Discrepancies Between *ALK* FISH and Capture Based NEOplus Diagnostics

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Background:

Research in recent years has unraveled several gene fusions driving tumor development in lung cancer. Especially adenocarcinomas of the lung harboring *ALK* and *ROS1* gene fusions exhibit striking sensitivity to ALK and ROS1 kinase inhibitors respectively, translating to dramatic responses in the clinic. Several different technologies are available to detect aberrant genomic structures. The most frequently used technologies include fluorescent *in situ* hybridization (FISH), currently considered as the "gold standard", immunohistochemistry (IHC) and hybrid capture based NGS sequencing.

Methods:

Here, we describe a selection of tumor samples showing discrepant results between fluorescent *in situ* hybridization and hybrid capture based NGS sequencing. These included samples with positive FISH but negative NEOplus as well as negative FISH and positive NEOplus results. In addition, we used response data of targeted therapies to evaluate the true genetic phenotype of the tumor.

Results:

Overall, several lung adenocarcinomas showed discrepant results if FISH and NEOplus data were compared. First, one sample was tested positive for *ALK* rearrangement using FISH which was not confirmed using NEOplus. In line with this finding, the tumor did not respond to ALK TKI treatment. Second, a total of 4 cases were fusion negative by FISH but positive by NEOplus. Three out of 4 *ALK* positive cases showed clinical response to ALK kinase inhibition, the clinical results for case number 4 are pending. Interestingly, one of these responding tumors was also negative for ALK expression using IHC.

In summary, we describe a selection of tumor samples with discrepant results for fusion detecting using FISH and NEOplus. Overall, in all of the cases for which clinical response data was available, tumor sensitivity was in line with the initial diagnosis generated by the NEOplus assay.