Detection of ALK Fusion Transcripts in FFPE Brazilian Lung Cancer Samples by NanoString Technology

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BACKGROUND: NanoString nCounter is a high-throughput digital system that uses direct single molecule imaging with multiplexed molecular barcodes to detect up to 800 targets in a single reaction. This new technology has the capability to detect nucleic acid targets (DNA, RNA, and small-RNAs) from cell lysates, FFPE and single cell samples. Recently, it has been used as tool to validate prognostic signatures in cancer with increased statistical power. Since NanoString can accurately count even poor quality RNA molecules, it has become possible to detect oncogenic gene fusion transcripts in FFPE samples.

HYPOTHESIS: To describe the standardization of NanoString technology for detection and analysis of ALK fusion transcripts in FFPE Brazilian lung cancer samples.

METHODS: In this study, 45 patients with lung cancer were included. Among these 45 cases, 15 were ALK-positive and 30 ALK-negative, as previously detected by FISH and immunohistochemistry. Furthermore, 2 lung cancer cell lines (H2228 as ALK-positive and CALU3 as ALK-negative) were also used as controls. RNA was isolated from three to four sections of 10 µm thick using RecoverAll Total Nucleic Acid Isolation kit (Life Technologies). A total of 50-100 ng of RNA was submitted to NanoString nCounter Elements protocol. The panel consists of 4 probes at 5' and 4 probes at 3' position of the fusion point (exon 20), in which the ratio 3'/5' of these probes suggest the presence of fusion. Moreover, the panel also contains specific fusion probes for EML4-ALK (5 variants), KIF5B-ALK and TGF-ALK fusions. Data analysis was performed using NanoString nSolver and R softwares.

RESULTS: A total of 3 samples (6.2%) were excluded from the analysis due to low positive control signals, all of them ALK-negative samples. Only 1 ALK-positive sample was not detected by this approach. Of the 14 ALK-positive samples detected, 8 (57.1%) presented the EML-ALK variant 1; 1 (7.2%) presented the EML-ALK variant 2; 2 (14.3%) exhibited the EML-ALK variant 3a; and 4 (21.4%) had no variant detected but presented high 3'/5' ratio, similarly to other positive samples.
Concluding, in this study, we optimized the use of the NanoString technology for detection of ALK fusion in lung FFPE samples. We observed that NanoString is a very sensitive method even in low quantity and poor quality RNA, detecting 93.3% of ALK-positivity and showing its great utility in routine ALK detection for therapeutic proposes.