Multiplex Detection of Clinically Relevant Mutations in Liquid Biopsies of Cancer Patients Using a Hybridization-Based Platform

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BACKGROUND: With the advent of precision oncology, liquid biopsies are quickly gaining acceptance in the clinical setting. However, in some cases, the amount of DNA isolated is insufficient for Next-Generation Sequencing (NGS) analysis. The nCounter platform could be an alternative, but it has never been explored for detection of clinically relevant alterations in fluids.

METHODS: Circulating-free DNA (cfDNA) was purified from blood, cerebrospinal fluid, and ascites of patients with cancer and analyzed with the nCounter 3 D Single Nucleotide Variant (SNV) Solid Tumor Panel, which allows for detection of 97 driver mutations in 24 genes.

RESULTS: Validation experiments revealed that the nCounter SNV panel could detect mutations at allelic fractions of 0.02–2% in samples with ≥5 pg mutant DNA/µL. In a retrospective analysis of 70 cfDNAs from patients with cancer, the panel successfully detected EGFR, KRAS, BRAF, PIK3CA, and NRAS mutations when compared with previous genotyping in the same liquid biopsies and paired tumor tissues (Cohen kappa of 0.96 (CI = 0.92–1.00) and 0.90 (CI = 0.74–1.00), respectively). In a prospective study including 91 liquid biopsies from patients with different malignancies, 90 yielded valid results with the SNV panel and mutations in EGFR, KRAS, BRAF, PIK3CA, TP53, NFE2L2, CTNNB1, ALK, FBXW7, and PTEN were found. Finally, serial liquid biopsies from a patient with NSCLC revealed that the semiquantitative results of the mutation analysis by the SNV panel correlated with the evolution of the disease.

CONCLUSIONS: The nCounter platform requires less DNA than NGS and can be employed for routine mutation testing in liquid biopsies of patients with cancer.

Introduction

Although genetic analysis of tumor tissue provides useful information for prognosis and treatment decision making, a significant percentage of patients with advanced-stage cancer cannot be biopsied or the amount of tumor tissue is insufficient for genetic analyses. In addition, repeated sampling for monitoring the course of the disease and detecting the emergence of mechanisms of resistance is frequently not feasible. Liquid biopsies constitute a minimally invasive, safe, and sensitive alternative in these cases; and are quickly gaining acceptance in the clinical setting (1–7).

Circulating-free DNA (cfDNA) purified from blood or other body fluids (8) is the most commonly used type of liquid biopsy. In patients with cancer, cfDNA contains a variable fraction of DNA originating in the tumor cells (circulating tumor DNA, or ctDNA) and can be used to identify clinically relevant mutations, amplifications, and gene fusions (4, 8–10), with polymerase chain reaction (PCR) based methods and targeted Next-Generation Sequencing (NGS) being the most frequently used techniques. The nCounter platform (NanoString Technologies) is a relatively novel technology initially developed for multiplex analysis of RNA molecules, and has been successfully applied for

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the detection of clinically relevant fusion transcripts and gene signatures in tumor tissues (11–13). In addition, a new hybridization probe chemistry has been developed for the detection of hotspot somatic variants in tumor tissue samples (14). However, despite the growing number of laboratories using nCounter, the platform has never been tested for the routine analysis of liquid biopsies.

In this study, we performed a retrospective validation on collection-stored cfDNA samples that revealed an excellent correlation of nCounter with other methodologies for mutation detection. Then, we prospectively analyzed fluids derived from patients with cancer and were able to detect a substantial number of relevant mutations, demonstrating that nCounter can be implemented in the clinical setting for the routine testing of liquid biopsies.

Material and Methods

PATIENTS AND CELL LINES
Fifteen liquid biopsy samples from healthy donors and 70 from patients with cancer were used for the retrospective validation of the nCounter Vantage 3D DNA Single Nucleotide Variant (SNV) Solid Tumor Panel (NanoString Technologies) (Table 1). All of them had been stored in a sample collection approved by the Spanish Ministry of Health (reference number C.0005039). Then, 91 liquid biopsies (Table 2) from 83 patients collected in 6 Spanish hospitals were analyzed with the same panel (see Tables 1 and 2 in the online Data Supplement). The study was carried out in accordance with the principles of the Declaration of Helsinki under an approved protocol of the institutional review board of Quirón Hospitals. Written informed consent was obtained from all patients and documented; samples were deidentified for patient confidentiality. Clinical information collected from each patient was limited to stage, gender, smoking status, and tumor histology. Cell lines with EGFR, KRAS, PIK3CA, BRAF, and NRAS mutations were used for analytical validation purposes and also as positive and negative controls (Supplemental Table 3).

DNA ISOLATION
Plasma samples (10 mL) were collected in sterile Vacutainer tubes (BD) and cerebrospinal-, pleural-, and ascitic-fluid samples (3–500 mL) in sterile containers. After 2 consecutive centrifugation steps (500g, 10 min), cfDNA was purified using the QIAsymphony® DSP Virus/Pathogen Midi Kit and a QIAsymphony robot (Qiagen), following the manufacturer’s instructions. Initial volume was 1.2 mL, final elution volume was 30 µL. DNA concentration was estimated using Qubit®. Finally, DNA from the cell lines was purified using the DNA Easy® extraction kit (Qiagen), according to the manufacturer’s instructions.

MUTATION DETECTION BY NCOUNTER
The nCounter Vantage 3D DNA SNV Solid Tumor Panel enables detection of 97 driver mutations in 24 clinically relevant genes (online Supplemental Table 4). For mutation detection using nCounter, 5 µL of purified cfDNA and a reference DNA (NanoString Technologies, provided with the panel) were subjected to a 21-cycle preamplification step in a Verity thermal cycler (Applied Biosystems), according to the manufacturer’s instructions. Amplified DNA was denatured at 95 °C for 10 minutes and hybridized at 65 °C for 18–24 hours with the SNV pool, which contains mutation- and exon-specific probes that bind to DNA independently of the presence of mutations. Capture, cleanup,
and digital data acquisition were performed using the nCounter Prep Station™ and Digital Analyzer™ (NanoString Technologies) (Supplemental Fig. 1).

**DATA ANALYSIS**

Count values were exported to Excel 2016 (Microsoft) using nSolver software v.4.0 (NanoString Technologies). For each mutation, samples with count values lower than the reference DNA were automatically considered negative and excluded from further analysis. The reference consists of a wild-type DNA that does not harbor any mutation and allows estimating the “background noise” counts for every mutation targeted by the kit. The remaining mutation-specific counts were normalized using the geometric mean of the exon counts of the corresponding gene in the same sample. The same procedure was applied to the count values derived from the reference DNA. Finally, the mutation-specific normalized counts of the samples were divided by the corresponding normalized counts of the reference DNA and the result was subjected to a base-2 logarithmic transformation to obtain the log MUT values. For every mutation in the SNV panel, the average log MUT of all the negative samples for this particular mutation included in the retrospective study plus 3 SD was established as the cut-off value for positivity. The only exceptions were KRAS mutations, where the mean plus 2 SDs was used.

**MUTATION TESTING BY PNA-Q-PCR AND NGS**

Samples used in the retrospective validation had been previously genotyped by quantitative PCR in presence of a peptide-nucleic acid (PNA-Q-PCR) or NGS (4, 8, 9, 15, 16). For details about these 2 techniques, see the Supplemental Methods.

**Results**

**ANALYTICAL VALIDATION**

First, we analyzed 15 cfDNA samples purified from the blood of healthy donors. All of them tested negative for the 97 mutations targeted by the Vantage 3D DNA SNV panel. Next, using DNA from 2 mutant cell lines, we found that 5 pg of mutated genomes per μL were sufficient to detect EGFR 15-bp deletions and KRAS G12C mutations (Supplemental Table 5). Serial dilutions of a mixture of DNAs from 13 mutant cell lines spiked into a pan-negative cfDNA were employed to determine the limit of detection of the panel. Mutations in EGFR, KRAS, NRAS, and PIK3CA were detected at allelic fractions between 0.02 and 2% (Supplemental Table 6). Finally, spiked samples of 2 mutant cell lines and a cfDNA purified from the blood of a patient who tested KRAS G12D positive were tested on different days by different operators, and the reproducibility of the SNV panel was found to be 100% (Supplemental Table 7).

**RETROSPECTIVE VALIDATION IN CLINICAL SAMPLES**

A total of 70 liquid biopsies from patients with cancer were used in the retrospective validation of the nCounter SNV panel. Most of them corresponded to plasma samples (n = 62), but sera (n = 3), ascites (n = 3), PE and CSF (n = 1 each) were also represented (Table 1). Regarding tumor types, the majority of samples were from patients with lung cancer (n = 49), followed by colorectal cancer (n = 20), melanoma (n = 20), and other malignancies (n = 5). All liquid biopsies in the retrospective cohort had been previously genotyped for EGFR, KRAS, BRAF, NRAS, and PIK3CA hotspot mutations by NGS or PNA-Q-PCR. The cfDNAs were reanalyzed using the nCounter SNV panel, the counts for each mutation were normalized, and positive and negative samples identified as explained in Methods (see also Supplemental Fig. 2). The results obtained for 3 representative hotspot mutations are shown in Fig. 1. In all cases, the distribution of the normalized counts was

| Characteristics               | N = 90 (%) |
|-------------------------------|------------|
| Tumor type and histology      |            |
| Lung cancer                   | 51 (56.6)  |
| Adenocarcinoma                | 42 (46.6)  |
| Squamous                      | 2 (2.2)    |
| Others                        | 7 (7.7)    |
| Colorectal cancer             | 20 (22.2)  |
| Adenocarcinoma                | 19 (21.1)  |
| Others                        | 1 (1.1)    |
| Breast cancer                 | 4 (4.4)    |
| Melanoma                      | 4 (4.4)    |
| Others                        | 11 (12.2)  |
| Collection time               |            |
| Basal                         | 62 (68.8)  |
| Progression                   | 8 (8.8)    |
| Follow up                     | 18 (20)    |
| Unknown                       | 2 (2.2)    |
| Type of fluid                 |            |
| Plasma                        | 87 (96.7)  |
| Pleural fluid                 | 1 (1.1)    |
| Cerebrospinal fluid           | 2 (2.2)    |
bimodal, with the mutant samples representing a different subpopulation.

The results of the previous cfDNA genotyping were used for comparison purposes (Fig. 2). For EGFR, KRAS, BRAF, NRAS, and PIK3CA mutation detection, nCounter and NGS/PNA-Q-PCR showed concordance rates ranging from 97.1 to 100% and Cohen kappas from 0.91 to 1.00 (Table 3). If the 5 genes were considered together, mutation status by nCounter showed an almost perfect agreement with previous genotyping, with only 4 discordant cases, 0.96 Cohen kappa (CI = 0.92–1.00), and 98.9% concordance (CI = 97.1–99.7%).

The 4 discordant samples were further investigated (Fig. 2). One corresponded to a plasma sample with a T790M in EGFR detected by PNA-Q-PCR at an extremely low allelic fraction (0.004%), well below the limit of detection of the nCounter SNV panel. The only discordant sample for BRAF had been positive for a V600K mutation by PNA-Q-PCR, with a 2.1% allelic fraction. The plasma sample had been stored for 4 years.

Fig. 1. Results of the mutation detection by nCounter in the retrospective cohort (n = 70) for 3 representative mutations, L858R in a) EGFR, b) G12C in KRAS, and c) V600E in BRAF. Left, individual normalized counts in the liquid biopsy samples, expressed as log MUT values. The dotted lines indicate the threshold. Right, distribution of the log MUT values.
Fig. 2. Heatmap of the liquid biopsies included in the retrospective cohort ($n = 70$). All samples were analyzed by nCounter and an alternative technique, either NGS or PNA-Q-PCR. Green, mutations detected by nCounter and the alternative technique. Blue, mutations detected only by nCounter. Pink, mutations detected only by the alternative technique.
at the moment of the nCounter analysis and showed very low exonic counts, indicating cfDNA degradation. Regarding the 2 samples discordant for KRAS, one corresponded to a plasma positive for a Q61L mutation by nCounter but negative by PNA-Q-PCR and one to a serum sample harboring a G12S mutation by PNA-Q-PCR at 0.12% allelic fraction, not detected by nCounter.

Paired tissue samples with complete genotyping results were available for 30 liquid biopsies included in the retrospective study. For EGFR, KRAS, BRAF, NRAS, and PIK3CA mutation detection, nCounter in liquid biopsy showed 71–100% sensitivity and 100% specificity vs paired tissue (Supplemental Table 8). When the 5 genes were considered together, mutation status in liquid biopsy by nCounter showed an almost perfect agreement with previous genotyping in tissue biopsies, with a Cohen kappa of 0.90 (CI = 0.74–1.00) and a 97.3% concordance (CI = 93.1–99.2%).

Finally, we compared the log MUT values obtained by nCounter with the allelic fractions previously found by NGS or PNA-Q-PCR in the same samples. For this analysis, we selected the KRAS-positive liquid biopsies and we found a linear correlation between the log MUT KRAS values and the log2 of the KRAS mutant allelic fractions derived from NGS or PNA-Q-PCR ($R^2 = 0.63$; Pearson $r = 0.80$; Supplemental Fig. 3).

**PROSPECTIVE ANALYSIS OF LIQUID BIOPSIES**

During a 6-month period (December 2018 to June 2019), liquid biopsy samples from 83 patients with cancer were collected, submitted to DNA extraction, and prospectively analyzed using the SNV nCounter panel. Six patients had 2 or more fluid samples available (Supplemental Table 1) bringing the total number of liquid biopsies tested to 91. In all cases, the concentration of purified cfDNA was less than 1 ng/mL, as measured by Qubit. Despite these low concentrations, only one of the 91 liquid biopsies showed very low exonic counts and was considered as not evaluable.

The characteristics of the 90 liquid biopsy samples finally included in the prospective study are presented in Table 2. The majority of them corresponded to plasma samples ($n = 87$), although 2 CSF and a PE were also included. Regarding the type of malignancy, most liquid biopsies were obtained from patients with lung cancer ($n = 51$); followed by colorectal (CRC) ($n = 20$), breast ($n = 4$), melanoma ($n = 4$), prostate ($n = 3$), and other tumors ($n = 8$), including thyroid, ovarian, pancreatic, sarcoma, and kidney cancer.

The results of the mutation analysis by nCounter are presented in Fig. 3A. Among the 51 fluid samples from patients with lung cancer, mutations in EGFR were found in 7 samples; 3 harbored exon 19 deletions, 3 exon 21 point mutations and one a G719A mutation in exon 18. Regarding KRAS mutations, 7 samples were positive for the G12 ($n = 4$), Q61 ($n = 2$), or G13 ($n = 1$) positions. Hotspot mutations in PIK3CA were found in 4 lung cancer liquid biopsies, 3 of them coming from the same patient (see next). Finally, 11 samples harbored mutations in a variety of genes, including PTEN, CTNNB1, GNAQ, NFE2L2, FGFR2, FBXW7, TP53, and ALK. The 2 liquid biopsies positive for ALK mutations corresponded to patients in progression to ALK targeted therapies. In the case of the patients with CRC, 2 out of 20 liquid biopsies were positive for KRAS mutations and one each for BRAF, NRAS, and CTNNB1. Finally, among samples collected from patients with other malignancies, a G12D mutation in NRAS was found in 2 serial samples from a melanoma patient in progression to BRAF/MEK inhibitors, while 2 consecutive liquid biopsies from a pancreatic cancer

### Table 3. Concordance of mutation detection by nCounter with NGS and PNA-Q-PCR in liquid biopsy samples. The 95% confidence intervals are indicated for the overall results.

| Genes* | EGFR | KRAS | BRAF | NRAS | PIK3CA | Overall |
|--------|------|------|------|------|--------|---------|
| No. of concordant results | 69   | 68   | 69   | 70   | 70     | 346     |
| No. of discordant results | 1    | 2    | 1    | 0    | 0      | 4       |
| Sensitivity | 100% | 93.3% | 100% | 100% | 100.0% | 94.7% (CI=85.4–98.9%) |
| Specificity | 98.0% | 98.2% | 98.3% | 100% | 100.0% | 99.7% (CI=98.1–100%) |
| Concordance | 98.6% | 97.1% | 98.6% | 100% | 100.0% | 98.9% (CI=97.1–99.7%) |
| Cohen kappa | 0.96 | 0.91 | 0.95 | 1.00 | 1.00 | 0.96 (CI=0.92–1.00) |

*Samples not carrying a mutation in a particular gene were used as negatives for this gene, independently of the mutational status of other genes.

Abbreviations: NGS—Next-Generation Sequencing; PNA-Q-PCR—quantitative PCR in presence of a quencher-labeled peptide nucleic acid.
patient harbored the I195T mutation in TP53 and a thyroid sample a Q61R mutation in NRAS.

Eighty-four of the 90 liquid biopsy samples in the prospective cohort had not been previously submitted to any kind of testing. The remaining 6 samples (4 blood and 2 CSFs) had been analyzed using liquid biopsy NGS panels, yielding invalid results. Interestingly, all of them were evaluable by nCounter, which detected drivers in 2, a KRAS G12D mutation in a blood sample, and a L1196M ALK resistance mutation in a CSF.

VALIDATION OF RESULTS OF THE PROSPECTIVE TESTING
A subset of 16 cfDNA samples from the prospective cohort with remaining material was subsequently submitted to NGS for validation purposes. The subset included samples from the 11 patients carrying mutations in genes not validated in the retrospective part of the study; such as TP53 or ALK (Fig. 3, A). The NGS panel employed did not cover CTNNB1, FBXW7, FGFR2, or GNAQ and mutations in these genes could not be confirmed. For the rest of genes, NGS showed concordant results with nCounter for EGFR (n = 4), TP53 (n = 3), PIK3CA (n = 2), NRAS (n = 2), ALK (n = 1), and NFE2L2 (n = 1) mutations. Sequencing only failed to detect a PTEN mutation, while 2 samples were not evaluable due to insufficient material (Fig. 3, A and Supplemental Table 9).

MUTATION ANALYSIS OF SERIAL SAMPLES
A clinical case where serial liquid biopsies were collected will be described in further detail (Fig. 3, B). It corresponded to a patient with lung cancer, diagnosed in February 2016, harboring an EML4-ALK fusion and wild-type for EGFR, KRAS, BRAF, and PIK3CA in
tumor tissue at presentation. The patient started ceritinib on February 2016, which was replaced by alectinib 3 months later due to hepatic toxicity. The patient was in remission for more than 2 years, and the 4 serial blood samples obtained from April 2016 to June 2017 were pan-negative by the nCounter SNV panel. In contrast, 2 mutations were detected in a fifth sample collected in April 2018, E545K in PIK3CA and E79Q in NFE2L2, at log MUT values of 2.1 and 3.2, respectively. The patient showed radiological progression in multiple sites 4 months later, in August 2018. Alectinib was then replaced by brigatinib, a blood sample obtained in October revealed a substantial increase in the log MUT values of both mutations, which rose to 5.6 and 7.8. A subsequent radiological evaluation demonstrated lack of response to brigatinib. Atezolizumab was then administered, but the log MUT values further increased in blood, to 7.6 and 10.1; an evaluation of response on February 2019 revealed progression of the disease. Lorlatinib was finally started and the patient underwent a partial response that was accompanied by a substantial decrease in the log MUT values of the 2 mutations, which dropped to 2.2 and 4.0.

Five serial samples from the patient had remaining material after nCounter, and PIK3CA mutations were tested by PNA-Q-PCR for validation purposes. The results showed a good agreement with those previously obtained by nCounter (Supplemental Table 10).

**Discussion**

Precision oncology, based on the assessment of molecular markers predictive of treatment outcome, has transformed clinical practice for many types of cancer. Since tumor tissue is not always available or sufficient for genetic testing, liquid biopsies have quickly gained acceptance in the clinical setting (1–7). Initially, blood and other fluids from patients with cancer were mainly employed to detect clinically relevant mutations in EGFR, KRAS, NRAS, or BRAF using PCR-derived techniques targeting a limited number of exons (17). However, in the last few years, several NGS platforms have been adapted to the requirements of liquid biopsies and are being used by a growing number of laboratories.

The nCounter technology is a multiplex hybridization-based assay (18) that differs from NGS techniques, being based on direct counting of the RNA or DNA molecules (19). The technology has been adapted for the detection of mutations in DNA purified from tumor tissue (14) by the design of 3 types of probes (S, M, and T). S probes have 2 binding regions, one detecting the presence of the mutation and the other binding to a nearby wild-type sequence; while M probes act like signal attenuators of the wild-type sequences, and T probes facilitate detection (Supplemental Fig. 1). The nCounter technology has been widely used in research studies to simultaneously determine mRNA expression levels of hundreds of genes in biological samples (20, 21), including liquid biopsies of patients with cancer (22, 23). Some of these exploratory studies have led to the identification of expression-based signatures to discriminate malignant lung nodules (24) and predict outcome to immunotherapy in solid tumors (25) or drug sensitivity in prostate cancer (26). However, nCounter has never been used for routine testing of liquid biopsies of patients with cancer and the only signature in clinical use is the U.S. Food and Drug Administration-approved, tumor-tissue-based Prosigna, which determines the risk of recurrence in breast cancer (11, 12, 27).

Here we have described the validation of the nCounter SNV panel, which can detect mutations and small indels in 27 genes, for the genotyping of liquid biopsy samples; followed by the implementation of the assay for the prospective testing of blood and other fluids of patients with cancer. During the validation study, we found that 5 pg of mutant DNA, purified from 1.2 mL of blood or other body fluids, was sufficient for successful analysis. Regarding limits of detection, using spiking experiments with cell lines we found values of 0.02–2% allelic fraction. The concentration of cfDNA in the liquid biopsy samples used in our study ranged between 0.1 and 0.5 ng/μL, and 5 μL were loaded in the nCounter assay; meaning that the total cfDNA input was 500–2500 pg. Since 5 pg of mutant DNA are required, the minimum allelic fractions needed for mutation detection in liquid biopsy samples by nCounter would be 0.1–0.02%, coinciding with the values found in cell line experiments. In contrast, using cfDNA inputs lower than 500 pg could lead to higher limits of detection.

The limits of detection of the nCounter SNV panel compare favorably with the requirements of liquid biopsy NGS assays (Supplemental Table 11) and the low requirement of input material explains that, among the 91 liquid biopsies prospectively analyzed with the panel, only 1 (1.1%) sample could not be evaluated. Remarkably, valid results could be obtained for 2 CSF samples, which are usually collected at small volumes and contain particularly low amounts of cfDNA (8, 28, 29). One of them corresponded to a patient who was EML4-ALK positive progressing to targeted therapies, where a L1196M resistant mutation was identified and used for the selection of subsequent therapies. Of note, NGS had been previously attempted with these 2 CSFs but yielded invalid results due to insufficient DNA concentration.

Finally, comparison with results obtained in tissue biopsies revealed diagnostic sensibility and specificity of 84.6% and 100%, respectively. All these values are in
the range of those reported for liquid biopsy NGS platforms such as Guardant Health or Oncomine (Supplemental Table 11). One of the limitations of our study was that the number of paired tissue samples was limited and the confidence interval calculated for the diagnostic sensitivity had a wide range, from 66.4 to 93.8%. However, we were able to compare the results obtained by nCounter in all the liquid biopsies in the retrospective cohort with the previous genotyping of the same samples by NGS or PNA-Q-PCR, showing an almost perfect agreement, with 99% concordance and a 0.96 Cohen kappa (CI = 0.92–1.00).

During prospective testing, EGFR mutations were found in 7/51 liquid biopsy samples from the patients with NSCLC analyzed (13.7%) (30), a percentage in the range of the frequency described in European populations, while KRAS mutations were detected in another 7 patients; EGFR and KRAS mutations were mutually exclusive, as expected. In the case of CRC, KRAS, NRAS or BRAF mutations were found in 5 out of 21 (23.8%) of liquid biopsies. This relatively low prevalence can be explained in 2 ways, (a) a considerable number of the patients with CRC were stage I–III A, with less tumor burden than advanced patients; and (b) 3 out of 21 samples corresponded to samples of patients in response to therapy. Finally, results obtained in serial liquid biopsies indicate that the nCounter SNV panel could be used to follow the evolution of patients with cancer. Although allelic fractions as such cannot be estimated, the log MUT values were directly dependent on allelic fractions and could be easily calculated and monitored (Fig. 4).

The nCounter platform confers several advantages over NGS techniques for mutation detection in liquid biopsies. It requires a substantially lower amount of material, has a 24–48 h turnaround time with relatively short hands-on time, sample preparation is simple compared to NGS, and data analysis is straightforward and does not require bioinformatics expertise (Supplemental Fig. 1). The main disadvantage of the nCounter platform is that, not being a sequencing technique, it cannot detect mutations other than those contained in the SNV panel, although the panel can be customized. Detection of mutations by nCounter can be particularly useful in some settings. Examples include liquid biopsies with small volumes and/or low concentrations of cfDNA, such as CSF samples (8) or pleural and peritoneal lavages (16); liquid biopsies where NGS has failed; or patients with cancer who are in urgent need of genetic testing to determine whether they are eligible for targeted therapies. Also, the nCounter SNV panel is well suited for monitoring patients in response to therapy where repeated NGS of liquid biopsies would not be cost-effective and can be spared until progression.

In summary, we have demonstrated that the nCounter SNV panel, initially developed for tumor tissue samples, shows an analytical performance similar to NGS in liquid biopsies, requires less material, and can be implemented for multiplex detection of somatic mutations in the clinical setting. Our results also pave the way for testing the performance of nCounter for the detection of other relevant alterations in liquid biopsies from patients with cancer, such as gene fusions or expression levels of genes predictive of response to immunotherapy.

Supplemental Material
Supplemental material is available at Clinical Chemistry online.

Nonstandard Abbreviations NGS, Next-Generation Sequencing; SNV, single nucleotide variant; cfDNA, circulating-free DNA; NSCLC, nonsmall cell lung cancer; CSF, cerebrospinal fluid; PE, pleural effusion; ctDNA, circulating tumor DNA; FFPE, formalin-fixed, paraffin embedded; PNA-Q-PCR, quantitative PCR in presence of a peptide nucleic acid; WT, wild-type.

Human Genes: EGFR, epidermal growth factor receptor; KRAS, KRAS proto-oncogene, GTPase; BRAF, B-Raf proto-oncogene, serine/threonine kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TP53, tumor protein p53; NFE2L2, nuclear factor, erythroid 2 like 2; CTNNB1, catenin beta 1; ALK, ALK receptor tyrosine kinase; FBXW7, F-box and WD repeat domain containing 7; PTEN, phosphatase and tensin homolog.

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