by Vogelstein and Kinzler in 1999, allowed accurate identification and quantification of rare mutations [20]. A technique based on dPCR named BEAMing (beads, emulsion, amplification, and magnetics) was tested in patients with different stages of colorectal cancer [9,13]. The assay's ability to quantify absolute ctDNA level allowed it to serve as a marker for disease dynamics.

The advent of next-generation sequencing (NGS) allowed high throughput interrogation of large numbers of template molecules and genetic loci simultaneously. However, conventional NGS platforms are associated with an error rate that varies from 0.05% to 1%, making them unsuitable for detecting rare mutations [21]. Several technologies have been developed to address this high error rate. The Safe-Sequencing System (Safe-SeqS) utilized molecular barcoding to amply each uniquely tagged DNA template and allowed the differentiation between background sequencing error and actual mutations [21]. Safe-SeqS has been applied to detect cancer mutations in various clinical samples [22–25], including plasma ctDNA in 8 different malignancies, including lung cancer [26]. Commonly applied assays for ctDNA analysis are listed in Table 1.

Table 1
Current technologies/methodologies for ctDNA detection.

Technology	Platform	Type of genetic alterations	Limit of detection	Advantages	Limitations	Reference
Real-Time PCR	ARMS	Candidate mutation	1%	– Cost-effective – Simple workflows	– Limited sensitivity – Detects only known mutations	[76,157]
	MS-PCR	Candidate gene	0.1%	– Quick turnaround	– Detects only specific CpG islands	[41,44,158]
Digital PCR	ddPCR	Candidate gene	0.001–0.01%	– High sensitivity	– Detects only specific loci	[88,99,159]
	BEAMing	Candidate gene	0.01%	– Quick turnaround	– Less effective for identifying rearrangements	[9,160]
NGS Targeted-NGS	TAm-Seq	Candidate gene	0.02%	– High sensitivity	– Detects only specific loci	[161,162]
	Safe-SeqS	Candidate gene, CNV	0.1%		– Longer turnaround	[17,22]
	CAPP-Seq (capture-based)	Candidate gene, CNV, rearrangements	0.004%		– Higher cost	[39 163]
	Bias-Corrected Targeted NGS	Candidate gene, CNV, rearrangements	> 0.1%		– Require bioinformatics	[149,164,165]
WES		Whole exon	Vary	– Does not require prior knowledge of the mutations	– Long turnaround – Require bioinformatics	[17,45]

ARMS: Amplification-Refractory Mutation System; MS-PCR: methylation-specific PCR; ddPCR: Droplet Digital PCR; BEAMing: beads, emulsions, amplification and magnetics; Tam-Seq: tagged-amplicon sequencing; Safe-SeqS: Safe-sequencing system; CAPP-Seq: CAncer Personalized Profiling by deep Sequencing; WES: whole exon sequencing.

Clinical applications of ctDNA

Current clinical utility of ctDNA analysis in lung cancer is summarized in Table 2

Early detection of disease

Despite the advances in lung cancer care, lung cancer is still the leading cause of cancer death worldwide, accounting for more than 9 million deaths annually worldwide [27]. The high mortality rate in lung cancer can be partly attributed to the late stage of diagnosis, as the 5-year survival rate drops substantially for stage III and IV disease [28]. While lung cancer incidence is rising in histological subtypes that are less related to smoking, the current screening guidelines were only for heavy-smokers [29–31]. There is a substantial unmet need for lung cancer screening in the non-smoking population. Liquid biopsy eliminates the risk of radiation exposure from radiographic screening and could be obtained more frequently. In the following section, we summarize the current evidence on utilizing cfDNA, ctDNA analysis, and multi-analyte blood tests that incorporate cfDNA/ctDNA testing for lung cancer screening.

Quantification of cfDNA without targeting specific tumor mutation

Table 2
Summary of current clinical utility of ctDNA analysis.

Clinical scenario	Current status of clinical utility	Major challenges	Potential solutions
Early detection	– For study purpose – Multi-analyte blood screening trials ongoing	– Low sensitivity – False-positive results – Cost-effectiveness for large-scale screening – Identifying population(s) that benefit from screening	– Multi-analyte blood test to achieve higher accuracy – Evaluate screening performance in clinical trials
Prediction of prognosis	For study purpose	– Cost-effectiveness compared to existing markers/staging system – Generalizability	– Larger-scale studies to determine whether ctDNA adds prognostic value to TMN staging – Prognostic value studied separately in each lung cancer subtype
Monitor of response and MRD	For study purpose	– False-positive results – Clinical implication of MRD is unclear	– Generate more evidence on the clinical implications of MRD in lung cancer
Identification of genetic determinants for targeted therapy	– cobas® v2 approved by FDA for ctDNA testing – ctDNA tests integrated in clinical trials	– Sensitivity is insufficient to replace tissue tests – Implication of subclinical progression based on ctDNA is unclear	– Development of cost-effective, easily-implemented ddPCR or optimized-NGS assays for routine practice – Integrate liquid biopsy into clinical trials
Applications in immune checkpoint blockade	For study purpose	– Accuracy for estimating MMR/MSI and TMB – Cost-effectiveness for routine practice – Competing modalities for assessing early response (i.e. nuclear imaging)	– ctDNA analysis for predicting or monitoring response validated in clinical trials

MRD: miminal residual disease; MMR: mismatch repair deficiency; MSI: microsatellite instability; TMB: tumor mutation burden; ddPCR: droplet digital PCR; NGS: next generation sequencing.

is a potential test for lung cancer screening. When using human telomerase reverse transcriptase (hTERT) as a marker for cfDNA quantification, the concentration of cfDNA was found significantly higher in lung cancer patients [32–34]. The screening performance of cfDNA quantification using qRT-PCR was tested in 1035 heavy smokers without lung cancer. In the study, the participants underwent annual computed tomography (CT) screening for five years. Participants who developed lung cancer during monitoring had a higher cDNA concentration at surgery compared to their baseline, but the cfDNA level at enrollment did not differ between subsequently diagnosed cases and cancer-free subjects [35]. The major limitation of utilizing cfDNA for cancer screening is that it is not tumor-specific and prone to false-positive results. Some benign medical conditions, such as infection, inflammation, and autoimmune disease were found to be associated with higher cfDNA levels [36–39].

Lung cancer-specific driver mutation are identified in approximately 60–70% of lung cancer cases [40–42]. Driver mutations are ubiquitous in tumor cells and are generally not present in normal cells, making them potential markers for disease detection. A study which analyzed 640 patient specimens of various cancer types reported that only 47% of stage I cancers have detectable ctDNA levels by dPCR compared to 82% in stage IV disease [22]. Another study achieved a 50% sensitivity for detecting stage I lung cancer by using a personalized gene panel. The median number of genes used for detection per patient was four [43]. In general, subclinical cancer detection by identifying common mutations in ctDNA has high specificity but low sensitivity. Increasing the number of targeted mutations in the assay could potentially improve sensitivity, but could suffer from cost-effectiveness issues.

Methylation of certain ctDNA regions could also be potential markers for early disease detection. Serum DNA methylation of MGMT, p16INK4a, RASSF1A, DAPK, RAR-b were found in lung cancer [44–48]. However, the frequency of methylation at a single locus is generally too low for diagnostic purposes [45,46,48]. Combining several methylation markers with or without lung cancer-specific mutations have been reported to increase the sensitivity of lung cancer detection [45–48].

Utilizing ctDNA for detection of preclinical lung cancer remains challenging. ctDNA may only represent a small fraction of cfDNA and may not be representative of the localized tumor [49]. The concentration of ctDNA is also lower in early-stage disease compared to that in late stages [22,43,49,50]. Furthermore, the genetic and epigenetic composition of lung cancer is heterogeneous across patients [51,52]. These ctDNA properties impede the sensitivity of ctDNA-based screening.

Given that neither cfDNA concentration, cancer-specific mutations or methylation in ctDNA alone provide sufficient sensitivity for early cancer detection, newer screening assays focused on combining multiple blood markers to increase accuracy. CancerSEEK, a blood test that assesses tumor-specific mutations in cfDNA and eight circulating protein, was set to detect eight common cancers [26]. Among the 104 stage I to III lung cancer patients tested, the sensitivity for detecting the disease was 59%. The performance of another test developed by GRAIL, which involved targeted sequencing, whole-genome sequencing, and methylation analysis, was tested in 124 stage I to III lung cancer patients [53]. The test had similar sensitivity (59%, 95% CI 47–70%) to CancerSEEK. While these tests demonstrated more promising results, their performance would need to be validated by large-scale studies. The low prevalence of lung cancer in the general population make the positive predictive value very low even with highly sensitive and specific methods. Therefore, it is unlikely that blood tests be used for universal lung cancer screening in the near future. Identifying high-risk groups for screening is equally essential as advancing technology for early detection.

Prediction of prognosis

The association between circulating DNA concentration and lung cancer stage found in prior studies indicates the potential prognostic value of cfDNA and ctDNA [22,43,49,50]. A study assessed the relationship between cfDNA concentration and overall survival in 73 lung cancer patients. When using 20 ng/ml as cutoff, patients with higher cfDNA concentration had an unfavorable overall survival after adjusted for disease stage (Hazard ratio [HR] for death: 3.77, 95% CI 1.16–12.28, P = 0.028) [34]. In the cfDNA and computed tomography lung cancer screening study mentioned in the previous section, a higher cfDNA concentration at diagnosis was significantly associated with poorer 5-year survival (P = 0.007) [35]. In a longitudinal analysis of blood samples from 134 patients with advanced lung adenocarcinoma enrolled in a randomized control trial, patients with pre-treatment cfDNA in the higher tertile had significantly short survival compared with those in the lower tertile (Log-rank test P = 0.030). Another study, in which quantitative Real-Time PCR targeting four genes was used to detect ctDNA, found that high concentration of the targeted genes was

associated with poor prognosis in stage IIIb and stage IV non-small cell lung cancer (NSCLC) patients (P = 0.006) [54]. The results demonstrate that cfDNA or ctDNA concentration could be a supplementary prognostic indicator to disease stage in lung cancer. However, most studies did not account for important factors that could affect the outcome, including performance status, subsequent treatment, and molecular or genetic subtypes of lung cancer. As the classification of lung cancer, mainly NSCLC is based on molecular and genetic testing [55], the prognostic value of cfDNA and ctDNA should be evaluated separately in each of the lung cancer subtypes.

Monitoring treatment response and detecting residue disease

cfDNA or ctDNA are desirables tool for real-time disease burden and treatment response monitoring for their short half-life and ease of accessibility compared to repeated image assessment or tissue biopsy [13,49,56]. Minimal residue disease (MRD) is an important indicator for relapse and poor prognosis in leukemia; MRD detection could facilitate the decision on giving more aggressive or salvage treatment [57,58]. The clinical implication of MRD in lung cancer is less established, but detection of MRD potentially help clinicians determine the intensity of monitoring, and lead to a timely diagnosis of clinically actionable disease. Two studies compared the pre and post-surgery detection rate of ctDNA mutation in patients with localized NSCLC and found that the frequency of ctDNA was significantly lower after surgery [59,60]. Thus, ctDNA could reflect the disease burden and serve as a marker of MRD. In a study that aimed to validate CAncer Personalized Profiling by deep Sequencing (CAPP-seq), a targeted-NGS assay, for prognosis prediction, three early NSCLC cases experienced extended disease-free periods after ctDNA became undetectable following curative treatment [43]. In a 24-patient subgroup of the Tracking Cancer Evolution Through Therapy (TRACERx) trial, 13 of the 14 NSCLC patients who experienced recurrence after surgery had a detectable ctDNA level before radiographic confirmation (median of 70 days preceding image diagnosis). Only one of the ten disease-free patients had a persistently detectable ctDNA. The study used patient-specific targeted-NGS panels for ctDNA detection [61]. Another study reported that 94% of the localized lung cancer cases, which subsequently had disease recurrence was positive for ctDNA by CAPP-seq on the first blood examination after curative treatment [62]. The detection of MRD preceded radiographic progression by a median of 5.2 months. While these evidence support MRD monitoring and risk stratification via ctDNA, the low detection rate of ctDNA before curative treatment in localized NSCLC and possible false positive findings raise concern on its clinical application. Besides, whether the detection of MRD in NSCLC translates into survival benefit remains unclear.

Monitoring treatment response using ctDNA had been studied in patients with NSCLC, particularly those with actionable mutations. ctDNA *EGFR* mutation levels were found to increase drastically within 24 h after initiation of EGFR TKI, suggesting TKI-induced cell death. The ctDNA *EGFR* mutation dropped to undetectable levels one week after treatment [63]. In the same study, six out of the seven patients who had a clinical response were absent of *EGFR* mutation ctDNA at eight weeks after treatment; a rise of ctDNA *EGFR* mutation can be detectable two months preceding clinical progression. The correlation between lowering of ctDNA and the clinical or radiographic response was confirmed in two other studies. In a study that analyzed the dynamics of ctDNA from 41 patients with advanced NSCLC enrolled in a phase II trial of pertuzumab and erlotinib, a decrease of ctDNA was associated with radiographic response confirmed by CT and fluorodeoxyglucose- positron emission tomography (FDG-PET) in patients with *EGFR* mutation [64]. Another study used the cobas® kit for ctDNA *EGFR* mutation detection in patients with metastatic NSCLC who received gemcitabine and erlotinib or erlotinib monotherapy in a phase III trial. In both arms, patients who are *EGFR* mutation-positive at baseline and still have detectable *EGFR* mutation in blood before start of cycle 3 (8 weeks after treatment) had a significantly unfavorable progression-free survival (PFS) and overall survival (OS) compared to those whose ctDNA *EGFR* mutation turned negative [65]. A similar correlation between dynamics of ctDNA and clinical outcomes was observed in NSCLC. In 15 histopathological confirmed Anaplastic lymphoma kinase (ALK)-positive advanced NSCLC cases, patients without detectable ALK mutation by capture-based NGS in the plasma had a slightly longer PFS [66]. A recent study, which used a 566-gene hybrid-capture NGS assay to detect ctDNA ALK mutation in 22 ALK-positive NSCLC patients, found that the dynamics of ALK mutation allele fraction was consistent with the response to treatment and disease progression on image studies [67]. Overall, it is possible to use the dynamics of ctDNA driver mutation level to predict response to treatment and even longer-term outcomes. Given that current evidence was generated from small sample groups and not under well-controlled settings, its generalizability in clinical practice remains questionable.

Identification of genetic determinants for molecular targeted therapy

Lung cancer, particularly advanced NSCLC is longer treated as a single disease entity but instead based on genetic or molecular profiling. Molecular targeted therapies for NSCLC with *EGFR*, *ALK*, *ROS1*, and *BRAF* mutation have been proven effective and approved by the US Food and Drug Administration (FDA) [68–76]. Understanding of the mechanisms of resistance in *EGFR* and *ALK*-positive NSCLC after first-line therapy also led to the development of new generations of TKIs that target acquired mutation [77,78]. Currently, testing of these mutations requires tumor tissue. The procedure for obtaining tumor tissue in advanced lung cancer patients is invasive and not always feasible. As genetic or molecular diagnosis became part of advanced NSCLC treatment, there is a demand for non-invasive diagnostic tools.

EGFR-mutant NSCLC

Identifying the genetic determinants for targeted therapy by ctDNA is most widely applied in *EGFR*-mutant NSCLC. Two companion, real-time PCR-based *EGFR* mutation diagnostic tests for EGFR TKIs, therascreen® and cobas®, were approved by the US FDA [79] (Table 3). The cobas® *EGFR* mutation test was originally approved for testing NSCLC tumor tissue for actionable *EGFR* mutations. In 2016, the US FDA expanded the indication of cobas® test v2 for the detection of *EGFR* exon 19 deletions, L858R, and T790M in plasma samples [80]. It is the first liquid biopsy test approved for use by the US FDA. The approval was based on the results from the randomized, phase III ENSURE trial, which compared the efficacy and safety of erlotinib versus gemcitabine

Table 3
US Food and Drug Administration-approved *EGFR* mutation testing assays [167].

Assay	Technology	No. mutations tested	Sample	Drugs indicated for detected mutations
therascreen® *EGFR* RGQ PCR kit (Qiagen Manchester Ltd.)	Scorpion® ARMS real-time PCR	21	FFPE	Gefitinib – exon 19 deletions, L858RAfatinib – exon 19 deletions, L858R
cobas® *EGFR* Mutation Test v2 (Roche Molecular Systems, Inc.)	Real-time PCR	42	FFPE	Erlotinib – exon 19 deletions, L858R Osimertinib – T790M
			Plasma	Erlotinib – exon 19 deletions, L858R

Abbreviations: ARMS = amplification refractory mutation system, No. = number, FFPE = formalin-fixed, paraffin-embedded tissue.

and cisplatin in treatment-naive advanced NSCLC patients [81]. Tissue and plasma of the 517 patient screened for the ENSURED trial were tested for *EGFR* mutation using the FDA-approved plasma *EGFR* test. In patients with positive *EGFR* mutation in the tumor tissue, 76.7% were also *EGFR* mutation-positive in the plasma. Of the tissue-negative patients, 98.2% were plasma-negative [80].

Acquired *EGFR* T790M gatekeeper mutation is the dominant mechanism of resistance to EGFR-TKI, and can be targeted by osimertinib [82,83]. Detection of *EGFR* T790M at disease progression is pivotal for determining subsequent treatment. The concordance between plasma and tissue T790M mutation status using the cobas® test was reported from the AURA trials. In the AURA phase I trial, the positive percent agreement (PPA) between plasma and tissue results was 73% for all activating mutations and 64% for T790M [84]. In the 551 patients screened for the AURA extension and AURA2 trial who provided matched tissue and plasma samples, the PPA and negative percent agreement (NPA) for T790M status was 90% and 98%, respectively [85]. The randomized, phase III AURA3 trial also incorporated ctDNA T790M testing as part of the analysis. Among the patients who had both tissue and plasma tested for T790M status by the FDA-approved plasma *EGFR* test, the PPA and NPA was 51% (95% CI 46–57) and 77% (95% CI 71–83), respectively. In the subgroup that is positive for both plasma and tissue T790M, the treatment efficacy comparing osimertinib to chemotherapy was consistent with that of the intended to treat population [86]. These studies indicate that when the tissue test results were set as the gold standard, the plasma test had higher specificity than sensitivity. To avoid false negative, the FDA recommends performing tissue EGFR mutation testing in patients whose plasma test is negative [80].

Although the US FDA did not approve the therascreen® test for testing plasma samples, the concordance between its plasma and tissue test results was reported in the single-arm, gefitinib IFUM trial [87]. The concordance rate, sensitivity and specificity for therascreen® plasma test in the 652 matched samples were 94.3%, 65.7%, and 99.8%, respectively. In the real-world setting, the concordance rate between plasma and tissue/cytology test results using the therascreen® test was 95% from the non-interventional ASSESS study [88].

EGFR mutations other than L858R and exon 19 deletions are considered uncommon EGFR mutations, and the sensitivity to EGFR-TKIs varies across these mutations [89]. The FDA recently broadens the indication of afatinib to advanced NSCLC with uncommon, non-resistant EGFR mutations (S768I, L861Q, and/or G719X) based on data from a combined analysis of three randomized trials [90]. While both therascreen® and cobas® tests are capable of detecting these uncommon mutations, they were not approved for this purpose in either tissue or plasma samples (Table 2).

Besides the FDA-approved plasma *EGFR* test, BEAMing [91], droplet digital PCR (ddPCR) [92], multiplex enrichment PCR [93–95], and other amplification refractory mutation system (ARMS) assays [96] were commonly used to detect EGFR mutations in ctDNA. The sensitivity for detecting *EGFR* mutation in ctDNA of these assays varies across studies, but in general, digital genomic approaches (ddPCR, BEAMing) have higher sensitivity compared to non-digital approaches (ARMS) for tracking mutations at low frequencies [97,98]. Several ongoing observational and interventional studies continue to validate the assays mentioned above for ctDNA driver mutation detection in different clinical settings [98].

ALK-positive NSCLC

ALK-positive advanced NSCLC is another area where molecular-targeted therapy has had great success. The US FDA approved five TKIs, including crizotinib, ceritinib, alectinib, brigatinib, and loratinib for the treatment of *ALK*-positive advanced NSCLC. Unlike EGFR-mutated NSCLC that has a dominant resistance mutation, *ALK*-positive NSCLC has several common secondary mutations (i.e. C1156Y, G1202R, I1171T, S1206Y, and E1210K) [99,100]. As the spectrum of targeted mutations differs across ALK inhibitors [101,102], it is essential to monitor the evolution of *ALK* mutation profile during treatment. A Japanese group developed a two-step ddPCR-based assay for detecting and monitoring secondary *ALK* mutations in plasma [103]. The two steps involve screening for 10 *ALK* mutations and tracking of mutation clone by mutation-specific probe after identifying the mutation. Among four patients who progressed on crizotinib, a secondary mutation: G1202R, was detected in one of the patients. The mutation was consistent with that from the metastatic brain tumor, and the frequency of the mutation in cfDNA aligned with the clinical course.

While highly sensitive dPCR-based ctDNA detection methods more effectively detect ctDNA mutations, designing PCR primers for rearrangement events such as *EML4-ALK* could be challenging [66]. Capture-based NGS is an alternative for detecting *ALK* rearrangement. Two studies compared the *ALK* mutation status in plasma identified by captured-cased NGS to that from paired tissue samples confirmed by immunohistochemistry (IHC) and in situ fluorescence hybridization (FISH) [66,104]. The sensitivity was 79.2% and 54.2% in the two studies, respectively; the specificity was 100% in both studies. The sensitivity was higher in patients with distant metastasis than those without, and higher in pre-treatment compared to post-treatment samples in patients followed longitudinally. One of the studies identified secondary mutations in a patient who progressed on crizotinib, indicating the potential application on monitoring resistance to ALK inhibitors [104]. Another study used a commercially available, NGS-based Guardant360 (G360; Guardant Health) panel to detect *ALK* mutation in cfDNA [105]. The G360 panel was able to identify resistance mutation in 16 of the 31 patients (52%) with known or presumed *ALK*-positive patients. Given that these assays have excellent specificity, it could serve as a complementary diagnostic and monitoring test when tumor tissue is not available.

Liquid biopsy via ctDNA *ALK*-mutation testing could offer some advantage over tissue tests in diagnosis and determining mechanism of resistance. The false-negative rate for detecting *ALK* fusion of standard IHC (Ventana D5F3) and FISH tissue test could be as high as 35% when NGS is used as the benchmark [106]. In the study that utilized G360 to detect plasma *ALK* mutation, five patients who were tested negative for *ALK* mutation in tissue but positive in plasma responded to crizotinib [105]. In the same study, 43% of the patients whose secondary mutation was detected in plasma had more than one resistance mutation. In contrast, only 12% of the 48 tumor tissues from patients who progressed on an ALK inhibitor had compound mutations [101]. These data do not necessarily indicate that liquid biopsy confers better sensitivity, but reflect intratumoral and intertumoral heterogeneity. Given the complexity of the clonal evolution of *ALK*-positive NSCLC, while ctDNA-based liquid biopsy may not replace tissue diagnosis, it provides additional information for personalized treatment.

Clinical utility of ctDNA analysis in immune checkpoint blockade

Following molecular targeted therapy, immune checkpoint blockade is another paradigm shift in lung cancer treatment. Immune checkpoint blockade improved the survival of patients with advanced NSCLC without actionable mutation and patients with extensive-stage SCLC when compared to chemotherapy in randomized controlled trials [107–114]. In contrast to molecular targeted therapy, which achieves cytocidal or cytostatic activity by inhibiting molecules that are pivotal for cell proliferation, the anti-cancer effect of immune checkpoint blockade is via activation of the exhausted T-cells [115]. Given the unique mechanism of action of immune checkpoint blockade, we discuss the clinical utility of ctDNA analysis in immune checkpoint blockade in a separate section.

Identifying high-responders

A proportion of NSCLC patients experience durable response to immune checkpoint inhibitors and have long progression-free and

overall survival periods. Recently, a lot of attention was on identifying responders to immune checkpoint blockade. Several biomarkers were found to be associated with response to immune checkpoint inhibitors, including PD-L1 expression, tumor infiltrating lymphocytes (TILS), tumor mutation burden (TMB), mismatch repair deficiency (dMMR), serum markers (i.e. LDH, eosinophil count, certain lymphocyte ratio), and radiographic markers [108,116–120]. The US FDA also approved immune checkpoint inhibitors in patient groups with high response tendency based on PD-L1 expression and mismatch repair deficiency status [121,122].

ctDNA-based liquid biopsy tests have the potential for predicting response by detection of TMB and dMMR. High TMB, defined by various cutoffs ranging from ≥5 to ≥20 mutations per Mb [123], is associated with a higher number of neoantigens, and leads to higher chances of neoantigen-specific cytotoxic lymphocytes activation by checkpoint blockade [117,124]. Several studies have reported that high TMB was associated with higher objective response rate, PFS and/or OS in lung cancer [117,125–127]. The allele frequency of certain tumor mutations in cfDNA could be very low; thus the sequencing depth is essential when estimating TMB from blood samples.

Whole exome sequencing (WES)-based analysis is a feasible approach for assessing TMB in blood samples (bTMB) [128–130]. Despite the sensitivity for detecting tumor mutations were relatively low in these studies, bTMB was correlated with TMB. However, the cost of high-depth WES is probably too high for routine clinical practice; targeted sequencing is considered a more realistic approach to estimate bTMB. A study analyzed the mutation burden from 97 paired blood (analyzed by G360 panel) and tumor samples (were analyzed by the FoundationOne assay), including 53 from NSCLC patients [131]. The number of mutations was significantly different (paired *t*-test $p < 0.05$) between blood and tissue samples. The number of mutations was poorly correlated between the two types of samples (Pearson Correlation $r = 0.24–0.33$). However, other studies demonstrated better concordance between bTMB and TMB by targeted-sequencing. A group used a targeted, capture-based NGS panel to analyze the mutation burden in ctDNA and compared to paired tumor samples (TMB determined by WES) from 56 patients with advanced NSCLC. The correlation between bTMB and TMB was higher than that in the prior study ($r = 0.8$). The correlation was stronger in patients with stage IV disease than those with stage IIIB disease. Similar results were demonstrated in a study that compared bTMB with TMB (assessed by targeted sequencing of 394 genes) form 259 NSCLC patients. The overall agreement and PPA were 81% and 64%, respectively. The PPA dropped to 17% when narrowing the number of genes to 62 [132].

The predictive value of bTMB for clinical outcomes was evaluated in patients who received atezolizumab in two randomized trials (OAK and POLAR trial) [133]. Using bTMB ≥ 16 single nucleotide variants (SNVs) out of the 394 genes in the FoundationOne panel as cutoff value yielded the most significant benefit from atezolizumab compared to docetaxel (HR for progression: 0.57, 95% CI 0.33–0.99) in the POLAR trial. Therefore, bTMB ≥ 16 was selected as the cutoff for high bTMB and validated in the OAK study. Patients with bTMB ≥ 16 had a more substantial improvement in PFS (HR 0.65 vs 0.95) and OS (0.64 vs 0.73) compared to the intention to treat population in the OAK trial. A dose response between bTBM and benefit from atezolizumab was also observed. The predictive value of bTBM for PFS and OS was better than tumor PD-L1 expression in this study population.

To sum-up, evidence supports the correlation between bTMB and TMB in lung cancer. High-depth WES is generally preferred for estimating TMB from bTMB, but targeted sequence that covers a sufficient number of targets is a lower-cost alternative. While there has been consensus on the definition of high TMB, the cut-off value of bTMB for predicting response to immune checkpoint blockade is unclear and may vary across assays.

In 2017, the US FDA approved pembrolizumab for any solid cancer that features dMMR [121]. It is the first anti-cancer therapy that was approved based on a biomarker rather than cancer type. In clinical practice, dMMR is assessed by microsatellite instability (MSI) status, which is commonly identified by PCR-based assay targeting specific microsatellites or expression of MMR-related proteins in the tumor tissue. Since MMR status is associated high response rate to immune checkpoint blockade [134], there is a demand for blood-based MMR/MSI tests in patients with insufficient tumor tissue or whose tissue cannot be obtained. Existing PCR-based microsatellite assay suffers from sensitivity issues when testing blood samples, as they are incapable of consistently detect low allele frequency ctDNA targets [123,135]. ddPCR-based assays and specific enriched techniques for detecting alterations in microsatellite in ctDNA could overcome the limitations of current tests [136,137]. High-Depth NGS for detecting MMR gene mutation or methylation could be another feasible solution [138]. Of note, none of these blood-based MSI/MMR assays was tested in samples of lung cancer patients. The majority of dMMR tumors have high TMB [139]; therefore, the methods for assessing bTMB described earlier are alternatives for testing MSI in blood.

Assessment of response to immune checkpoint blockade

The response pattern in patients treated with immune checkpoint inhibitors is different from that observed in patients who received chemotherapy or targeted therapy. Approximately 5–10% of the patients treated with immune checkpoint inhibitors experience initial progression and are misclassified as progressive disease by RECIST criteria before tumor shrinkage or stabilization of the disease; this phenomenon is referred as pseudoprogression or atypical response [140–143]. It is presumably caused by inflammation around the tumor that is induced by anti-tumoral immune response [140,144]. In contrast, 10–30% of the patients receiving immune checkpoint blockade develop hyperprogression, which is defined as ≥a 2-fold increase in tumor growth rate after initiation of therapy [145–148], and may need timely salvage therapy. To confirm response or progression may require a substantial period for observation. The median time to response in NSCLC trial patients treated with immune checkpoint blockade ranged from 2.1 to 3.3 months and could take up to more than eight months to confirm response for some patients [108–111,149]. Having a marker that could determine response to checkpoint blockade early in the treatment course could guide clinical decisions.

ctDNA potentially serve as an early response marker and differentiate pseudoprogression from true progression. In a proof of concept study that assessed the change of ctDNA at eight weeks after nivolumab or pembrolizumab from baseline in 15 cancer patients (including 10 NSCLC patients), most patients with undetectable ctDNA had durable response to checkpoint blockade [150]. In the study, ctDNA level was examined by ddPCR or bidirectional pyrophosphorolysis-activated polymerization PCR in patients with known mutations, while targeted-NGS was applied to those whose mutation status was unavailable. Another study utilized a multigene NGS assay for ctDNA quantification, and defined ctDNA response as having ≥a 50% decrease in specific mutation allele frequency. In 28 NSCLC patients who received immune checkpoint blockade, the median time to ctDNA response was shorter compared to radiographic response (24.5 days versus 72.5 days); a ctDNA response was associated with favorable PFS (HR 0.29, 95% CI 0.09–0.89, P = 0.03), and OS (HR 0.17, 95% CI, 0.05–0.62, P = 0.007) [151]. Response to immune checkpoint blockade by ctDNA could be determined as early as two weeks into treatment. A study analyzed paired blood and tissue samples from 14 NSCLC patients treated with nivolumab by a 53-gene NGS assay; only mutations that were presented in both tissue and blood were quantified. Four patients who had a decrease in the allelic frequency of the major mutation at two weeks all experienced durable response. However, only 7 of the 14 patients had detectable mutation(s) shared between blood and tissue and could be included in the analyses [152]. ctDNA is also capable of tracking resistance to immune checkpoint blockade. In a study that utilized NGS-based targeted error-correction sequencing for dynamic monitoring

ctDNA in 14 NSCLC patients found that a recrudescence in ctDNA levels was indicative of acquired resistance to immune checkpoint blockade [153]. Overall, dynamics of ctDNA during immune checkpoint blockade is a potential biomarker for predicting response preceding radiographic assessment, and for differentiating pseudoprogression from true progression. Limited by the sensitivity of the current assays, only patients who have detectable ctDNA can benefit from these approaches.

Clinical applications in small cell lung cancer

Small cell lung cancer (SCLC) is distinct from NSCLC in terms of biology, prognosis and treatment, and thus is discussed in this separate section. Similar to the results from studies focusing on NSCLC [32–34], the cfDNA concentration in patients with SCLC was found to be significantly higher compared to that in healthy individuals [154]. *TP53* and *RB1* are inactivated in nearly all patients with SCLC [155]. Despite also presented in NSCLC, the allelic frequency of *TP53* and *RB1* mutation in the plasma were found to be higher in SCLC compared to NSCLC, and therefore have potential as specific blood markers for SCLC [156]. In a study that used targeted sequencing to assess the *TP53* mutation rate in plasma samples from SCLC patients and healthy individuals, 49% of the plasma samples from SCLC patients were positive for *TP53* mutation compared to 11% of samples from healthy individuals. Probably due to clonal hematopoiesis, *TP53 mutation* was found presented in cfDNA in a proportion of non-cancer individuals in prior published data along with this study [157,158]; therefore, *TP53* alone is insufficient for diagnostic purposes. A few studies that attempted to validate multigene assays for early detection or monitoring of cancer included a small number of SCLC patients [22,43,62], but it is challenging to evaluate the performances of such assays in SCLC owing to the small sample sizes. A more recent study utilized a 14 gene assay, which included frequently mutated genes in SCLC (*TP53, RB1, PTEN, NOTCH, MYC, PIK3CA, KIT, BRAF*), for longitudinal ctDNA analysis in 27 patients with SCLC [156]. Disease-specific mutation(s) was identified in 23 out of the 27 patients (85%). In 140 plasma samples, the most commonly identified mutations occurred in *TP53* (70%) and *RB1* (52%). The dynamics of the mutation allele frequency was consistent with the patients' radiographic response, and was able to detect occult disease progression as early as two months prior to radiographic progression. The study demonstrated that response to treatment and possibly clonal evolution of SCLC could be monitored via ctDNA analysis. However, as no molecular-targeted therapy has been approved in SCLC, clinical applications of ctDNA analysis in this area remains limited.

Recently, three immune checkpoint inhibitors: atezolizumab, nivolumab, and pembrolizumab have been approved by the FDA for the treatment of SCLC. Given the generally high number of somatic mutations in SCLC tumors, TMB could be a potential marker for response to immune checkpoint [155]. In a phase I/II trial assessing the efficacy of nivolumab alone and nivolumab combined with ipilimumab in recurrent SCLC, patients were classified into high, mid and low mutation burden. The response rate, PFS and 1-year survival rate were more favorable in patients with high TMB ($\geq$248 mutations) [159,160]. However, the association between TMB and outcome was less apparent when TMB was assessed in plasma samples. In the phase III front-line atezolizumab trial for extensive stage SCLC, the benefit of adding atezolizumab to chemotherapy was similar across bTMB subgroups [114]. In this study, bTMB was assessed by an assay with proven capability of predicting outcome in NSCLC patients treated with atezolizumab [133]. More data is required to determine the clinical value of ctDNA analysis in predicting response and outcomes to immune checkpoint blockade in patients with SCLC.

Future directions

Today, liquid biopsy is on the horizon and may lead to a paradigm shift in cancer treatment. Among the liquid biopsy approaches (i.e. ctDNA, CTC, circulating RNA, microRNA, serum metabolites, exosomes), ctDNA or cfDNA analysis is technically more feasible to be carried out in larger scales and integrated into routine clinical practice. Despite promising evidence on utilizing ctDNA for early detection, risk stratification, identification of genetic determinants, response assessment, and resistance monitoring in lung cancer has been emerging, there are many challenges to overcome.

The development of dPCR, ddPCR, and optimized NGS approaches has substantially increased the sensitivity of detecting ctDNA. However, as the overall ctDNA levels are low in early-stage diseases [22,43,49,50], the sensitivity of these tests remains suboptimal for screening or early detection purposes Bettegowda:2014jx}[43,45–48]. False-positive findings that are related to the tests' limited specificity or specific clinical conditions (i.e. clonal hematopoiesis of indeterminate potential) could lead to serious consequences when applied to clinical practice, and should not be overlocked [15,17]. High-depth NGS assays with a sufficient number of targets for cross-validation may overcome these issues, but are still too costly for routine clinical practice [49]. A more practical approach to improve the accuracy for disease detection is to incorporate more than one blood biomarkers in the assay. At least two multi-biomarker assays for early-stage cancer detection are now tested in large-scale clinical studies [161].

For late-stage disease, the major challenge for ctDNA analysis is translating the evidence on actionable mutations detection, response assessment, and resistance identification to clinical practice. The approval of the cobas® test for detecting *EGFR* mutations in blood was a milestone in lung cancer treatment, as it was the first liquid biopsy test for clinical use approved by the FDA [80]. While liquid biopsy is currently part of the standard of care in *EGFR*-mutant NSCLC, most of the patients with other subtypes of lung cancer still cannot benefit from liquid biopsy. The majority of the studies described in this review used in-house assays or commercially available kits that were not approved for clinical purposes, thus cannot be widely applied in clinical practice. To push these cutting-edge ctDNA assays through clinical approval will require biomarker-integrated clinical trials. We expect to see more clinical trials having a design similar to that of the AURA3 study [86,162], in which ctDNA analysis was part of the trial protocol.

ctDNA analysis also help advance scientific knowledge of lung cancer. Limited by the current technology, liquid biopsy is unlikely to replace tissue biopsy in the near future, but provides valuable information on tumor heterogeneity and clonal evolution. The clonal evolution of lung cancer was found to be branching rather than linear [61], and the degree of dominance of a tumor clone influenced the response to treatment [124]. Longitudinal analysis of ctDNA overcomes the spatial and temporal limitations of a single tissue biopsy and pushes the frontier of our understanding of tumor biology. Past studies have been able to discover new resistance mechanisms to targeted therapy in *EGFR*-mutant and *ALK*-positive NSCLC via ctDNA analysis [78,163–166]. Therefore, in the upcoming future, ctDNA analysis is expected to take a pivotal role in the development of novel lung cancer therapies.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

[1] Acad Sci Paris Les PM, 1948. l"homme de chez de sanguin de nucléiques du plasma d"acides de Metais P. n.d.
[2] Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646–50.
[3] Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989;46:318–22. https://doi.org/10.1159/000226740.
[4] Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin

and mechanism of circulating DNA: Apoptosis and active DNA release. Clin Chim Acta; Int J Clin Chem 2001;313:139–42.
[5] Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61:1659–65.
[6] Stroun M, Lyautey J, Lederrey C, Mulcahy HE, Anker P. Alu repeat sequences are present in increased proportions compared to a unique gene in plasma/serum DNA: evidence for a preferential release from viable cells? Ann N Y Acad Sci 2001;945:258–64.
[7] Sikora K, Bedin C, Vicentini C, Malpeli G, D'Angelo E, Sperandio N, et al. Evaluation of cell-free DNA as a biomarker for pancreatic malignancies. Int J Biol Markers 2015;30:136–41. https://doi.org/10.5301/jbm.5000088.
[8] Choi J-J, Reich CF, Pisetsky DS. The role of macrophages in the in vitro generation of extracellular DNA from apoptotic and necrotic cells. Immunology 2005;115:55–62. https://doi.org/10.1111/j.1365-2567.2005.02130.x.
[9] Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci 2005;102:16368–73. https://doi.org/10.1073/pnas.0507904102.
[10] Lo YMD, Chan KCA, Sun H, Chen EZ, Jiang P, Lun FMF, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med 2010;2. https://doi.org/10.1126/scitranslmed.3001720. 61ra91–1.
[11] Heitzer E, Auer M, Hoffmann EM, Pichler M, Gasch C, Ulz P, et al. Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer. Int J Cancer 2013;133:346–56. https://doi.org/10.1002/ijc.28030.
[12] Diaz LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579–86. https://doi.org/10.1200/JCO.2012.45.2011.
[13] Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985–90. https://doi.org/10.1038/nm.1789.
[14] Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell 2012;150:264–78. https://doi.org/10.1016/j.cell.2012.06.023.
[15] Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20:1472–8. https://doi.org/10.1038/nm.3733.
[16] Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477–87. https://doi.org/10.1056/NEJMoa1409405.
[17] Hu Y, Ulrich BC, Supplee J, Kuang Y, Lizotte PH, Feeney NB, et al. False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 2018;24:4437–43. https://doi.org/10.1158/1078-0432.CCR-18-0143.
[18] Kimura H, Kasahara K, Kawaishi M, Kunitoh H, Tamura T, Holloway B, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non–small-cell lung cancer. Clin Cancer Res 2006;12:3915.
[19] Center for Drug Evaluation and Research, Research. Approved Drugs - cobas EGFR Mutation Test v2; 2016.
[20] Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci USA 1999;96:9236.
[21] Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci USA 2011;108:9530.
[22] Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6. https://doi.org/10.1126/scitranslmed.3007094. 224ra24–4.
[23] Wang Y, Springer S, Mulvey CL, Silliman N, Schaefer J, Sausen M, et al. Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas. Sci Transl Med 2015;7:293ra104.
[24] Kinde I, Bettegowda C, Wang Y, Wu J, Agrawal N, Shih I-M, et al. Evaluation of DNA from the papanicolaou test to detect ovarian and endometrial cancers. Sci Transl Med 2013;5:167ra4.
[25] Wang Y, Li L, Douville C, Cohen JD, Yen T-T, Kinde I, et al. Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Sci Transl Med 2018;10:eaap8793.
[26] Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359:926–30. https://doi.org/10.1126/science.aar3247.
[27] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424. https://doi.org/10.3322/caac.21492.
[28] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67:7–30. https://doi.org/10.3322/caac.21387.
[29] Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers–a different disease. Nature Publ Group 2007;7:778–90. https://doi.org/10.1038/nrc2190.
[30] Lortet-Tieulent J, Soerjomataram I, Ferlay J, Rutherford M, Weiderpass E, Bray F. International trends in lung cancer incidence by histological subtype: adenocarcinoma stabilizing in men but still increasing in women. Lung Cancer 2014;84:13–22. https://doi.org/10.1016/j.lungcan.2014.01.009.
[31] Moyer VA. Screening for Lung Cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med 2014;160:330–8. https://doi.org/10.7326/M13-2771.
[32] Sozzi G, Conte D, Leon M, Ciricione R, Roz L, Ratcliffe C, et al. Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol 2003;21:3902–8. https://doi.org/10.1200/JCO.2003.02.006.
[33] Paci M, Maramotti S, Bellesia E, Formisano D, Albertazzi L, Ricchetti T, et al. Circulating plasma DNA as diagnostic biomarker in non-small cell lung cancer. Lung Cancer 2009;64:92–7. https://doi.org/10.1016/j.lungcan.2008.07.012.
[34] Catarino R, Coelho A, Araújo A, Gomes M, Nogueira A, Lopes C, et al. Circulating DNA: diagnostic tool and predictive marker for overall survival of NSCLC patients. PLoS ONE 2012;7:e38559https://doi.org/10.1371/journal.pone.0038559.
[35] Sozzi G, Roz L, Conte D, Mariani L, Andriani F, Vullo Lo S, et al. Plasma DNA quantification in lung cancer computed tomography screening: five-year results of a prospective study. Am J Respir Crit Care Med 2009;179:69–74. https://doi.org/10.1164/rccm.200807-1068OC.
[36] Moreira VG, Prieto B, Rodríguez JSM, Alvarez FV. Usefulness of cell-free plasma DNA, procalcitonin and C-reactive protein as markers of infection in febrile patients. Ann Clin Biochem 2010;47:253–8. https://doi.org/10.1258/acb.2010.009173.
[37] Weerakoon KG, McManus DP. Cell-Free DNA as a diagnostic tool for human parasitic infections. Trends Parasitol 2016;32:378–91. https://doi.org/10.1016/j.pt.2016.01.006.
[38] Fatouros IG, Destouni A, Margonis K, Jamurtas AZ, Vrettou C, Kouretas D, et al. Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtraining. Clin Chem 2006;52:1820–4. https://doi.org/10.1373/clinchem.2006.070417.
[39] Bartoloni E, Ludovini V, Alunno A, Pistola L, Bistoni O, Crinò L, et al. Increased levels of circulating DNA in patients with systemic autoimmune diseases: A possible marker of disease activity in Sjögren's syndrome. Lupus 2011;20:928–35. https://doi.org/10.1177/0961203311399606.
[40] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12:175–80. https://doi.org/10.1016/S1470-2045(10)70087-5.
[41] Sholl LM, Aisner DL, Varella-Garcia M, Berry LD, Dias-Santagata D, Wistuba II, et al. Multi-institutional oncogenic driver mutation analysis in lung adenocarcinoma: the lung cancer mutation consortium experience. J Thorac Oncol 2015;10:768–77. https://doi.org/10.1097/JTO.0000000000000516.
[42] Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI's Lung Cancer Mutation Consortium (LCMC). J Clin Oncol 2016;29. https://doi.org/10.1200/jco.2011.29.18_suppl.cra7506. CRA7506–6.
[43] Newman AM, Bratman SV, To J, Wynne JF, Eclov NCW, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548–54. https://doi.org/10.1038/nm.3519.
[44] Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999;59:67–70.
[45] Bearzatto A, Conte D, Frattini M, Zaffaroni N, Andriani F, Balestra D, et al. p16(INK4A) Hypermethylation detected by fluorescent methylation-specific PCR in plasmas from non-small cell lung cancer. Clin Cancer Res 2002;8:3782–7.
[46] Fujiwara K, Fujimoto N, Tabata M, Nishii K, Matsuo K, Hotta K, et al. Identification of epigenetic aberrant promoter methylation in serum DNA is useful for early detection of lung cancer. Clin Cancer Res 2005;11:1219–25.
[47] Hsu H-S, Chen T-P, Hung C-H, Wen C-K, Lin R-K, Lee H-C, et al. Characterization of a multiple epigenetic marker panel for lung cancer detection and risk assessment in plasma. Cancer 2007;110:2019–26. https://doi.org/10.1002/cncr.23001.
[48] Shivapurkar N, Gazdar AF. DNA methylation based biomarkers in non-invasive cancer screening. Curr Mol Med 2010;10:123–32.
[49] Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Publ Group 2017;17:223–38. https://doi.org/10.1038/nrc.2017.7.
[50] Couraud S, Vaca-Paniagua F, Villar S, Oliver J, Schuster T, Blanché H, et al. Noninvasive diagnosis of actionable mutations by deep sequencing of circulating free DNA in lung cancer from never-smokers: a proof-of-concept study from BioCAST/IFCT-1002. Clin Cancer Res 2014;20:4613–24. https://doi.org/10.1158/1078-0432.CCR-13-3063.
[51] Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121–34. https://doi.org/10.1016/j.cell.2012.08.024.
[52] Pietanza MC, Ladanyi M. Bringing the genomic landscape of small-cell lung cancer into focus. Nat Genet 2012;44:1074–5. https://doi.org/10.1038/ng.2415.
[53] Klein EA, Hubbel E, Maddala T, Oncol AAJC. Development of a comprehensive cell-free DNA (cfDNA) assay for early detection of multiple tumor types: The Circulating Cell-free Genome Atlas (CCGA) study. J Clin Oncol 2018;2018(36):12021–31.
[54] Sher Y-P, Shih JY, Yang P-C, Roffler SR, Chu Y-W, Wu C-W, et al. Prognosis of non-small cell lung cancer patients by detecting circulating cancer cells in the peripheral blood with multiple marker genes. Clin Cancer Res 2005;11:173–9.
[55] Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018;553:446–54. https://doi.org/10.1038/nature25183.
[56] Yao W, Mei C, Nan X, Hui L. Evaluation and comparison of in vitro degradation kinetics of DNA in serum, urine and saliva: A qualitative study. Gene 2016;590:142–8. https://doi.org/10.1016/j.gene.2016.06.033.
[57] Brüggemann M, Raff T, Flohr T, Gökbuget N, Nakao M, Droese J, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006;107:1116–23. https://doi.org/10.1182/blood-2005-07-2708.
[58] Pui C-H, Pei D, Raimondi SC, Coustan-Smith E, Jeha S, Cheng C, et al. Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with Response-Adapted therapy. Leukemia 2017;31:333–9. https://doi.org/10.1038/leu.2016.234.
[59] Guo N, Lou F, Ma Y, Li J, Yang B, Chen W, et al. Circulating tumor DNA detection

in lung cancer patients before and after surgery. Sci Rep 2016;6:33519. https://doi.org/10.1038/srep33519.
[60] Chen K, Zhang J, Guan T, Yang F, Lou F, Chen W, et al. Comparison of plasma to tissue DNA mutations in surgical patients with non-small cell lung cancer. J Thoracic Cardiovas Surgery 2017;154(1123–1131):e2https://doi.org/10.1016/j.jtcvs.2017.04.073.
[61] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545:446–51. https://doi.org/10.1038/nature22364.
[62] Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discovery 2017;7:1394–403. https://doi.org/10.1158/2159-8290.CD-17-0716.
[63] Riediger AL, Dietz S, Schirmer U, Meister M, Heinzmann-Groth I, Schneider M, et al. Mutation analysis of circulating plasma DNA to determine response to EGFR tyrosine kinase inhibitor therapy of lung adenocarcinoma patients. Sci Rep 2016;6:33505. https://doi.org/10.1038/srep33505.
[64] Punnoose EA, Atwal S, Liu W, Raja R, Fine BM, Hughes BGM, et al. Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. Clin Cancer Res 2012;18:2391–401. https://doi.org/10.1158/1078-0432.CCR-11-3148.
[65] Mok T, Wu Y-L, Lee JS, Yu CJ, Sriuranpong V, Sandoval-Tan J, et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. Clin Cancer Res 2015;21:3196–203. https://doi.org/10.1158/1078-0432.CCR-14-2594.
[66] Cui S, Zhang W, Xiong L, Pan F, Niu Y, Chu T, et al. Use of capture-based next-generation sequencing to detect ALK fusion in plasma cell-free DNA of patients with non-small-cell lung cancer. Oncotarget 2017;8:2771–80. https://doi.org/10.18632/oncotarget.13741.
[67] Dagogo-Jack I, Brannon AR, Ferris LA, Campbell CD, Lin JJ, Schultz KR, et al. Tracking the evolution of resistance to ALK tyrosine kinase inhibitors through longitudinal analysis of circulating tumor DNA. JCO Precis Oncol 2018;2018:1–14. https://doi.org/10.1200/PO.17.00160.
[68] Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8. https://doi.org/10.1056/NEJMoa0909530.
[69] Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–46. https://doi.org/10.1016/S1470-2045(11)70393-X.
[70] Sequist LV, Yang JC-H, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327–34. https://doi.org/10.1200/JCO.2012.44.2806.
[71] Yang JC-H, Wu Y-L, Schuler M, Sebastian M, Popat S, Yamamoto N, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 2015;16:141–51. https://doi.org/10.1016/S1470-2045(14)71173-8.
[72] Crizotinib versus Chemotherapy in Advanced ALK-Positive Lung Cancer. N Engl J Med 2015; 373: p. 1582–2. doi: 10.1056/NEJMx150036.
[73] Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, Kim D-W, et al. Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2017;377:829–38. https://doi.org/10.1056/NEJMoa1704795.
[74] Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018;378:113–25. https://doi.org/10.1056/NEJMoa1713137.
[75] Shaw AT, Solomon BJ. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 2015;372:683–4. https://doi.org/10.1056/NEJMc1415359.
[76] Planchard D, Smit EF, Groen HJM, Mazieres J, Besse B, Helland Å, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. Lancet Oncol 2017;18:1307–16. https://doi.org/10.1016/S1470-2045(17)30679-4.
[77] Cross DAE, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an Irreversible EGFR TKI, Overcomes T790M-Mediated Resistance to EGFR Inhibitors in Lung Cancer. Cancer Discovery 2014;4:1046–61. https://doi.org/10.1158/2159-8290.CD-14-0337.
[78] Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, Dougherty B, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med 2015;21:560–2. https://doi.org/10.1038/nm.3854.
[79] Administration UFADAUFAD, 2015. List of cleared or approved companion diagnostic devices (in vitro and imaging tools); n.d.
[80] Administration UFAD. Cobas EGFR mutation test v2.; 2016.
[81] Wu YL, Zhou C, Liam C-K, Wu G, Liu X, Zhong Z, et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label ENSURE study. Ann Oncol 2015;26:1883–9. https://doi.org/10.1093/annonc/mdv270.
[82] Yun C-H, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong K-K, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci USA 2008;105:2070–5. https://doi.org/10.1073/pnas.0709662105.
[83] Jänne PA, Yang JC-H, Kim D-W, Planchard D, Ohe Y, Ramalingam SS, et al. AZD9291 in EGFR inhibitor-resistant non–small-cell lung cancer. N Engl J Med 2015;372:1689–99. https://doi.org/10.1056/NEJMoa1411817.
[84] Karlovich C, Goldman JW, Sun J-M, Mann E, Sequist LV, Konopa K, et al. Assessment of EGFR mutation status in matched plasma and tumor tissue of NSCLC patients from a phase I study of rociletinib (CO-1686). Clin Cancer Res 2016;22:2386–95. https://doi.org/10.1158/1078-0432.CCR-15-1260.
[85] Jenkins S, Yang JC-H, Ramalingam SS, Yu K, Patel S, Weston S, et al. Plasma ctDNA analysis for detection of the EGFR T790M mutation in patients with advanced non-small cell lung cancer. J Thorac Oncol 2017;12:1061–70. https://doi.org/10.1016/j.jtho.2017.04.003.
[86] Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376:629–40. https://doi.org/10.1056/NEJMoa1612674.
[87] Douillard J-Y, Ostoros G, Cobo M, Ciuleanu T, Cole R, McWalter G, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014;9:1345–53. https://doi.org/10.1097/JTO.0000000000000263.
[88] Reck M, Hagiwara K, Han B, Tjulandin S, Grohé C, Yokoi T, et al. ctDNA determination of EGFR mutation status in european and japanese patients with advanced NSCLC: the ASSESS study. J Thorac Oncol 2016;11:1682–9. https://doi.org/10.1016/j.jtho.2016.05.036.
[89] Wu T-H, Hsiue EH-C, Lee J-H, Lin C-C, Yang JC-H. New data on clinical decisions in NSCLC patients with uncommon EGFR mutations. Expert Rev Respir Med 2017;11:51–5. https://doi.org/10.1080/17476348.2017.1267569.
[90] Yang JC-H, Sequist LV, Geater SL, Tsai C-M, Mok TSK, Schuler M, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol 2015;16:830–8. https://doi.org/10.1016/S1470-2045(15)00026-1.
[91] Taniguchi K, Uchida J, Nishino K, Kumagai T, Okuyama T, Okami J, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res 2011;17:7808–15. https://doi.org/10.1158/1078-0432.CCR-11-1712.
[92] Yung TKF, Chan KCA, Mok TSK, Tong J, To K-F, Lo YMD. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital PCR in non-small cell lung cancer patients. Clin Cancer Res 2009;15:2076–84. https://doi.org/10.1158/1078-0432.CCR-08-2622.
[93] He C, Liu M, Zhou C, Zhang J, Ouyang M, Zhong N, et al. Detection of epidermal growth factor receptor mutations in plasma by mutant-enriched PCR assay for prediction of the response to gefitinib in patients with non-small-cell lung cancer. Int J Cancer 2009;125:2393–9. https://doi.org/10.1002/ijc.24653.
[94] Brevet M, Johnson ML, Azzoli CG, Ladanyi M. Detection of EGFR mutations in plasma DNA from lung cancer patients by mass spectrometry genotyping is predictive of tumor EGFR status and response to EGFR inhibitors. Lung Cancer 2011;73:96–102. https://doi.org/10.1016/j.lungcan.2010.10.014.
[95] Zhao X, Han R-B, Zhao J, Wang J, Yang F, Zhong W, et al. Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I-IV non-small cell lung cancer patients. Respiration 2013;85:119–25. https://doi.org/10.1159/000338790.
[96] Sundaresan TK, Sequist LV, Heymach JV, Riely GJ, Jänne PA, Koch WH, et al. Detection of T790M, the acquired resistance EGFR mutation, by tumor biopsy versus noninvasive blood-based analyses. Clin Cancer Res 2016;22:1103–10. https://doi.org/10.1158/1078-0432.CCR-15-1031.
[97] Buder A, Tomuta C, Filipits M. The potential of liquid biopsies. Curr Opin Oncol 2016;28:130–4. https://doi.org/10.1097/CCO.0000000000000267.
[98] Kwapisz D. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? Ann Transl Med 2017;5:46–56. https://doi.org/10.21037/atm.2017.01.32.
[99] Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734–9. https://doi.org/10.1056/NEJMoa1007478.
[100] Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. Sci Trans Med 2012;4. https://doi.org/10.1126/scitranslmed.3003316.
[101] Gainor JF, Dardaei L, Yoda S, Friboulet L, Leshchiner I, Katayama R, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer. Cancer Discov 2016;6:1118–33. https://doi.org/10.1158/2159-8290.CD-16-0596.
[102] Aubry A, Galiacy S, Allouche M. Targeting ALK in Cancer: Therapeutic Potential of Proapoptotic Peptides. Cancers (Basel) 2019;11:275. https://doi.org/10.3390/cancers11030275.
[103] Yoshida R, Sasaki T, Umekage Y, Tanno S, Ono Y, Ogata M, et al. Highly sensitive detection of ALK resistance mutations in plasma using droplet digital PCR. BMC Cancer 2018;18:1136. https://doi.org/10.1186/s12885-018-5031-0.
[104] Wang Y, Tian P-W, Wang W-Y, Wang K, Zhang Z, Chen B-J, et al. Noninvasive genotyping and monitoring of anaplastic lymphoma kinase (ALK) rearranged non-small cell lung cancer by capture-based next-generation sequencing. Oncotarget 2016;7:65208–17. https://doi.org/10.18632/oncotarget.11569.
[105] McCoach CE, Blakely CM, Banks KC, Levy B, Chue BM, Raymond VM, et al. Clinical utility of cell-free DNA for the detection of ALK fusions and genomic mechanisms of ALK inhibitor resistance in non-small cell lung cancer. Clin Cancer Res 2018;24:2758–70. https://doi.org/10.1158/1078-0432.CCR-17-2588.
[106] Ali SM, Hensing T, Schrock AB, Allen J, Sanford E, Gowen K, et al. Comprehensive genomic profiling identifies a subset of crizotinib-responsive ALK-rearranged non-

small cell lung cancer not detected by fluorescence in situ hybridization. Oncologist 2016;21:762–70. https://doi.org/10.1634/theoncologist.2015-0497.
[107] Herbst RS, Baas P, Kim D-W, Felip E, Pérez-Gracia JL, Han J-Y, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. The Lancet 2016;387:1540–50. https://doi.org/10.1016/S0140-6736(15)01281-7.
[108] Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375:1823–33. https://doi.org/10.1056/NEJMoa1606774.
[109] Frederickson AM, Arndorfer S, Zhang I, Lorenzi M, Insinga R, Arunachalam A, et al. Pembrolizumab plus chemotherapy for first-line treatment of metastatic nonsquamous non-small-cell lung cancer: a network meta-analysis. Immunotherapy 2019;11:407–28. https://doi.org/10.2217/imt-2018-0193.
[110] Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. 2015; 373: p. 123–35. doi: 10.1056/NEJMoa1504627.
[111] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627–39. https://doi.org/10.1056/NEJMoa1507643.
[112] Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med 2018;378:2288–301. https://doi.org/10.1056/NEJMoa1716948.
[113] Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, Pawel von J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255–65. https://doi.org/10.1016/S0140-6736(16)32517-X.
[114] Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 2018;379:2220–9. https://doi.org/10.1056/NEJMoa1809064.
[115] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64. https://doi.org/10.1038/nrc3239.
[116] Teng MWL, Ngiow SF, Ribas A, Smyth MJ. Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res 2015;75:2139–45. https://doi.org/10.1158/0008-5472.CAN-15-0255.
[117] Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science 2015;348:124–8. https://doi.org/10.1126/science.aaa1348.
[118] Tavaré R, Escuin-Ordinas H, Mok S, McCracken MN, Zettlitz KA, Salazar FB, et al. An effective immuno-PET imaging method to monitor CD8-dependent responses to immunotherapy. Cancer Res 2016;76:73–82. https://doi.org/10.1158/0008-5472.CAN-15-1707.
[119] Nishino M, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol 2017;14:655–68. https://doi.org/10.1038/nrclinonc.2017.88.
[120] Hopkins AM, Rowland A, Kichenadasse G, Wiese MD, Gurney H, McKinnon RA, et al. Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers. Br J Cancer 2017;117:913–20. https://doi.org/10.1038/bjc.2017.274.
[121] Page UFADAUFADAH, 2017. FDA approves first cancer treatment for any solid tumor with a specific genetic feature; n.d.
[122] Administration UFAD. Pembrolizumab (KEYTRUDA) checkpoint inhibitor; 2016.
[123] Cabel L, Proudhon C, Romano E, Girard N, Lantz O, Stern M-H, et al. Clinical potential of circulating tumour DNA in patients receiving anticancer immunotherapy. Nat Rev Clin Oncol 2018;15:639–50. https://doi.org/10.1038/s41571-018-0074-3.
[124] McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9. https://doi.org/10.1126/science.aaf1490.
[125] Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 2017;16:2598–608. https://doi.org/10.1158/1535-7163.MCT-17-0386.
[126] Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 2019;51:202–6. https://doi.org/10.1038/s41588-018-0312-8.
[127] Chae YK, Davis AA, Raparia K, Agte S, Pan A, Mohindra N, et al. Association of tumor mutational burden with DNA repair mutations and response to anti-PD-1/PD-L1 therapy in non-small-cell lung cancer. Clinical Lung Cancer 2019;20(88–96):e6https://doi.org/10.1016/j.cllc.2018.09.008.
[128] Goodall J, Mateo J, Yuan W, Mossop H, Porta N, Miranda S, et al. Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. Cancer Discovery 2017;7:1006–17. https://doi.org/10.1158/2159-8290.CD-17-0261.
[129] Koeppel F, Blanchard S, Jovelet C, Genin B, Marcaillou C, Martin E, et al. Whole exome sequencing for determination of tumor mutation load in liquid biopsy from advanced cancer patients. PLoS ONE 2017;12:e0188174https://doi.org/10.1371/journal.pone.0188174.
[130] Jovelet C, Ileana E, Le Deley M-C, Motté N, Rosellini S, Romero A, et al. Circulating cell-free tumor DNA analysis of 50 genes by next-generation sequencing in the prospective MOSCATO trial. Clin Cancer Res 2016;22:2960–8. https://doi.org/10.1158/1078-0432.CCR-15-2470.
[131] Davis AA, Chae YK, Agte S, Pan A, Simon NI, Taxter TJ, et al. Comparison of tumor mutational burden (TMB) across tumor tissue and circulating tumor DNA (ctDNA). J Clin Oncol 2017;35:e23028–38. https://doi.org/10.1200/JCO.2017.35.15_suppl.e23028.
[132] Fabrizio D, Lieber D, Malboeuf C, Silterra J, White E, Coyne M, et al. Abstract 5706: A blood-based next-generation sequencing assay to determine tumor mutational burden (bTMB) is associated with benefit to an anti-PD-L1 inhibitor, atezolizumab. Cancer Res 2018;78:5706–16. https://doi.org/10.1158/1538-7445.AM2018-5706.
[133] Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24:1441–8. https://doi.org/10.1038/s41591-018-0134-3.
[134] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20. https://doi.org/10.1056/NEJMoa1500596.
[135] Buza N, Ziai J, Hui P. Mismatch repair deficiency testing in clinical practice. Expert Rev Mol Diagn 2016;16:591–604. https://doi.org/10.1586/14737159.2016.1156533.
[136] Stern M-H, Silveira AB, Bieche I, Melaabi S, Cabel L, Buecher B, et al. Abstract 4599: detecting MSI phenotype in circulating blood DNA. Cancer Res 2018;78:4599–609. https://doi.org/10.1158/1538-7445.AM2018-4599.
[137] Ladas I, Yu F, Leong KW, Fitarelli-Kiehl M, Song C, Ashtaputre R, et al. Enhanced detection of microsatellite instability using pre-PCR elimination of wild-type DNA homo-polymers in tissue and liquid biopsies. Nucleic Acids Res 2018;46:e74–84. https://doi.org/10.1093/nar/gky251.
[138] Srinivasan P, Tran C, Reichel J, Patel JA, Hasan M, Meng F, et al. Abstract 3656: Detecting MSI in plasma: Implications for early detection of lynch associated tumors. Cancer Res 2018;78:3656–66. https://doi.org/10.1158/1538-7445.AM2018-3656.
[139] Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34. https://doi.org/10.1186/s13073-017-0424-2.
[140] Chiou VL, Burotto M. Pseudoprogression and immune-related response in solid tumors. J Clin Oncol 2015;33:3541–3. https://doi.org/10.1200/JCO.2015.61.6870.
[141] Hodi FS, Hwu W-J, Kefford R, Weber JS, Daud A, Hamid O, et al. Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with pembrolizumab. J Clin Oncol 2016;34:1510–7. https://doi.org/10.1200/JCO.2015.64.0391.
[142] Queirolo P, Spagnolo F. Atypical responses in patients with advanced melanoma, lung cancer, renal-cell carcinoma and other solid tumors treated with anti-PD-1 drugs: A systematic review. Cancer Treat Rev 2017;59:71–8. https://doi.org/10.1016/j.ctrv.2017.07.002.
[143] Tazdait M, Mezquita L, Lahmar J, Ferrara R, Bidault F, Ammari S, et al. Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. Eur J Cancer 2018;88:38–47. https://doi.org/10.1016/j.ejca.2017.10.017.
[144] Di Giacomo AM, Danielli R, Guidoboni M, Calabrò L, Carlucci D, Miracco C, et al. Therapeutic efficacy of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with metastatic melanoma unresponsive to prior systemic treatments: clinical and immunological evidence from three patient cases. Cancer Immunol Immunother 2009;58:1297–306. https://doi.org/10.1007/s00262-008-0642-y.
[145] Saâda-Bouzid E, Defaucheux C, Karabajakian A, Coloma VP, Servois V, Paoletti X, et al. Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. Ann Oncol 2017;28:1605–11. https://doi.org/10.1093/annonc/mdx178.
[146] Kim Y, Kim CH, Kim HS, Sun J-M, Ahn JS, Ahn M-J, et al. Hyperprogression after immunotherapy: Clinical implication and genomic alterations in advanced non-small cell lung cancer patients (NSCLC). J Clin Oncol 2018;36:9075–85. https://doi.org/10.1200/JCO.2018.36.15_suppl.9075.
[147] Fuentes-Antrás J, Provencio M, Díaz-Rubio E. Hyperprogression as a distinct outcome after immunotherapy. Cancer Treat Rev 2018;70:16–21. https://doi.org/10.1016/j.ctrv.2018.07.006.
[148] Soria F, Beleni AI, D'Andrea D, Resch I, Gust KM, Gontero P, et al. Pseudoprogression and hyperprogression during immune checkpoint inhibitor therapy for urothelial and kidney cancer. World J Urol 2018;36:1703–9. https://doi.org/10.1007/s00345-018-2264-0.
[149] Rizvi NA, Mazieres J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol 2015;16:257–65. https://doi.org/10.1016/S1470-2045(15)70054-9.
[150] Cabel L, Riva F, Servois V, Livartowski A, Daniel C, Rampanou A, et al. Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study. Ann Oncol 2017;28:1996–2001. https://doi.org/10.1093/annonc/mdx212.
[151] Goldberg SB, Narayan A, Kole AJ, Decker RH, Teysir J, Carriero NJ, et al. Early assessment of lung cancer immunotherapy response via circulating tumor DNA. Clin Cancer Res 2018;24:1872–80. https://doi.org/10.1158/1078-0432.CCR-17-1341.
[152] Iijima Y, Hirotsu Y, Amemiya K, Ooka Y, Mochizuki H, Oyama T, et al. Very early response of circulating tumour-derived DNA in plasma predicts efficacy of nivolumab treatment in patients with non-small cell lung cancer. Eur J Cancer 2017;86:349–57. https://doi.org/10.1016/j.ejca.2017.09.004.
[153] Anagnostou V, Forde PM, White JR, Niknafs N, Hruban C, Naidoo J, et al. Dynamics of tumor and immune responses during immune checkpoint blockade in non-small cell lung cancer. Cancer Res 2019;79:1214–25. https://doi.org/10.

1158/0008-5472.CAN-18-1127.
[154] Board RE, Williams VS, Knight L, Shaw J, Greystoke A, Ranson M, et al. Isolation and extraction of circulating tumor DNA from patients with small cell lung cancer. Ann N Y Acad Sci 2008;1137:98–107. https://doi.org/10.1196/annals.1448.020.
[155] George J, Lim JS, Jang SJ, Cun Y, Ozretić L, Kong G, et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524:47–53. https://doi.org/10.1038/nature14664.
[156] Almodovar K, Iams WT, Meador CB, Zhao Z, York S, Horn L, et al. Longitudinal cell-free DNA analysis in patients with small cell lung cancer reveals dynamic insights into treatment efficacy and disease relapse. J Thorac Oncol 2018;13:112–23. https://doi.org/10.1016/j.jtho.2017.09.1951.
[157] Gormally E, Vineis P, Matullo G, Veglia F, Caboux E, Le Roux E, et al. TP53 and KRAS2 mutations in plasma DNA of healthy subjects and subsequent cancer occurrence: a prospective study. Cancer Res 2006;66:6871–6. https://doi.org/10.1158/0008-5472.CAN-05-4556.
[158] Krimmel JD, Schmitt MW, Harrell MI, Agnew KJ, Kennedy SR, Emond MJ, et al. Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic TP53 mutations in noncancerous tissues. Proc Natl Acad Sci USA 2016;113:6005–10. https://doi.org/10.1073/pnas.1601311113.
[159] Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol 2016;17:883–95. https://doi.org/10.1016/S1470-2045(16)30098-5.
[160] Fernández-Cuesta L, Perdomo S, Avogbe PH, Leblay N, Delhomme TM, Gaborieau V, et al. Identification of circulating tumor DNA for the early detection of small-cell lung cancer. EBioMedicine 2016;10:117–23. https://doi.org/10.1016/j.ebiom.2016.06.032.
[161] Early detection: a long road ahead. Nature Publ Group 2018; 18: p. 401–1. doi: 10.1038/s41568-018-0021-8.
[162] Wu Y-L, Jenkins S, Ramalingam S, Han J-Y, Delmonte A, Hsia T-C, et al. MA08.03 osimertinib vs platinum-pemetrexed for T790M-mutation positive advanced NSCLC (AURA3): plasma ctDNA analysis. J Thoracic Oncol 2017;12:S386. https://doi.org/10.1016/j.jtho.2016.11.436.
[163] Ou S-HI, Cui J, Schrock AB, Goldberg ME, Zhu VW, Albacker L, et al. Emergence of novel and dominant acquired EGFR solvent-front mutations at Gly796 (G796S/R) together with C797S/R and L792F/H mutations in one EGFR (L858R/T790M) NSCLC patient who progressed on osimertinib. Lung Cancer 2017;108:228–31. https://doi.org/10.1016/j.lungcan.2017.04.003.
[164] Oztan A, Fischer S, Schrock AB, Erlich RL, Lovly CM, Stephens PJ, et al. Emergence of EGFR G724S mutation in EGFR-mutant lung adenocarcinoma post progression on osimertinib. Lung Cancer 2017;111:84–7. https://doi.org/10.1016/j.lungcan.2017.07.002.
[165] Ou S-HI, Lee TK, Young L, Fernandez-Rocha MY, Pavlick D, Schrock AB, et al. Dual occurrence of ALK G1202R solvent front mutation and small cell lung cancer transformation as resistance mechanisms to second generation ALK inhibitors without prior exposure to crizotinib. Pitfall of solely relying on liquid re-biopsy? Lung Cancer 2017;106:110–4. https://doi.org/10.1016/j.lungcan.2017.02.005.
[166] Puig O, Yang JC-H, Ou S-HI, Chiappori A, Chao BH, Belani CP, et al. Pooled mutation analysis for the NP28673 and NP28761 studies of alectinib in ALK+ non-small-cell lung cancer (NSCLC). J Clin Oncol 2017;34:9061–71. https://doi.org/10.1200/JCO.2016.34.15_suppl.9061.
[167] Hsiue EH-C, Lee J-H, Lin C-C, Yang JC-H. Profile of the therascreen® EGFR RGQ PCR kit as a companion diagnostic for gefitinib in non-small cell lung cancer. Expert Rev Mol Diagn 2016;16:1251–7. https://doi.org/10.1080/14737159.2016.1248414.