Detection of gene fusions and exon skipping events in lung FFPE samples with Oncomine Precision Assay on Ion Torrent Genexus™ System

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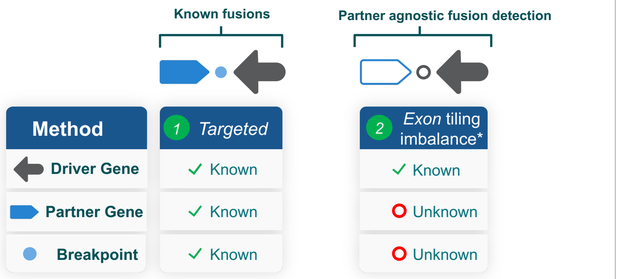
INTRODUCTION

Gene fusions and exon skipping events play an important oncogenic role in non-small cell lung cancer (NSCLC). Here we employed the Oncomine Precision Assay (OPA) for sequencing of 1032 archived research FFPE (Formalin-Fixed Paraffin-Embedded) lung samples using the Genexus™ integrated sequencing platform. The RNA assay strategy is aimed at providing a wide scope for studying known oncogenic fusions and exon skip variants, as well as a method for detection of fusions in a partner agnostic manner. We summarize the findings that include detected samples with oncogenic fusions in tyrosine kinase genes *ALK*, *RET*, *ROS1* as well as *MET* exon14 skipping, and demonstrate the novel fusion detection capabilities of the panel with Exon tiling imbalance (Fig. 1).



** Available for ALK, FGFR1,2,3, NTRK1,2,3, and RET fusion drivers*

Figure 1. *FusionSync* combined approach for detection of known and novel gene fusions.

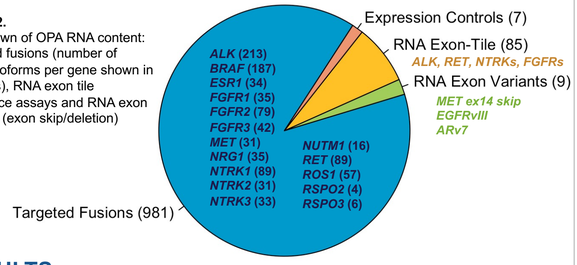
MATERIALS AND METHODS

RNA libraries were prepared from 1032 unique lung research FFPE samples, and were sequenced with Oncomine Precision Assay Ion AmpliSeq™ HD RNA panel (Fig. 2) (developed for use on both tissue and liquid biopsy research samples with low input RNA):

- The panel targets 981 known fusion isoforms across 16 oncogenic driver genes
- Targets *MET* exon 14 skipping, *EGFRvIII* (exon deletion) and *ARv7* and utilizes an end-to-end bioinformatic solution for reporting these intragenic variants in sample types where they may be detected
- Supports partner agnostic fusion detection in *ALK*, *RET*, *NTRK1,2,3* and *FGFR1,2,3 genes*
- Additional WT assays in the panel are utilized to assess library quality (7 assays in house keeping genes) and normalize coverage levels for intragenic assays in *MET, EGFR, AR* (6 assays)
- Results are assessed bioinformatically with a framework that includes detailed genomic annotations of the fusion breakpoint and generates interpretable fusion calls and report in the Genexus™ software.

Oncomine Precision Assay (OPA) – RNA content

Figure 2.
Breakdown of OPA RNA content: Targeted fusions (number of fusion isoforms per gene shown in brackets), RNA exon tile Imbalance assays and RNA exon Variants (exon skip/deletion)



RESULTS

Lung FFPE research samples: filtering and summary of findings

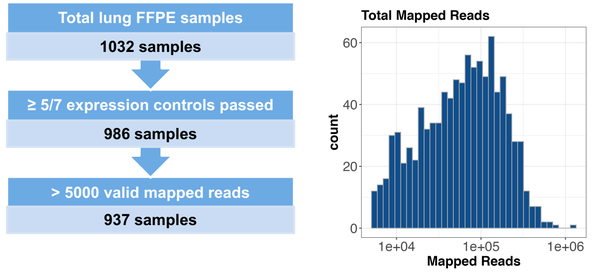


Figure 3. Left: Count of Total and unique lung FFPE samples and sample quality filtering. Right: histogram of number of filtered valid mapped reads to OPA RNA panel

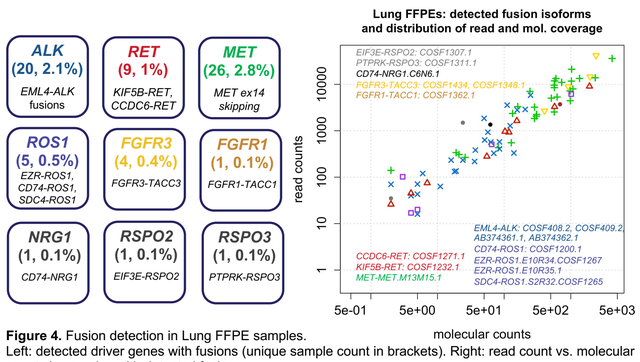


Figure 4. Fusion detection in Lung FFPE samples.
Left: detected driver genes with fusions (unique sample count in brackets). Right: read count vs. molecular counts in samples with detected fusions

Lung samples with *MET* ex14 skip RNA detection and DNA splice site evidence

	Valid Controls	Total RNA Mapped Reads	isoform	Read. Counts	Mol. Counts	Splice site variant: DNA Evidence	
						SNV/indel	Allele. Freq.
1	7	152458	MET-MET.M13M15.1	21135	1030	COSM6108462	0.42
2	7	83587	MET-MET.M13M15.1	13355	599	COSM29636	0.22
3	7	98379	MET-MET.M13M15.1	11738	1022	chr7:116412046|A>T	0.48
4	7	340835	MET-MET.M13M15.1	11016	730	COSM6108462	0.32
5	7	302302	MET-MET.M13M15.1	9587	564	COSM6108462	0.05
6	7	192983	MET-MET.M13M15.1	7187	976	chr7:116412046|A>T	0.4
7	7	35595	MET-MET.M13M15.1	7040	244	COSM6108461	0.31
8	7	55441	MET-MET.M13M15.1	5327	623	chr7:116412046|A>T	0.38
9	7	139808	MET-MET.M13M15.1	5042	510	chr7:116412043|G>A	0.35
10	7	285315	MET-MET.M13M15.1	4430	982	COSM6108460	0.29
11	7	21810	MET-MET.M13M15.1	3312	160	COSM6108461	0.38
12	6	8699	MET-MET.M13M15.1	3226	286	COSM6438141	0.71
13	7	506286	MET-MET.M13M15.1	2353	134	chr7:116411902|G>A	0.32
14	7	20805	MET-MET.M13M15.1	1840	285	chr7:116412045|T>A	0.16
15	7	8955	MET-MET.M13M15.1	550	128	COSM6924587	0.31
16	6	13724	MET-MET.M13M15.1	338	19	chr7:116412043|G>A	0.3
17	7	71973	MET-MET.M13M15.1	308	21	chr7:116412045|T>G	0.05

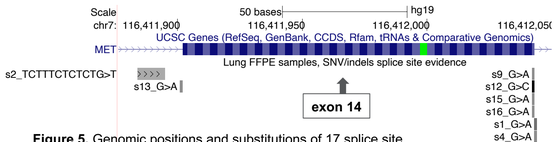


Figure 5. Genomic positions and substitutions of 17 splice site SNVs/indels in samples with positive RNA detection of *MET* exon 14 skip variant (*MET-MET*.M13M15.1). Variants congregate in the 5p and 3p ends of *MET* exon 14. (AF≥0.05; tick intensity marks AF in the range 0.05-0.71)

Table 1. High RNA coverage provides a strong and sufficient evidence for MET ex-14 skip detection. A total of 26 samples with *MET* ex-14 skip were detected (>150 reads; >10 mol.counts). In the table, splice site SNV/indel was detected in 17/26 samples with Allele frequency ≥0.05. The remaining samples had poor DNA quality (3) or might have intronic splice mutations not covered by the assay (6).

Exon tiling imbalance score distributions in *ALK* and *RET*

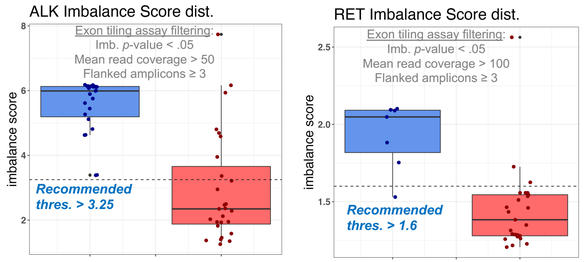


Figure 6. Distributions of imbalance scores computed in positive (blue) and negative (red) fusion samples passing exon-tiling quality filters (described in grey) using RNA exon tiling assay, for partner agnostic fusion detection. Left: *ALK*, 17/20 *ALK* fusion samples passed exon tiling filters with imb.score>3.25. Right: *RET*, 7/9 *RET* fusion samples passed exon tiling filters with imb.score>1.6

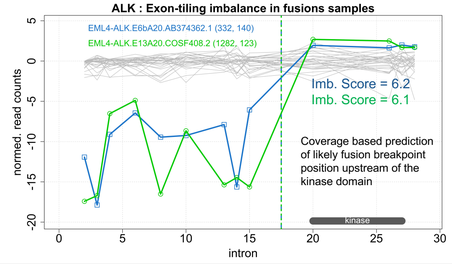


Figure 7. Example of Exon-tiling imbalance results in 2 *ALK* fusion samples (blue, green; read and molecular coverage per isoform shown in brackets). Normal FFPE samples (grey) are used to normalize exon-junction mRNA read coverage and predict de novo breakpoint positions (vertical dashed lines).

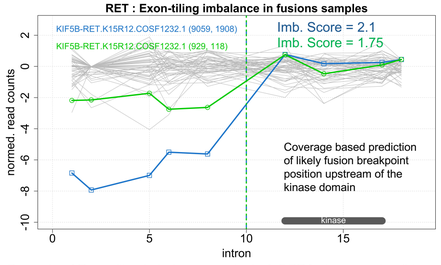


Figure 8. Example of Exon-tiling imbalance results in 2 *RET* fusion samples (blue, green; read and molecular coverage per isoform shown in brackets). Normal FFPE samples (grey) are used to normalize exon-junction mRNA read coverage and predict de novo breakpoint positions (vertical dashed lines).

CONCLUSIONS

We used the OPA RNA AmpliSeq HD panel to demonstrate detection of fusions and exon skipping rearrangements, as well as the potential for novel fusion detection with Exon tiling expression imbalance, in a research cohort of Lung FFPEs.

OPA RNA panel and the bioinformatic analysis in the Genexus™ software provide a powerful research tool for detection of oncogenic fusions and rearrangements at varying archived sample qualities, while retaining a simple workflow and fast turn-around time.