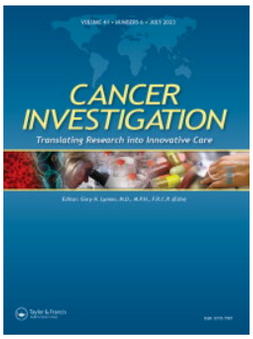

Cancer Investigation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icnv20

The Utility of ctDNA in Lung Cancer Clinical Research and Practice: A Systematic Review and Meta-Analysis of Clinical Studies

Xuezheng Sun, Page Abrahamson, Nicholas Ballew, Linda Kalilani, Kelesitse Phiri, Kelly F. Bell, Alexander Slowley, Magdalena Zajac, Erin Hofstatter, Alexander Stojadinovic, Angela Silvestro, Zebin Wang, Amine Aziez & Solange Peters

To cite this article: Xuezheng Sun, Page Abrahamson, Nicholas Ballew, Linda Kalilani, Kelesitse Phiri, Kelly F. Bell, Alexander Slowley, Magdalena Zajac, Erin Hofstatter, Alexander Stojadinovic, Angela Silvestro, Zebin Wang, Amine Aziez & Solange Peters (2023) The Utility of ctDNA in Lung Cancer Clinical Research and Practice: A Systematic Review and Meta-Analysis of Clinical Studies, Cancer Investigation, 41:6, 571-592, DOI: 10.1080/07357907.2023.2220820

To link to this article: https://doi.org/10.1080/07357907.2023.2220820

CANCER INVESTIGATION
2023, VOL. 41, NO. 6, 571–592
https://doi.org/10.1080/07357907.2023.2220820

Taylor & Francis
Taylor & Francis Group

REVIEW

The Utility of ctDNA in Lung Cancer Clinical Research and Practice: A Systematic Review and Meta-Analysis of Clinical Studies

Xuezheng Sun[a], Page Abrahamson[b], Nicholas Ballew[c], Linda Kalilani[a], Kelesitse Phiri[c], Kelly F. Bell[c], Alexander Slowley[d], Magdalena Zajac[d], Erin Hofstatter[e], Alexander Stojadinovic[c]*, Angela Silvestro[e], Zebin Wang[e]*, Amine Aziez[f] and Solange Peters[g]

[a]GSK, Research Triangle Park, NC, USA; [b]Salmon Bay Consulting, Seattle, WA, USA; [c]GSK, Collegeville, PA, USA; [d]GSK, London, England, UK; [e]GSK, Waltham, MA, USA; [f]GSK, Zug, Switzerland; [g]Department of Oncology, Lausanne University Hospital, Lausanne, Switzerland

ABSTRACT

This systematic review with embedded meta-analysis aimed to evaluate the clinical utility of circulating tumor DNA (ctDNA) in lung cancer. After screening and review of the Embase database search, 111 studies from 2015 to 2020 demonstrated ctDNA's value in prognostication/monitoring disease progression, mainly in patients with advanced/metastatic disease and non–small cell lung cancer. ctDNA positivity/detection at any time point was associated with shorter progression-free survival and overall survival, whereas ctDNA clearance/decrease during treatment was associated with a lower risk of progression and death. Validating these findings and addressing challenges regarding ctDNA testing integration into clinical practice will require further research.

ARTICLE HISTORY
Received 11 April 2023
Accepted 29 May 2023

KEYWORDS
Biomarker; circulating tumor DNA; non-small cell lung cancer; small cell lung cancer; lung cancer

Introduction

Released by cancer cells through apoptosis, lysis of circulating tumor cells, and active secretion, circulating tumor DNA (ctDNA) constitutes a small fraction of cell-free DNA (cfDNA) in blood (1,2). Unlike tumor tissue sampling, which is limited by invasiveness and associated risks, tissue accessibility, sampling frequency, and cost, ctDNA offers a convenient, minimally invasive method to measure tumor burden and genomic profile. Additionally, because of the short half-life in circulation, ctDNA is considered a "real-time" snapshot of tumor activity (2). These features make ctDNA a promising molecular biomarker in oncology and have triggered interest in ctDNA utility during the past decade. Recent technological advances in the detection and quantification of low-abundance ctDNA have accelerated the progress of integrating ctDNA testing into clinical decision-making in cancer care. Recently published US Food and Drug Administration draft guidance and the European Society for Medical Oncology recommendations on ctDNA utility in cancer further confirm the value and potential of ctDNA while also highlighting the knowledge gaps and data limitations in its clinical utility (3,4). Within the field, there is substantial interest in refining study designs and standardizing assessment methods to improve the level of evidence for the different ctDNA utilities in early- and late-stage disease (3–5).

ctDNA biology, evaluation techniques, and potential applications have been reviewed in numerous publications (2,6–9). As the treatment landscape for lung cancer has increasingly shifted toward the use of targeted therapies and immunotherapies (10–12), there has been substantial interest in using ctDNA for the detection of specific gene mutations to inform treatment selection. Multiple actionable mutations have been

CONTACT Xuezheng Sun amy.x.sun@gsk.com Oncology Epidemiology, Value Evidence and Outcomes, R&D Global Medical, GSK, 5 Moore Dr, Research Triangle Park, NC 27709, USA.
*Author was present at GSK at the time the analysis was conducted.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/07357907.2023.2220820

identified in non–small cell lung cancer (NSCLC), including mutations in *EGFR*, *KRAS*, and *ALK* (11). ctDNA-mediated detection of these mutations has the potential to help direct targeted therapy selection or monitor treatment response in situations where biopsies are not feasible.

Previous systematic reviews on ctDNA in lung cancer have primarily focused on diagnostic accuracy, evaluating the concordance between ctDNA results and tissue-based testing (13–16). Another systematic review in NSCLC focused on the association between ctDNA and response to immune checkpoint inhibitors (ICIs) (17). The literature on ctDNA utility within small cell lung cancer (SCLC) is more limited, with 1 systematic review that examined the role of ctDNA for disease monitoring and genomic profiling (18). Although these analyses furnish valuable insights, they do not provide information on the bigger picture of how ctDNA is being used across lung cancer in this rapidly developing field. This systematic review aimed to comprehensively summarize data on the utility of ctDNA from published clinical studies of lung cancer. The findings detail the current landscape of ctDNA applications in lung cancer, serving as a foundation to inform study design development and result interpretation.

Methods

Literature identification and eligibility criteria

A systematic literature review was performed using the Embase database to identify published literature on lung cancer and ctDNA from January 1, 2015, to December 31, 2020. The final search terms were "('lung cancer'/exp OR 'lung cancer') AND ('ctDNA' or 'circulating tumor DNA' or 'cell-free tumor DNA') AND [english]/-lim AND [2015–2020]/py". No limitations were placed on lung cancer type or disease stage.

Full-text original clinical research articles were included; review/meta-analysis articles and non-English articles were excluded. Conference abstracts, editorials, commentaries, case reports, and study protocols were excluded because of incompleteness of data or small sample sizes. Articles were included for studies in which ctDNA was linked to clinical features (eg, tumor stage) or outcomes (eg, progression-free survival). Articles that were limited to the following topics were considered out of scope for this review and therefore excluded: cfDNA, specimens other than blood/plasma/serum, ctDNA analytic validation (eg, descriptive analysis of ctDNA dynamics or correlation with tumor tissue DNA), or assessment of the tumor burden without relating ctDNA to tumor characteristics or clinical outcomes. However, articles that measured the genomic alterations of specific molecular tumor-related alterations in cfDNA were included. Initial selection based on title and abstract was performed by 2 independent reviewers; any disagreements on inclusion/exclusion were resolved by a third reviewer or consensus-based discussion. One reviewer also identified additional articles using reference lists from systematic review or meta-analysis reports on the same or a similar topic.

Data collection and extraction

One reviewer extracted data from included articles into a predefined extraction Excel table, covering article information (authors, title, publication year), study design (study period, location, research/clinical settings, study population, sample size), tumor characteristics (lung cancer subtype, stage), and ctDNA-related information (platform type, test timing, clinical utility). Extracted data were quality checked by a second reviewer.

The ctDNA utilizations proposed by Wan and colleagues and Narayan and colleagues were adapted (Table 1) (2,19). Studies were classified into 3 categories based on ctDNA testing aims, the timing of ctDNA tests, and endpoints/outcomes in the analyses: (1) diagnosis (early diagnosis, screening), (2) prognostication (detection, profiling, prognostication), and (3) monitoring (monitoring disease progression, treatment response, or genomic evolution using longitudinal ctDNA samples). Because disease history and ctDNA dynamics are a continuous course, categories were not mutually exclusive. Studies

Table 1. Description of ctDNA clinical utilities in lung cancer.

Utility	Aims	Timing of ctDNA Test	Endpoints/Outcomes
Diagnosis (early diagnosis, screening)	Detect ctDNA in general population or high-risk noncancer populations to identify patients with lung cancer	Cross-sectional or longitudinal before clinical lung cancer diagnosis	Cancer incidence, prediction accuracy, etc
Prognostication (detection, profiling, prognostication)	Characterize ctDNA qualitatively or quantitatively in patients with lung cancer to predict clinical prognosis	Cross-sectional in disease/treatment journey (eg, at diagnosis, after surgery, after systemic therapy)	Cancer characteristics, recurrence, progression, death, etc
Monitoring (monitoring disease progression, treatment response, or genomic evolution)	Capture ctDNA dynamics (ie, change in repeated measures) during/after treatment qualitatively or quantitatively in patients with lung cancer, and correlate these features with progression, treatment response, or tumor evolution	Longitudinal through patient treatment journey (eg, during/after neoadjuvant treatment, adjuvant treatment, or systemic treatment)	Treatment response, recurrence, progression, death, prediction accuracy, lead time, changes in ctDNA genomic profile, etc

ctDNA: circulating tumor DNA.
Adapted from the ctDNA utilizations proposed by Wan et al.[2] and Narayan et al.[19]

that fulfilled more than 1 predefined category were reported in multiple categories.

Meta-analysis

A meta-analysis was conducted to quantitively review and synthesize the association of ctDNA with clinical outcomes for patients with lung cancer. Studies that reported hazard ratios (HRs) with corresponding 95% CIs for the association between ctDNA and progression-free survival (PFS) or overall survival (OS) were included in the meta-analyses. ctDNA detection was assessed as a binary variable (positive vs. negative or decrease/clearance yes vs. no). For longitudinal ctDNA assessments, results were grouped according to 3 broad categories (before, during, or after treatment). The definition of ctDNA decrease/-clearance varied across studies in terms of the timing and number of ctDNA assessments. However, because of the small number of studies, all definitions of ctDNA decrease/clearance were grouped together and treated as the same. Separate analyses were performed for the detection of *EGFR* mutation(s) in ctDNA. Although mutations in other tumor genes were of interest, data were insufficient for meta-analyses.

Pooled estimates were calculated using fixed-effect and random-effect models (20), respectively, depending on the p value (cut point, 0.05) and I^2 for the test of heterogeneity (>50%). Results were presented in forest plots. Stratified analyses were performed by potential effect modifiers, including ctDNA assessment timing (before, during, or after treatment), study type (observational study vs. clinical trial), lung cancer histology subtype (NSCLC vs. other), and disease stage (advanced/metastatic vs. early) when adequate data were available (≥3 studies in a category). The appropriateness of the assumptions made for the analyses was assessed by performing the I^2 test of heterogeneity and using meta-regression to assess effect modifiers (21,22). Egger tests (when study number was >10) and funnel plots were used to assess publication bias. All statistical tests were 2-sided with α of 0.05. All analyses were performed using R version 4.1.0 with the "meta" and "metafor" packages.

Results

Study selection

A total of 2200 publications were identified in the initial Embase search (Figure 1). Of the 238 articles selected from the initial screening for full-text review, 101 articles were selected for inclusion. Ten additional articles were identified by reviewing the reference lists for recent selected meta-analysis or review articles (23–25). The final review included 111 articles (Table 2).

Study and patient characteristics

Table 3 summarizes the characteristics for all 111 included studies. ctDNA was increasingly studied during 2015–2020, with 60 studies (54.1%) published in 2019 or 2020. Overall, 52.3% of included studies were conducted in Asia-Pacific, 25.2% in European countries, and 19.8% in the United States or Canada. Most studies (78.4%) used an

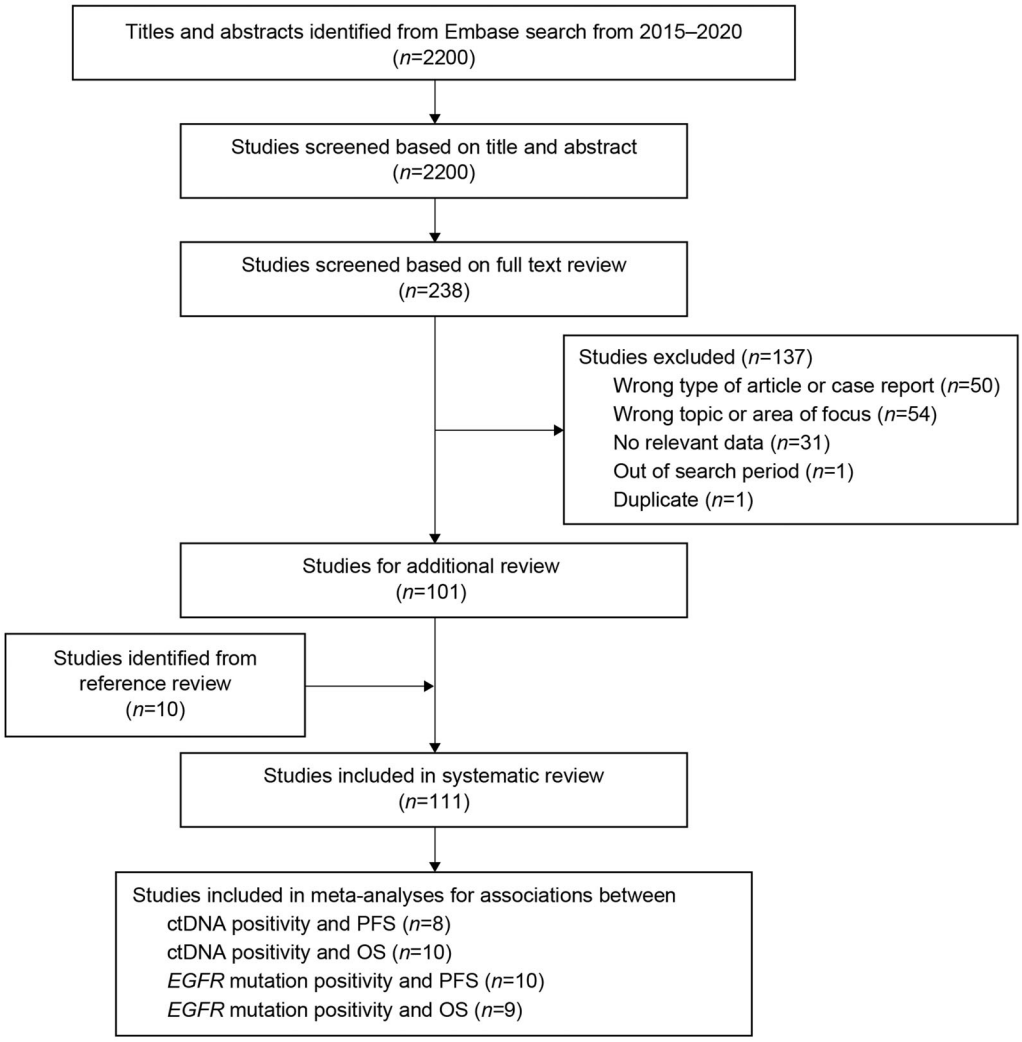

Figure 1. PRISMA diagram. All records were screened and reviewed by 2 independent human reviewers. ctDNA: circulating tumor DNA; EGFR: epidermal growth factor receptor; OS: overall survival; PFS: progression-free survival.

observational design, with clinical trials constituting 21.6% of the studies. The number of patients who underwent ctDNA testing differed significantly across studies, ranging from 8 to 1070, with 39.6% of studies including fewer than 50 patients with ctDNA data. Of the included studies, 73.0% were conducted among patients with advanced/metastatic disease (Table 3). Most studies (79.3%) included patients with NSCLC.

ctDNA measurement

Although the number of ctDNA tests per patient depended on the study aims and design, 52.3% of the studies measured ctDNA in each patient (or a subset of patients) multiple times (Supplementary Table 1). In the identified studies, tumor-informed testing was used less frequently (2.7%) than tumor-naïve testing (97.3%). Prognostication was the most common utility (76.6%), followed by monitoring progression/treatment response (47.7%). Only 3 studies (2.7%) assessed ctDNA for early cancer diagnosis.

ctDNA for early cancer diagnosis and screening

Three studies used ctDNA as a screening biomarker for the diagnosis of lung cancer (Supplementary Table 2) (26–28). Studies were in the exploratory stage and generally focused on diagnostic accuracy compared with pathological diagnosis, with varied results for sensitivity and specificity.

Table 2. Basic characteristics of the 111 studies included in the literature review.

First author	Year	Location	Study Type	Subtype	Tumor Stage[a]	Sample Size[b]	Assay Type	ctDNA Testing Number[c]	Utility
Abbosh C	2017	Multiple	Observational	NSCLC	I–III	100	Tumor-informed	Single	Evolution
Aldea M	2019	France	Observational	NSCLC	IV	247	Tumor-naïve	Multiple	Prognostication Monitoring
Alegre E	2016	Spain	Observational	NSCLC	I–IV	36	Tumor-naïve	Multiple	Prognostication Monitoring
Anagnostou V	2019	USA	Observational	NSCLC	Metastatic	40	Tumor-naïve	Multiple	Monitoring
Balgkouranidou I	2016	Greece	Observational	NSCLC	I–IV	44	Tumor-naïve	Single	Prognostication
Beagan JJ	2020	Netherlands	Observational	NSCLC	Metastatic	20	Tumor-naïve	Single	Prognostication
Buder A	2020	Austria	Observational	NSCLC	Metastatic	108	Tumor-naïve	Single	Prognostication
Buttitta F	2020	Italy	Observational	NSCLC	III–IV	64	Tumor-naïve	Multiple	Monitoring
Chabon JJ	2016	USA	Clinical trial	NSCLC	Advanced	43	Tumor-informed	Multiple	Monitoring
Chae YK	2019	USA	Observational	NSCLC	III–IV	136	Tumor-naïve	Single	Prognostication
Chaudhuri AA	2017	USA	Observational	Any LC	I–III	40	Tumor-naïve	Multiple	Diagnosis Prognostication Monitoring
Chen C	2020	China	Observational	NSCLC	I	163	Tumor-naïve	Single	Diagnosis
Chen K	2019	China	Observational	Any LC	I–IV	26	Tumor-naïve	Multiple	Monitoring
Chia BSH	2020	Singapore	Observational	Any LC	I–III	9	Tumor-naïve	Single	Prognostication
Chiou C-C	2020	China	Observational	NSCLC	IV	11	Tumor-naïve	Multiple	Monitoring
Cho M-S	2020	South Korea	Observational	NSCLC	I–IV	36	Tumor-naïve	Single	Prognostication
Corradetti MN	2019	USA	Clinical trial	Any LC	III	24	Tumor-naïve	Single	Prognostication
Dagogo-Jack I	2018	USA	Observational	Adenocarcinoma	I–IV	22	Tumor-naïve	Single	Monitoring
Del Re M	2017	Italy	Observational	NSCLC	IIIB, IV	49	Tumor-naïve	Single	Prognostication
Demuth C	2018	Denmark	Observational	NSCLC	III–IV	40	Tumor-naïve	Multiple	Monitoring
Dietz S	2020	Germany	Observational	NSCLC	Metastatic	73	Tumor-naïve	Single	Prognostication
Ding PN	2019	Australia	Observational	NSCLC	IV	28	Tumor-naïve	Multiple	Monitoring
Duan J	2020	China	Clinical trial	Adenocarcinoma	IV	180	Tumor-naïve	Multiple	Prognostication Monitoring
Ebert EBF (A)	2020	Denmark	Observational	NSCLC	Advanced	82	Tumor-naïve	Multiple	Prognostication Monitoring
Ebert EBF (B)	2020	Denmark	Observational	NSCLC	Advanced	98	Tumor-naïve	Multiple	Prognostication Monitoring
Gandara DR	2018	Multiple	Clinical trial	NSCLC	Advanced	1070	Tumor-naïve	Single	Prognostication
Giroux Leprieur E	2020	France	Observational	NSCLC	Advanced	8	Tumor-naïve	Multiple	Monitoring
Giroux Leprieur E	2018	France	Observational	NSCLC	Advanced	15	Tumor-naïve	Multiple	Prognostication Monitoring
Goldberg SB	2018	USA	Observational	NSCLC	Metastatic	28	Tumor-naïve	Multiple	Prognostication Monitoring
Guibert N	2019	France	Clinical trial	NSCLC	IIIB, IV	86	Tumor-naïve	Multiple	Prognostication
Guibert N	2016	France	Observational	Adenocarcinoma	Metastatic	32	Tumor-naïve	Single	Prognostication
Guo D	2020	China	Observational	NSCLC	I–IV	64	Tumor-naïve	Multiple	Monitoring
Guo N	2016	China	Observational	NSCLC	I–IV	41	Tumor-naïve	Multiple	Monitoring
Han JY	2016	South Korea	Clinical trial	NSCLC	IIIB, IV	208	Tumor-naïve	Single	Prognostication
Han X	2019	China	Observational	Adenocarcinoma	III–IV	32	Tumor-naïve	Multiple	Monitoring
He J (A)	2017	China	Observational	Adenocarcinoma	III/IV	120	Tumor-naïve	Multiple	Prognostication
He J (B)	2017	China	Clinical trial	NSCLC	Advanced	200	Tumor-naïve	Multiple	Prognostication Monitoring
Hellmann MD	2020	USA	Observational	NSCLC	IV	31	Tumor-informed	Single	Prognostication
Herbreteau G	2020	France	Clinical trial	SCLC	Limited or extensive[d]	68	Tumor-naïve	Single	Prognostication
Horn GYF	2020	Hong Kong	Observational	NSCLC	IV	20	Tumor-naïve	Multiple	Monitoring
Horn L	2019	USA	Clinical trial	NSCLC	IV	76	Tumor-naïve	Single	Prognostication
Iijima Y	2017	Japan	Observational	NSCLC	Metastatic	14	Tumor-naïve	Multiple	Prognostication Monitoring
Imamura F	2016	Japan	Observational	NSCLC	III–IV	52	Tumor-naïve	Single	Prognostication
Isaksson S	2019	Sweden	Observational	NSCLC	I–III	146	Tumor-naïve	Single	Prognostication
Iwama E	2018	Japan	Observational	NSCLC	IIIB, IV	35	Tumor-naïve	Multiple	Prognostication

(*continued*)

Table 2. Continued.

First author	Year	Location	Study Type	Subtype	Tumor Stage[a]	Sample Size[b]	Assay Type	ctDNA Testing Number[c]	Utility
Iwama E	2017	Japan	Clinical trial	Adenocarcinoma	IIIB, IV	35	Tumor-naïve	Multiple	Prognostication Monitoring
Jia J	2018	China	Observational	NSCLC	IV	150	Tumor-naïve	Multiple	Prognostication Monitoring
Jiang J	2020	Germany	Observational	NSCLC	III–IV	71	Tumor-naïve	Multiple	Prognostication
Jiang T	2017	China	Clinical trial	NSCLC	IIIB, IV	48	Tumor-naïve	Multiple	Prognostication
Kim T	2019	South Korea	Observational	Adenocarcinoma	I–IV	81	Tumor-naïve	Single	Prognostication
Kuang PP	2020	China	Observational	NSCLC	IB-III	38	Tumor-naïve	Multiple	Prognostication Monitoring
Lee JY	2016	South Korea	Observational	NSCLC	IV	81	Tumor-naïve	Multiple	Prognostication Monitoring
Lee Y	2018	South Korea	Observational	NSCLC	Advanced	57	Tumor-naïve	Multiple	Prognostication Monitoring
Leung M	2020	UK	Observational	Any LC	I–IV	192	Tumor-naïve	Single	Prognostication
Lin X	2019	China	Observational	NSCLC	III–IV	36	Tumor-naïve	Single	Prognostication
Liu Y	2020	China	Observational	Squamous cell	IA–IV	26	Tumor-naïve	Single	Prognostication
Liu Z	2019	China	Observational	NSCLC	Advanced	853	Tumor-naïve	Single	Prognostication
Mastoraki S	2021	USA	Observational	NSCLC	I–IV	139	Tumor-naïve	Single	Prognostication
Mezquita L	2020	France	Observational	NSCLC	I–IV	128	Tumor-naïve	Multiple	Prognostication
Minari R	2020	Italy	Observational	NSCLC	Advanced	120	Tumor-naïve	Single	Prognostication
Moding EJ	2020	USA	Clinical trial	NSCLC	II–III	65	Tumor-naïve	Multiple	Prognostication Monitoring
Mok T	2015	China	Clinical trial	NSCLC	IIIB, IV	447	Tumor-naïve	Multiple	Prognostication Monitoring
Moon SM	2020	South Korea	Observational	NSCLC and SCLC	I–IV	99	Tumor-naïve	Single	Diagnosis
Nardo G	2020	Italy	Observational	NSCLC	I–IV	122	Tumor-naïve	Single	Prognostication
Nong J	2018	China	Observational	SCLC	Limited or extensive[d]	22	Tumor-naïve	Multiple	Prognostication Monitoring
Ohara S	2020	Japan	Observational	Any LC	IIA–IIIA	20	Tumor-naïve	Multiple	Prognostication
O'Kane GM	2019	Canada	Observational	NSCLC	IV	72	Tumor-naïve	Single	Prognostication
Ortiz-Cuaran S	2020	France	Clinical trial	NSCLC	Metastatic	78	Tumor-naïve	Multiple	Prognostication Monitoring
Oxnard GR	2016	Taiwan	Clinical trial	NSCLC	Advanced	271	Tumor-naïve	Single	Prognostication
Park C-K	2019	South Korea	Clinical trial	NSCLC	Metastatic	80	Tumor-naïve	Single	Prognostication
Pécuchet N	2016	France	Observational	NSCLC	IIIB, IV	105	Tumor-naïve	Multiple	Prognostication
Pender A	2020	Canada	Clinical trial	NSCLC	Advanced	177	Tumor-naïve	Single	Prognostication
Peng M	2020	China	Observational	NSCLC	I–IV	75	Tumor-naïve	Multiple	Prognostication
Peng X	2019	China	Observational	Any LC	I–IV	31	Tumor-naïve	Single	Prognostication
Phallen J	2019	USA	Observational	NSCLC	I–IV	28	Tumor-naïve	Multiple	Monitoring
Piotrowska Z	2015	USA	Clinical trial	Any LC	Advanced	25	Tumor-naïve	Single	Prognostication Monitoring
Provencio M	2018	Spain	Observational	NSCLC	III–IV	41	Tumor-naïve	Multiple	Monitoring
Raja R	2018	Worldwide	Observational	NSCLC	III–IV	100	Tumor-naïve	Multiple	Monitoring
Rao C	2018	China	Observational	NSCLC	IV	34	Tumor-naïve	Single	Prognostication
Reckamp KL	2020	USA	Observational	NSCLC	III–IV	76	Tumor-naïve	Single	Prognostication
Remon J	2017	France	Observational	NSCLC	Advanced	48	Tumor-naïve	Single	Prognostication
Romero A	2020	Spain	Observational	NSCLC	IIIB, IV	22	Tumor-naïve	Multiple	Monitoring
Schwaederlé MC	2017	USA	Observational	Adenocarcinoma	Advanced	88	Tumor-naïve	Single	Prognostication
Song Y	2020	China	Observational	NSCLC	III–IV	248	Tumor-naïve	Multiple	Prognostication Monitoring
Spence T	2021	Canada	Observational	NSCLC	Metastatic	343	Tumor-naïve	Multiple	Monitoring
Sueoka-Aragane N	2015	Japan	Observational	NSCLC	Advanced	89	Tumor-naïve	Multiple	Prognostication
Taus Á	2018	Spain	Observational	Adenocarcinoma	III–IV	33	Tumor-naïve	Multiple	Monitoring
Thompson JC	2016	USA	Observational	NSCLC	III–IV	102	Tumor-naïve	Single	Prognostication
Tsui DWY	2018	Singapore	Clinical trial	NSCLC	IV	50	Tumor-naïve	Multiple	Prognostication Monitoring
Uchida J	2016	Japan	Observational	NSCLC	IV	57	Tumor-naïve	Multiple	Monitoring
Usui K	2019	Japan	Observational	NSCLC	Advanced	122	Tumor-naïve	Multiple	Monitoring
Wang J	2017	China	Observational	NSCLC	IV	22	Tumor-naïve	Single	Prognostication
Wang W	2017	China	Observational	NSCLC	IV	75	Tumor-naïve	Single	Prognostication

(*continued*)

Table 2. Continued.

First author	Year	Location	Study Type	Subtype	Tumor Stage[a]	Sample Size[b]	Assay Type	ctDNA Testing Number[c]	Utility
Wang Y	2019	China	Observational	NSCLC	I–IV	464	Tumor-naïve	Single	Prognostication
Wang Z	2018	China	Clinical trial	Adenocarcinoma	IV	183	Tumor-naïve	Multiple	Prognostication Monitoring
Wang Z	2019	China	Observational	NSCLC	III–IV	84	Tumor-naïve	Single	Prognostication
Wei Z	2017	China	Clinical trial	NSCLC	IIIB, IV	200	Tumor-naïve	Multiple	Prognostication Monitoring
Wu Y-L	2019	China	Clinical trial	NSCLC	Advanced	169	Tumor-naïve	Single	Prognostication
Xing P	2019	China	Observational	Adenocarcinoma	Metastatic	36	Tumor-naïve	Multiple	Prognostication Monitoring
Yang W	2020	China	Observational	NSCLC	I	82	Tumor-naïve	Multiple	Monitoring
Yang X	2016	China	Observational	NSCLC	IV	73	Tumor-naïve	Multiple	Prognostication Monitoring
Yu HA	2020	USA	Clinical trial	NSCLC	Metastatic	49	Tumor-naïve	Multiple	Monitoring
Zhang C	2017	USA	Observational	NSCLC	Advanced	45	Tumor-naïve	Single	Monitoring
Zhang EW	2020	USA	Observational	NSCLC	Metastatic	75	Tumor-naïve	Single	Prognostication
Zhang H	2018	China	Observational	NSCLC	III–IV	160	Tumor-naïve	Single	Prognostication
Zhang S	2018	China	Observational	NSCLC	III–IV	307	Tumor-naïve	Single	Prognostication
Zheng D	2016	China	Observational	NSCLC	Advanced	117	Tumor-naïve	Single	Prognostication Monitoring
Zheng Q	2020	China	Observational	NSCLC	Advanced	54	Tumor-naïve	Single	Prognostication
Zhou Q	2016	China	Clinical trial	NSCLC	IIIB, IV	78	Tumor-naïve	Multiple	Prognostication
Zhou Y	2018	China	Observational	NSCLC	I–IV	212	Tumor-naïve	Single	Prognostication
Zhu Y-J	2017	China	Observational	NSCLC	III–IV	57	Tumor-naïve	Multiple	Prognostication Monitoring

LC: lung cancer; NSCLC: non–small cell lung cancer; SCLC: small cell lung cancer; UK: United Kingdom; US: United States.

[a]There were no standardized definitions for "early stage," "advanced stage," or "metastatic stage." Typically, early stage included stages I, IIA, IIB, and IIIA; advanced stage included locally advanced and stage IV; metastatic disease includes initial stage IV or metastatic disease with initial diagnosis of early stage.

[b]Sample size was the number of patients who underwent ctDNA testing, not overall size of the study patient population.

[c]Number of times within the study that ctDNA was sampled and tested.

[d]Not further defined.

Table 3. Characteristics of the 111 studies included in the review.

Characteristic	Studies, *n* (%)
Study characteristics	
Publication year	
2015	3 (2.7)
2016	15 (13.5)
2017	15 (13.5)
2018	18 (16.2)
2019	22 (19.8)
2020	38 (34.2)
Region	
Asia-Pacific	58 (52.3)
Europe	28 (25.2)
United States and Canada	22 (19.8)
Multiple countries	3 (2.7)
Study design	
Clinical trial	24 (21.6)
Observational study	87 (78.4)
Sample size[a]	
<50	44 (39.6)
50–100	33 (29.7)
≥101	34 (30.6)
Patient characteristics	
Tumor stage[b]	
Stage I	2 (1.8)
Stage I–III	5 (4.5)
Stage II/III	2 (1.8)
Stage III	1 (0.9)
Stage I–IV	20 (18.0)
Stage III/IV	29 (26.1)
Advanced	21 (18.9)
Stage IV or "metastatic"	31 (27.9)
Tumor subtype	
Any non–small cell lung cancer	88 (79.3)
Adenocarcinoma only	11 (9.9)
Squamous cell carcinoma only	1 (0.9)
Small cell lung cancer only	2 (1.8)
Any lung cancer (not defined)	9 (8.1)

ctDNA: circulating tumor DNA.
[a]Number of patients who underwent ctDNA testing, not the size of the study population.
[b]Categories represent the descriptions provided in the studies.

ctDNA for prognostication

Linking ctDNA to prognosis was the most common utility identified among the included studies. Most studies of ctDNA detection and lung cancer prognosis focused on PFS or OS, with worse survival outcomes for patients with detected ctDNA at all disease stages. The association between detection of ctDNA and tumor characteristics, such as stage and subtype, has not been extensively studied in patients with lung cancer. Results from studies reporting these associations have been mixed.

ctDNA positivity before treatment

Many studies focused on the implications of ctDNA positivity/detection before treatment (eg, surgery, chemotherapy, targeted therapy) in patients with all disease stages combined (10 studies, Supplementary Table 3) (29–38), nonmetastatic disease (stages I–III combined, 7 studies, Supplementary Table 4) (39–45), and advanced/metastatic disease (45 studies, Supplementary Table 5) (42,46–88). The association between detectable ctDNA before treatment and baseline tumor features (eg, tumor size, type of metastasis) was not consistently reported. Several studies reported higher ctDNA levels with advanced disease stage; this association was not consistently observed, perhaps owing in part to the inclusion of relatively few patients with early-stage disease (30–34,36,42,49). Similarly, several studies reported ctDNA positivity or a higher concentration of ctDNA associated with specific metastatic sites or the number of metastatic sites, but results were sparse and inconsistent (32,33,49–52,86,87).

Most studies reported shorter OS or PFS associated with ctDNA positivity and/or higher concentrations of ctDNA, regardless of lung cancer stage (Supplementary Tables 4 and 5) (39–88). Among studies that evaluated overall ctDNA positivity (not specific genetic mutations in ctDNA) before treatment, only 2 studies did not find an association between overall ctDNA positivity/detection and survival outcomes (44,71). Most of the studies that evaluated mutations in a single predefined mutated oncogene (eg, T790M resistance mutation in the *EGFR* gene, *KRAS*) did not find an association between pretreatment ctDNA and prognosis (34,46,54,59,63,67,68). One study found patients with a low pretreatment T790M/*EGFR* ratio had significantly less tumor shrinkage (47). Three of the studies that reported no association between detectable pretreatment ctDNA and subsequent outcomes were clinical trials (54,67,68). Four studies focused on the association between the methylation of specific genes (*KMT2C*, *SOX17*, *SHOX2*) and prognosis and reported improved survival outcomes with lower levels of methylation (30,35,42,45). Ten studies analyzed the association between pretreatment ctDNA and the overall response rate, disease control rate, or tumor response; however, the results were not consistent (38,46,48,60,70,75,77,79,84,85).

ctDNA during or after treatment

The prognostic value of ctDNA assessed cross-sectionally during treatment, after treatment, or

specifically at the time of radiographic progression was evaluated in patients with lung cancer in 24 studies (Supplementary Table 6) (37,44,45,70,81,86, 89–105). Two studies were conducted in patients with nonmetastatic lung cancer; in both studies, a higher risk of recurrence was observed for patients with detectable ctDNA after surgery or chemotherapy compared with patients with undetectable ctDNA after surgery/chemotherapy (44,105).

The remaining 22 studies included only patients with advanced/metastatic lung cancer. Most studies consistently reported an association between ctDNA positivity during/after treatment and reduced survival (PFS, OS, or relapse-free survival [RFS]) or treatment response. All 8 studies that assessed overall ctDNA positivity during/after treatment in patients with advanced/metastatic disease found worse outcomes for patients with detectable ctDNA.

Most studies (12/14, 86%) that focused on specific genes (*EGFR*, *BRAF*, *KRAS*, methylated *SHOX2*) in ctDNA assessed during/after treatment observed an association between the detection of variants in these genes and worse survival outcomes. One study of 20 patients with metastatic disease treated with second- or third-line EGFR-tyrosine kinase inhibitor (TKI)-targeted therapy found 50% of non-responders had *EGFR* detected in ctDNA after treatment compared with none with *EGFR* detected among the ongoing treatment responders (89). An additional study of 120 patients with advanced disease treated with a first- or second-generation TKI found that detection of the *EGFR* T790M resistance mutation during treatment was associated with progression at extra-thoracic metastatic sites ($p = 0.008$) and bone disease ($p = 0.003$) (91). However, 2 studies found no association between the detection of the T790M resistance mutation in *EGFR* and patient outcomes (93,97).

ctDNA assessed at nonuniform or unclear time points

Ten additional studies included patients with cross-sectional ctDNA collected at nonuniform or unclear time points across the treatment spectrum (Supplementary Table 7) (106–115). As with the cross-sectional studies noted above, a consistent association between ctDNA detection or higher levels of ctDNA was associated with worse survival outcomes. A study of 128 patients with stage I–IV NSCLC with *ALK* assessed before treatment with a TKI, or at the radiographic follow-up evaluation reported a shorter OS for those with $\geq$1 *ALK* mutations than for those with no ctDNA detected; OS and PFS were worse for those with $\geq$2 *ALK* mutations than for those with a single *ALK* mutation (111).

Association between ctDNA positivity at any time point and clinical outcomes in patients with advanced disease (meta-analysis)

To evaluate the association between ctDNA positivity at certain time points and clinical outcomes, we included studies that reported an HR and 95% CI for the association between ctDNA positivity and PFS and OS in a meta-analysis. Among the 8 estimates from 6 studies ($n = 622$) included, patients with lung cancer who were positive for ctDNA had a significantly shorter PFS than those with no ctDNA detected (HR, 2.34; 95% CI, 1.89–2.89; Figure 2(A)). A stronger association was observed for the 5 observational studies (HR, 2.99; 95% CI, 2.27–3.94) than for the 3 clinical trials (HR, 1.67; 95% CI, 1.21–2.31; Supplementary Table 8). Similar results were observed for 10 studies ($n = 743$) specifically reporting the detection of the *EGFR* alterations in ctDNA (HR, 2.19; 95% CI, 1.78–2.68; Figure 2(B)) and among 4 studies ($n = 337$) evaluating the *EGFR* T790M resistance mutation in ctDNA (HR, 2.55; 95% CI, 1.67–3.90; Supplementary Figure 1). However, a stronger association between *EGFR* and PFS was observed for the studies assessing the detection of *EGFR* during treatment (HR, 4.29; 95% CI, 2.77–6.67) than for the studies that assessed *EGFR* before treatment (HR, 1.82; 95% CI, 1.37–3.29; Supplementary Table 8). No significant evidence of publication bias was found in these analyses ($p > 0.05$; Supplementary Figure 2). Subgroup analyses were performed by study design, ctDNA test timing, lung cancer subtype, and disease stage and are summarized in Supplementary Table 8; results were similar to overall positivity results for both ctDNA overall and *EGFR* mutations, with positivity associated with shorter PFS and OS.

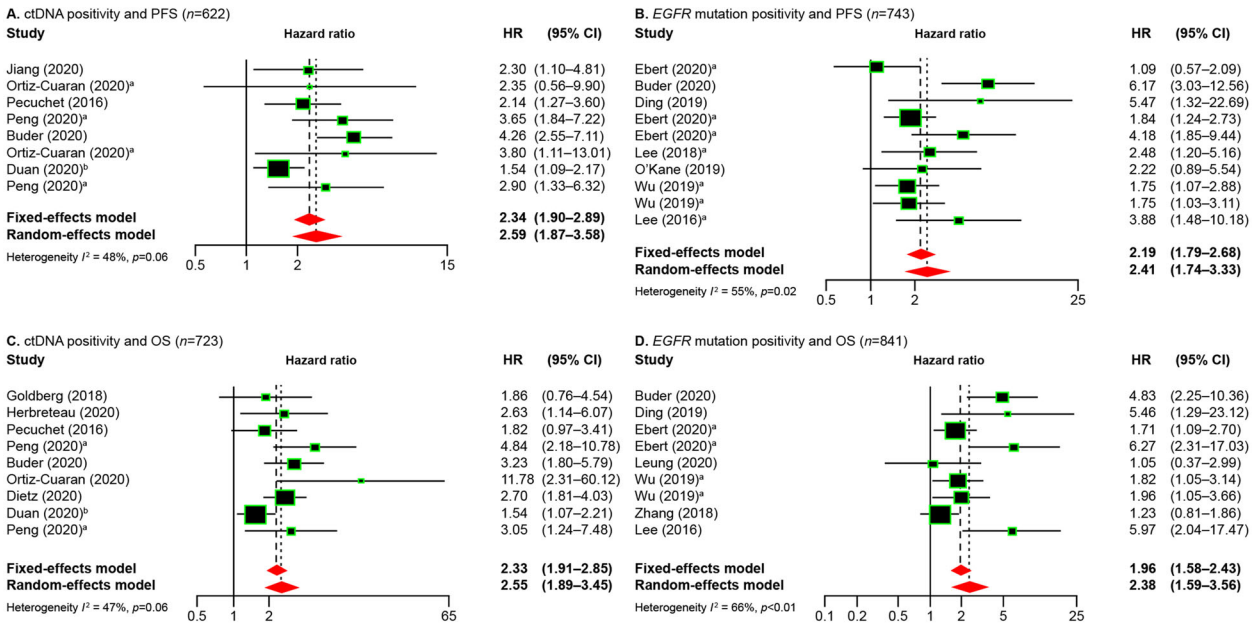

Figure 2. Meta-analysis of ctDNA positivity, PFS, and OS. (A) Meta-analysis of ctDNA positivity and PFS ($n = 622$). (B) Meta-analysis of *EGFR* mutation positivity and PFS ($n = 743$). (C) Meta-analysis of ctDNA positivity and OS ($n = 723$). (D) Meta-analysis of *EGFR* mutation positivity and OS ($n = 841$). [a]If a study measured the cross-sectional association at different time points, the HR at each time point was assumed independently and entered the meta-analysis separately, which is reflected by multiple records from 1 study in the forest plots. The assumption was assessed and supported by sensitivity analysis where 1 study only contributed 1 record in meta-analysis. [b]HR was adjusted for baseline status. ctDNA: circulating tumor DNA; HR: hazard ratio; OS: overall survival; PFS: progression-free survival.

Among 9 estimates from 8 studies ($n = 723$) with data available for ctDNA positivity and OS, patients with positive ctDNA had a significantly shorter OS than those with no detected ctDNA (HR, 2.33; 95% CI, 1.91–2.85; Figure 2(C)). A stronger association was observed for the 6 observational studies (HR, 2.72; 95% CI, 2.12–3.50) than for the 3 clinical trials (HR, 1.81; 95% CI, 1.31–2.50; Supplementary Table 8). No differences were observed for ctDNA timing (before vs. during treatment) or for analyses limited to patients with advanced/metastatic disease or those with the NSCLC subtype. Similar results were observed for 9 studies ($n = 841$) specifically reporting circulating mutations in *EGFR* (HR, 1.96; 95% CI, 1.58–2.43; Figure 2(D)). Stratified analyses showed few significant differences by study design (Supplementary Table 3). A stronger association between *EGFR* positivity and shorter OS was observed for *EGFR*-mutation detection during treatment (HR, 5.34; 95% CI, 3.05–9.34) than for detection before treatment (HR, 1.64; 95% CI, 1.30–2.07; Supplementary Table 8). However, the small number of studies within each stratum precludes drawing strong conclusions. The funnel plot for the *EGFR* positivity analysis shows asymmetry, which may indicate the presence of publication bias ($p = 0.0311$); little evidence of publication bias was observed in the other OS analyses.

Longitudinal ctDNA for monitoring disease progression or treatment response

Longitudinal ctDNA assessment was used in evaluating ctDNA dynamics during treatment, evaluating prognosis, determining lead time compared with radiographic progression, and assessing changes to the genomic profile of tumors.

ctDNA dynamics

In 8 studies, ctDNA detection was assessed for changes at $\geq$ 2-time points during the treatment period without evaluating the associations with clinical outcomes (Supplemental Table 9) (51,58,65,116–120). These studies described ctDNA changes in a variety of ways, which makes it challenging to compare results. In

general, these studies show a decrease in ctDNA positivity during or after treatment compared with before treatment, or a higher proportion of patients with detectable ctDNA in those with progressive disease (PD) than in those with stable disease (SD). For example, among 20 patients with stage IV NSCLC who received either a TKI or chemotherapy, 65% had detectable ctDNA before treatment, which subsequently decreased for patients with SD (35%) and increased for those with PD (80%) (117). Another study of 41 patients with stage I–IV NSCLC reported 8.9% of patients with ctDNA positivity before surgery, which decreased to 0.28% after surgery (116).

ctDNA for monitoring tumor progression

The association between ctDNA collected at multiple time points in relation to lung cancer outcomes was evaluated in 7 studies of patients with nonmetastatic lung cancer or for all disease stages combined, and in 30 studies of patients with advanced/metastatic disease. In each of the 7 studies evaluating the association between ctDNA monitoring and clinical outcomes in patients with the nonmetastatic disease (or all stages combined), more favorable survival (longer PFS, OS, or RFS) was observed for patients with ctDNA clearance, ctDNA decreases, or no detectable ctDNA observed across the treatment and/or follow-up periods (Supplementary Table 10) (26,43,45,105,121–123). Each study evaluated multigene ctDNA using a next-generation sequencing (NGS) platform; no studies focused on specific genes such as *EGFR*.

ctDNA monitoring studies conducted in patients with advanced/metastatic lung cancer consistently reported improved survival outcomes for patients with ctDNA clearance, ctDNA decreases, and those who remained with undetectable ctDNA across the monitoring period (Supplementary Table 11) (46,48,50,54,56,58,66–71,73,77,79,81,87,95,101,106,124–133). Among these studies, 18 evaluated the change/clearance of a specific gene in ctDNA, *EGFR* or *EGFR* T790M, and the remaining 12 studies evaluated overall ctDNA clearance/decreases without a specific gene focus. Several studies ($n = 8$) evaluated the association between ctDNA changes and radiological response; in each study, ctDNA clearance/decrease/absence was associated with improved tumor response outcomes (48,70,71,79,81,126,128,129).

Association between ctDNA clearance/decrease and clinical outcomes (meta-analysis)

For the meta-analysis of longitudinal ctDNA, we included studies that reported an HR and 95% CI for the association between ctDNA clearance/decrease during treatment and PFS or OS. The change in ctDNA was defined in a variety of ways, including any decrease (67,95) or >50% decrease (71) in ctDNA concentration from before to after treatment; above vs. below median change in detected ctDNA quantity from baseline and at follow-up during treatment (68); increased ctDNA or no change vs. complete clearance in ctDNA (45,46,132); and ctDNA disappearance at 4 weeks (126), 8 weeks (56), or the start of the third therapy cycle (129) during systemic treatment. ctDNA clearance/decrease was associated with a lower risk of progression in 8 studies (Figure 3(A); HR, 0.24; 95% CI, 0.19–0.31) and a lower risk of death in 8 studies (Figure 3(B); HR, 0.40; 95% CI, 0.27–0.60). However, there was evidence of publication bias ($p = 0.0005$) based on the funnel plot analysis for the OS analysis.

Subgroup analyses of ctDNA clearance/change and PFS did not show differences when limited to observational studies or advanced/metastatic disease (Supplementary Table 12). For the OS analysis, the association between improved survival outcomes and ctDNA clearance/change was stronger in observational studies (HR, 0.21; 95% CI, 0.13–0.36) than in clinical trials (HR, 0.62; 95% CI, 0.49–0.78; Supplementary Table 12). There was no difference in the OS analysis when limiting the analysis to studies of patients with advanced/metastatic lung cancer.

Lead time of ctDNA-detected progression compared with radiographic progression

The lead time or concordance for detection of ctDNA progression compared with radiographic progression after treatment was reported in 12 studies; 5 studies focused specifically on circulating *EGFR* and/or *EGFR* T790M, with the remaining 7 studies evaluating the detection ctDNA (Supplementary

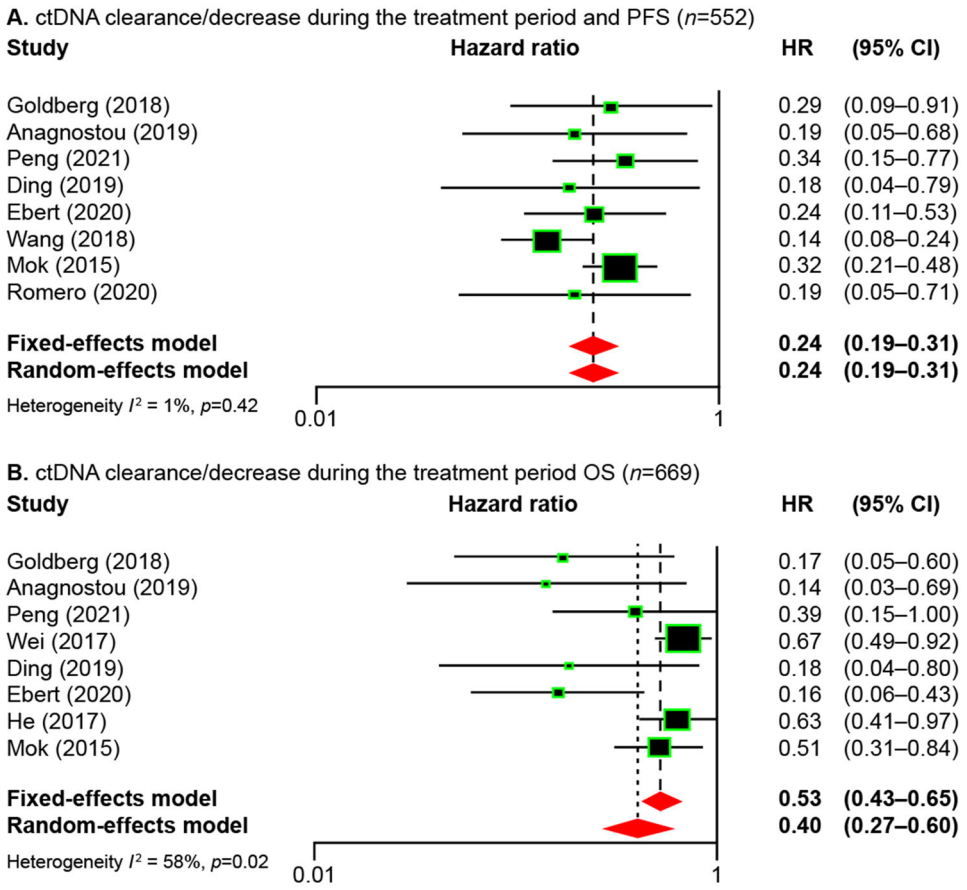

Figure 3. Meta-analysis of ctDNA clearance/decrease during the treatment period and clinical outcomes. (A) Meta-analysis of ctDNA clearance/decrease during the treatment period and PFS ($n = 552$). (B) Meta-analysis of ctDNA clearance/decrease during the treatment period and OS ($n = 669$). ctDNA: circulating tumor DNA; HR: hazard ratio; OS: overall survival; PFS: progression-free survival.

Table 13) (26,31,43,45,54,65,71,86,99,124,127,132). Most studies (8/12; 67%) were conducted in patients with advanced/metastatic disease. The average lead time varied widely between studies (17 days to 12.6 months) in both patients with early-stage and advanced/metastatic disease. The variation may reflect differences in frequency or timing (eg, weekly, monthly, pre-/post-treatment only), ctDNA assessments (overall positivity or specific alterations), radiographic imaging (eg, every 2 months, every 5–10 weeks), and the study populations (eg, treatment type, early stage vs. metastatic), making it difficult to compare results across studies. However, each of these studies demonstrated that ctDNA may be a useful tool for detecting disease progression earlier than standard radiographic methods.

Assessing tumor evolution and resistance by changes in ctDNA genomic profile

Clonal evolution, or the acquisition of new mutations in response to selective pressures, is increasingly recognized to explain tumor heterogeneity and therapeutic resistance (134). Longitudinal profiling of ctDNA in serial monitoring provides an attractive, cost-effective, non-invasive monitoring technique to describe tumor genomic clonal evolution in response to treatment in a real-time manner, and consequently contribute to understanding the mechanisms of acquired drug resistance. Several studies have evaluated the prognostic effect of newly acquired mutations during TKI treatment, with *EGFR* T790M most frequently evaluated. Additional studies evaluated small samples of patients during treatment for several different ctDNA-based genetic changes. These ctDNA-based genetic changes included the acquisition of new mutations during TKI treatment (122,135) or chemotherapy (36), increases in gene copy numbers or losses of tumor suppressor genes during ICI therapy (136), and the frequency of C > G and C > A substitutions during chemotherapy (77).

In this review, 1 clinical study examined genetic evolution with systemic approaches (eg, evolutionary trees). In the TRACERx study, Abbosh and colleagues detected subclonal single-nucleotide variants in early-stage NSCLC and mapped them back to multiregion exome sequencing (M-Seq)–derived tumor phylogenetic trees (137). A limited number of additional studies described changes in the detection of specific genes in ctDNA, which may indicate genomic evolution in response to treatment. For example, a study of 43 patients with advanced NSCLC evaluated the copy number gains in specific genes (eg, *MET*, *EGFR*) from before treatment with third-generation EGFR-TKI, rociletinib, to the time of PD; patients with shorter PFS were more likely to have copy number gains (47).

Discussion

In this systematic review, we summarized the data on ctDNA clinical utility in lung cancer, focusing on 3 key areas: diagnosis, prognostication, and monitoring. Among these, prognostication was by far the most studied utility for ctDNA in lung cancer. In meta-analyses, both overall ctDNA positivity and the presence of *EGFR* mutations within ctDNA were associated with worse PFS and OS outcomes. The second most common ctDNA utility in lung cancer was disease monitoring. The association between ctDNA dynamics and clinical outcomes was evaluated by a meta-analysis, in which ctDNA clearance/decrease was associated with a lower risk of progression and death. In terms of diagnosis or other applications within ctDNA monitoring, such as detecting residual disease or disease relapse, assessment of treatment efficacy, or identification of treatment resistance mechanisms, the data in the literature are limited and/or mixed. As such, although these results are promising, the existing data are insufficient to formulate definitive conclusions or recommendations for use, and additional research is needed. The least studied utility was diagnosis (3 of 111 studies), and many key issues need be addressed before ctDNA can be applied for cancer screening and early detection.

Despite the consistent association between ctDNA and prognosis in patients with lung cancer, several important limitations need to be addressed in future studies. A critical limitation is the small sample size in most studies, with almost 70% of studies having ctDNA data for fewer than 100 patients. Consistent with this, many studies were not statistically powered to detect associations between ctDNA results and outcomes in subgroups where patients were stratified by factors with the potential to contribute to heterogeneity (eg, lung cancer subtype or stage). Further, the small sample sizes also limited the independent prognostic value of the results because it was not possible to estimate and remove potential confounding variables (eg, stage). Sample sizes were especially small in studies measuring ctDNA dynamics longitudinally for monitoring disease progression and/or treatment response. For studies with few patients (ie, <20) with ctDNA encompassing hundreds of cancer-related or other genes measured at 2 or more time points, it is challenging to determine the true association between ctDNA changes and prognosis. Moreover, most of the identified studies simply estimated the association of ctDNA with clinical outcomes at multiple time points individually or ctDNA change over 2 time points. Consequently, they cannot address important questions, such as quantitatively characterizing ctDNA dynamics throughout the disease course or identifying the optimal timing and frequency of blood sampling for the intended purpose of the study and whether any adjustments need to be made depending on cancer subtype.

Most of the studies evaluated were conducted in patients with advanced/metastatic NSCLC. There were limited prognostication data in patients with early-stage lung cancer or other histological subtypes. Because only 2 of the included studies focused exclusively on patients with SCLC, the utility of ctDNA for predicting prognosis in patients with this aggressive lung cancer is less clear (36,84). Besides prognostication, 3 studies used ctDNA for the detection of molecular residual disease (26,43,137). These studies suggest that ctDNA analysis, particularly using highly sensitive tumor-informed assays, can identify recurrence earlier than radiographic

imaging. If confirmed, ctDNA for molecular detection has the potential to inform and improve patient identification and stratification for treatment selection. A more comprehensive review covering topics beyond our study, such as technical considerations and major barriers for use in clinical practice, on ctDNA molecular residual disease detection in patients with early-stage NSCLC, was published recently (138).

Several limitations should be considered when interpreting the findings of this systematic literature review. First was the heterogeneity across studies. Within each of the 3 clinical applications or articles that addressed similar research questions, studies vary significantly by study design, lung cancer histological type, stage, tumor burden, location, treatment, the timing of ctDNA testing, features of ctDNA assays, and in the pre-analytic or analytic parameters of the ctDNA analysis. For example, in studies that assessed a single gene mutation, some studies estimated the mutation status using digital PCR assays (24), whereas others used NGS (139). In addition, the timing of ctDNA sampling varied, with some studies testing for *EGFR* mutations before (45,51) or during treatment (66,90,126). The differences across studies reflect the complexity of ctDNA research but also highlight the importance of standardizing guidelines when ctDNA becomes part of standard clinical practice. In meta-analyses, when the overall prognostication associations with PFS and OS were estimated, we did not detect heterogeneity by stage, histological type, or ctDNA measurement timing. However, this result may be due to sparse data in certain subgroups and the underpowered analyses. Moreover, there might be other factors that were not evaluated but that also contributed to the associated heterogeneity. Another limitation of the embedded meta-analyses was that not all prognostic studies reported HRs. Of the 85 studies on prognostication, only 26 studies qualified for meta-analyses. Many studies, particularly those with small sample sizes, simply reported the median survival time for patients with detected vs. those with no detected ctDNA alterations. The lack of HR data in these studies may have biased the pooled HRs.

Our study covered a wide range of ctDNA clinical utility and synthesized the data largely based on the availability of published studies. Therefore, some interesting topics were not reviewed in detail. For example, as an actionable alteration in advanced NSCLC, ctDNA *EGFR* mutations have been extensively studied and reviewed previously (140–142). However, this review focused on the association of ctDNA *EGFR* mutations, at both a single time point or longitudinally with clinical outcomes. Beyond that, several studies also demonstrated the value of *EGFR* or *EGFR* T790M mutation in monitoring EGFR-TKI treatment and treatment resistance (122,135). However, because data were usually presented descriptively or on a patient-by-patient basis, with large interstudy differences, we did not summarize these findings, despite their clinical relevance.

Although ctDNA testing has been increasingly used and studied in recent years, ctDNA is unlikely to replace tumor tissue DNA testing for lung cancer screening and diagnosis or for the detection of disease progression in the short-term. However, ctDNA will play an important role in lung cancer as oncology advances precision medicine. This systematic review of 111 studies with embedded meta-analyses demonstrated that ctDNA is a sensitive biomarker, reflecting tumor burden and dynamics, that also has prognostic value. These factors make ctDNA a promising tool for predicting clinical outcomes and tracking treatment responses. Large-scale, prospective clinical trials are needed to further validate these findings. Future studies will evaluate the potential for improved patient outcomes or cost savings of ctDNA applications compared with standard clinical approaches to assess treatment response, detect treatment resistance, individualize therapy, and predict outcomes.

Acknowledgments

Medical writing and editorial assistance, funded by GSK (Waltham, Massachusetts) and coordinated by Hasan Jamal, MSc, of GSK, were provided by Betsy C. Taylor, PhD, CMPP, and Jennifer Robertson, PhD, of Ashfield MedComms, an Inizio company.

Disclosure statement

SX, NB, LK, PK, KB, A Slowley, MZ, EH, A Silvestro, and AP are current employees of GSK.

A Stojadinovic and ZW were employees of GSK at the time the analysis was conducted.

PA reports consulting services to GSK and Merck Sharp & Dohme Corp.

SP reports institutional financial support for clinical trials from Amgen, AstraZeneca, Biodesix, Boehringer Ingelheim, Bristol Myers Squibb, Clovis, GSK, Illumina, Lilly, Merck Serono, Merck Sharp & Dohme, Mirati, Novartis, Pfizer, Phosplatin Therapeutics, and Roche/Genentech; and consultation/advisory role for AbbVie, Amgen, AstraZeneca, Bayer, Beigene, Biocartis, Boehringer Ingelheim, Bristol Myers Squibb, Clovis, Daiichi Sankyo, Debiopharm, ecancer, Eli Lilly, Elsevier, Fishawack, Foundation Medicine, Illumina, Imedex, Incyte, IQVIA, Janssen, Medscape, Merck Sharp & Dohme, Merck Serono, Merrimack, Novartis, Oncology Education, PharmaMar, Phosplatin Therapeutics, PER, Pfizer, PRIME, Regeneron, RMEI, Roche/Genentech, RTP, Sanofi, Seattle Genetics, and Takeda; and institutional fees for talks in a company's organized public events for AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, ecancer, Eli Lilly, Illumina, Imedex, Medscape, Merck Sharp & Dohme, Novartis, PER, Pfizer, Prime, Roche/Genentech, RTP, Sanofi, and Takeda.

Funding

This study (OneCDP 218463 and 214962) was supported by GSK; GSK participated in the study design; conducting the study; and data collection, management, and interpretation.

ORCID

Xuezheng Sun http://orcid.org/0000-0003-1804-4215

Data availability statement

As a systematic literature review and meta-analysis, this manuscript contains no original data.

References

1. Husain H, Velculescu VE. Cancer DNA in the circulation: the liquid biopsy. JAMA. 2017;318(13):1272–1274. doi:10.1001/jama.2017.12131.
2. 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 Rev Cancer. 2017;17(4):223–238. doi:10.1038/nrc.2017.7.
3. U.S. Department of Health and Human Services Food and Drug Administration. Draft guidance for industry: use of circulating tumor DNA for early-stage solid tumor drug development. Rockville (MD): Food and Drug Administration; 2022.
4. Pascual J, Attard G, Bidard F-C, Curigliano G, De Mattos-Arruda L, Diehn M, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO precision medicine working group. Ann Oncol. 2022;33(8):750–768. doi:10.1016/j.annonc.2022.05.520.
5. Friends of Cancer Research. Assessing the use of ctDNA as an early endpoint in early-stage disease (white paper). Friends of Cancer Research Annual Meeting. 2021 [cited 2021 Mar 14]. Available from: https://friendsofcancerresearch.org/wp-content/uploads/Assessing_Use_of_ctDNA_Early_Endpoint_Early-Stage_Disease-1.pdf.
6. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer. 2020;1(3):276–290. doi:10.1038/s43018-020-0043-5.
7. Dasari A, Morris VK, Allegra CJ, Atreya C, Benson AB, Boland P, et al. ctDNA applications and integration in colorectal cancer: an NCI Colon and rectal-anal task forces whitepaper. Nat Rev Clin Oncol. 2020;17(12):757–770. doi:10.1038/s41571-020-0392-0.
8. De Rubis G, Rajeev Krishnan S, Bebawy M. Liquid biopsies in cancer diagnosis, monitoring, and prognosis. Trends Pharmacol Sci. 2019;40(3):172–186. doi:10.1016/j.tips.2019.01.006.
9. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. Arch Pathol Lab Med. 2018;142(10):1242–1253. doi:10.5858/arpa.2018-0901-SA.
10. Yang S, Zhang Z, Wang Q. Emerging therapies for small cell lung cancer. J Hematol Oncol. 2019;12(1):47. doi:10.1186/s13045-019-0736-3.
11. Majeed U, Manochakian R, Zhao Y, Lou Y. Targeted therapy in advanced non-small cell lung cancer: current advances and future trends. J Hematol Oncol. 2021;14(1):108. doi:10.1186/s13045-021-01121-2.
12. Jones GS, Baldwin DR. Recent advances in the management of lung cancer. Clin Med . 2018;18(Suppl 2):s41–s46. doi:10.7861/clinmedicine.18-2-s41.
13. Esagian SM, Grigoriadou GI, Nikas IP, Boikou V, Sadow PM, Won J-K, et al. Comparison of liquid-based to tissue-based biopsy analysis by targeted next generation sequencing in advanced non-small cell lung cancer: a comprehensive systematic review. J Cancer Res Clin Oncol. 2020;146(8):2051–2066. doi:10.1007/s00432-020-03267-x.
14. Passiglia F, Rizzo S, Di Maio M, Galvano A, Badalamenti G, Listì A, et al. The diagnostic accuracy of circulating tumor DNA for the detection of EGFR-T790M mutation in NSCLC: a systematic

review and meta-analysis. Sci Rep. 2018;8(1):13379. doi:10.1038/s41598-018-30780-4.
15. Sebastião MM, Ho RS, de Carvalho JPV, Nussbaum M. Diagnostic accuracy of next generation sequencing panel using circulating tumor DNA in patients with advanced non-small cell lung cancer: a systematic review and meta-analysis. J Health Econ Outcomes Res. 2020;7(2):158–163. doi:10.36469/jheor.2020.17088.
16. Zhou S, Huang R, Cao Y. Detection of epidermal growth factor receptor mutations in peripheral blood circulating tumor DNA in patients with advanced non-small cell lung cancer: A PRISMA-compliant meta-analysis and systematic review. Medicine (Baltimore)). 2020;99(40):e21965. doi:10.1097/MD.0000000000021965.
17. Wang H, Zhou F, Qiao M, Li X, Zhao C, Cheng L, et al. The role of circulating tumor DNA in advanced non-small cell lung cancer patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis. Front Oncol. 2021;11:671874. doi:10.3389/fonc.2021.671874.
18. Pizzutilo EG, Pedrani M, Amatu A, Ruggieri L, Lauricella C, Veronese SM, et al. Liquid biopsy for small cell lung cancer either de novo or transformed: systematic review of different applications and meta-analysis. Cancers. 2021;13(9):2265. doi:10.3390/cancers13092265.
19. Narayan P, Ghosh S, Philip R, Barrett JC, McCormack RT, Odegaard JI, et al. State of the science and future directions for liquid biopsies in drug development. Oncologist. 2020;25(9):730–732. doi:10.1634/theoncologist.2020-0246.
20. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods. 2010;1(2):97–111. doi:10.1002/jrsm.12.
21. Jansen JP, Cope S. Meta-regression models to address heterogeneity and inconsistency in network meta-analysis of survival outcomes. BMC Med Res Methodol. 2012;12:152. doi:10.1186/1471-2288-12-152.
22. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–1558. doi:10.1002/sim.1186.
23. Boonstra PA, Wind TT, van Kruchten M, Schuuring E, Hospers GAP, van der Wekken AJ, et al. Clinical utility of circulating tumor DNA as a response and follow-up marker in cancer therapy. Cancer Metastasis Rev. 2020;39(3):999–1013. doi:10.1007/s10555-020-09876-9.
24. Carvalho Â, Ferreira G, Seixas D, Guimarães-Teixeira C, Henrique R, Monteiro FJ, et al. Emerging lab-on-a-chip approaches for liquid biopsy in lung cancer: status in CTCs and ctDNA research and clinical validation. Cancers. 2021;13(9):2101. doi:10.3390/cancers13092101.
25. Freitas C, Sousa C, Machado F, Serino M, Santos V, Cruz-Martins N, et al. The role of liquid biopsy in early diagnosis of lung cancer. Front Oncol. 2021;11:634316. doi:10.3389/fonc.2021.634316.
26. 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 Discov. 2017;7(12):1394–1403. doi:10.1158/2159-8290.CD-17-0716.
27. Chen C, Huang X, Yin W, Peng M, Wu F, Wu X, et al. Ultrasensitive DNA hypermethylation detection using plasma for early detection of NSCLC: a study in Chinese patients with very small nodules. Clin Epigenetics. 2020;12(1):39. doi:10.1186/s13148-020-00828-2.
28. Moon SM, Kim J-H, Kim SK, Kim S, Kwon H-J, Bae J-S, et al. Clinical utility of combined circulating tumor cell and circulating tumor DNA assays for diagnosis of primary lung cancer. Anticancer Res. 2020;40(6):3435–3444. doi:10.21873/anticanres.14329.
29. Alegre E, Fusco JP, Restituto P, Salas-Benito D, Rodríguez-Ruiz ME, Andueza MP, et al. Total and mutated EGFR quantification in cell-free DNA from non-small cell lung cancer patients detects tumor heterogeneity and presents prognostic value. Tumour Biol. 2016;37(10):13687–13694. doi:10.1007/s13277-016-5282-9.
30. Balgkouranidou I, Chimonidou M, Milaki G, et al. SOX17 promoter methylation in plasma circulating tumor DNA of patients with non-small cell lung cancer. Clin Chem Lab Med. 2016;54(8):1385–1393.
31. Chen K, Zhao H, Shi Y, Yang F, Wang LT, Kang G, et al. Perioperative dynamic changes in circulating tumor DNA in patients with lung cancer (DYNAMIC). Clin Cancer Res. 2019;25(23):7058–7067. doi:10.1158/1078-0432.CCR-19-1213.
32. Cho MS, Park CH, Lee S, Park HS. Clinicopathological parameters for circulating tumor DNA shedding in surgically resected non-small cell lung cancer with EGFR or KRAS mutation. PLoS One. 2020;15(3):e0230622. doi:10.1371/journal.pone.0230622.
33. Kim T, Kim EY, Lee SH, Kwon DS, Kim A, Chang YS. Presence of mEGFR ctDNA predicts a poor clinical outcome in lung adenocarcinoma. Thorac Cancer. 2019;10(12):2267–2273. doi:10.1111/1759-7714.13219.
34. Leung M, Freidin MB, Freydina DV, Von Crease C, De Sousa P, Barbosa MT, et al. Blood-based circulating tumor DNA mutations as a diagnostic and prognostic biomarker for lung cancer. Cancer. 2020;126(8):1804–1809. doi:10.1002/cncr.32699.
35. Liu Y, Feng Y, Hou T, Lizaso A, Xu F, Xing P, et al. Investigation on the potential of circulating tumor DNA methylation patterns as prognostic biomarkers for lung squamous cell carcinoma. Transl Lung

Cancer Res. 2020;9(6):2356–2366. doi:10.21037/tlcr-20-1070.
36. Nong J, Gong Y, Guan Y, Yi X, Yi Y, Chang L, et al. Circulating tumor DNA analysis depicts subclonal architecture and genomic evolution of small cell lung cancer. Nat Commun. 2018;9(1):3114. doi:10.1038/s41467-018-05327-w.
37. Peng X, Liu X, Xu L, Li Y, Wang H, Song L, et al. The mSHOX2 is capable of assessing the therapeutic effect and predicting the prognosis of stage IV lung cancer. J Thorac Dis. 2019;11(6):2458–2469. doi:10.21037/jtd.2019.05.81.
38. Wang Y, Xie S, He B. Effect of EGFR gene polymorphism on efficacy of chemotherapy combined with targeted therapy for non-small cell lung cancer in Chinese patients. Am J Cancer Res. 2019;9(3):619–627.
39. Chia BSH, Nei WL, Charumathi S, Fong KW, Tan MH. Baseline plasma EGFR circulating tumour DNA levels in a pilot cohort of EGFR-mutant limited-stage lung adenocarcinoma patients undergoing radical lung radiotherapy. Case Rep Oncol. 2020;13(2):896–903. doi:10.1159/000508932.
40. Corradetti MN, Torok JA, Hatch AJ, Xanthopoulos EP, Lafata K, Jacobs C, et al. Dynamic changes in circulating tumor DNA during chemoradiation for locally advanced lung cancer. Adv Radiat Oncol. 2019;4(4):748–752. doi:10.1016/j.adro.2019.05.004.
41. Isaksson S, George AM, Jönsson M, Cirenajwis H, Jönsson P, Bendahl P-O, et al. Pre-operative plasma cell-free circulating tumor DNA and serum protein tumor markers as predictors of lung adenocarcinoma recurrence. Acta Oncol. 2019;58(8):1079–1086. doi:10.1080/0284186X.2019.1610573.
42. Mastoraki S, Balgkouranidou I, Tsaroucha E, Klinakis A, Georgoulias V, Lianidou E. KMT2C promoter methylation in plasma-circulating tumor DNA is a prognostic biomarker in non-small cell lung cancer. Mol Oncol. 2021;15(9):2412–2422. doi:10.1002/1878-0261.12848.
43. Moding EJ, Liu Y, Nabet BY, Chabon JJ, Chaudhuri AA, Hui AB, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. Nat Cancer. 2020;1(2):176–183. doi:10.1038/s43018-019-0011-0.
44. Ohara S, Suda K, Sakai K, Nishino M, Chiba M, Shimoji M, et al. Prognostic implications of preoperative versus postoperative circulating tumor DNA in surgically resected lung cancer patients: a pilot study. Transl Lung Cancer Res. 2020;9(5):1915–1923. doi:10.21037/tlcr-20-505.
45. Peng M, Huang Q, Yin W, Tan S, Chen C, Liu W, et al. Circulating tumor DNA as a prognostic biomarker in localized non-small cell lung cancer. Front Oncol. 2020;10:561598. doi:10.3389/fonc.2020.561598.
46. Ebert EBF, McCulloch T, Holmskov Hansen K, Linnet H, Sorensen B, Meldgaard P. Clearing of circulating tumour DNA predicts clinical response to osimertinib in EGFR mutated lung cancer patients. Lung Cancer. 2020;143:67–72. doi:10.1016/j.lungcan.2020.03.020.
47. Chabon JJ, Simmons AD, Lovejoy AF, Esfahani MS, Newman AM, Haringsma HJ, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun. 2016;7:11815. doi:10.1038/ncomms11815.
48. Ebert EBF, McCulloch T, Hansen KH, Linnet H, Sorensen B, Meldgaard P. Clearing of circulating tumour DNA predicts clinical response to first line tyrosine kinase inhibitors in advanced epidermal growth factor receptor mutated non-small cell lung cancer. Lung Cancer. 2020;141:37–43. doi:10.1016/j.lungcan.2019.12.016.
49. Imamura F, Uchida J, Kukita Y, Kumagai T, Nishino K, Inoue T, et al. Monitoring of treatment responses and clonal evolution of tumor cells by circulating tumor DNA of heterogeneous mutant EGFR genes in lung cancer. Lung Cancer. 2016;94:68–73. doi:10.1016/j.lungcan.2016.01.023.
50. Jia J, Huang B, Zhuang Z, Chen S. Circulating tumor DNA as prognostic markers for late stage NSCLC with bone metastasis. Int J Biol Markers. 2018;33(2):222–230. doi:10.1177/1724600817753576.
51. Lee Y, Park S, Kim WS, Lee JC, Jang SJ, Choi J, et al. Correlation between progression-free survival, tumor burden, and circulating tumor DNA in the initial diagnosis of advanced-stage EGFR-mutated non-small cell lung cancer. Thorac Cancer. 2018;9(9):1104–1110. doi:10.1111/1759-7714.12793.
52. Park CK, Cho HJ, Choi YD, Oh IJ, Kim YC. A phase II trial of osimertinib in the second-line treatment of non-small cell lung cancer with the EGFR T790M mutation, detected from circulating tumor DNA: LiquidLung-O-Cohort 2. Cancer Res Treat. 2019;51(2):777–787. doi:10.4143/crt.2018.387.
53. Remon J, Caramella C, Jovelet C, Lacroix L, Lawson A, Smalley S, et al. Osimertinib benefit in EGFR-mutant NSCLC patients with T790M-mutation detected by circulating tumour DNA. Ann Oncol. 2017;28(4):784–790. doi:10.1093/annonc/mdx017.
54. Tsui DWY, Murtaza M, Wong ASC, Rueda OM, Smith CG, Chandrananda D, et al. Dynamics of multiple resistance mechanisms in plasma DNA during EGFR-targeted therapies in non-small cell lung cancer. EMBO Mol Med. 2018;10(6):e7945. doi:10.15252/emmm.201707945.
55. Wang J, Bai H, Hong C, Wang J, Mei TH. Analyzing epidermal growth factor receptor mutation status changes in advanced non-small-cell lung cancer at different sampling time-points of blood

within one day. Thorac Cancer. 2017;8(4):312–319. doi:10.1111/1759-7714.12443.
56. Wang Z, Cheng Y, An T, Gao H, Wang K, Zhou Q, et al. Detection of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial. Lancet Respir Med. 2018;6(9):681–690. doi:10.1016/S2213-2600(18)30264-9.
57. Wu Y-L, Zhou C, Lu S, Qin S, Pan H, Wu G, et al. Erlotinib versus gemcitabine/cisplatin in Chinese patients with EGFR mutation-positive advanced non-small-cell lung cancer: crossover extension and post-hoc analysis of the ENSURE study. Lung Cancer. 2019;130:18–24. doi:10.1016/j.lungcan.2019.01.016.
58. Xing P, Han X, Wang S, Liu Y, Yang S, Hao X, et al. Co-mutational assessment of circulating tumour DNA (ctDNA) during osimertinib treatment for T790M mutant lung cancer. J Cell Mol Med. 2019;23(10):6812–6821. doi:10.1111/jcmm.14565.
59. Zhang H, He B, Cui J, Zhao M, Zhang Z. Comparison of circulating DNA from plasma and urine for EGFR mutations in NSCLC patients. Cancer Biomark. 2018;23(3):427–436. doi:10.3233/CBM-181511.
60. Zheng Q, Hong S, Huang Y, et al. EGFR T790M relative mutation purity predicts osimertinib treatment efficacy in non-small cell lung cancer patients. Clin Transl Med. 2020;9(1):17.
61. Guibert N, Pradines A, Farella M, Casanova A, Gouin S, Keller L, et al. Monitoring KRAS mutations in circulating DNA and tumor cells using digital droplet PCR during treatment of KRAS-mutated lung adenocarcinoma. Lung Cancer. 2016;100:1–4. doi:10.1016/j.lungcan.2016.07.021.
62. Han JY, Choi JJ, Kim JY, Han YL, Lee GK. PNA clamping-assisted fluorescence melting curve analysis for detecting EGFR and KRAS mutations in the circulating tumor DNA of patients with advanced non-small cell lung cancer. BMC Cancer. 2016;16:627. doi:10.1186/s12885-016-2678-2.
63. Iwama E, Sakai K, Azuma K, Harada D, Nosaki K, Hotta K, et al. Exploration of resistance mechanisms for epidermal growth factor receptor-tyrosine kinase inhibitors based on plasma analysis by digital polymerase chain reaction and next-generation sequencing. Cancer Sci. 2018;109(12):3921–3933. doi:10.1111/cas.13820.
64. Piotrowska Z, Niederst MJ, Karlovich CA, Wakelee HA, Neal JW, Mino-Kenudson M, et al. Heterogeneity underlies the emergence of EGFRT790 wild-type clones following treatment of T790M-positive cancers with a third-generation EGFR inhibitor. Cancer Discov. 2015;5(7):713–722. doi:10.1158/2159-8290.CD-15-0399.
65. Zhu Y-J, Zhang H-B, Liu Y-H, Zhang F-L, Zhu Y-Z, Li Y, et al. Estimation of cell-free circulating EGFR mutation concentration predicts outcomes in NSCLC patients treated with EGFR-TKIs. Oncotarget. 2017;8(8):13195–13205. doi:10.18632/oncotarget.14490.
66. Duan J, Xu J, Wang Z, Bai H, Cheng Y, An T, et al. Refined stratification based on baseline concomitant mutations and longitudinal circulating tumor DNA monitoring in advanced EGFR-mutant lung adenocarcinoma under gefitinib treatment. J Thorac Oncol. 2020;15(12):1857–1870. doi:10.1016/j.jtho.2020.08.020.
67. He J, Tan W, Tang X, Ma J. Variations in EGFR ctDNA correlates to the clinical efficacy of afatinib in non small cell lung cancer with acquired resistance. Pathol Oncol Res. 2017;23(2):307–315. doi:10.1007/s12253-016-0097-y.
68. Wei Z, Wang W, Shu Z, Zhou X, Zhang Y. Correlation between circulating tumor DNA levels and response to tyrosine kinase inhibitors (TKI) treatment in non-small cell lung cancer. Med Sci Monit. 2017;23:3627–3634. doi:10.12659/msm.902265.
69. Yang X, Zhuo M, Ye X, Bai H, Wang Z, Sun Y, et al. Quantification of mutant alleles in circulating tumor DNA can predict survival in lung cancer. Oncotarget. 2016;7(15):20810–20824. doi:10.18632/oncotarget.8021.
70. Giroux Leprieur E, Herbretau G, Dumenil C, Julie C, Giraud V, Labrune S, et al. Circulating tumor DNA evaluated by next-generation sequencing is predictive of tumor response and prolonged clinical benefit with nivolumab in advanced non-small cell lung cancer. Oncoimmunology. 2018;7(5):e1424675. doi:10.1080/2162402X.2018.1424675.
71. 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(8):1872–1880. doi:10.1158/1078-0432.CCR-17-1341.
72. Guibert N, Jones G, Beeler JF, Plagnol V, Morris C, Mourlanette J, et al. Targeted sequencing of plasma cell-free DNA to predict response to PD1 inhibitors in advanced non-small cell lung cancer. Lung Cancer. 2019;137:1–6. doi:10.1016/j.lungcan.2019.09.005.
73. Iwama E, Sakai K, Azuma K, Harada T, Harada D, Nosaki K, et al. Monitoring of somatic mutations in circulating cell-free DNA by digital PCR and next-generation sequencing during afatinib treatment in patients with lung adenocarcinoma positive for EGFR activating mutations. Ann Oncol. 2017;28(1):136–141. doi:10.1093/annonc/mdw531.
74. Lee JY, Qing X, Xiumin W, Yali B, Chi S, Bak SH, et al. Longitudinal monitoring of EGFR mutations in plasma predicts outcomes of NSCLC patients treated with EGFR TKIs: Korean Lung Cancer Consortium

(KLCC-12-02). Oncotarget. 2016;7(6):6984–6993. doi:10.18632/oncotarget.6874.

75. Oxnard GR, Thress KS, Alden RS, Lawrance R, Paweletz CP, Cantarini M, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol. 2016;34(28):3375–3382. doi:10.1200/JCO.2016.66.7162.
76. Sueoka-Aragane N, Katakami N, Satouchi M, Yokota S, Aoe K, Iwanaga K, et al. Monitoring EGFR T790M with plasma DNA from lung cancer patients in a prospective observational study. Cancer Sci. 2016;107(2):162–167. doi:10.1111/cas.12847.
77. Han X, Han Y, Tan Q, Huang Y, Yang J, Yang S, et al. Tracking longitudinal genetic changes of circulating tumor DNA (ctDNA) in advanced lung adenocarcinoma treated with chemotherapy. J Transl Med. 2019;17(1):339. doi:10.1186/s12967-019-2087-9.
78. Horn L, Whisenant JG, Wakelee H, Reckamp KL, Qiao H, Leal TA, et al. Monitoring therapeutic response and resistance: analysis of circulating tumor DNA in patients with ALK + lung cancer. J Thorac Oncol. 2019;14(11):1901–1911. doi:10.1016/j.jtho.2019.08.003.
79. 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;86:349–357. doi:10.1016/j.ejca.2017.09.004.
80. Liu Z, Xie Z, Zhao S, Ye D, Cai X, Cheng B, et al. Presence of allele frequency heterogeneity defined by ctDNA profiling predicts unfavorable overall survival of NSCLC. Transl Lung Cancer Res. 2019;8(6):1045–1050. doi:10.21037/tlcr.2019.12.10.
81. Ortiz-Cuaran S, Mezquita L, Swalduz A, Aldea M, Mazieres J, Leonce C, et al. Circulating tumor DNA genomics reveal potential mechanisms of resistance to BRAF-targeted therapies in patients with BRAF-mutant metastatic non-small cell lung cancer. Clin Cancer Res. 2020;26(23):6242–6253. doi:10.1158/1078-0432.CCR-20-1037.
82. Reckamp KL, Patil T, Kirtane K, Rich TA, Espenschied CR, Weipert CM, et al. Duration of targeted therapy in patients with advanced non-small-cell lung cancer identified by circulating tumor DNA analysis. Clin Lung Cancer. 2020;21(6):545.e541–552.e541. doi:10.1016/j.cllc.2020.06.015.
83. 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(9):1441–1448. doi:10.1038/s41591-018-0134-3.
84. Herbreteau G, Langlais A, Greillier L, Audigier-Valette C, Uwer L, Hureaux J, et al. Circulating tumor DNA as a prognostic determinant in small cell lung cancer patients receiving atezolizumab. JCM. 2020;9(12):3861. doi:10.3390/jcm9123861.
85. Jiang T, Li X, Wang J, Su C, Han W, Zhao C, et al. Mutational landscape of cfDNA identifies distinct molecular features associated with therapeutic response to first-line platinum-based doublet chemotherapy in patients with advanced NSCLC. Theranostics. 2017;7(19):4753–4762. doi:10.7150/thno.21687.
86. Pécuchet N, Zonta E, Didelot A, Combe P, Thibault C, Gibault L, et al. Base-position error rate analysis of next-generation sequencing applied to circulating tumor DNA in non-small cell lung cancer: a prospective study. PLOS Med. 2016;13(12):e1002199. doi:10.1371/journal.pmed.1002199.
87. Song Y, Hu C, Xie Z, Wu L, Zhu Z, Rao C, et al. Circulating tumor DNA clearance predicts prognosis across treatment regimen in a large real-world longitudinally monitored advanced non-small cell lung cancer cohort. Transl Lung Cancer Res. 2020;9(2):269–279. doi:10.21037/tlcr.2020.03.17.
88. Wang Z, Duan J, Cai S, Han M, Dong H, Zhao J, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel. JAMA Oncol. 2019;5(5):696–702. doi:10.1001/jamaoncol.2018.7098.
89. Beagan JJ, Bach S, van Boerdonk RA, van Dijk E, Thunnissen E, van den Broek D, et al. Circulating tumor DNA analysis of EGFR-mutant non-small cell lung cancer patients receiving osimertinib following previous tyrosine kinase inhibitor treatment. Lung Cancer. 2020;145:173–180. doi:10.1016/j.lungcan.2020.04.039.
90. Buder A, Hochmair MJ, Setinek U, Pirker R, Filipits M. EGFR mutation tracking predicts survival in advanced EGFR-mutated non-small cell lung cancer patients treated with osimertinib. Transl Lung Cancer Res. 2020;9(2):239–245. doi:10.21037/tlcr.2020.03.02.
91. Minari R, Mazzaschi G, Bordi P, Gnetti L, Alberti G, Altimari A, et al. Detection of EGFR-activating and T790M mutations using liquid biopsy in patients with EGFR-mutated non-small-cell lung cancer whose disease has progressed during treatment with first- and second-generation tyrosine kinase inhibitors: a multicenter real-life retrospective study. Clin Lung Cancer. 2020;21(5):e464–e473. doi:10.1016/j.cllc.2020.02.021.
92. O'Kane GM, Liu G, Stockley TL, Shabir M, Zhang T, Law JH, et al. The presence and variant allele fraction of EGFR mutations in ctDNA and development of resistance. Lung Cancer. 2019;131:86–89. doi:10.1016/j.lungcan.2019.03.019.
93. Del Re M, Bordi P, Petrini I, Rofi E, Mazzoni F, Belluomini L, et al. Patients with NSCLC may

display a low ratio of p.T790M vs. activating EGFR mutations in plasma at disease progression: implications for personalised treatment. Oncotarget. 2017; 8(49):86056–86065. doi:10.18632/oncotarget.20947.

94. Pender A, Hughesman C, Law E, Kristanti A, McNeil K, Wong S, et al. EGFR circulating tumour DNA testing: identification of predictors of ctDNA detection and implications for survival outcomes. Transl Lung Cancer Res. 2020;9(4):1084–1092. doi: 10.21037/tlcr-19-581.
95. Romero A, Serna-Blasco R, Alfaro C, Sánchez-Herrero E, Barquín M, Turpin MC, et al. ctDNA analysis reveals different molecular patterns upon disease progression in patients treated with osimertinib. Transl Lung Cancer Res. 2020;9(3):532–540. doi: 10.21037/tlcr.2020.04.01.
96. Wang W, Song Z, Zhang Y. A comparison of ddPCR and ARMS for detecting EGFR T790M status in ctDNA from advanced NSCLC patients with acquired EGFR-TKI resistance. Cancer Med. 2017; 6(1):154–162. doi:10.1002/cam4.978.
97. Zhang S, Zhu L, Xia B, Chen E, Zhao Q, Zhang X, et al. Epidermal growth factor receptor (EGFR) T790M mutation identified in plasma indicates failure sites and predicts clinical prognosis in non-small cell lung cancer progression during first-generation tyrosine kinase inhibitor therapy: a prospective observational study. Cancer Commun (Lond)). 2018; 38(1):28. doi:10.1186/s40880-018-0303-2.
98. Nardo G, Carlet J, Marra L, Bonanno L, Boscolo A, Dal Maso A, et al. Detection of low-frequency KRAS mutations in cfDNA from EGFR-mutated NSCLC patients after first-line EGFR tyrosine kinase inhibitors. Front Oncol. 2020;10:607840. doi:10.3389/fonc.2020.607840.
99. Zheng D, Ye X, Zhang MZ, Sun Y, Wang JY, Ni J, et al. Plasma EGFR T790M ctDNA status is associated with clinical outcome in advanced NSCLC patients with acquired EGFR-TKI resistance. Sci Rep. 2016;6:20913. doi:10.1038/srep20913.
100. Zhou Q, Yang J-J, Chen Z-H, Zhang X-C, Yan H-H, Xu C-R, et al. Serial cfDNA assessment of response and resistance to EGFR-TKI for patients with EGFR-L858R mutant lung cancer from a prospective clinical trial. J Hematol Oncol. 2016;9(1):86. doi:10.1186/s13045-016-0316-8.
101. Demuth C, Winther-Larsen A, Madsen AT, Meldgaard P, Sorensen BS. A method for treatment monitoring using circulating tumour DNA in cancer patients without targetable mutations. Oncotarget. 2018;9(57):31066–31076. doi:10.18632/oncotarget.25779.
102. Hellmann MD, Nabet BY, Rizvi H, Chaudhuri AA, Wells DK, Dunphy MPS, et al. Circulating tumor DNA analysis to assess risk of progression after long-term response to PD-(L)1 blockade in NSCLC. Clin Cancer Res. 2020;26(12):2849–2858. doi:10.1158/1078-0432.CCR-19-3418.
103. Zhang EW, Dagogo-Jack I, Kuo A, Rooney MM, Shaw AT, Digumarthy SR. Association between circulating tumor DNA burden and disease burden in patients with ALK-positive lung cancer. Cancer. 2020;126(20):4473–4484. doi:10.1002/cncr.33118.
104. Jiang J, Adams H-P, Lange M, Siemann S, Feldkamp M, McNamara S, et al. Plasma-based longitudinal mutation monitoring as a potential predictor of disease progression in subjects with adenocarcinoma in advanced non-small cell lung cancer. BMC Cancer. 2020;20(1):885. doi:10.1186/s12885-020-07340-z.
105. Kuang P-P, Li N, Liu Z, Sun T-Y, Wang S-Q, Hu J, et al. Circulating tumor DNA analyses as a potential marker of recurrence and effectiveness of adjuvant chemotherapy for resected non-small-cell lung cancer. Front Oncol. 2020;10:595650. doi:10.3389/fonc.2020.595650.
106. Aldea M, Hendriks L, Mezquita L, Jovelet C, Planchard D, Auclin E, et al. Circulating tumor DNA analysis for patients with oncogene-addicted NSCLC with isolated central nervous system progression. J Thorac Oncol. 2020;15(3):383–391. doi:10.1016/j.jtho.2019.11.024.
107. Chae YK, Davis AA, Agte S, Pan A, Simon NI, Iams WT, et al. Clinical implications of circulating tumor DNA tumor mutational burden (ctDNA TMB) in non-small cell lung cancer. Oncologist. 2019;24(6): 820–828. doi:10.1634/theoncologist.2018-0433.
108. He J, Tan W, Ma J. Circulating tumor cells and DNA for real-time EGFR detection and monitoring of non-small-cell lung cancer. Future Oncol. 2017; 13(9):787–797. doi:10.2217/fon-2016-0427.
109. Lin X, Dong W, Lai X, Feng W, Yu X, Gu Q, et al. The clinical value of circulating tumor DNA detection in advanced non-small cell lung cancer. Transl Cancer Res. 2019;8(1):170–179. doi:10.21037/tcr.2019.01.20.
110. Zhou Y, Ma Y, Shi H, Du Y, Huang Y. Epidermal growth factor receptor T790M mutations in non-small cell lung cancer (NSCLC) of Yunnan in southwestern China. Sci Rep. 2018;8(1):15426. doi:10.1038/s41598-018-33816-x.
111. Mezquita L, Swalduz A, Jovelet C, et al. Clinical relevance of an amplicon-based liquid biopsy for detecting ALK and ROS1 fusion and resistance mutations in patients with non-small-cell lung cancer. JCO Precis Oncol. 2020;4:PO.19.00281.
112. Rao C, Nie L, Miao X, Xu Y, Li B, Zhang T. The clinical characteristics and prognostic analysis of Chinese advanced NSCLC patients based on circulating tumor DNA sequencing. Onco Targets Ther. 2018;11:337–344. doi:10.2147/OTT.S154589.
113. Schwaederlé MC, Patel SP, Husain H, Ikeda M, Lanman RB, Banks KC, et al. Utility of genomic assessment of blood-derived circulating tumor DNA

(ctDNA) in patients with advanced lung adenocarcinoma. Clin Cancer Res. 2017;23(17):5101–5111. doi:10.1158/1078-0432.CCR-16-2497.

114. Thompson JC, Yee SS, Troxel AB, Savitch SL, Fan R, Balli D, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. Clin Cancer Res. 2016; 22(23):5772–5782. doi:10.1158/1078-0432.CCR-16-1231.
115. Dietz S, Christopoulos P, Yuan Z, Angeles AK, Gu L, Volckmar A-L, et al. Longitudinal therapy monitoring of ALK-positive lung cancer by combined copy number and targeted mutation profiling of cell-free DNA. EBioMedicine. 2020;62:103103. doi:10.1016/j.ebiom.2020.103103.
116. 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. doi:10.1038/srep33519.
117. Ho GYF, Wang T, Kwok H-H, Rasul R, Peila R, Guzman M, et al. Longitudinal multi-gene panel assessment of circulating tumor DNA revealed tumor burden and molecular characteristics along treatment course of non-small cell lung cancer. Transl Lung Cancer Res. 2020;9(5):1873–1884. doi: 10.21037/tlcr-20-675.
118. Spence T, Perera S, Weiss J, Grenier S, Ranich L, Shepherd F, et al. Clinical implementation of circulating tumour DNA testing for EGFR T790M for detection of treatment resistance in non-small cell lung cancer. J Clin Pathol. 2021;74(2):91–97. doi:10.1136/jclinpath-2020-206668.
119. Uchida J, Imamura F, Kukita Y, Oba S, Kumagai T, Nishino K, et al. Dynamics of circulating tumor DNA represented by the activating and resistant mutations in epidermal growth factor receptor tyrosine kinase inhibitor treatment. Cancer Sci. 2016; 107(3):353–358. doi:10.1111/cas.12860.
120. Usui K, Yokoyama T, Naka G, Ishida H, Kishi K, Uemura K, et al. Plasma ctDNA monitoring during epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor treatment in patients with EGFR-mutant non-small cell lung cancer (JP-CLEAR trial). Jpn J Clin Oncol. 2019;49(6):554–558. doi:10.1093/jjco/hyz023.
121. Guo D, Yang L, Yang J, Shi K. Plasma cell-free DNA methylation combined with tumor mutation detection in prognostic prediction of patients with non-small cell lung cancer (NSCLC). Medicine. 2020; 99(26):e20431. doi:10.1097/MD.0000000000020431.
122. Phallen J, Leal A, Woodward BD, Forde PM, Naidoo J, Marrone KA, et al. Early noninvasive detection of response to targeted therapy in non-small cell lung cancer. Cancer Res. 2019;79(6):1204–1213. doi:10.1158/0008-5472.CAN-18-1082.
123. Yang W, You N, Jia M, Yeung S-CJ, Ou W, Yu M, et al. Undetectable circulating tumor DNA levels correlate with low risk of recurrence/metastasis in postoperative pathologic stage I lung adenocarcinoma patients. Lung Cancer. 2020;146:327–334. doi: 10.1016/j.lungcan.2020.06.009.
124. Buttitta F, Felicioni L, Lorito AD, Cortellini A, Irtelli L, Brocco D, et al. Early prediction of resistance to tyrosine kinase inhibitors by plasma monitoring of EGFR mutations in NSCLC: a new algorithm for patient selection and personalized treatment. Oncotarget. 2020;11(11):982–991. doi:10.18632/oncotarget.27517.
125. Chiou CC, Wang CL, Luo JD, Liu CY, Ko HW, Yang CT. Targeted sequencing of circulating cell free DNA can be used to monitor therapeutic efficacy of tyrosine kinase inhibitors in non-small cell lung cancer patients. Cancer Genomics Proteomics. 2020; 17(4):417–423. doi:10.21873/cgp.20200.
126. Ding PN, Becker TM, Bray VJ, Chua W, Ma YF, Lynch D, et al. The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: a prospective study. Lung Cancer. 2019;134:187–193. doi:10.1016/j.lungcan.2019.06.021.
127. Provencio M, Torrente M, Calvo V, Pérez-Callejo D, Gutiérrez L, Franco F, et al. Prognostic value of quantitative ctDNA levels in non small cell lung cancer patients. Oncotarget. 2018;9(1):488–494. doi:10.18632/oncotarget.22470.
128. Taus Á, Camacho L, Rocha P, Hardy-Werbin M, Pijuan L, Piquer G, et al. Dynamics of EGFR mutation load in plasma for prediction of treatment response and disease progression in patients with EGFR-mutant lung adenocarcinoma. Clin Lung Cancer. 2018;19(5): 387–394.e382. doi:10.1016/j.cllc.2018.03.015.
129. Mok T, Wu Y-L, Lee JS, Yu C-J, 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(14): 3196–3203. doi:10.1158/1078-0432.CCR-14-2594.
130. Yu HA, Schoenfeld AJ, Makhnin A, Kim R, Rizvi H, Tsui D, et al. Effect of osimertinib and bevacizumab on progression-free survival for patients with metastatic EGFR-mutant lung cancers: a phase 1/2 single-group open-label trial. JAMA Oncol. 2020;6(7):1048–1054. doi:10.1001/jamaoncol.2020.1260.
131. Zhang C, Wei B, Li P, Yang K, Wang Z, Ma J, et al. Prognostic value of plasma EGFR ctDNA in NSCLC patients treated with EGFR-TKIs. PLOS One. 2017; 12(3):e0173524. doi:10.1371/journal.pone.0173524.
132. 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(6):1214–1225. doi:10.1158/0008-5472.CAN-18-1127.

133. Raja R, Kuziora M, Brohawn PZ, Higgs BW, Gupta A, Dennis PA, et al. Early reduction in ctDNA predicts survival in patients with lung and bladder cancer treated with durvalumab. Clin Cancer Res. 2018; 24(24):6212–6222. doi:10.1158/1078-0432.CCR-18-0386.
134. Amirouchene-Angelozzi N, Swanton C, Bardelli A. Tumor evolution as a therapeutic target. Cancer Discov. 2017;7(8):805–817.
135. 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(2):1–14. doi:10.1200/PO.17.00160.
136. Giroux Leprieur E, Hélias-Rodzewicz Z, Takam Kamga P, Costantini A, Julie C, Corjon A, et al. Sequential ctDNA whole-exome sequencing in advanced lung adenocarcinoma with initial durable tumor response on immune checkpoint inhibitor and late progression. J Immunother Cancer. 2020; 8(1):e000527. doi:10.1136/jitc-2020-000527.
137. 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(7655):446–451. doi:10.1038/nature22364.
138. Pellini B, Szymanski J, Chin RI, Jones PA, Chaudhuri AA. Liquid biopsies using circulating tumor DNA in non-small cell lung cancer. Thorac Surg Clin. 2020;30(2):165–177. doi:10.1016/j.thorsurg.2020.01.005.
139. Herath S, Sadeghi Rad H, Radfar P, Ladwa R, Warkiani M, O'Byrne K, et al. The role of circulating biomarkers in lung cancer. Front Oncol. 2021;11: 801269. doi:10.3389/fonc.2021.801269.
140. Zhang Y-L, Yuan J-Q, Wang K-F, Fu X-H, Han X-R, Threapleton D, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. Oncotarget. 2016;7(48):78985–78993. doi:10.18632/oncotarget.12587.
141. Zhao Y, Liu J, Cai X, Pan Z, Liu J, Yin W, et al. Efficacy and safety of first line treatments for patients with advanced epidermal growth factor receptor mutated, non-small cell lung cancer: systematic review and network meta-analysis. BMJ. 2019;367:l5460. doi:10.1136/bmj.l5460.
142. Biaoxue R, Shuanying Y. Tissue or blood: which is more suitable for detection of EGFR mutations in non-small cell lung cancer? Int J Biol Markers. 2018; 33(1):40–48. doi:10.5301/ijbm.5000256.