Review Article

Multiplexed detection of predictive fusions and splicing variants in RNA from lung cancer tissue samples using a hybridization-based platform: narrative review

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Background and Objective: Cancer is one of the leading causes of disease-related casualties worldwide. More than 2 million new cases of lung cancer were detected in 2021 and this malignancy was the most common cause of cancer related death with 1.80 million casualties. Personalized medicine has revolutionized the therapeutic landscape of some hematological malignancies and solid tumors, particularly non-small cell lung cancer (NSCLC). Rearrangements of anaplastic lymphoma receptor tyrosine kinase, *ROS* protooncogene 1, receptor tyrosine kinase, *RET* proto-oncogene and neurotrophic receptor tyrosine kinase genes and *MET* proto-oncogene receptor tyrosine kinase, exon 14 splicing are present in 1–9% of NSCLC patients and their correct identification is key to select targeted therapies. NSCLC patients presenting these types of alterations can receive tyrosine kinase inhibitors (TKIs), which have demonstrated improved clinical benefit compared with standard chemotherapy. The nCounter system, a hybridization-based platform from NanoString Technology, has been tested in recent years for the detection of fusions and splicing variants in NSCLC. In this literature review, we summarize the published studies in this area.

Methods: We performed a search narrative of the scientific literature in PubMed database and selected all the articles in English from origin until October 5th, 2020 where nCounter was used for fusion and splicing variant detection.

Key Content and Findings: nCounter has been demonstrated to be a useful tool for fusion and splicing variant testing in NSCLC in the clinical setting. The technique has several advantages such as a fewer processing steps, short turnaround time and less hands-on time compared with gold standard methods [fluorescence in situ hybridization (FISH), immunohistochemistry (IHC)] or next-generation sequencing (NGS). In addition, it can be easily employed in formalin-fixed paraffin-embedded (FFPE) tumor samples and requires low quantities of tissue. Finally, nCounter has shown high sensitivity and specificity compared with gold standard methods for detection of clinically relevant fusions and splicing variants.

Conclusions: nCounter can be employed in the clinical setting for the detection of splicing variants and fusion transcripts in NSCLC.

Keywords: Lung cancer; gene fusions; splicing; hybridization-based platform

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Introduction

Cancer of the lung is one of the most common malignancies and the first cause of cancer-related deaths, representing almost 25% (1). Around 84% of lung tumors are adenocarcinomas, squamous cell carcinomas and large cell carcinomas, which are grouped as non-small cell lung cancers (NSCLCs). Several types of genetic alterations have been demonstrated to be oncogenic and are referred to as drivers, including point mutations, deletions, insertions and gene fusions. The 45% of driver alterations in NSCLC are somatic mutations in the *KRAS* proto-oncogene (*KRAS*), epidermal growth factor receptor (*EGFR*) and *B-Raf* proto-oncogene (*BRAF*) genes, while oncogenic gene fusions and splicing variants are present in 5–10% of patients.

Fusion gene and splicing variant occur when two different genes are juxtaposed or when particular exons of a mRNA are processed in different combinations, respectively. The most common are anaplastic lymphoma receptor tyrosine kinase (*ALK*), *ROS* protooncogene 1, receptor tyrosine kinase (*ROS1*), *RET* proto-oncogene (*RET*) and neurotrophic receptor tyrosine kinase (*NRTK1/2/3*) fusions and the *MET* proto-oncogene, receptor tyrosine kinase splicing (*MET*Δex14) variant being mutually exclusive with other drivers (2). The development of the first tyrosine kinase inhibitors (TKIs) targeting *ALK* fusions represented a breakthrough advance in the NSCLC treatment landscape in the last decade. Several pre-clinical and clinical studies have demonstrated the clinical benefit of targeted therapies with TKIs in patients with *ALK*, *ROS1*, *NTRK1/2/3*, *RET* fusions rearrangements or *MET*Δex14 splicing variant. These benefits include increased objective response rates (ORR), progression-free survival (PFS) and overall survival (OS) compared with chemotherapy and TKIs are currently the standard of care in first line treatment of the NSCLC patients harboring the alterations mentioned above. However, due to the emergence of drug resistance, patients ultimately relapse to TKIs and new generation inhibitors have been developed, targeting some mechanisms of resistance (3-6) (*Table 1*).

The first ALK inhibitor (ALKi) approved by the Food and Drug Administration (FDA) for metastatic NSCLC was crizotinib in 2011, which targets *ALK*, *ROS1* and c-*MET* (3). Two second-generation ALKis, ceritinib and alectinib, obtained FDA approval in 2014 and 2015 for patients progressing to crizotinib or intolerant to it (7). Based on the results of the randomized phase III ALEX trial, alectinib was also approved in November of 2017 for treatment-naïve *ALK*-positive patients (8). Thereupon, the FDA authorized brigatinib for those patients who had failed prior *ALK*i treatment (9,13). In this fast-growing therapeutic landscape, highly potent third generation ALKis, such as lorlatinib, have been recently developed to treat acquired resistance, improve the control of the disease, and target central nervous system (CNS) disease (10).

Regarding the rest of oncogenic fusions, *ROS1* patients are currently treated with two inhibitors, crizotinib and entrectinib, that bind to ROS1 fusion protein (3,5,11). In the case of RET, the first multi-kinase inhibitors tested were cabozantinib, vandetanib and lenvatinib, with contrasting results. More recently, two selective RET inhibitors, selpercatinib and pralsetinib, demonstrated better clinical efficacy and good tolerability, being approved in 2020 (4,14,15). Finally, the kinase inhibitors larotrectinib and entrectinib were approved by the FDA in 2018 and 2019, respectively, for the treatment of patients with *NTRK1-3*

Table 1 Summary of inhibitors approved for fusion-positive NSCLC patients

Target	Alteration	Frequency	Drug	Reference
ALK	Fusion	5–7%	Crizotinib	(3)
			Ceritinib	(7)
			Alectinib	(8)
			Brigatinib	(9)
			Lorlatinib	(10)
ROS1	Fusion	1–2%	Crizotinib	(3)
			Ceritinib	(7)
			Entrectinib	(5,11)
			Lorlatinib	(10)
RET	Fusion	1–2%	Selpercatinib	(4)
			Pralsetinib	(4)
NTRK	Fusion	1%	Larotrectinib	(6)
			Entrectinib	(5,11)
METΔex14	Splicing variant	3–4%	Crizotinib	(3)
			Capmatinib	(12)
			Tepotinib	(12)

NSCLC, non-small cell lung cancer; *ALK*, anaplastic lymphoma receptor tyrosine kinase; *ROS1*, *ROS* protooncogene 1, receptor tyrosine kinase; *RET*, *RET* proto-oncogene; *NTRK*, neurotrophic receptor tyrosine kinase genes; *MET*, *MET* proto-oncogene receptor tyrosine kinase.

Table 2 The search strategy summary

Items	Specification
Date of search	2012/08/24–2020/11/27
Databases and other sources searched	PubMed
Search terms used	See Table S1
Timeframe	2012–2021
Inclusion and exclusion criteria	Inclusion criteria: research articles and reviews about nCounter technology for fusion and splicing detection in NSCLC in FFPE tissue
	Exclusion criteria: articles that have no performed the technique in FFPE tissue
Selection process	It was conducted independently by Ana Giménez-Capitán and Miguel Ángel Molina-Vila, all authors attended a meeting to discuss the literature selection and obtained the consensus

FFPE, formalin-fixed paraffin-embedded; NSCLC, non-small cell lung cancer.

fusion-positive solid tumors (6,11,16,17).

In the case of *MET* exon 14 skipping mutation, several MET TKIs have been developed and are currently being tested in clinical trials (18-25). Two type Ib MET TKIs, tepotinib and capmatinib, have recently been approved by the FDA for the treatment of NSCLC patients harboring *METΔex14* (12).

Although there are several publications of fusion detection using the nCounter methodology, the perception is that this platform has not managed to establish itself as a benchmark. In most clinical trials, the use of technologies such as next-generation sequencing (NGS), immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) is preferred or often required for fusion detection. However, our laboratory has been using nCounter for several years and we have observed that this technology outperforms NGS (26) and should be universally accepted for testing fusions and splicing variants in tumor samples. Consequently, we performed a narrative review of the scientific literature about fusion and splicing variant detection using nCounter to support this point and we present the following article in accordance with Narrative Review reporting checklist (available at https://jxym.amegroups.com/article/view/10.21037/jxym-22-6/rc).

Methods

We performed a search narrative of the scientific literature in the PubMed database using the keywords "nCounter" and "fusion" and "non-small cell lung cancer" or "nCounter" and "splicing variant" and "non-small cell lung cancer". The articles listed after both searches were individually examined, and those actually describing the use of nCounter for fusion and splicing variant detection were selected (*Table 2* and Table S1).

The nCounter technology

The nCounter is a hybridization-based platform (NanoString Technologies, Seattle, WA, USA) based in a fluorescent barcode that enables direct detection of hundreds (≤800) of different target molecules in a single assay. The technology can be used for gene expression profiling, detection of fusion and alternative splicing transcripts or protein analysis, can be easily incorporated into the diagnostic routine and is cost-effective compared to alternative techniques. Regarding gene expression and detection of altered transcripts, the panels can be commercial or custom-made.

The technology can be adapted for simultaneous analysis of multiple fusion transcripts, using a dual strategy aimed to detect possible imbalances in the 3'/5' expression of the wild type (WT) sequences and specific fusion junction targets (27). The nCounter protocol has 3 basic steps: (I) the RNA is hybridized with the specific probe pairs (reporter probe and capture probe); (II) the tripartite structure coated with streptavidin is bound to the surface of the sample cartridge and reporters are aligned by an electric current and immobilized for data collection; (III) fluorescent barcodes are counted by a digital analyzer, RNAs are identified and

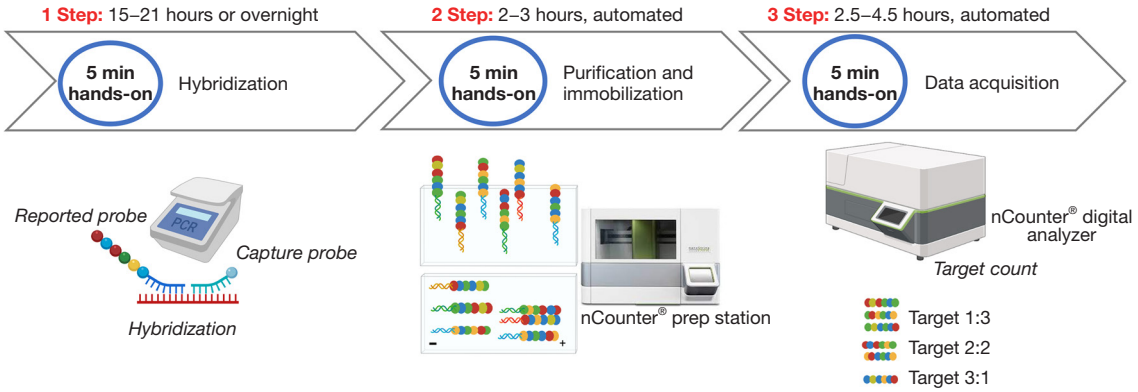

Figure 1 nCounter system workflow (BioRender illustration software).

counts tabulated (27-29) (*Figure 1*).

The technique has several advantages compared with gold standard methods such as FISH and IHC or other techniques such as NGS, such as a short turnaround time and needs less hands-on time (*Table 3*). In addition, it requires low amounts of RNA, which can be easily purified from a single tissue or cytology slide with a minimum area of 1.1 mm^2 (27). This aspect is particularly relevant in the case of NSCLC patients, since biopsies are often scarce or the only sample available is a cytological specimen. Often the mRNA from formalin-fixed paraffin-embedded (FFPE) is degraded and with this system the sample can be direct measure without amplification step avoiding any bias. All of these considerations made an attractive platform for the clinical setting implementation (30). The main disadvantage of nCounter is that many laboratories only dispose of NGS and do not have the technology and the required equipment available. At the technical level, an advantage of NGS over nCounter is that NGS can determine the specific sequence of the fusion point and detect any deviation from the standard sequence, while nCounter cannot.

In this review, we will summarize the studies published using nCounter for the detection of fusion genes in NSCLC, which are summarized in *Table 4*. The same table also presents the sensitivity and specificity of the nCounter results versus orthogonal techniques such as FISH or IHC.

Detection of *ALK*, *ROS1* and *RET* gene fusions by nCounter

In 2012, Suehara and colleagues were the first group to report the detection of *ALK*, *ROS1* and *RET* fusion using nCounter technology (35). The study included 75 lung adenocarcinoma RNA samples; 6 extracted from frozen tissue and 69 from FFPE blocks. Each sample was analyzed using 100 to 200 ng of total RNA using 5'/3' imbalance probes targeting two selected regions of 100 base pairs (pb) for each gene under study. Using serial dilutions of RNA from cell lines, they first determined that the positive tumor cell content should be >25% for the fusion to be detectable. In the case of the 75 samples, the nCounter assay correctly identified 24/24 positive cases. Furthermore, they identified aberrant 5' to 3' ratios in *ROS1* and *RET* of novel Golgi associated PDZ and coiled-coil motif containing (*GOPC*) *GOPC-ROS1* and kinesin family member 5B (*KIF5B*) *KIF5B-RET* fusions (35).

Next, Lira *et al.* [2013] developed an nCounter assay able to identify specific *ALK* fusions, which included 8 pairs of imbalance probes and 7 pairs of probes for *ALK* known fusion variants. The assay was validated in RNA (500 ng) isolated from 10 µm sections of FFPE blocks from 67 NSCLC samples, 34 positive and 33 negative (29), and was found to be highly concordant with FISH and IHC.

In 2014, the same group modified the technology for simultaneous screening of *ALK*, *ROS1* and *RET* fusions. The new assay included 24 probe pairs targeting wild-type 3' and 5' regions of *ALK*, *ROS1*, and *RET* and 27 fusion-specific probe pairs. The assay was validated in 295 NSCLC specimens, *ALK* results were 100% and 97.8% concordant with FISH and IHC, respectively. Regarding *ROS1* and *RET*, they observed 100% concordance with FISH (36).

In 2017, our group validated nCounter for routine detection of fusion transcripts (27). Our codeset included 24 imbalance probe pairs targeting *ALK*, *ROS1* and *RET*; and 23 fusion-specific probe pairs. Using FFPE blocks derived from cell lines, we determined 25 ng of total RNA with >10% tumor cell content was sufficient for the detection of fusion transcripts. The assay was retrospectively

Table 3 Comparison of NanoString nCounter with Illumina MiSeq RNA-Seq, ThermoFisher Ion AmpliSeq RNA Fusion, Agenda Bioscience MassArray, IHC and FISH assay properties

Characteristics	NanoString nCounter	Illumina MiSeq RNA-Seq	ThermoFisher Ion AmpliSeq RNA Fusion	Agena Bioscience MassArray	IHC	FISH
Panel	Elements Custom Panel or Vantage 3D™ Lung Fusion Panel	TruSight RNA fusion panel	RNA fusion lung cancer research panel V2	Lung FUSION™ Panel	Not apply	Not apply
Processing steps	RNA extraction, hybridization, purification and scan, data analysis*	RNA extraction, reverse transcribe sample, fragmentation, cDNA library preparation, sequencing, data analysis	RNA extraction, reverse transcribe sample, fragmentation, cDNA library preparation, sequencing, data analysis	RNA extraction, reverse transcribe sample, PCR amplification, PCR primer extension, SpectroCHIP Array and Clean Resin, data analysis	Cut FFPE tissue, automatic hybridization, slide evaluation	Cut FFPE tissue, deparaffinization, tissue pretreatment, hybridization, washing, slide evaluation
Input requirements	6–50 ng total RNA*	10 ng total RNA	10 ng total RNA	10–40 ng of cDNA	A slide of FFPE tissue	A slide of FFPE tissue
Sensitivity	<1 copy/cell	<1 copy cell	<1 copy cell	<1 copy cell	50–100 cells	50–100 cells
Specificity	Design of Capture and Reporter probes	Rely on data analysis	Primer design	Primer design	Rely on antibody to be used	Rely on probes to be used
Assay time	24 hours*	2.5 days	2 days	8hours	24 hours	48 hours
Hands-on time	15 min*	11 hours	45 min (using Ion Chef)	1 hour	1 hour	3 hours
Up to sample per assay	12	8 samples per run	16 samples per Ion 318 Chip	96	1	1
Genes in the panel	Custom personalized up 800 transcripts Vantage 3D™ Lung Fusion Panel: *ALK, RET, ROS1, NTRK1*	Targeting 507 genes	*ALK, RET, ROS1, NTRK1*	*ALK, RET, ROS1*	NA	NA
Number of genes or transcripts detected	Custom Panel up to 800 genes or Commercial Vantage 3D™ Lung Fusion Panel has 63 probes: 35 for specific fusion detection and 24 for positional gene expression imbalance detection	Gene fusion panel targeting 507 cancer-associated fusion genes and 7,690 exons	Over 70 transcripts	31 transcripts	1	1
Analysis software	Manual or nSolver™ analysis software	RNA fusion analysis module	Ion Reporter™ Software	MassArray analysis software	Manual analysis, pathology specialist	Manual analysis, pathology specialist

*, advantages. NA, not applicable; RNA-Seq, RNA sequencing; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; FFPE, formalin-fixed paraffin-embedded; *ALK*, anaplastic lymphoma receptor tyrosine kinase; *ROS1*, *ROS* protooncogene 1, receptor tyrosine kinase; *RET*, *RET* proto-oncogene; *MET*, *MET* proto-oncogene receptor tyrosine kinase.

Table 4 Summary of nCounter sensibility and specificity *vs.* gold standard techniques

Study (author, year, country)	Alteration	Type of nCounter panel	nCounter sensibility *vs.* FISH/IHC/PCR/NGS	nCounter specificity *vs.* FISH/IHC/RT-PCR/NGS
Lira *et al.*, 2013, Korea (29)	*ALK*	Custom panel, Elements assay	FISH: 100% and IHC: 97.8%	FISH and IHC: 98.8%
	ROS1		FISH: 100%	FISH: 100%
	RET		FISH: 100%	FISH: 100%
Reguart *et al.*, 2017, Spain (27)	*ALK*	Custom panel, Elements assay	FISH: 87.5% and IHC: 98.5%	FISH: 84.9% IHC: 97.2 %
	RET		Not reported	not reported
	ROS1		FISH: 85.9% and IHC: 87.2%	FISH: 96.1% and IHC: 88.3%
Lindquist *et al.*, 2017, Sweden (31)	*ALK*	Custom panel, Elements assay	FISH: 100%	FISH: 100%
	RET		FISH: 100%	FISH: 100%
	ROS1		FISH: 100%	FISH: 100%
Rogers *et al.*, 2017, Australia (32)	*ALK*	Custom panel, Elements assay	FISH: 94%	FISH: 97%
	ROS1		FISH: 100%	FISH: 100%
	RET		Not reported	FISH: 100%
Evangelista *et al.*, 2017, Brazil (33)	*ALK*	Custom panel, Elements assay	FISH and/or IHC: 100%	FISH and/or IHC: 100%
Aguado C *et al.*, 2021, Spain (26)	*METΔex14*	Custom panel, Elements assay	RT-PCR: 54.2%	RT-PCR: 100%
			NGS: 100%	NGS: 98.4%
Elfving *et al.*, 2021, Sweden (34)	*NTRK*	TruSight Tumor 170 RNA assay	No concordance with IHC	No concordance with IHC

ALK, anaplastic lymphoma receptor tyrosine kinase; *ROS1*, *ROS* protooncogene 1, receptor tyrosine kinase; *RET*, *RET* proto-oncogene; *MET*, *MET* proto-oncogene receptor tyrosine kinase; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; FFPE, formalin-fixed paraffin-embedded; NGS, next-generation sequencing; IHC, immunohistochemistry.

validated in 108 FFPE samples from advanced NSCLC patients of them, 98 were successfully analyzed by nCounter (91%), which identified 55 fusion positive cases (32 *ALK*, 21 *ROS1*, and two *RET*). nCounter results were highly concordant with IHC (98.5%, CI 91.8–99.7) and FISH [87.5%, confidence interval (CI): 79.0–92.9] for *ALK*. Regarding *ROS1*, nCounter showed a similar agreement with IHC and FISH (87.2% and 85.9%).

Three additional groups published in 2017 their experiences in detection of *ALK*, *ROS1* and *RET* fusions by nCounter. Lindquist *et al.* analyzed a Swedish cohort comprising 169 FFPE lung cancer blocks. The RNA was 100 to 250 ng and 80% of samples yielded valid results. Five *ALK*, two *ROS1* and three *RET* positive cases were detected, agreement with FISH was 100% (31). Rogers *et al.* compared three platforms with FISH; nCounter, a Lung Fusion array (Agena Bioscience, San Diego, CA, USA) and a NGS fusion panel (Thermo Fisher Scientific, Waltham, MA, USA) (29,36). Valid results by nCounter were obtained for 48/51 surgically resected NSCLC samples; 17 tested were positive for *ALK*, two for *ROS1* and one for *RET*. Overall agreement with FISH was 96% for nCounter, compared to 94% for the array and 86% for the NGS panel (32). Finally, Evangelista *et al.* tested the nCounter *ALK*-fusion panel developed by Lira *et al.* in 43 FFPE lung cancer biopsies from a Brazilian cohort (29,36). A total of 100 ng RNA was used for the analysis. The assay detected 13 *ALK*-positive samples with 100% agreement with FISH and/or IHC (33).

Detection of MET and NTRK alterations by nCounter

Li *et al.* [2016] pioneered the detection *METΔex14* transcripts by nCounter, incorporating to the Lira assay

probes for *MET* exons 13 and 14. When used to analyze an Asian population cohort (n=271), the assay detected 20 gene *ALK* fusions (7.4%), six *ROS1* (2.2%) and *RET* (2.2%) fusions and seven *METΔex14* skipping (2.5%) (37).

In 2020, our group performed an extensive retrospective validation of nCounter for the detection of *MET* alterations, not only *METΔex14* but also MET overexpression. Of the 474 advanced NSCLC samples analyzed, 422 (89%) yielded valid results by nCounter, which identified 13 patients (3%) with METΔex14 and 15 (3.2%) overexpressing MET. The two subgroups displayed distinct phenotypes and rarely coexisted with other drivers. NGS failed to detect 3/8 (37.5%) *METΔex14* samples positive by nCounter (26). Regarding patients with overexpressing *MET* mRNA, 92% had *MET* amplification by FISH and/or NGS. However, three FISH-negative patients showed high *MET* RNA expression by nCounter, one of them received MET TKI treatment deriving clinical benefit.

Next, our group performed a prospective study to demonstrate the feasibility and usefulness of embedding the RNA tissue-based nCounter panel described by Aguado *et al.* (26) in the clinical routine. In a cohort of 224 advanced NSCLC patients, nCounter testing yielded an informative result in 207 patients (92%). Driver alterations for *ALK* (n=7, 4%) and *METΔex14* (n=9, 5%) were detected and patients treated with ALK or MET TKIs based on the nCounter results (38).

Novaes *et al.* (39) published in 2021 a new study in a Brazilian cohort lung of 142 FFPE lung adenocarcinoma samples, incorporating specific probes for *NRTK1* fusion detection. Of them, 134 (94.4%) yielded valid results. *ALK* rearrangements were detected in 6.5% samples (21/325), while the frequency observed for *RET* and *ROS1* rearrangements was 0.6% (2/325) and 0.3% (1/325), respectively. *NTRK1* fusion results were not reported (39).

A more extensive study for *NTRK* rearrangements was published in 2021 by Elfving *et al.* comparing detection by IHC assay with nCounter and NGS (TruSight Tumor 170 RNA assay, Illumina, San Diego, CA, USA). A total of 688 NSCLC samples were first stained with the pan-TRK antibody clone EPR17341. Positive cases were further analyzed by the other techniques. However, nCounter or NGS could not confirm an *NTRK* fusion in any of the IHC positive cases (34).

In summary, all the studies conclude that nCounter platform is particularly useful for fusions and splicing variants detection. However, some of the published articles offer limited evidence at this respect and only a few of them report an extensive validation of the technique, not only using FFPE blocks obtained from cell lines but also comparing the nCounter results with gold standard techniques (NGS, FISH, IHC) in FFPE tumor samples [i.e., (27,29,36); see *Table 4*]. Also, the minimum amount of tissue sample, the limit of detection, the sensitivity and the specificity of nCounter for fusion and splicing variant detection are all described, being these data particularly useful for the reproducible implementation of the technique in the clinical setting.

Summary

The nCounter technique has demonstrated high sensitivity and specificity for detection of clinically relevant fusions and splicing variants compared with gold standard (FISH, IHC) and can be easily implemented in the clinical setting for multiplex detection of these alterations. nCounter can be used in FFPE tumor samples, requires low quantities of RNA, has a short turnaround time and needs less hands-on time than other techniques.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (Available at https://jxym.amegroups.com/article/view/10.21037/jxym-22-6/coif). The series "Predictive Molecular Pathology in Lung Cancer" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 Search terms used

Search terms used
("Fusion variants in lung cancer" [Mesh]) and "nCounter" [Mesh]
("Splicing variants in lung cancer" [Mesh]) and "nCounter" [Mesh]
("Lung cancer" [Mesh]) and "nCounter" [Mesh]
("*ALK*, *ROS1*, *RET* fusions" [Mesh]) and "nCounter" [Mesh]
("*MET* ex 14 skipping" [Mesh]) and "nCounter" [Mesh]
"Fusion variants in FFPE tissue" [Mesh]
"*MET* ex 14 skipping in FFPE tissue" [Mesh]

ALK, anaplastic lymphoma receptor tyrosine kinase; ROS1, ROS protooncogene 1, receptor tyrosine kinase; RET, RET proto-oncogene; MET, MET proto-oncogene receptor tyrosine kinase; FFPE, frozen formalin paraffin tissue.

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