The purpose of our article\(^1\) 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 gene panel 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.\(^2\) FISH is considered a quite expensive and laboratory-intensive technique, with a low power of multiplexing.\(^3\) 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.\(^4,5\) 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.\(^4,5\) In this way, the interpretation of multiplex FISH could be even more complex. So, 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.\(^4\) 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.\(^5\) We also agree with the fact that the 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.\(^6\)

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.\(^3\) 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.

Greta Ali, MD\(^2;\) Rossella Bruno, PhD\(^2;\) Gabriella Fontanini, MD\(^2,3\)

1 Unit of Pathological Anatomy and 2 Program of Pleuropulmonary Pathology, Azienda Ospedaliero Universitaria Pisana, AOUP, Pisa, Italy; 3 Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy


Accepted for publication July 17, 2018.

The authors have no relevant financial interest in the products or companies described in this article.


In Reply.—We thank the authors for their interest in our review\(^1\) and for their comments.