Detection of fusion oncogenes in routinely collected biorepository samples

Zoran Gatalica[1]*, Inga Rose[1], Nataliya Kuzmova[1], Semir Vranic[2]

[1] Reference Medicine, Phoenix, AZ, [2] College of Medicine, QU Health, Qatar University, Doha, Qatar
*Presenting author: zoran@referencemedicine.com

AACR SPECIAL CONFERENCE IN CANCER RESEARCH: FUSION-POSITIVE CANCER: FROM DISCOVERY TO THERAPY
January 13 - 15, 2026
Hilton Philadelphia at Penn's Landing
Philadelphia, Pennsylvania

Introduction

Biorepository tumor samples linked to clinical data are indispensable for biomarker discovery and translational cancer research. Oncogenic fusions are highly specific and therapeutically actionable targets, yet their detection remains challenging due to biological diversity and low prevalence in most solid tumors (≈1–4%), with the notable exception of prostate adenocarcinoma (*TMPRSS2::ERG* >30%). Systematic, cost-effective screening of archived samples could accelerate fusion-driven research and therapeutic development.

Methods and Samples

We examined formalin-fixed, paraffin-embedded (FFPE) tumor samples from a commercial biorepository (Reference Medicine, Phoenix, AZ) using a workflow that included expert pathology review, whole-slide scanning, construction of multi-tumor tissue microarrays (TMAs), immunohistochemistry (IHC), and genomic profiling with commercial next-generation sequencing (NGS) panels.

More than 500 tumors were reviewed; selected cases of NSCLC (n=68) were cored into TMAs. IHC for ALK, ROS1, HER2, ALK, BRAF, MET, and pTRK proteins was performed on TMAs.

Results

- Two (2) of sixty-eight (68) screened NSCLC cases were positive by IHC for ROS1 and ALK proteins.
- Thirty-eight (38) of the cases were further profiled using Next Generation Sequencing (NGS) utilizing various commercially available panels such as Burning Rock OncoScreen Plus, Illumina TSO500 (complete DNA mutation and RNA fusion analysis), and Archer FusionPlex Comprehensive Thyroid & Lung RNA-Seq (a targeted panel of gene fusions).
- The subsequent NGS sequencing confirmed both IHC positive results (*EZR::ROS1* fusion and a *EML4::ALK* fusion, respectively).
- All other tested cases were negative by NGS, in concordance with the IHC data.

Table 1. An immunohistochemistry (IHC) screening panel of six antibodies was used (ALK, ROS1, pTRK, BRAF, MET, and HER2).

Biomarker	Oncogenic alteration	Role of IHC	Preferred confirmatory test	Predictive therapies	Pitfalls (%)
ROS1	ROS1 fusions (EZR–ROS1, CD74–ROS1)	Good screen; treat any positive as presumptive	RNA fusion NGS (preferred) or FISH	Crizotinib, entrectinib, taletrectinib	Confirm all IHC positives; rare (~1–2%)
HER2 (ERBB2)	ERBB2 mutations (Ex20ins); ± amplification	IHC 3+ not sufficient in lung	DNA/RNA NGS; FISH for amplification	Trastuzumab deruxtecan	Actionability driven by mutation, not IHC alone (1-2%)
ALK	ALK fusions (EML4–ALK)	Useful screen	RNA fusion NGS or FISH	Alectinib, lorlatinib	Prefer NGS confirmation (2-5%)
BRAF	V600E (others exist)	VE1 can screen V600E	DNA NGS or PCR	Dabrafenib + trametinib (V600E)	Non-V600 differ in evidence (1-2%)
MET (c-MET)	Exon 14 skipping; amplification	Overexpression not predictive for Ex14	RNA/DNA NGS (Ex14); FISH for amp	Capmatinib, tepotinib	Copy-number thresholds matter (1-2%)
pan-TRK (NTRK1/2/3)	NTRK1-3 fusions	Useful screen; background staining pitfalls	RNA fusion NGS (preferred) or RT-PCR; FISH context-dependent	Larotrectinib, Entrectinib	Confirm all IHC positives (1%)

Figures 1-2. Hematoxylin and Eosin-stained (H&E) and ROS1 IHC stained (left and right, respectively) tissue microarray containing 34 NSCLC samples.

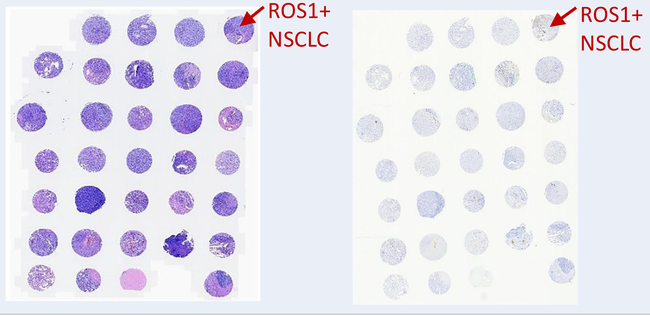


Figure 3. Hematoxylin and Eosin-stained (H&E) of ROS1+ NSCLC case.

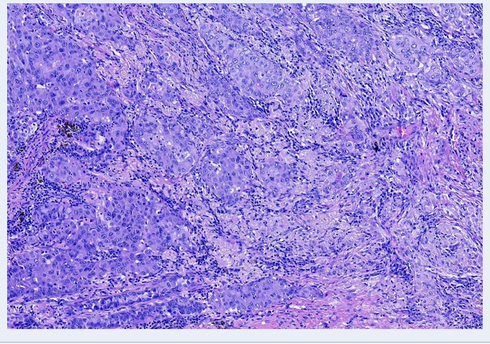
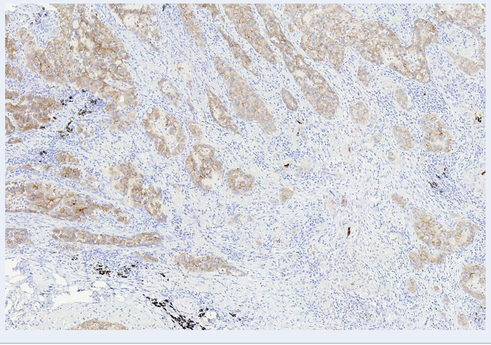


Figure 4. ROS1+ by immunohistochemistry (Rabbit mAb #3287 by Cell Signaling)



Conclusions and Key Takeaways

- ***The study illustrates a cost-effective approach to identifying rare fusion events in a biorepository of NSCLC.***
- ***Excluding manpower hours (pathology review, generation of tissue microarray/TMA/), the cost for NGS testing of 34 (which was the size of one TMA generated) lung cancers would cost a minimum of $20k based on the best commercial price per tested panel, whereas a single IHC staining for ROS1 costs $50 (<$2 per sample). With this approach, ROS1 fusion was identified for a fraction of the cost of NGS if each sample was tested.***
- ***A similar IHC approach using mutation-specific Abs (e.g., BRAFV600E, ALK, ARv7, IDH1) can also be used for NSCLC and other tumors (e.g., melanoma, gliomas, prostate cancer).***
- ***The approach allows for better utilization of existing samples and a lower cost per sample.***

For additional information
contact Inga Rose at
inga@referencemedicine.com
Reference Medicine, Phoenix, AZ

Or visit:
www.ReferenceMedicine.com