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Intact or broken-apart RNA: an alternative concept for ALK fusion screening in non-small cell lung cancer (NSCLC).

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Abstract

Anaplastic lymphoma kinase (ALK) break-apart fluorescent in situ hybridization (FISH) is currently used in diagnostics for the selection of non-small cell lung cancer (NSCLC) patients to receive crizotinib. We evaluated ALK status in NSCLC with a novel ALK mRNA test based on the break-apart FISH concept, which we called break-apart transcript (BAT) test. ALK5' and ALK3' transcript patterns were established with qPCR for ALK-expressing controls including fusion-negative neuroblastomas, as well as fusion-positive anaplastic large cell lymphomas and NSCLC. The BAT test was evaluated on 271 RNA samples from routinely processed paraffin NSCLC tissues. Test results were compared with ALK FISH (n=121), immunohistochemical (IHC) analysis (n=86), and automated quantitative analysis (AQUA, n=83). On the basis of the nonoverlapping ALK BAT patterns in ALK-expressing controls ($P<0.0001$), 8/174 adenocarcinomas (4.6%) among 259 informative NSCLC were predicted as fusion positive. Overall concordance for paired method results was high (94.1% to 98.8%) but mainly concerned negative prediction because of the limited availability of positive-matched cases. Tumors with 100% cytoplasmic IHC staining of any intensity (n=3) were positive for AQUA, FISH, and BAT test; tumors with lower IHC positivity and different staining patterns were AQUA-negative. Upon multiple reevaluations, ALK gene status was considered as originally misinterpreted by FISH in 3/121 cases (2.5%). Tumors with >4 ALK gene copies were associated with longer overall survival upon first-line chemotherapy. In conclusion, application of the ALK BAT test on routinely processed NSCLC tissues yields the same fusion partner independent information as ALK break-apart FISH but is more robust and cost-effective. The BAT concept may be considered for the development of further drug-predictive translocation tests.