

Peer Review File

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Comment 1: In the text, the authors report that a not negligible amount of starting material is required to successfully perform molecular analysis. Please, could the authors discuss this point at the sight of scant samples available in diagnostic setting?

Reply 1: We have discussed this point in the methods section of the manuscript and we have included new references.

Change in the text: Page 7 lines 125-134

Comment 2: Please, could the authors improve the quality of the figure 1?

Reply 2: We create a new figure with better quality

Change in the text: New Figure 1

Comment 3: Please, could the authors report if positive results were also confirmed by orthogonal technologies?

Reply 3: In most of the studies reviewed in our manuscript, positive results by nCounter were confirmed by orthogonal techniques such as FISH or IHC. We have added a new table (Table 3) showing the results of these comparisons and detailing the sensibility and specificity of nCounter versus the different orthogonal techniques.

Change in the text: New Table 3, Page 7 Line 136

Comment 4: The authors should make a table with the different current studies using the hybridization-based platforms and showing also which methods were used in these studies as gold standard methods (FISH, DNA and/or RNA NGS, even ALK IHC.) and the specificity and the sensitivity of these different comparative studies.

Reply 4: See reply to comment 3, reviewer A. We have added a new table (Table 3) comparing the sensibility and specificity of nCounter versus gold standard methods in the studies review in our article.

Change in the text: New Table 3, Page 7, Line 136

Comment 5: The authors should make a table with the pros and the cons of using the hybridization-based platform and describing the limitations of this approach comparatively with other methods for gene fusions identification.

Reply 5: We have created a table (Table 2) comparing the requirements of the techniques used for fusion and splicing variant detection. In the table, we highlight the advantages that nCounter offer in comparison with the others techniques

Change in the text: New Table 2 and Page 7, line 128

Comment 6: What were the associations between oncogenic gene fusions and splicing variants and the tumorigenesis and development of NSCLC? Please supplement in the introduction.

Reply 6: We have added in the introduction a brief explanation about how fusions and splicing variants can occur in cancer cells

Change in the text: Page 4, Line 60-61

Comment 7: What were the advantages of target therapy in the treatment of lung cancer? Please state in the introduction.

Reply 7: We have added in the introduction a brief explanation about the advantages of targeted therapy in the treatment of lung cancer, and we have included appropriate references

Change in the text: Page4, line 65-70

Comment 8: For target drugs, whether there were drug-resistant during the treatment? Please state in the review.

Reply 8: We have added a comment and some references in the introduction about emergence of resistance to targeted therapy in cancer patient.

Change in the text: Page4, line 70-73

Comment 9: How many oncogenic genes could be detected by nCounter technology? And how about the detective cost and accuracy rate of nCounter technology? Please state in the review.

Reply 9: We have added a paragraph detailing the number of genes and mentioning the cost of nCounter. In addition, as mentioned in the replies to reviewers A and B, we have added a table comparing sensitivity and specificity of nCounter with gold standard techniques

Change in the text: Page 6-7, line 111-116 and Table 2

Comment 10: How about the prospect of the applications of nCounter technology? Please supplement in the review.

Reply 10: We have added this information in the additional paragraph mentioned above.

Change in the text: Page 6-7, line 111-1116