NanoString System Versus Multiplex Fluorescent In Situ Hybridization for Gene Fusion Diagnosis in Lung Cancer Cytology Samples: Competing or Complementary Methods?

To the Editor—With the increased number of gene fusions (ie, ALK and ROS1, but also RET, NTRK 1/2/3, and NRG1) to analyze in non–small cell lung cancer (NSCLC) samples, including small/poor-cell/cytology ones, we fully agree with Ali et al1 that there is an urgent need for multiplex methods that can screen a large array of clinically informative targets with minimal tissue samples. In this field, the NanoString system reviewed by Ali et al1 is very interesting and promising compared with the classic gold standard fluorescent in situ hybridization (FISH) tests. Ali et al1 argued that FISH is expensive, requires technical expertise that may not be readily available in a histopathology laboratory, and is not amenable to multiplexed analysis.1 We intend to provide an alternative point of view about these drawbacks of FISH testing in NSCLC samples.

Ali et al1 said that FISH testing is an expensive and labor-intensive complex analysis. In fact, alternative molecular analyses are not clearly less expensive. For example, in France, the cost of a FISH test is €95.2, whereas a NanoString transcriptomic analysis could cost more than €2000.2 The cost of NanoString system equipment is also greater than that used to perform FISH analyses, limiting to date its implementation in histopathology laboratories. In terms of labor-intensity, the full process of a FISH analysis takes about 24 hours, with an interpretation step requiring only some minutes. FISH also allows the interpretation of samples with few tumor cells, including cytology samples that can be recognized among nontumor cells by trained pathologists, whereas the detection of low percentages of rearranged alleles could be challenging using methods requiring DNA/RNA extraction.3

According to Ali et al,1 FISH is not amenable to multiplexed analysis. Nevertheless, besides classical break-apart probes designed to analyze a single gene, multiplexed FISH probes are now developed to concurrently analyze several oncogenes as ALK and ROS1 genes to date, using different fluorescent probe combinations. For example, the 4-color Vysis ALK/ROS1 Dual Break Apart probe kit (Abbott Molecular, Chicago, Illinois) and the 3-color FlexISH ALK/ROS1 Distinctive Probe (ZytoVision, GmbH, 

Design and interpretation algorithm of a multiplexed ALK, ROS1, and RET break-apart 4-color fluorescent in situ hybridization probe. A, Design of the probe. The ALK probe is labeled in 5′-green and 3′-red, the ROS1 probe is labeled in 5′-green and aqua and 3′-red and aqua, and the RET probe is labeled in 5′-green and gold and 3′-red and gold. B, Interpretation algorithm. A first interpretation using red and green filters (eg, combined in a dual-band red-green filter) permits the identification of the cases without rearrangement (<15% positive nuclei) or with potential gene rearrangement with at least 15% of positive tumor nuclei (ie, nuclei with either 5′-green–3′-red split signals or isolated 3′-red signals). In case of potential gene rearrangement, further analysis using aqua and gold filters would permit the identification of the rearranged gene: no colocalization of positive signals with aqua or gold signals means an ALK rearrangement, whereas colocalization of positive signals with aqua or gold signals means ROS1 or RET rearrangements, respectively.
The purpose of our article was to evaluate the role of the NanoString system in the analysis of gene fusions in lung cytology. We never assessed whether NanoString system is cheaper than fluorescence in situ hybridization (FISH). Indeed, to compare the cost of a 1-gene test with that of an entire panel, it can be misleading. It is not appropriate to refer to NanoString “transcriptomic analysis” and to report costs without specifying the exact number of genes included in the panel. It is generally accepted that NanoString can be more cost-effective than FISH, particularly in terms of assessable targets and data interpretation. FISH is considered a quite expensive and labor-intensive technique, with a low power of multiplexing.

Currently, multiplex FISH assays for lung cancer are commercially available only for the simultaneous analysis of 2 fusion genes, ALK and ROS1. However, we agree on the possibility to design and develop a multiplex probe to concurrently analyze up to 3 oncogenes, but we have some concerns about the interpretation of data. Problems related to FISH interpretation have been widely reported and discussed over the years. Undoubtedly, FISH is considered the gold standard for the analysis of gene fusions, but literature data indicate that it is prone both to false-negative and false-positive results and to a significant interobserver variability. FISH analyses are evaluated by pathologists and can suffer from some degree of subjectivity. Moreover, in cases with nuclear overlapping, crush artifact, or technical limitations, FISH analysis may be uninterpretable. In this way, the interpretation of multiplex FISH could be even more complex. In our opinion, a few minutes are not always sufficient to interpret FISH even for highly skilled pathologists.

On the other hand, NanoString data interpretation is fully automated and objective thanks to dedicated analysis software. In addition, concerning the analysis of gene fusions, in comparison to FISH, NanoString system also allows us to recognize the most frequent and known fusion variants, which can influence the response to tyrosine kinase inhibitors. We also agree with the fact that the detection of low percentages of rearranged alleles could be challenging, but it was reported that a tumor cell content of 10% can be sufficient to detect gene fusions by NanoString. In conclusion, we believe that FISH is a valuable technique with a crucial role in the clinical practice of lung cancer, but the introduction of new diagnostic systems could be advantageous. Particularly, NanoString can provide an alternative molecular diagnostic approach that can help to control costs, eliminate unnecessary testing, and improve turnaround time. Anyway, it always has to be considered that the adoption of a specific technique depends on local resources, expertise of the laboratory, number of cases per year, and local reimbursement policy.