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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

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Abstract

Purpose—To explore whether targeted next generation sequencing (NGS) of liquid biopsy in advanced non-small cell lung cancer (NSCLC) could potentially overcome the innate problems that arise with standard tissue biopsy, like intratumoral heterogeneity and the inability to obtain adequate samples for analysis.

Methods—The Scopus, Cochrane Library, and MEDLINE (via PubMed) databases were searched for studies with matched tissue and liquid biopsies from advanced NSCLC patients, analyzed with targeted NGS. The number of mutations detected in tissue biopsy only, liquid biopsy only, or both was assessed and the positive percent agreement (PPA) of the two methods was calculated for every clinically relevant gene.

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Conflict of interest The authors report no conflict of interest.

Results—A total of 644 unique relevant articles were retrieved and data were extracted from 38 studies fulfilling the inclusion criteria. The sample size was composed of 2000 mutations tested in matched tissue and liquid biopsies derived from 1141 patients. No studies analyzed circulating tumor cells. The calculated PPA rates were 53.6% (45/84) for ALK, 53.9% (14/26) for BRAF, 56.5% (13/23) for ERBB2, 67.8% (428/631) for EGFR, 64.2% (122/190) for KRAS, 58.6% (17/29) for MET, 54.6% (12/22) for RET, and 53.3% (8/15) for ROS1. We additionally recorded data for 65 genes that are not recommended by current guidelines for mutational testing. An extra category containing results of unspecified genes was added, with a PPA rate of 55.7% (122/219).

Conclusion—Despite many advantages, liquid biopsy might be unable to fully substitute its tissue counterpart in detecting clinically relevant mutations in advanced NSCLC patients. However, it may serve as a helpful tool when making therapeutic decisions. More studies are needed to evaluate its role in everyday clinical practice.

Keywords

NGS; ctDNA; NSCLC; EGFR mutations; ALK fusion gene; ROS1 translocation; BRAF testing

Introduction

The advent of tyrosine kinase inhibitors (TKIs) has been a milestone in the treatment of advanced non-small cell compared to previous standard therapy (Vestergaard et al. 2018). Current guidelines have already adopted their use as first- and second-line treatment options (Hanna et al. 2017; Ettinger et al. 2018; Planchard et al. 2018). TKIs are designed to interrupt specific molecular pathways that promote tumor survival and growth (Levy et al. 2012). Genetic tumor profiling is, therefore, necessary to reveal mutations that enhance those pathways, allowing oncologists to effectively match TKI agents to every patient's individual tumor molecular landscape. This rapid shift towards personalized medicine is reflected by the most recent diagnostic recommendations. Mutational testing of genes including EGFR, ALK, ROS1, and BRAF is currently considered standard for all patients with advanced NSCLC, regardless of their individual characteristics (Kerr et al. 2014; Kalemkerian et al. 2018; Lindeman et al. 2018). In addition, further testing for genes including ERBB2, RET, MET, and KRAS is also encouraged as part of a more extensive laboratory workup, when available, due to their implications in tumor prognosis and treatment response against newer promising targeted agents (Kerr et al. 2014; Kalemkerian et al. 2018; Lindeman et al. 2018).

The current gold standard method for genetic tumor profiling is tissue biopsy. However, its use remains problematic for numerous reasons. Lung biopsy is an invasive procedure with a notoriously high incidence of both major and minor complications (Overman et al. 2013; Heerink et al. 2017). In addition, tissue biopsy yields inadequate DNA material for genetic analysis at a significant rate (VanderLaan et al. 2014). Intratumoral heterogeneity is also a well-established phenomenon (Vogelstein et al. 2013) that inherently limits its accuracy in capturing a complete snapshot of the tumor's mutational status, with potentially different results from different biopsy sites. Apart from the initial tumor genetic profiling, frequent monitoring for the emergence of new mutations or genetic modifications is also vital in detecting treatment resistance and managing it accordingly, as in the case of the EGFR

T790M mutation and the use of third-line TKIs such as osimertinib (Mok et al. 2016). The aforementioned characteristics among others, make serial tissue biopsies contextual and potentially functionally nonrepresentative of the overall mutational burden. As a consequence, using tissue biopsy as a guiding tool for targeted therapy may limit its full efficacy.

Liquid biopsy is a promising complement/alternative to the classic tissue biopsy. By assessing blood samples for the presence of either circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA), it provides the necessary genetic material to perform tumor mutational analysis (Mayo-de-Las-Casas et al. 2018). Unlike many tissue biopsies, liquid biopsies are far less cumbersome with greatly reduced risk for complications. Performing repeated biopsies over time is, therefore, more feasible and may represent the overall tumor mutational burden accurately (Santarpia et al. 2016). However, liquid biopsy is not yet widely adopted as a standard diagnostic method because of inadequate data supporting its use (Kalemkerian et al. 2018; Lindeman et al. 2018). As a result, guidelines suggest it as an alternative to tissue biopsy only in settings where tissue availability becomes the limiting factor for molecular testing (Kalemkerian et al. 2018; Lindeman et al. 2018).

While current recommendations support mutational testing only for a few key cancer-driver genes, it is already evident that obtaining a more complete mutational profile is most likely the future direction of targeted therapy. NSCLC displays a heterogeneous mutational profile across different patients (Tan et al. 2014). Overall, current NSCLC clinical trials assess the mutational status of over 190 different genes, according to the My Cancer Genome online database (https://www.mycancergenome.org). Next generation sequencing (NGS) is a method of DNA analysis that allows parallel sequencing of numerous small DNA fragments, thus making concurrent testing for a very wide mutational gene panel possible (Sabour et al. 2017). It is currently deemed as an acceptable sequencing method for various instances during NSCLC mutational testing (Lindeman et al. 2018). Therefore, combining liquid biopsy with NGS offers the potential to obtain a comprehensive tumor genetic profile through minimally invasive means. Generally, NGS can either be directed towards specific genes through a predetermined gene panel (targeted NGS) or towards the whole genome or exome of the patient. The former represents a more appealing choice for daily clinical practice, through lower cost, faster results and higher sensitivity with lower detection thresholds (El Achi et al. 2019).

The objective of this systematic review is to examine whether liquid biopsy is a suitable alternative to tissue biopsy mutational testing, by reviewing studies that compare matched targeted NGS-analyzed tissue and liquid biopsy samples of advanced NSCLC patients.

Methods

Search and study selection

We accessed relevant articles by searching through the MEDLINE (via PubMed), Scopus, and Cochrane Library databases. The following search algorithm was applied to the MEDLINE and Scopus electronic databases: “(next generation sequencing) AND (liquid biopsy OR circulating OR cfDNA OR ctDNA OR CTC) AND lung”. Cochrane Library was

searched by combining the search terms “next-generation sequencing” and “lung”. No publication date or any other type of filters were used. In addition, we manually searched through the references of eligible articles for any relevant articles not already included in the original search. The last search was performed on April 24th, 2020. Two researchers (S.E. and G.G.) performed the initial screening independently and assessed studies for eligibility. Any disagreements were resolved by reaching a consensus. We initially screened the abstracts of all studies and accepted those that included NSCLC in their objective and contained liquid biopsy and NGS as part of their methodology. Review articles were excluded during the screening process.

In the process of assessing the eligibility of studies that passed screening, we first excluded: (1) case reports, (2) articles written in any language other than English and (3) studies that employed whole-genome NGS instead of targeted NGS. We subsequently included studies that: (1) involved human subjects (and not cell lines or artificial samples), (2) included patients with advanced stage NSCLC (stage IIIb, stage IV according to the AJCC Staging System), (3) included both tissue and liquid biopsies in their methodologies, (4) had matched tissue and liquid biopsies and (5) had NGS performed in both tissue and liquid biopsy samples. Finally, eligible studies were excluded from the final analysis if they presented data in a way that did not allow us to properly extract it. For example, we omitted articles with mixed data from different types of cancers other than the subtypes of NSCLC, different NSCLC stages or results that didn’t take matched samples into account.

Data collection

To collect data from studies included in the final analysis, we created specialized spreadsheets via Microsoft Excel®. Two researchers (S.E. and G.G.) extracted all data independently. Their results were compared for all relative parameters and disagreements were resolved by reaching a consensus. We examined all papers included in the final analysis for the following parameters: (1) number of mutations detected in both tissue and liquid biopsy via targeted NGS, (2) number of mutations detected in tissue but not liquid biopsy via targeted NGS, and (3) number of mutations detected in liquid but not tissue biopsy via targeted NGS and (4) total number of patients that we extracted data from (not necessarily equal to the total of number patients in the study). We also recorded identifying study characteristics, such as (5) the country where each study was conducted and (6) the year of publication, in addition to the technical specifications of the NGS assays employed for the analysis of both liquid and tissue biopsies, including (7) the sequencing platform, (8) the gene panel, (9) the average coverage and (10) any allele frequency or copy number alteration threshold for mutation calling as set by the authors.

The mycancergenome.org database was used as a reference point to determine the clinical relevance of each individual gene. Only genes whose mutational status has been an eligibility criterion for clinical trials, regarding either NSCLC or one of its subtypes (e.g. lung adenocar-cinoma, squamous cell lung carcinoma), were selected. In those cases where mutations were clearly indicated as driver or non-driver, we recorded data from the former category only. In studies where the authors did not compile the results from matched tissue and liquid biopsy samples themselves, we extracted data by matching results from the

available supplementary data tables. We applied any individual allele frequency confidence thresholds for variant calling set by the authors in cases where it was clearly stated. All available data were grouped by gene only, rather than by each individual mutation and different mutations of the same gene were categorized together.

During our analysis, we only included data derived from advanced NSCLC patients that were the result of targeted NGS. We did not consider data from patients with other malignancies, early stage NSCLC or data derived from any method other than targeted NGS in cases where they were co-presented with relevant results. Moreover, if patients were characterized as stage III instead of IIIa or IIIb, they were not included during data extraction. To avoid introducing bias by recording duplicate data, we skipped data collection from identical patient populations already included in the analysis through a different study. Between studies with identical patient populations, we favored those that presented the greatest amount of extractable data. We also omitted any data where cytologic analysis of pleural fluid was used in lieu of primary tumor biopsy, but accepted tumor biopsies from either primary or metastatic sites. It should be noted that we did not apply any limit to the time difference between tissue and plasma sampling. Finally, studies with extractable data of interest for less than two patients were excluded from the final qualitative synthesis.

Statistical analysis

We compared the performance of tissue biopsy and liquid biopsy, by calculating the positive percent agreement (PPA) between the two. Since tissue NGS analysis was not validated in every study included in the final analysis, it could not be considered as a reference method but rather as a best alternative to liquid biopsy NGS. Therefore, sensitivity, specificity, positive predictive value, and negative predictive value were inappropriate comparison measures in this instance and were not calculated. In addition, data where no mutation was found in both tissue and liquid biopsy NGS were often omitted by the authors or could not be reliably extracted based on the information provided. Thus, negative percent agreement, despite being an appropriate statistical measure, could not be calculated.

Results

Study selection

A total of 780 relevant articles were retrieved by searching the literature. The last search was performed on April 24th, 2020. After removing 136 duplicate studies, we screened the titles and abstracts of 644 articles. Of them, 420 were deemed irrelevant, while 224 were determined to be relevant and their full-text articles were assessed for eligibility. After assessment against the preset inclusion and exclusion criteria, 155 articles were excluded: 4 were written in a non-English language, 21 were case reports, 7 employed whole genome instead of targeted NGS, 6 did not involve human subjects, 16 enrolled only early stage NSCLC patients, 31 lacked either tissue or liquid biopsies, 4 had non-matched tissue and liquid biopsy samples, and 40 utilized a method other than NGS for either of the two biopsy types. An additional 26 studies did not provide enough information to unequivocally determine their eligibility and thus had to be excluded as well. The remaining 69 studies fulfilled all eligibility criteria and were selected for the data extraction process. Twenty-one

of them presented their data in a manner that did not allow selective extraction of relevant data only. Data were extracted from 48 studies. Five of them were found to enroll populations that had already been included in the analysis through a different study and their results were not counted. Another five studies had extractable data for only $n = 1$ patient and were also excluded from the data synthesis. The final analysis was performed on the remaining 38 studies. The complete process is summarized in Fig. 1.

Individual data and data synthesis

A total of 38 non-case report studies that compared matched tissue and liquid biopsy samples of advanced NSCLC patients were individually analyzed and their data were subsequently synthesized (Couraud et al. 2014; Thress et al. 2015; Vanni et al. 2015; Kaisaki et al. 2016; Pécuchet et al. 2016; Paweletz et al. 2016; Rachiglio et al. 2016; Villaflor et al. 2016; Schwaederlé et al. 2017; Xu et al. 2017; Yao et al. 2017; Iwama et al. 2017; Dagogo-Jack et al. 2018; Veldore et al. 2018; Yang et al. 2018; Li et al. 2018; Liu et al. 2018; Vollbrecht et al. 2018; Garcia et al. 2018; Toor et al. 2018; McCoach et al. 2018; Guo et al. 2018; Hu et al. 2018; Jin et al. 2018; Sabari et al. 2018; Papadopoulou et al. 2019; Tong et al. 2019; Lam et al. 2019; Aggarwal et al. 2019; Ge et al. 2019; Wu et al. 2019; Chen et al. 2019; Supplee et al. 2019; Tang et al. 2019; Streubel et al. 2019; Horn et al. 2019; Pritchett et al. 2019; Tran et al. 2019). The technical aspects and characteristics of every study included in the final analysis are presented in Table 1. The sample size was composed of 2000 mutations tested in matched biopsies derived from 1141 patients. Data were collected for 74 different gene categories of interest. It should be noted that all 38 eligible studies employed cfDNA/ctDNA analysis as their liquid biopsy method. No studies utilized analysis of CTCs instead, despite being included during the initial screening, as they were deemed ineligible for other reasons.

Genes recommended for mutational testing by current guidelines

As previously mentioned, the most recent guidelines recommend mutational testing in advanced NSCLC for the genes ALK, BRAF, ERBB2 (HER2), EGFR, KRAS, MET, RET and ROS1(Kerr et al. 2014; Kalemkerian et al. 2018; Lindeman et al. 2018). The sample sizes were 84 samples for ALK, 26 for BRAF, 23 for ERBB2, 631 for EGFR, 190 for KRAS, 29 for MET, 22 for RET, and 15 for ROS1. Cumulative PPA rates were calculated to be as follows: 53.6% (45/84) for ALK, 53.9% (14/26) for BRAF, 56.5% (13/23) for ERBB2, 67.8% (428/631) for EGFR, 64.2% (122/190) for KRAS, 58.6% (17/29) for MET, 54.6% (12/22) for RET, and 53.3% (8/15) for ROS1. The results of every individual study are comprehensively presented in Fig. 2.

Genes not recommended for mutational testing by current guidelines

Data were also recorded for 65 genes of clinical interest that are not yet recommended by current guidelines for mutational testing. Total sample sizes for all these genes in addition to the eight genes recommended for testing by the current guidelines are presented in Fig. 3, while cumulative PPA rates are calculated and displayed in Fig. 4. Additional data regarding the PPA results from every individual study can be found in Supplemental Fig. 1.

Unspecified genes

An extra category was added for cases where the authors did not provide a detailed breakdown of their results for every gene but their data were otherwise eligible for inclusion and data extraction. This category contained 219 samples, with 132 samples coming from Pritchett et al. (Pritchett et al. 2019), 86 samples coming from Aggarwal et al. (Aggarwal et al. 2019) and 1 sample from Couraud et al. (Couraud et al. 2014). The cumulative PPA for this category was calculated at 55.7% (122/219).

Discussion

To our knowledge, this is the first systematic review in the literature comparing targeted NGS analysis of plasma and tissue biopsies in advanced NSCLC. Our findings support the notion that targeted NGS in liquid biopsy falls short in detecting mutations compared to NGS in tissue biopsy in these patients. Most genes provided inadequate sample sizes to draw any reliable conclusions. Exceptions were EGFR and TP53, constituting together more than half of the overall sample size (n = 2000) used in the analysis, with 631 and 432 individual-tested mutations respectively. Both displayed similar PPA rates of 67.8% (428/631) for EGFR and 63.2% (273/432) for TP53. On the other hand, genes like ALK, BRAF, ERBB2, KRAS, MET and ROS1 that are currently included in the mutational testing guidelines all had significantly smaller sample sizes and, with the exception of KRAS, all provided unsatisfactory PPA rates of less than 60%. These data seem to be in accordance with the previously reported suboptimal performance of targeted NGS detecting gene translocations in ctDNA, as the breaking point each time might not be included in the limited NGS panels applied for liquid biopsies (Schram et al. 2017). Given the current data, NGS liquid biopsy seems unable to completely substitute its tissue biopsy counterpart, regardless of the gene being tested. Despite this, moderately satisfactory results as in the case of EGFR may solidify its position as a useful tool to complement tissue biopsy in certain clinical scenarios.

As NGS analysis in plasma is generally less sensitive than in tissue biopsy (Rolfo et al. 2018; Li et al. 2019), there was no surprise that a significant number of cases in our review revealed specific mutations during tissue biopsy molecular genotyping and failed to do so in their associated liquid biopsy. Mutation analysis on tissues is a well-standardized procedure where, in contrast to ctDNA, morphologic correlation to enrich tumor DNA is feasible; enrichment is achieved with macro-microdissection of areas containing a high percentage of tumor cells to reach the high initial DNA load necessary for optimal tumor genotyping (Shiau et al. 2014). On the other hand, ctDNA is only a tiny fraction of the total cell-free DNA and negative mutation testing might mean anything from true absence or low release of tumor DNA into the blood (e.g. due to response to therapy), a technical error of the NGS assay itself or a mutation not covered by the selected plasma assay (NGS panels for plasma can be less comprehensive than for tissue molecular analysis) (Weber et al. 2014; Merker et al. 2018; Rolfo et al. 2018; Aggarwal et al. 2019).

In contrast, this review also reported cases that showed rare specific mutations in the blood but not in the associated tissue biopsy, although this finding was less common. Although considered the current standard of care, tissues may fail to provide adequate DNA load for tumor genotyping in a significant number of cases. This may be due to insufficient sampling

or sufficient sampling of low tumor cellularity or poor quality caused by chemical degradation (VanderLaan et al. 2014; Hagemann et al. 2015). Such failures are more common when CT-guided transthoracic core biopsies or bone biopsies are performed (VanderLaan et al. 2014). In addition, ctDNA may reflect intratumoral heterogeneity better than tissue-derived DNA, as the former may contain circulating apoptotic/necrotic DNA derived from tumor clones residing in both primary and distant sites, thus could reveal mutation(s) undetected from a tumor biopsy that lacks the relevant clones (spatial heterogeneity). In cases that plasma was collected after tumor biopsy, discordance could also be due to tumor evolution in time reflected in ctDNA but not in the tissue (temporal heterogeneity) (Weber et al. 2014; Siravegna et al. 2017). Of interest, mutations detected during ctDNA analysis might be irrelevant to lung cancer (e.g. clonal hemopoiesis; other benign/premalignant/malignant processes) (Chae and Oh 2019). Similar to false negative, NGS ctDNA assays can also result in false positive results (Rolfo et al. 2018).

When looking at the technical specifications of the NGS assays used for tissue and liquid biopsy analysis, it becomes evident that there was significant heterogeneity, not only across different studies but also within the studies themselves. This finding strongly reflects the lack of standardization in NGS assays (Jennings et al. 2017), which might be hindering its widespread implementation into daily practice. Molecular coverage can be a significant factor affecting the sensitivity of NGS assays (Petrackova et al. 2019). Many studies in our analysis employed coverage of less than 1,000x, especially in the analysis of tissue samples, and while no standard parameters have been established, it may be considered inadequate (Jennings et al. 2017; Petrackova et al. 2019), and could thus explain some of the false-negative results in tissue biopsies. On the other hand, there is a trade-off between the amount of genes that can be covered and the analytical sensitivity achieved by high molecular coverage, which is especially important in liquid biopsy analysis (Karachaliou et al. 2015). Consequently, studies that utilized very wide gene panels may had to compromise depth and sensitivity, while others may have had to limit their gene selection, resulting in liquid biopsy samples with false negative findings in both cases.

In an era where advanced NSCLC patients are mostly diagnosed with small biopsies or cytology rather than surgery or do not undergo biopsy at all for causing discomfort or potential minor/major complications, liquid biopsy incorporation into clinical practice may be exceptionally helpful for patient management (VanderLaan et al. 2014; Travis et al. 2015; Heerink et al. 2017; Siravegna et al. 2017; Mayo-de-Las-Casas et al. 2018). CtDNA tumor genotyping could be a vital supplement or alternative due to its minimally invasive nature, allowing for routine genetic follow-up (Santarpia et al. 2016). In treatment-naive advanced NSCLC patients, ctDNA status has been associated with both survival and response to first- or second-line TKIs (Ai et al. 2016; Pécuchet et al. 2016; Phallen et al. 2019). In advanced NSCLC patients on TKIs that present with disease progression, ctDNA mutational analysis can identify the mechanisms of resistance and predict patient benefit from specific targeted treatments, such as third-line TKIs in the case of T790M mutation (Thress et al. 2015; Vollbrecht et al. 2018; Iwama et al. 2018). In advanced NSCLC patients on immunotherapy, liquid biopsy has also been reported to predict response to treatment (Giroux Leprieur et al. 2018; Rizvi et al. 2018). Besides ctDNA, CTCs could also be of value for monitoring

disease, assessing prognosis and predict response to TKIs, chemotherapy or immunotherapy in NSCLC patients (Chinniah et al. 2019; Gallo et al. 2019; Tamminga et al. 2019).

Of interest, several studies have used diverse polymerase chain reaction (PCR) methods (e.g. droplet digital PCR; allele-specific PCR), rather than NGS, on liquid biopsy to assess prognosis, response to treatment and resistance in advanced NSCLC patients and compared plasma-based results with the ones on tissue biopsy (Weber et al. 2014; Karachaliou et al. 2015; Zhu et al. 2015; Lee et al. 2016; Sundaresan et al. 2016). PCR is cheaper, requires less technical expertise (e.g. no bioinformatics support), and has more rapid turnaround time than NGS; it is thus ideal when a selected treatment is urgent, e.g. osimertinib in cases of T790M mutation (Sacher et al. 2016; Postel et al. 2018; Rolfo et al. 2018). However, it is much less comprehensive than NGS, being able to detect only one or just a few mutations; in contrast, NGS can provide a more complete tumor profile detecting single-nucleotide variants, insertions/deletions, translocations, and amplifications (Hagemann et al. 2015; Postel et al. 2018; Rolfo et al. 2018). Although NGS has traditionally been considered less sensitive than PCR, authors in recent reports, which reflect improvement in the NGS technology and standardization, describe similar sensitivity for the detection of driver and resistance mutations (Li et al. 2019) or an even better performance of NGS in the case of T790M (Dono et al. 2019).

This review has several limitations. Most of the studies included were retrospective or heterogeneous in their design. Studies used variable NGS panels which could differ between plasma analyses across different studies, but also between plasma and tissue analysis within the same study. Meanwhile, many of them focused on a limited gene spectrum. As a result, not all genes are covered uniformly in our cumulative results and data for a significant number of genes are extremely limited. Both older and recent studies were included, although the latter reported higher sensitivity/specificity, possibly because of the improvements in NGS technology and standardization. The authors also applied diverse cut-offs to report variants in their NGS experiments. In many studies, plasma and tissue were not collected at the same time (time difference between them ranged from 0 days to many months). Lastly, our total patient population included both treatment-naive and patients under TKI treatment, ranging from adequate responders to first-line treatment to patients progressing after multiple TKI trials. Thus, the results from different studies are not directly comparable to one another.

The latest guidelines support the use of ctDNA mutation analysis in cases when tissue biopsy is not performed or provides inadequate DNA for analysis (Kalemkerian et al. 2018; Lindeman et al. 2018). More evidence is needed to support its use in treatment-naive patients or patients under TKI that undergo progression, e.g. due to EGFR T790M or C797S mutations, and there is the recommendation to follow-up a negative result with reflex tissue-based testing whenever possible (Kalemkerian et al. 2018; Lindeman et al. 2018; Rolfo et al. 2018; Li et al. 2019). In this direction, recent studies have attempted to provide more clinical validity/utility of testing the ctDNA of advanced NSCLC patients with NGS (Laufer-Geva et al. 2018; Sabari et al. 2018; Leighl et al. 2019; Aggarwal et al. 2019; Li et al. 2019). Dual plasma and tissue-based targeted NGS testing detects more mutations than each one

separately, thus more patients can be treated with targeted therapies (Leighl et al. 2019; Aggarwal et al. 2019).

An inherent weakness of NGS is the failure to detect epigenetic modifications affecting gene expression without altering the base sequence (Fernandez-Marmiesse et al. 2017). The techniques used to reveal these modifications may significantly degrade the already limited genetic material available for sequencing (Gai and Sun 2019). As a result, choosing sequencing over other applications of the isolated tumor genetic material may still provide an incomplete picture of the mechanisms involved in treatment response and tumor behavior, even if both tissue and liquid biopsy are utilized.

Conclusion

In conclusion, most advanced NSCLC patients are unresectable and are diagnosed with small biopsies or cytology, both of which may have insufficient DNA for molecular analysis. This systematic review showed that targeted NGS in plasma shows inferior performance in detecting mutations compared to tissue biopsy in advanced NSCLC patients. However, given the fact that technology around ctDNA mutation analysis with NGS will most likely continue to be improving the years to come, accumulating evidence in the form of prospective studies, randomized clinical trials and systematic reviews/meta-analyses will soon result in re-evaluation of ctDNA clinical validity and utility in advanced NSCLC. Targeted NGS testing on ctDNA has the potential to become a highly accurate diagnostic modality for the presence of actionable mutations in treatment-naïve and resistant to TKIs patients or for the selection for clinical trials, as shown in the most recent publications on the field. The field is rapidly evolving, but current retrospective data show a synergistic and clinical utility to both methodologies when clinically feasible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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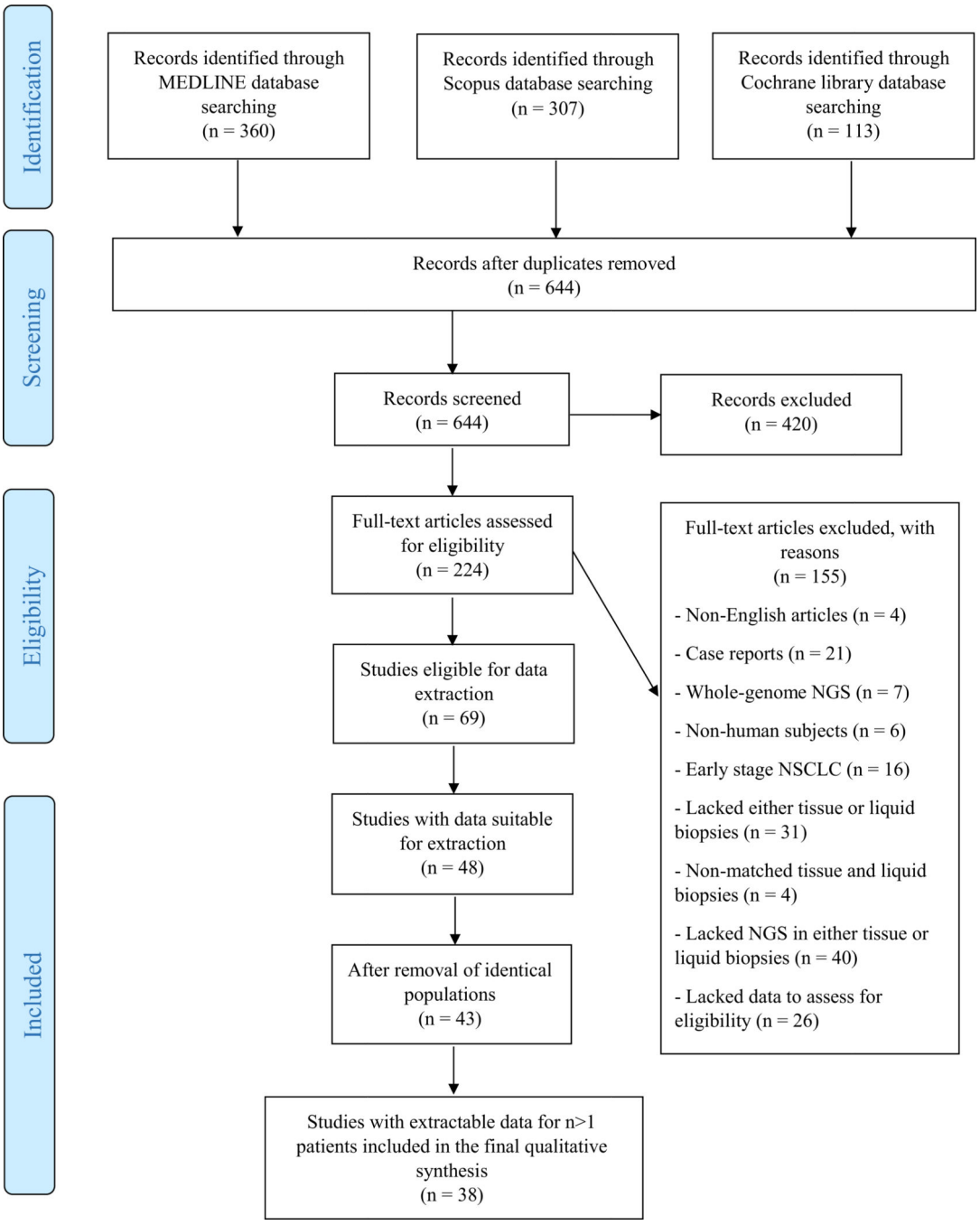

Fig. 1.

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram of study selection

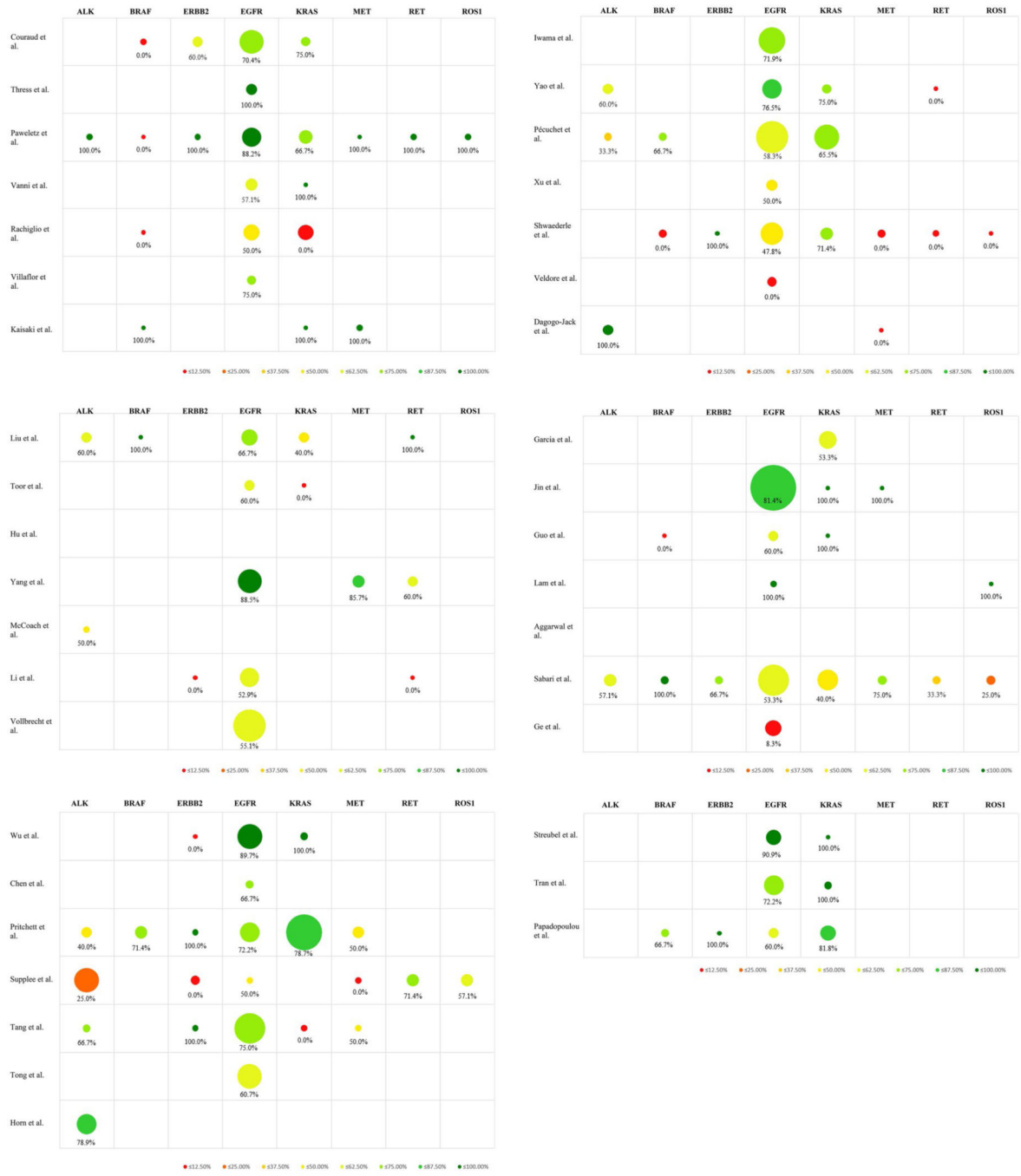

Fig. 2.

Positive percent agreement (PPA) rates of genes recommended for mutational testing. A table representation combining a bubble chart of the sample sizes with a heat-map representation of the PPA rates of every individual study included in the analysis, in chronological order, according to the date of first online publication. Only genes recommended for mutational testing by the most recent guidelines are displayed

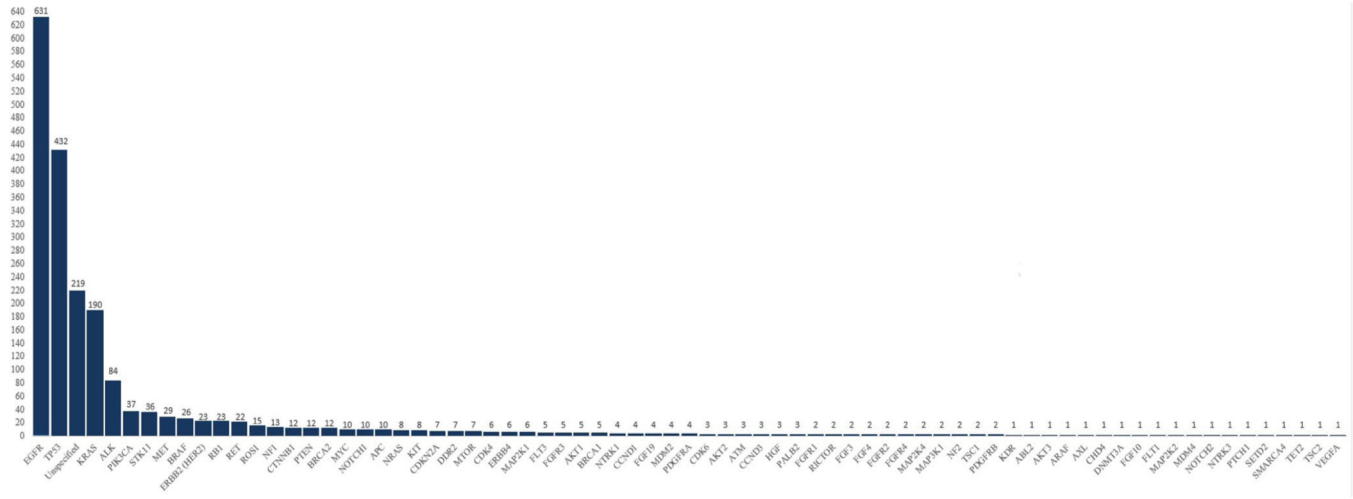

Fig. 3.

Gene sample sizes. A bar chart representation of the total sample sizes of every gene included in the final analysis

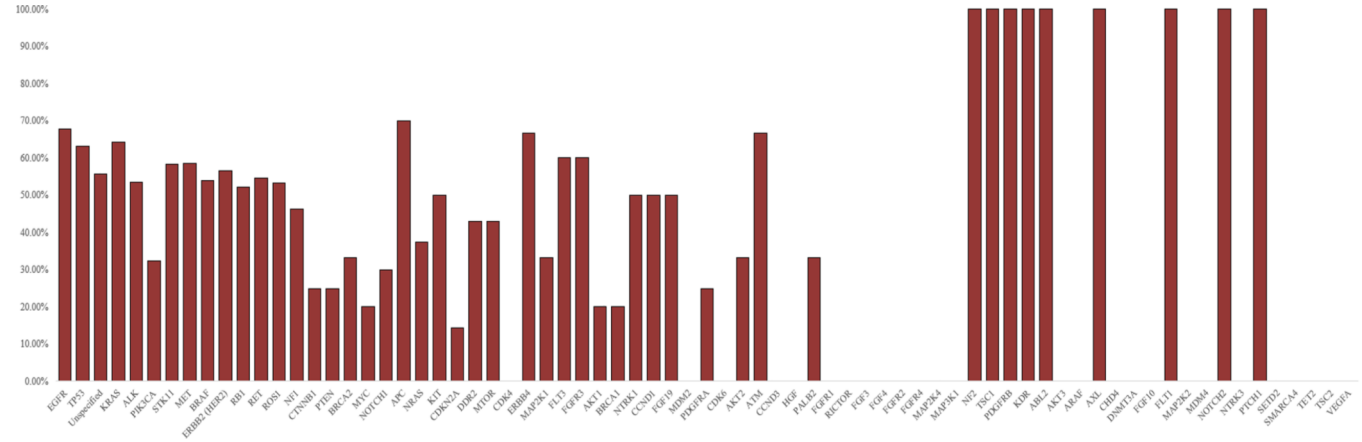

Fig. 4.

Gene cumulative positive percent agreement (PPA) rates. A bar chart representation of the cumulative PPA rates for every gene, when taking into account all studies included in the final analysis

Table 1

Technical specifcations of the next-generation sequencing assays used in all studies included in the analysis, in chronological order, according to the date of frst online publication

Author	Country	Tissue NGS platform	Tissue NGS panel	# of genes	Coverage	Detection limit	Liquid biopsy NGS platform	Liquid biopsy NGS panel	# of genes	Coverage	Detection limit	# of patients
Couraud et al. (2014)	France	IonTorrent PGM sequencer	Custom	5	1000×	5%	IonTorrent PGM sequencer	Custom	5	10,000 ×	0.13%	38
Thress et al. 2015	USA	Illumina MiSeq	Vall d'Hebron Institute of Oncology	57	3000 ×	3%	Illumina HiSeq	Qiagen GeneRead Lung Cancer v1	20	30,000 ×	N/A	2
Paweletz et al. (2016)	USA	N/A	N/A	N/A	N/A	N/A	Illumina	N/A	N/A	N/A	N/A	31
Vanni et al. 2015	Italy	IonTorrent PGM sequencer	Ion AmpliSeq Colon and Lung Cancer Panel v1	22	> 500 ×	5%	IonTorrent PGM sequencer	Ion AmpliSeq Colon and Lung Cancer v1	22	> 500 ×	5%	2
Rachiglio et al. (2016)	Italy	IonTorrent PGM sequencer	Oncomine Solid Tumour DNA kit	22	N/A	2%	IonTorrent PGM sequencer	Oncomine Solid Tumour DNA kit	22	N/A	2%	19
Villaflor et al. 2016	USA	N/A	Multiple	< 10 to > 300	N/A	N/A	Illumina HiSeq 2500	Guardant360	54/68	10,000 ×	0.10%	4
Kaisaki et al. 2016	UK	IonTorrent PGM sequencer	Ion Ampliseq Cancer Hotspot v1/v2	46/50	N/A	0.50%	IonTorrent PGM sequencer	Ion	46/50	N/A	0.50%	3
Iwama et al. 2017	Japan	Ion Proton Sequencer	Ion AmpliSeq Colon and Lung Cancer Panel v2	22	N/A	N/A	Ion Proton Sequencer	Ion AmpliSeq Colon and Lung Cancer v2	22	N/A	0.50%	32
Yao et al. 2017	China	Illumina HiSeq	N/A	40	855.17 ×	0.20%	Illumina HiSeq	N/A	40	2022.29 ×	0.20%	25
Pécuchet et al. (2016)	France	Ion Proton Sequencer	Ion AmpliSeq Colon and Lung Cancer v2	32	N/A	0.1%[a], 0.3%[b]	Ion Proton Sequencer	Ion AmpliSeq Colon and Lung Cancer v2	32	N/A	0.1%[a], 0.3%[b]	108
Xu et al. 2017	China	Illumina HiSeq 2500	Accu-Act	61	> 500 ×	N/A	Illumina HiSeq 2500	Accu-Act	61	7000 ×	N/A	5
Shwaederle et al. (2017)	USA	N/A	Foundation-One CDx	324	N/A	N/A	N/A	Guardant360	54/68/70	15,000 ×	N/A	26

Author	Country	Tissue NGS platform	Tissue NGS panel	# of genes	Coverage	Detection limit	Liquid biopsy NGS platform	Liquid biopsy NGS panel	# of genes	Coverage	Detection limit	# of patients
Veldore et al. 2018	India	Illumina HiSeq 2500	N/A	N/A	~ 100,000 ×	0.01%	Illumina HiSeq 2500	N/A	N/A	~ 100,000 ×	0.01%	4
Dagogo-Jack et al. (2018)	USA	HiSeq	Foundation One; Oncopanel	N/A	N/A	N/A	Illumina HiSeq 2500	PanCancer v3	566	1195 ×	0.50%	10
Liu et al. 2018	China	Ion Proton Sequencer	Beijing Genomics Institute Lung Cancer	146	372 ×	1%	Ion Proton Sequencer	Beijing Genomics Institute Lung Cancer	146	938 ×	0.01%	25
Toor et al. 2018	USA	N/A	Caris; Paradigm	592; 125	N/A	N/A	HiSeq 2500	Genomics	68–73	N/A	N/A	8
Hu et al. 2018	USA	Ion Proton	Genomics	275–443	N/A	N/A	Ion Proton	Institute	275–443	N/A	N/A	14
Yang et al. 2018	China	Illumina NextSeq 500	Institute	63	500 ×	5%	Illumina NextSeq 500	Lung	63	3000 ×	0.30%	56
McCoach et al. (2018)	USA	N/A	N/A	N/A	N/A	N/A	Illumina HiSeq 2500	Guardant360	70a/3b/18d/6c	10,000 ×	0.04%[a], 0.02%[b], 0.04%[c], 2.12[d]	2
Li et al. 2018	China	Illumina NextSeq 500	N/A	168	2000 ×	N/A	Illumina NextSeq 500	N/A	168	10,000 ×	2.25[d, e], 2.50[d] / 1.75[d, e]	13
Vollbrecht et al. (2018)	Germany	IonTorrent S5 XL	Ion AmpliSeq Colon and Lung Cancer v2	32	N/A	N/A	IonTorrent S5 XL	Ion AmpliSeq Colon and Lung Cancer v2; Oncomine Lung cfDNA	32; 11	N/A	N/A	21
Garcia et al. 2018	France	IonTorrent PGM sequencer	Custom AmpliSeq	N/A	N/A	N/A	Illumina NextSeq 500	SWIFT-56G Oncology	56	> 500 ×	0.5%	15
Jin et al. 2018	China	N/A	GeneseeqOne Pan-Cancer	416	600 ×	5%	N/A	GeneseeqOne Pan-Cancer	416	2000 ×	0.1%	69
Guo et al. 2018	China	Illumina NextSeq 500	Illumina NextSeq 500	22	2000 ×	N/A	Illumina NextSeq 500	Lung Cancer	17	20,000 ×	0.1%[e], 0.2%	10
Lam et al. 2019	USA	IonTorrent PGM sequencer	IonTorrent AmpliSeq Cancer;	46; 143	N/A	N/A	N/A	Guardant360	54/68/70	8,000–15,000 ×	N/A	2

Author	Country	Tissue NGS platform	Tissue NGS panel	# of genes	Coverage	Detection limit	Liquid biopsy NGS platform	Liquid biopsy NGS panel	# of genes	Coverage	Detection limit	# of patients
			Oncomine Cancer									
Aggarwal et al. (2019)	USA	N/A	Center for Personalized Diagnostics; Comprehensive Solid Tumor HaloPlexHS v2.0; Penn Precision	47; 153; 20	N/A	N/A	Illumina Hi-Seq 2500	Guardant360	73	N/A	N/A	86
Sabari et al. 2018	USA	N/A	MSKIMPACT	468	500–1000 ×	N/A	Illumina NextSeq	ResBio ctDx-Lung; Archer REVEAL ctDNA 28	21; 28	N/A	0.10%	106
Ge et al. 2019	China	Illumina NextSeq 500, HiSeq 4000, MiSeq, MiniSeq	ddCAP on tissue; OncoAim	10; 59	N/A	5%	Illumina NextSeq 500	ddCAP	10	N/A	5%	13
Wu et al. 2019	China	Illumina HiSeq 4000	GeneseeqOne	416	800 ×	–	Illumina HiSeq 4000	GeneseeqOne	416	3000 ×	2.00[d], 0.65[d]	48
Chen et al. 2019	China	Illumina NovaSeq 6000	Genecast, Beijing, China	1407	2400 ×	2%	Illumina NovaSeq 6000	Genecast, Beijing, China	1407	3000 ×	0.20%	3
Pritchett et al. 2019	USA	Illumina NextSeq 500, Illumina MiSeq	ArcherDx FusionPlex Solid Tumor	52	N/A	N/A	Illumina NextSeq 500	InVisionFirst	36	N/A	N/A	178
Supplee et al. 2019	USA	N/A	N/A	N/A	N/A	N/A	Illumina NextSeq 500	Guardant360 v14.0–17.0; ctDx-Lung kit	-; 20	N/A	–	13
Tang et al. 2019	China	Illumina NextSeq 500	N/A	56	N/A	N/A	Illumina NextSeq 500	N/A	56	N/A	N/A	47
Tong et al. 2019	China	Illumina HiSeq 4000	GeneseeqOne	416	1000 ×	0.50%	Illumina HiSeq 4000	GeneseeqOne	416	10,000 ×	0.10%	28
Horn et al. 2019	USA	Illumina NextSeq	N/A	12	N/A	N/A	Illumina NextSeq	N/A	12	N/A	N/A	18

Author	Country	Tissue NGS platform	Tissue NGS panel	# of genes	Coverage	Detection limit	Liquid biopsy NGS platform	Liquid biopsy NGS panel	# of genes	Coverage	Detection limit	# of patients
Streubel et al. 2019	Germany	IonTorrent S5XL	Ion AmpliSeq Colon and Lung Cancer	32	N/A	N/A	IonTorrent S5XL	Oncomine Lung cfDNA	11	N/A	0.10%	10
Tran et al. 2019	Vietnam	Illumina NextSeq 550	N/A	4	N/A	1%	Illumina NextSeq	N/A	4	10,000 ×	1%	21
Papadopoulou et al. 2019	GreecIonTorrent PGM sequencer4000	Custom Ion AmpliSeq	23	N/A	N/A	IonTorrent PGM sequencer	Custom Ion AmpliSeq	23	N/A	N/A	26	

N/A not available; # of genes=number of genes; # of patients= number of patients included in our analysis

[a] Single Nucleotide Variants

[b] Indels

[c] Fusions

[d] Copy Number Alterations

[e] Hotspot mutations