Analytical validation of solid tumor fusion gene detection in a comprehensive NGS-based clinical cancer genomic test

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Background

As the number of clinically actionable cancer genes grows and the size of most diagnostic biopsies decreases, next-generation sequencing (NGS) becomes increasingly attractive as a diagnostic tool, as it can detect all classes of genomic alterations in all cancer genes in a single test. However, for NGS to achieve its full utility in the clinic, robust analytical validation and performance comparison against established detection methodologies are required for each class of targetable genomic alteration. Previously, we reported on the development and validation of an NGS-based diagnostic test for accurate detection of clinically-relevant genomic alterations across all exons of 287 cancer genes in routine FFPE specimens. Here, we present validation of fusion gene detection in the test, enabled by hybrid-selection and deep sequencing of commonly rearranged introns in 19 genes.

Methods

Foundation Medicine's NGS-based cancer assay

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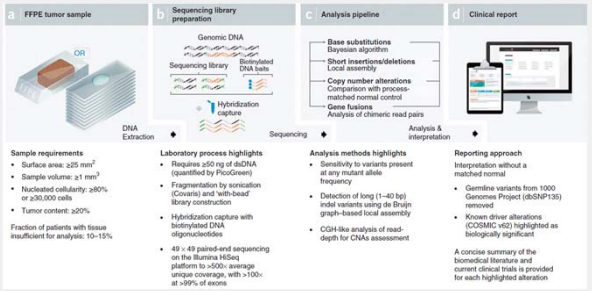
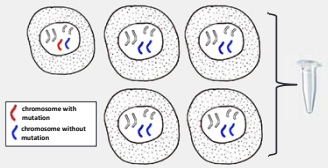


Fusion gene detection using hybrid-selection and deep sequencing of introns

Genomic rearrangements are identified by analyzing chimeric read pairs, read pairs which map to separate chromosomes, or at greater distance than expectation. Pairs are clustered by genomic coordinate, and clusters containing at least five chimeric pairs (3 for known fusions) are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (MQ>30) and distribution of alignment positions (sd>10). Rearrangements are annotated for predicted function (e.g., creation of fusion gene)

Fusion gene detection validation: Cell-line models



20% Cellular Fraction (10% allele frequency)

Tumor cell-lines selected for analysis	Known gene-fusion
SU-DHL-1	*NPM1-ALK*
NCI-H2228	*EML4-ALK*
HCC-78	*SLC34A2-ROS1*
LC-2/ad	*CCDC6-RET*
NCI-H660	*TMPRSS2-ERG*

These 5 cell lines were mixed into 22 variably sized pools such that each fusion was represented at 20%, 25%, 33%, 50%, and 100% cellular fraction at least once, for a total of 32 gene fusion test cases

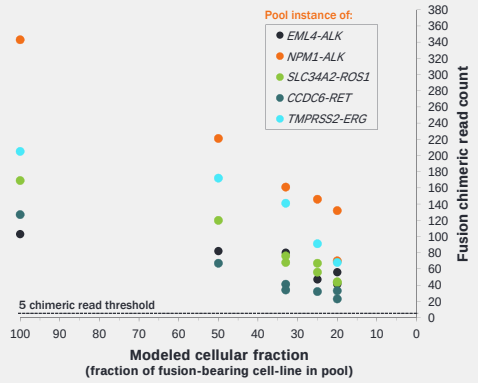
Fusion gene detection validation: Clinical FFPE specimens

FM NGS assay precision (reproducibility)	
Genes tested	*RET* (x2), *ALK* (x2), *ROS1* (x2), *FGFR3*, *BRAF*, *EGFR (vIII - intragenic)*, *ERG*
# of specimens tested	10
# replicates	5 (3 intra + 2 inter plate)
# total assays	50
Sample tested	Aliquots of originally extracted DNA

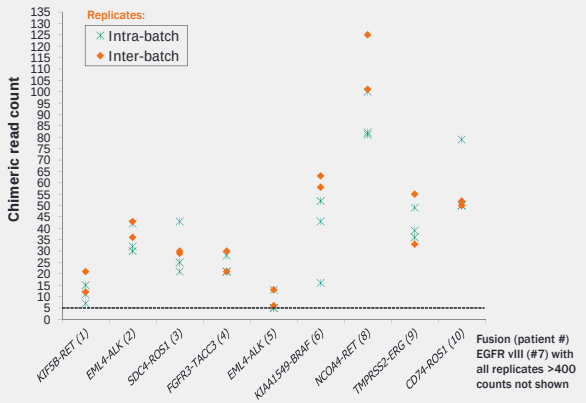
Concordance with standard of care (SOC) clinical testing	
Gene tested	*ALK* rearrangement
SOC assay	Abbott Vysis FISH
# of total samples	45 (from MSKCC)
# of FISH positive samples	22
Sample tested on FM NGS assay	DNA from new 4x10µm unstained sections from original FFPE block

Results

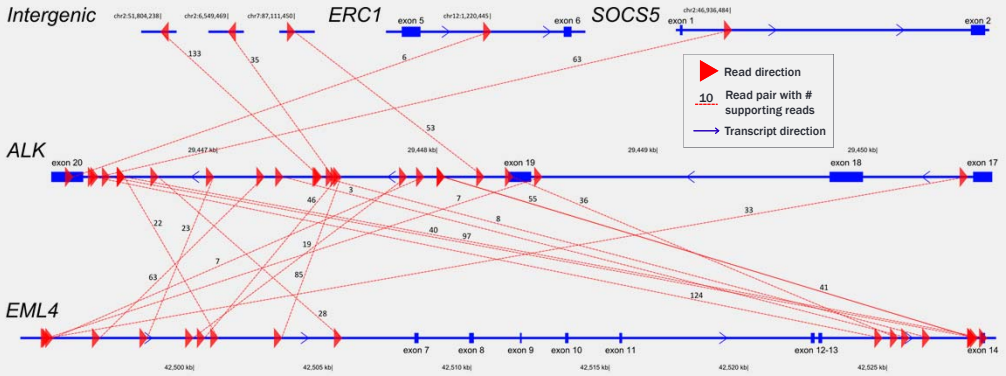
Cell-line models demonstrate fusion detection sensitivity at 20% cellular fraction and above



Repeated testing of clinical FFPE specimens demonstrates detection reproducibility within and across assay batches



All 22 *ALK* FISH+ rearrangements were observed by NGS despite significant variability in breakpoint locations



Validation analysis	Result summary
Cell-line models	All 32/32 fusions detected (sensitivity 100%, 95% CI 89%-100%), with no false positive calls
ALK FISH concordance	Of the 22 *ALK* FISH (+) cases, all were detected, including two marginal cases (<5 reads and low MQ) that were (+) by NGS as both partners of the fusion event were known (i.e. *EML4/ALK*). In the 23 FISH (-) cases, a single novel gene fusion (*SOCS5-ALK*) discovered with >60 chimeric reads
NGS assay precision	All alterations were detected in all replicates
Survey of 724 clinical FFPE lung adenocarcinomas	5% *ALK*, 3% *RET*, and 2% *ROS1* rearrangement frequency respectively, in line with published data

Conclusions

- We present rigorous validation of targeted fusion gene detection for solid tumors in an NGS-based test for use in clinical oncology
- Performance of the NGS-based test (FoundationOne™) matches current standard of care assays for rearrangements, while efficiently assessing multiple relevant markers
- Given the ability to detect a broader range of genomic alterations than currently available technologies with high accuracy from small biopsies, this type of testing can become a direct component of patient care and potentially expand targeted treatment options