Plasma cell-free DNA genotyping is an alternative to tissue genotyping, particularly when tissue specimens are insufficient or unavailable, and provides critical information that can be used to guide treatment decisions in managing patients with non-small cell lung cancer (NSCLC). In this article, we review the evolution of plasma cfDNA genotyping from an emerging concept, through development of analytical methods, to its clinical applications as a standard-of-care tool in NSCLC.

The number of driver or resistance mutations recommended for testing in NSCLC continues to increase. Because of the expanding list of therapeutically relevant variants, comprehensive testing to investigate larger regions of multiple genes in a single run is often preferable and saves on time and cost, compared with performing serial single-gene assays. Recent advances in nucleic acid next-generation sequencing have led to a rapid expansion in cfDNA genotyping technologies. Analytic assays that have received regulatory approval are now routinely used as diagnostic companions in the setting of metastatic NSCLC. As the demand for plasma-based technologies increases, more regulatory approvals of cfDNA genotyping assays are expected in the future.

Plasma cfDNA genotyping is currently aiding oncologists in the delivery of personalized care by facilitating matching of patients with targeted therapy and monitoring emergence of resistance to therapy. Further advances underway to increase assay sensitivity and specificity will potentially expand the use of plasma cfDNA genotyping in early cancer detection, monitoring response to therapy, detection of minimal residual disease, and evaluation of tumor mutational burden in NSCLC. The Oncologist 2021;26:e1812–e1821

Implications for Practice: Plasma cell-free DNA (cfDNA) genotyping offers an alternative to tissue genotyping, particularly when tissue specimens are insufficient or unavailable. Advances in cfDNA genotyping technologies have led to analytic assays that are now routinely used to aid oncologists in the delivery of personalized care by facilitating matching of patients with targeted therapy and monitoring emergence of resistance to therapy. Further advances currently underway to increase assay sensitivity and specificity will potentially expand the use of plasma cfDNA genotyping in early cancer detection, monitoring response to therapy, detection of minimal residual disease, and evaluation of tumor mutational burden in non-small cell lung cancer.
available, including alterations in epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ROS oncogene 1 (ROS1), B-Raf proto-oncogene (BRAF), MET proto-oncogene (MET), RET proto-oncogene (RET), and neurotrophic receptor tyrosine kinase (NTRK). The Kirsten rat sarcoma viral oncogene homolog (KRAS) gene mutation KRAS G12C is another oncogenic driver mutation that is now actionable based on the recent approval of a targeted therapy for KRAS G12C-mutant NSCLC by the U.S. Food and Drug Administration (FDA) [4]. Another emerging actionable biomarker is EBBB2 (HER2) [1–3]. Guidelines also recommend testing for the expression level of tumor-derived programmed cell death ligand 1 (PD-L1) [1–3]. The number of driver or resistance mutations recommended to be tested in NSCLC continues to increase [5]. Because of the expanding list of therapeutic targets, broader comprehensive testing to investigate larger regions of multiple genes in a single run is preferable and saves time and cost, compared with performing serial single-gene assays [5].

Genotyping has historically been performed using formalin-fixed, paraffin-embedded specimens from tissue biopsies [1–3], which often requires substantial amounts of tissue to achieve the minimum DNA or RNA input required for assay sensitivity. Small biopsy specimens with limited tumor cellularity might only be sufficient for morphological diagnosis, histologic subtype classification, and PD-L1 staining, but the tissue might be insufficient in quantity and/or quality for genotyping. Although tissue genotyping is considered the gold standard for molecular profiling [3, 5–7], this is often not feasible for all patients and tumor types [8]. This is particularly relevant in lung cancer in which the tumor site may be difficult to access and obtaining adequate tissue specimens for comprehensive genotyping often necessitates invasive procedures. In NSCLC specifically, tissue biopsy has been shown to be inadequate for molecular testing [8–10].

Liquid biopsy involves the analysis of tumor-derived material in body fluids, including blood, urine, saliva, pleural effusion, and cerebrospinal fluid [11, 12]. The most tested analyte in blood is plasma cell-free DNA (cfDNA), composed of DNA fragments that are passively released from apoptotic or necrotic cells or released from digestion of cells by phagocytes [8, 13–15]. Plasma cfDNA contains circulating tumor DNA (ctDNA), which is a subset of DNA specifically shed from tumor cells [16]. The amount of ctDNA in plasma cfDNA varies depending on the tumor type, tumor stage and burden, tumor location, vascularization, apoptotic rate, metastatic potential of the cancer cells, and factors affecting the patient’s blood volume [8, 15, 16]. The half-life of ctDNA in the bloodstream varies from approximately 16 minutes to 2.5 hours, making ctDNA a “real-time” molecular marker of disease [17, 18]. Other tested analytes in blood include tumor-derived extracellular vesicles (EVs), circulating tumor cells (CTCs), messenger RNA (mRNA), and microRNA (miRNA). EVs are lipid bilayer-encapsulated vesicles of ~30 to 2,000 nm that contain DNA, RNA, proteins, and lipids from the tumor of origin and are believed to play a critical role in intercellular communication [19–21]. CTCs are detectable in some but not all cancers. Although EVs and CTCs are promising liquid biopsy analytes, they are not yet routinely used for clinical genotyping in NSCLC. mRNA and miRNA are currently still being evaluated as biomarkers in the research setting [22] and are not yet used for molecular testing in the clinic.

Plasma cfDNA genotyping technologies have recently advanced and increased the ability to identify oncogenic driver mutations. They are now routinely used to aid oncologists in the delivery of personalized care by facilitating matching of patients with targeted therapy and monitoring emergence of resistance to therapy. Tumor-specific biomarkers that can be identified in plasma include somatic point mutations, insertions/deletions (indels), amplifications, gene fusions, mRNA splice variants, and tumor proteins [12]. In this article, we review the evolution of plasma cfDNA genotyping from an emerging concept, through development of analytical methods, to its clinical applications as a standard-of-care tool in NSCLC.

**Evolution of Analytical Methods for cfDNA Genotyping**

Advances in nucleic acid-based cfDNA genotyping have lead to the development of several analytical methods to identify somatic driver or resistance mutations (Table 1). These methods range from polymerase chain reaction (PCR)-based approaches to broader coverage next-generation sequencing (NGS).

PCR-based approaches can detect DNA alterations by amplifying small DNA regions of interest known as hotspots (Table 1). These assays can be used to detect driver mutations, such as those found in EGFR, KRAS, or BRAF [23–25]. The assays can also be used to identify the emergence of predefined treatment-resistant clones in blood, often several weeks before imaging methods can confirm clinical progression, and to monitor emergence of specific mutations over the course of treatment [24, 26–28].

NGS, or high throughput sequencing or massively parallel sequencing, uses distinct approaches to the biochemistry of DNA sequencing to simultaneously perform millions of sequencing reactions. NGS is designed to investigate large regions of multiple genes in a single run and can detect somatic mutations, including single-nucleotide variations (SNVs), copy number variations (CNVs), indels, and gene fusions [11, 26]. NGS platforms include whole genome sequencing, whole exome sequencing, hybrid capture panels, and amplicon sequencing panels (Table 1). The broad spectrum of genomic information from NGS surpasses that from PCR and fluorescence in situ hybridization techniques combined. However, the increased number of genes evaluable by NGS often reduces sensitivity, sometimes leading to the option of selecting PCR assays to maximize sensitivity for detection of a patient-specific single-nucleotide variant. More recently, cfDNA genotyping assays that combine PCR and NGS technologies have been developed to maximize on the advantages of both platforms.

**FDA-Approved Assays for Plasma cfDNA Genotyping in NSCLC**

Several plasma cfDNA genotyping assays for NSCLC are in clinical practice, including PCR- and NGS-based assays. Most
### Table 1. Analytical methods for plasma cfDNA genotyping

| Assay category | Assay technology/name | Analysis scale | Method | Limit of detection (as % of cfDNA) | Advantages |
|----------------|------------------------|----------------|--------|-----------------------------------|------------|
| Quantitative PCR | qPCR | Single mutations or panels of known and well-characterized mutations | Preferentially amplifies rare mutant DNA molecules | ~0.1%–1% | Highly sensitive and specific. Low turnaround time. |
| | cobas EGFR Mutation Test v2 (FDA and EMA approved) | | | | |
| | therascreen EGFR Plasma KRQ, PCR kit | | | | |
| | PANAMutyper R EGFR kit | | | | |
| Digital PCR | ddPCR | Single locus or multiplexed assays | Partitions target DNA into different reactions for massively parallel qPCR | ~0.04%–0.1% | Highly sensitive and specific. |
| | Bio-Rad QX200 ddPCR Dx system | | | | |
| | BEAMing | | | | |
| | OncoBEAM EGFR kit | | | | |
| NGS whole genome and exome sequencing | Whole genome sequencing | Genome wide Exome wide | NGS of whole genome or whole exome | ~10% for whole genome ~5% for whole exome | Evaluation of entire genome or exome can lead to discovery of new targets. Exome sequencing allows rapid aneuploidy assessment with lower cost than whole genome sequencing. Discovery of mechanisms of resistance. |
| | Roche/454 | | | | |
| | Ion Torrent: Proton/PGM | | | | |
| | Illumina Sequencing (Solexa) | | | | |
| | SOLiD | | | | |
| Hybrid capture-based NGS | Targeted NGS sequencing | Targeted sequencing of captured regions of the genome | Subset of exome is hybridized to biotinylated probes and captured for NGS analysis | ~0.001%–0.5% | Highly sensitive. Simultaneous detection of predetermined genes of interest. Comprehensive detection of known and unknown mutations. Lower cost and less bioinformatics data compared with whole genome sequencing. |
| | Guardant360 CDx (FDA approved) | | | | |
| | Foundation One Liquid CDx (FDA approved) | | | | |
| | Resolution dsDX Lung | | | | |
| | CAPP-Seq | | | | |
| | TEC-Seq | | | | |
| Multiplex PCR-based NGS | Targeted NGS sequencing | Targeted sequencing of predefined regions | PCR amplification enriches targets before NGS analysis | ~0.01–2.0% | Highly sensitive. |
| | TAm-Seq | | | | |
| | Enhanced TAm-Seq | | | | |
| | Safe-SeqS | | | | |
| | Natera | | | | |
| Combination approaches (including DNA + biomarkers) | CAPP-Seq + GRP | Single locus to genome wide | Combines different ctDNA detection methods, sometimes including protein biomarkers | Variable | Improved detection compared with standard ctDNA analysis alone in certain settings. |
| | CancerSEEK | | | | |
| | UrOSEEK | | | | |

*Abbreviations: BEAMing, beads, emulsion, amplification and magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; ddPCR, digital droplet polymerase chain reaction; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; NGS, next-generation sequencing; PGM, personal genome machine; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; qPCR, quantitative polymerase chain reaction; Safe-SeqS, Safe-Sequence System; SOLiD, sequencing by oligonucleotide ligation and detection; TAm-Seq, tagged-amplicon deep sequencing.*
are laboratory-developed tests (LDTs) [29] and are performed in Clinical Laboratory Improvement Amendments (CLIA)–certified laboratories and monitored by the Centers for Medicare & Medicaid Services (CMS). As such, most tests in clinical practice are used in the market as LDTs, with a few that have received regulatory approval. A number of tests have received FDA approval as companion diagnostic (CDx) assays or have received FDA’s breakthrough device designation.

**Cobas EGFR Mutation Test**

The cobas EGFR Mutation Test v2 (Roche Molecular Systems Inc., Pleasanton, CA; https://diagnostics.roche.com) was the first commercially available plasma-genotyping test to receive FDA approval in June 2016 for the identification of patients with EGFR driver mutations who may benefit from treatment with tyrosine kinase inhibitors (TKIs) [30, 31]. The test kit is based on real-time quantitative PCR (qPCR), which differs from classic PCR in that the intensity of the fluorescent light emitted by the probes is read every cycle, allowing for an estimate of the quantity of the loaded sample to be derived based on the number of cycles needed to obtain a threshold fluorescent signal [32]. The cobas EGFR assay is designed to detect G719X substitutions in exon 18, deletion mutations in exon 19, T790M and S768I substitutions and insertions in exon 20, and L858R and L861Q substitutions in exon 21 and is used as a CDx test for erlotinib [30]. Clinical trials in patients with advanced NSCLC showed that patients with exon 19 deletions or L858R substitutions in exon 21 who were treated with erlotinib as first-line treatment were likely to experience clinical benefit compared with patients treated with chemotherapy [33, 34].

**Guardant360 CDx**

Guardant360 CDx (Guardant Health, Redwood City, CA; https://www.guardanthealth.com) is a commercially available targeted NGS panel that uses hybridization-capture technology coupled with NGS. A subset of the exome or predetermined DNA sequences of interest are hybridized to biotinylated probes and captured using streptavidin, and then the captured DNA is sequenced by NGS. To date, Guardant360 CDx can detect cDNA mutations (74 genes), amplifications (18 genes), fusions (6 genes), and indels (23 genes) [35, 36]. This includes detection of the National Comprehensive Cancer Network (NCCN) clinical guideline-recommended biomarker mutations (EGFR, ALK, ROS1, BRAF, RET, MET, NTRK, KRAS, and ERBB2 [HER2]) for NSCLC. The FDA granted Guardant360 CDx breakthrough device status in January 2018 [37] and approved it for comprehensive genomic profiling in patients with any solid malignant neoplasm and as a CDx for the EGFR inhibitor osimertinib in August 2020 [38]. In May 2021, the FDA approved Guardant360 CDx for comprehensive genomic profiling in adult patients with KRAS G12C-mutated locally advanced or metastatic NSCLC and as a CDx for the recently approved KRAS G12C inhibitor sotorasib [4, 39].

In a prospective cohort study involving 323 patients with NSCLC [8], the addition of plasma cfDNA genotyping to tissue-based genotyping markedly increased identification of targetable mutations, facilitating delivery of therapies that matched the patients’ DNA mutation. In 229 patients, actionable mutations were detected in 20.5% of patients with tissue genotyping alone, which increased to 35.8% with the use of Guardant360 CDx [8].

Guardant360 CDx was subsequently shown to have the same rate of biomarker detection as traditional tissue genotyping in the multi-institution, head-to-head, Noninvasive versus Invasive Lung Evaluation (NILE) study [40] that analyzed 282 patients with newly diagnosed advanced NSCLC. Biomarkers were detected in a higher proportion of patients with plasma cfDNA genotyping than with tissue genotyping (77 patients, 27.3% vs. 60 patients, 21.3%; p < .0001), with the cfDNA genotyping results delivered significantly faster than the tissue genotyping results (median, 9 days vs. 15 days) [40]. A recent retrospective analysis of samples from the FLAURA [41] and AURA3 [42] studies also showed that Guardant360 CDx had similar diagnostic performance to the cobas EGFR Mutation Test in identifying patients with NSCLC who were positive for EGFR exon 19 deletions and T790M and L858R substitutions eligible for treatment with osimertinib [43].

**FoundationOne Liquid CDx**

FoundationOne Liquid CDx (Foundation Medicine, Cambridge, MA; https://www.foundationmedicine.com) is a commercially available targeted NGS panel that uses hybridization-capture technology to detect more than 300 cancer-related genes and multiple genomic signatures such as tumor mutational burden (TMB) and microsatellite instability. In August 2020, the FDA approved the FoundationOne Liquid CDx for comprehensive genomic profiling in patients with any solid tumor and for use as a CDx assay to identify patients with NSCLC who may benefit from treatment with three first-line TKIs, gefitinib, osimertinib, and erlotinib [44]. Clinical validity of FoundationOne Liquid CDx as an aid in identifying patients with advanced NSCLC who may be eligible for treatment with the three first-line TKIs was established through a noninferiority study that compared FoundationOne Liquid CDx with the cobas EGFR Mutation Test in identifying EGFR exon 19 deletion and EGFR exon 21 L858R substitutions involving 177 samples from patients with NSCLC [45].

**Emerging Evidence to Support Use of Plasma cfDNA Genotyping in Oncology**

Tissue genotyping, coupled with imaging (CT and MRI), remains the standard of care in oncology. Nevertheless, plasma cfDNA genotyping has notable advantages over tissue genotyping. Blood draws are less invasive and less risky, making plasma cfDNA genotyping more appealing to clinicians and patients. The turnaround time for plasma cfDNA genotyping is less than that for tissue genotyping, as the latter requires wait time for scheduling and performing the biopsy and tissue processing. cfDNA genotyping delivered results faster than tissue genotyping in the NILE study (median, 9 days vs. 15 days) [40]. In another study [46], the median time from pathologic diagnosis to delivery of genotyping results was 3 days with cfDNA plasma NGS performed before or in parallel with the diagnostic procedure.
versus 18 days with cfDNA plasma NGS and 35.5 days with tissue NGS, respectively, performed at the end of the diagnostic procedure per the routine standard of care. Technological advances have made it possible for clinicians to process blood on-site or ship it to central laboratories for testing, limiting the earlier challenges of variable results because of sample collection and pretreatment conditions that have the potential to affect genotyping results. More importantly, plasma cfDNA genotyping provides information on the complete heterogeneity (both spatial and temporal) of the tumor as compared with a snapshot from a single needle biopsy. Furthermore, repeated sampling is more feasible with plasma cfDNA genotyping and allows real-time monitoring of treatment efficacy, development of resistance, and cancer progression [11, 47].

A few studies have shown concordance between results obtained from plasma and tissue genotyping [48]. A study that evaluated orthogonal plasma and tissue genotyping using NGS-based digital sequencing in >750 patients with solid tumors demonstrated high accuracy and specificity (>99% positive percent agreement and negative percent agreement and >92% positive predictive values) [35]. As already described in the section on Guardant360 CDx, the NILE study [40] demonstrated that Guardant360 CDx identified guideline-recommended biomarkers at a rate at least as high as tissue genotyping in untreated metastatic NSCLCs, with high concordance, lower turnaround time, and higher biomarker discovery rate [40], and a prospective cohort study of patients with NSCLC [8] demonstrated that addition of plasma cfDNA genotyping to tissue genotyping markedly improved identification of targetable mutations, facilitating delivery of therapies that matched the patients’ DNA mutation. A retrospective subanalysis of the IFUM trial [49] that evaluated whether ctDNA could be used as a surrogate for determination of EGFR status using PCR to analyze exon 19 deletions, L858R mutation, and T790M mutation in paired tissue and plasma samples showed an agreement of 94.3% between 652 matched tumor and plasma samples, independent of mutation subtype; the test’s sensitivity and specificity were 65.7% and 99.8%, respectively [49]. In a recent retrospective meta-analysis of 25 studies involving 4,881 lung cancer cases [50], the sensitivity and specificity of EGFR mutation as detected by PCR genotyping of plasma cfDNA compared with matched tissue genotyping were 65.3% and 98.2%, respectively.

A recent position publication by the International Association for the Study of Lung Cancer [11] recommends implementation of plasma cfDNA genotyping in the clinic in a number of relevant therapeutic settings and provides algorithms to aid practicing oncologists in making treatment decisions for patients with advanced, treatment-naive NSCLC and patients with progressive or recurrent NSCLC. According to a previously published algorithm developed as a tool to aid in clinical decisions [51], plasma cfDNA genotyping can be used to test for detectable driver alterations if tissue is unavailable or inadequate for comprehensive genotyping or can be performed concurrently with tissue sequencing when tissue samples are available. Given the concordance between results obtained from plasma cfDNA and tissue genotyping [8, 35, 40, 49, 50] and the previously published recommendations [11, 51], a consideration is to use plasma cfDNA genotyping as the initial method to assess specimens from patients with NSCLC and provide critical information that could potentially be used to guide clinical decisions in some situations. In cases in which results from plasma cfDNA genotyping are inconclusive, tissue genotyping can then be performed. However, the approach of initially using plasma cfDNA genotyping alone and then using tissue genotyping only if results from plasma genotyping are negative is not currently considered standard of care; this approach should be reserved for special situations, such as in the context of acquired resistance or in selected cases at diagnosis [51].

**Limitations of Plasma cfDNA Genotyping**

Several limitations must be taken into consideration in the use of plasma cfDNA genotyping. For advanced NSCLC, the presence of cfDNA has been reported in approximately 85% of cases [52]. However, treatment-naïve patients with slow-growing tumors may be at risk of false-negative findings [11]. A recent retrospective study that evaluated plasma samples from the FLAURA [41] and AURA3 [42] trials using the cobas EGFR Mutation Test demonstrated that EGFR exon 19 deletion and L858R and T790M mutations were not detected in plasma of a subset of patients identified to have EGFR-mutant NSCLC by tissue genotyping as not all tumors shed detectable levels of mutated ctDNA into systemic circulation [43]. Patients who have visceral or extrathoracic disease are more likely to have detectable ctDNA in blood [8]. Because of this inherent limitation, it is recommended that, although positive findings from plasma cfDNA genotyping are clinically actionable, negative findings should be considered inconclusive and warrant tissue genotyping [11]. Another consideration in the context of NGS is the potential for false-positive findings arising from somatic mutations in hematopoietic stem cells (clonal hematopoiesis of indeterminate potential; CHIP) [53–55]. Because most cfDNA fragments in blood originate from hematopoietic cells, these somatic mutations could be falsely identified as tumor-specific mutations. However, most current algorithms filter out CHIP false positives [53, 56]. Additionally, plasma genotyping does not capture histologic transformation, which is a phenotypic switch between tumor histologies that can occur in patients with oncogene addicted NSCLC leading to resistance to therapy in the absence of significant changes in mutational profile seen in ctDNA [57, 58].

Reimbursement for plasma cfDNA genotyping is currently limited. CMS proposes that the evidence is sufficient to expand coverage of NGS as a diagnostic laboratory test when performed in a CLIA-certified laboratory and in limited situations, restricting coverage to monitoring relapse, metastasis, or advanced stage III and IV cancers [59]. Accumulation of more extensive data from prospective trials of plasma cfDNA genotyping such as the NILE study [40], recommendations by clinical guidelines, and FDA approval of assays would likely increase the support of payors for plasma cfDNA genotyping and enable its broad adoption as standard of care in oncology.
Current Clinical Applications of Plasma cfDNA Genotyping

Current Clinical Applications of Plasma cfDNA Genotyping
Plasma cfDNA genotyping is routinely used in clinical practice as a complement to tissue genotyping to assist in guiding therapy at diagnosis and monitoring emergence of resistance to therapy [11] (Fig. 1).

Genotyping NSCLC at Diagnosis to Guide Therapy Decisions
In treatment-naïve patients, plasma cfDNA genotyping should be considered at the time of initial diagnosis in all patients who need tumor genotyping and is particularly recommended when tumor tissue is scarce or unavailable or when a significant delay (typically ≥2 weeks) is expected in obtaining tumor tissue or tissue sequencing results [11]. Current clinical guidelines [1–3] recommend testing treatment-naïve patients for known oncogenic driver alterations in EGFR, ALK, ROS1, BRAF, MET, RET, and NTRK, and if present, using one of several targeted therapies directed against the known gene mutations.

Monitoring Emergence of Resistance to Therapy
In addition to detecting mutations among treatment-naïve patients, plasma cfDNA genotyping can also be used to track the emergence of resistance mutations during therapy. Patients with EGFR-mutated or ALK-rearranged NSCLCs treated with the indicated TKIs have been shown to develop resistance mutations associated with disease progression, and to benefit from successive lines of TKIs that can specifically target these new mutations [11]. Monitoring for the appearance of resistance mutations requires repeated tumor sampling to assess the mechanism of resistance, which requires performing serial biopsies at the site of tumor progression and is more suited to plasma versus tissue genotyping [11, 60]. After identification of the specific resistance mutation, the next step is matching the mutations to the most appropriate treatment agent [60]. Plasma cfDNA genotyping offers the advantage of early, noninvasive detection of resistance mutations. A comparison of plasma cfDNA genotyping and CT imaging in detecting early progression as indicated by the emergence of the T790M mutation showed plasma cfDNA genotyping detecting the mutation earlier than CT by 51 days in one study [61] and 103 days in another [62]. Of note, longitudinal monitoring of changes in ctDNA using plasma cfDNA genotyping is not a standard approach outside the research setting. Even if plasma cfDNA genotyping identifies resistance mutations that indicate early progression, the current clinical recommendation is to first confirm radiographic and clinical progression before modifying therapy. As such, plasma cfDNA genotyping results can be used to plan subsequent lines of therapy but not for making decisions to change therapy.

Future Clinical Applications of Plasma cfDNA Genotyping
Technical advances in plasma cfDNA genotyping to increase sensitivity and specificity are opening new potential applications including use in early cancer detection, monitoring response to therapy, detection of minimal residual disease (MRD), and evaluation of TMB [11, 63] (Fig. 1). These uses are likely to become part of routine clinical practice in the coming years.

Early Cancer Detection
Per NCCN guidelines [1], there is currently insufficient evidence to support use of plasma cfDNA genotyping for establishing primary lung cancer diagnosis as ctDNA shedding is low in early stages of NSCLC and ctDNA is not reliably detected with currently available technologies [26, 64, 65]. However, as sensitivity and specificity improve, plasma cfDNA genotyping could ultimately be used in NSCLC early detection and diagnosis. Leading technologies in this field have been granted FDA breakthrough device designation (Table 2) and include the GRAIL multicancer early detection platform (GRAIL; Menlo Park, CA; https://grail.com/), the CancerSEEK platform (Thrive Earlier Detection, Cambridge, MA; https://thrivedetect.com/) for cancer diagnosis, and the Ivy-Gene CORE Test (Helio Health, Irvine, CA; https://www.heliohealth.com) for detecting variable ctDNA methylation patterns to confirm presence of early-stage cancers. Another promising technology is the DELFI technology that specifically detects alternations in nucleosomal fragmentation profiles in plasma cfDNA from patients with cancer [66].

The most advanced of the technologies in development is the GRAIL platform that employs DNA methylation signatures to detect early-stage cancers. DNA methylation is a biological mechanism that controls genomic instructions that are carried out in the body [67]. The platform uses targeted bisulfite sequencing and machine learning to detect cfDNA methylation patterns and identify those that are abnormally methylated, with the additional ability of determining the tissue of origin of the ctDNA [68]. Use of this platform for population scale mass cancer screening is currently being validated in four clinical trials that enrolled a combined 180,000 participants in North America and the...
Monitoring Response to Therapy

Tumors can change mutation patterns or acquire new mutations over time [69]. Pretreatment ctDNA levels have been shown to be prognostic and on-treatment ctDNA levels to be predictive for patients with NSCLC receiving immune checkpoint inhibitors [70, 71]. In this context, plasma cfDNA genotyping to detect changes in ctDNA levels can be used to monitor the molecular make up of a patient’s tumor over time. This has the potential of sparing patients from undergoing repeated invasive procedures to obtain tissue biopsy samples and enables monitoring of the patients’ state of disease systemically (as deciphered from biomarkers in the blood), and not just relying on results from a single, localized cell population represented by a needle biopsy [11].

Detection of Minimal Residual Disease

Detection of residual ctDNA following surgery or curative treatment can be used as a surrogate for MRD, predicting future relapse. ctDNA levels may be low following treatment and therefore require highly sensitive assays for detection. A few studies have demonstrated the ability of ctDNA to detect post-treatment MRD, which was shown to be prognostic [72, 73]. The TRACERx study in early-stage lung cancer [73] demonstrated that MRD indicative of recurrence could be detected in plasma at a median of 70 days before imaging-confirmed relapse, suggesting the utility of plasma cfDNA genotyping in this setting. However, ctDNA analysis for MRD detection is not yet approved for clinical practice.

Evaluation of Tumor Mutational Burden

Studies have shown that an increase in somatic mutations present in tumor cells increases the potential for...
recognition of the tumor cells by the immune system [74]. The presence of mutations in the tumor generates antigens that are not expressed by normal cells (neoantigens), and the higher the TMB, the more the tumor is likely to be immunogenic [74]. Novel assays to measure TMB from blood have been developed, and these have demonstrated agreement of TMB derived from plasma and tissue genotyping [75, 76]. Additionally, evidence has emerged that a high TMB is associated with increased clinical activity of inhibitors of programmed cell death (PD) 1 and its ligand PD-L1 in NSCLC [76, 77]. As such, plasma TMB has now become an important cfDNA genotyping marker and can potentially be used to stratify patients likely to respond to immunotherapy [63]. The FDA-approved FoundationOne Liquid CDx includes TMB assessment as part of the platform.

Need for Prospective Trials to Assess the Clinical Utility of Plasma cfDNA Genotyping

Data from prospective clinical trials of plasma cfDNA genotyping are very limited, with clinical trial data available only from the NILE study [40]. Most current data supporting the clinical utility of plasma cfDNA genotyping comes from retrospective observational studies. Going forward, there is a need for more prospective clinical trials to evaluate use of plasma cfDNA genotyping in the clinic.

CONCLUSION

Plasma cfDNA genotyping has considerable potential in improving the management of patients with NSCLC as it offers an alternative when tissue biopsy specimens are insufficient or unfeasible. It also provides information on both spatial and temporal dynamic changes in tumor profiles that can be used to guide treatment decisions. Plasma cfDNA genotyping is currently being pursued in NSCLC, with some assays having received regulatory approval and having been put into clinical use; additional assays are in the development and validation stages. More regulatory approvals of plasma cfDNA genotyping assays are expected as the demand for plasma-based technologies is increasing in oncology. Numerous studies have shown that plasma cfDNA genotyping is feasible in clinical practice. Broad adoption of plasma cfDNA genotyping as a standard-of-care tool in oncology practice depends on gathering prospective data to validate assays and identifying the most effective testing strategies to implement at different stages of NSCLC.

Plasma cfDNA genotyping has evolved from an emerging concept and is currently aiding oncologists in the delivery of personalized care by facilitating matching of patients with targeted therapy and monitoring emergence of resistance to therapy. Further advances currently underway to increase assay sensitivity and specificity will potentially expand the use of plasma cfDNA genotyping in early cancer detection, monitoring response to therapy, detection of MRD, and measurement of TMB in NSCLC.

ACKNOWLEDGMENTS

We thank Byeong Yoon, Ph.D. (Amgen Inc.) for critical review of the manuscript and Martha Mutomba (on behalf of Amgen Inc.) for medical writing assistance. This study was supported by Amgen Inc. in the form of medical writing, submission fee, and open access publication.

AUTHOR CONTRIBUTIONS

Conception/design: Jhanelle Gray, Jeffrey C. Thompson, Erica L. Carpenter, Elab Elkhouly, Charu Aggarwal

Collection and/or assembly of data: Jhanelle Gray, Jeffrey C. Thompson, Erica L. Carpenter, Elab Elkhouly, Charu Aggarwal

Data analysis and interpretation: Jhanelle Gray, Jeffrey C. Thompson, Erica L. Carpenter, Elab Elkhouly, Charu Aggarwal

Manuscript writing: Jhanelle Gray, Jeffrey C. Thompson, Erica L. Carpenter, Elab Elkhouly, Charu Aggarwal

Final approval of manuscript: Jhanelle Gray, Jeffrey C. Thompson, Erica L. Carpenter, Elab Elkhouly, Charu Aggarwal

DISCLOSURES

Jhanelle Gray: AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, GI Therapeutics, Merck, Pfizer, Novartis, Ludwig Institute for Cancer Research (RF); AstraZeneca, Blueprint Medicines, Bristol-Myers Squibb, EMD Serono, Merck KGaA, Invivata, Merck, Novartis (C/A). Jeffrey C. Thompson: AstraZeneca, Guardant Health (SAB); Erica L. Carpenter: Janssen, Becton Dickinson, United Health Group, Merck (C/A); Guardant Health, Immedex, AstraZeneca, FoxChase Cancer Center, Bristol-Myers Squibb (H); Menarini, VPS, American Gastroenterological Association, Immedex, AstraZeneca, Foundation Medicine, Gordon Conference, Americas Hepato-Pancreato-Biliary Association (Other—travel assistance). Ehab Elkhouly: Amgen (E), Amgen (OI). Charu Aggarwal: Merck, MacroGenics, Novartis, AstraZeneca (RF—institution); AstraZeneca, Blueprint, Celgene, Eli Lilly & Co., Merck, Daichi Sankyo (SAB).

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (OI) Expert testimony; (H) Honoraria received; (D) Ownership interests; (IP) Intellectual property rights/inventor/patient holder; (SAB) Scientific advisory board.

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