ALK gene rearrangement in NSCLC by NGS: an EQA case report

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Introduction

Rearrangement of the anaplastic lymphoma kinase (ALK) gene in non-small cell lung carcinoma (NSCLC) define a molecular subgroup of tumour characterised clinically by sensitivity to ALK tyrosine kinase inhibitors such as crizotinib. Reliable detection of ALK gene rearrangement is crucial for selecting patients eligible for crizotinib therapy. Gene rearrangements are commonly identified using fluorescence in-situ hybridisation (FISH) and immunohistochemistry (IHC). In the past decade, next generation sequencing (NGS) is gradually being introduced into clinical laboratories for its ability to screen for multiple molecular markers of NSCLC in a time efficient manner.

An external quality assurance (EQA) program was introduced in 2020 to monitor laboratory performance in diagnostic interpretation of ALK gene rearrangement analysis by FISH, IHC and molecular techniques. Forty-three laboratories were enrolled in this program and seven laboratories (16%) performed molecular detection of ALK fusion variants by NGS or reverse transcription PCR (RT-PCR). There was a 60% discordance within the molecular results reported in this survey where laboratories failed to detect the presence of an ALK fusion variant. In summary, we describe the outcome of an investigation to determine the ALK fusion variant in this case.

Method

- Two NSCLC cases were provided for ALK gene rearrangement analysis in the 2020 EQA survey. Laboratories enrolled in the Molecular exercise of this survey were provided with 2 x 3µm sections for each case.
- All participating laboratories were required to perform FISH, IHC and/or molecular analysis and provide a diagnostic interpretation of ALK gene rearrangement status or the absence/presence of an ALK fusion variant.
- Survey results were assessed against a consensus ALK gene rearrangement status confirmed by FISH and IHC. Case 1 was positive for ALK gene rearrangement; Case 2 was negative for ALK gene rearrangement.
- A generic report containing methods details and overall laboratory performance was issued to all participating laboratories.
- Molecular method details used by participating laboratories in the 2020 EQA survey are listed in Table 1.

Table 1. Method details of molecular techniques used.

Laboratory	Method	Library/Template Preparation Kit	Panel	Platform	Limit of Detection
1	NGS	Ion AmpliSeq Library Kit 2.0/Ion PI Hi-Q OT2 (Thermo Fisher Scientific)	Ion AmpliSeq RNA Lung Fusion Panel (Thermo Fisher Scientific)	Ion Proton System (Thermo Fisher Scientific)	~1%
2	NGS	Ion AmpliSeq Library Kit 2.0/Ion PGM Hi-Q View Chef Kit (Thermo Fisher Scientific)	Ion AmpliSeq RNA Lung Fusion Panel (Thermo Fisher Scientific)	Ion Torrent Personal Genome Machine (Thermo Fisher Scientific)	Fusion transcript detection from 20 or more fusion reads
3	NGS	Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific)	Oncomine Comprehensive Panel (Thermo Fisher Scientific)	Ion GeneStudio S5 System (Thermo Fisher Scientific)	Targeted gene fusions detected on 500 or more reads
4	NGS	Archer FusionPlex Lung (Archer Dx)	Archer FusionPlex Lung Panel (Archer Dx)	MiSeq System (illumina)	5%
5	RT-PCR	EML4-ALK Fusion Gene Detection Kit (Amoy Dx)	n/a	SLAN-96 PCR Real-Time PCR System (Zeesan)	25 copies/µL EML4-ALK fusion plasmid DNA

Results

- ALK gene rearrangement status reported by participating laboratories using IHC, FISH and/or molecular techniques (NGS/RT-PCR) for both cases are presented in Figures 1 and 2.
- Overall, discordant results (false negative) were noted in the molecular results reported for Case 1 (see Figure 1). Laboratories 1 and 2 detected the ALK fusion variant and were concordant. Laboratories 3, 4 and 5 did not detect the fusion and were discordant.
- To investigate the ALK fusion variant detected by 2/5 laboratories in Case 1, samples were sent to the Department of Anatomical and Cellular Pathology, Prince of Wales Hospital (Hong Kong) to determine the genotype by RNA and DNA NGS. Genotype data presented in this report was kindly provided by this laboratory to share their findings:

1. RNA sequencing using in-house capture panel:

This panel covers all coding exons of *ALK* gene such that it can detect the ALK fusions regardless of the fusion partner (the technology is similar to the illumina's RNA fusion panel). After sequencing, fusion calling was performed on STAR-Fusion and Manta on illumina BaseSpace and Manta on a local computer. Interestingly, STAR-Fusion and Manta on illumina BaseSpace did not detect any fusion. However, the Manta running on local computer detected the EML4-ALK fusion. The fusion was created by fusing the intron 5 of *EML4* gene to exon 20 of *ALK* gene.

2. DNA sequencing using in-house capture panel:

In order to delineate the DNA breakpoint of Case 1, DNA extracted from the sample was analysed with a capture panel which covered the intron 17-exon 24 of *ALK* gene. The data were aligned to hg19 by BWA and the fusion was called by Manta. The DNA sequencing called the same breakpoint as those in the RNA sequencing data.

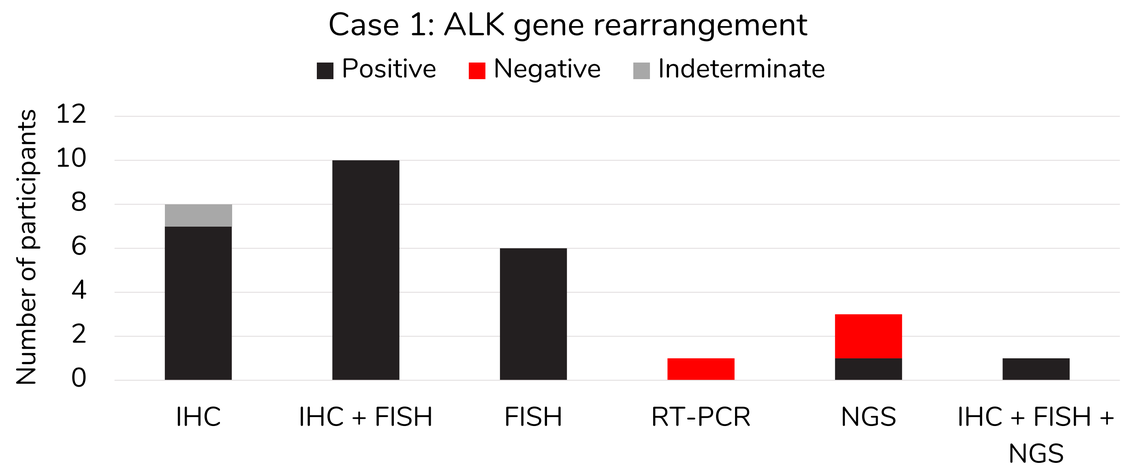

Figure 1. ALK gene rearrangement status reported for Case 1. Immunohistochemistry (IHC); fluorescent in-situ hybridisation (FISH); next generation sequencing (NGS); reverse transcription PCR (RT-PCR).

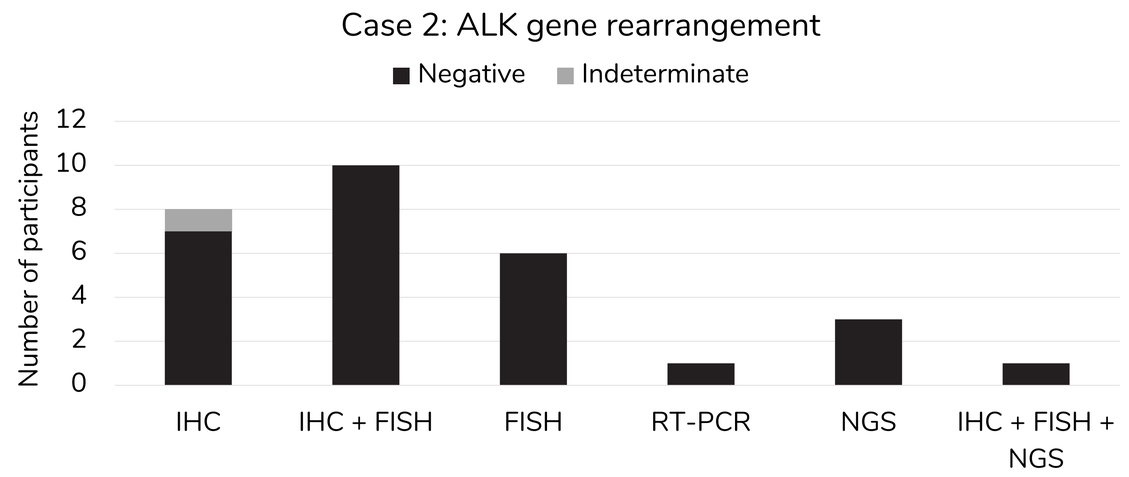

Figure 2. ALK gene rearrangement status reported for Case 2. Immunohistochemistry (IHC); fluorescent in-situ hybridisation (FISH); next generation sequencing (NGS); reverse transcription PCR (RT-PCR).

Conclusion

- It is likely that the panel/target screened by the discordant laboratories did not cover the ALK fusion variant present in Case 1.
- The data from Prince of Wales Hospital supported that there is an EML4-ALK fusion in Case 1. The fusion variant was created by fusing the intron 5 of *EML4* gene to exon 20 of *ALK* gene.
- Detection of this fusion variant can be very challenging, specifically for RNA sequencing.
- On the assay level, this fusion may be missed by amplicon sequencing since most of the amplicon sequencing assay targets the exon-exon boundary of the fusion genes. For example, this fusion variant may be missed by Archer's FusionPlex panel since the primer of the Archer's assay may be on or before breakpoint of the *ALK* gene such that the primer cannot detect the fusion.
- On the fusion calling level, this fusion may be filtered out by some stringent rule set of the variant callers.
- NSCLC patients with positive ALK gene rearrangement are unlikely to respond to crizotinib therapy; hence any false negative results will have significant implications in patient treatment.
- Continuous participation in EQA programs has been proven to improve laboratory performance.

References

Brisudova, A. and Skarda, J. 2020. Gene rearrangement detection by next-generation sequencing in patients with non-small cell lung carcinoma. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 164(2).

Selinger, C., Rogers, T.M., Russell, P.A., et al. 2013. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Modern Pathology*, 26.

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