Detection of a novel RET gene fusion in a non-small cell lung cancer patient using AMP chemistry

Peng Fang, Zhenyu Yan, Weihua Liu, Jennifer Biroschak, Paul Labrousse, Jennifer Wright, Cindy Spittle, Chad Galderisi, and Jin Li, MolecularMD Corp., Cambridge, MA and Portland, OR

Introduction

In lung cancer, several driver Receptor Tyrosine Kinases (RTKs) have been identified in gene fusions, including ALK, RET, ROS1, NTRK1, NRG1 and FGFR1/2/3. These oncogenic gene fusions have become either direct targets or biomarkers for molecular therapies for patients with lung cancer. Screening patients for the presence of these gene fusions would be critical for oncologists to select appropriate therapeutic intervention or for drug developers to design novel therapeutics and conduct clinical trials. However, detection of fusions, especially unknown fusions, from FFPE samples is challenging for many diagnostic techniques.

ArcherDX™ FusionPlex™ Lung Thyroid Panel is built on Anchored Multiplex PCR (AMP™) Chemistry (Figure 1), allowing detection of both novel and known fusions. The panel targets fusions involving the driver genes ALK, FGFR3, MET, NTRK1, NTRK3, PPARG, RET and ROS1 (Table 1).

Here we report the results from our study using the Archer Lung-Thyroid Panel. We identified and verified the presence of a novel fusion between Myosin Phosphatase Rho Interacting Protein (MPRIP) and RET oncogene, in an FFPE sample from a non-small cell lung cancer (NSCLC) patient.

Figure 1. Principle of Anchored Multiplex PCR

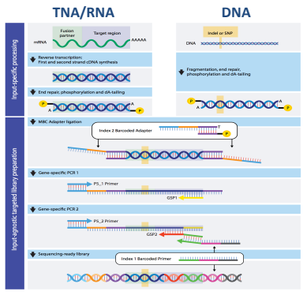


Table 1. Assay Targets of ArcherDX™ FusionPlex™ Lung Thyroid Panel

Gene	mRNA Accession#	Target Exons	Direction	Assay Type
ALK	NM_004304	19,20,21,22	5'	Fusion
FGFR3	NM_000142	16,17,intron18	5'	Fusion
MET	NM_000245	15 (exon14skipping)	5'	Mutation
NTRK1	NM_001007792	8,10,11,12,	5'	Fusion
NTRK3	NM_002530	13,14,15,16	5'	Fusion
NTRK3	NM_001007156	15	5'	Fusion
PPARG	NM_005037.5	1,2,3	5'	Fusion
RET	NM_020975.4	8,9,10,11,12,13	5'	Fusion
ROS1	NM_002944.2	31,32,33,34,35,36,37	5'	Fusion

Table 2. NSCLC FFPE clinical sample data

Sample Name	Tumor Content	Age at Case Start	Gender	Histological Type	pTNM T	pTNM N	pTNM M	Stage
MMD-2-2-194	20%	66	M	non-keratinizing squamous cell carcinoma	T2a	N0	M0	IB
MMD-2-2-195	25%	76	M	keratinizing squamous cell carcinoma	T2b	N2	M0	III A
MMD-2-2-196	20%	62	M	keratinizing squamous cell carcinoma	T1b	N0	M0	IA
MMD-2-2-197	30%	56	M	adenocarcinoma	T3	N1	M0	III A
MMD-2-2-198	40%	59	F	adenocarcinoma with acinar type	T2b	N2	M0	III A
MMD-2-2-199	35%	76	M	adenocarcinoma	T3	N0	M0	IIB
MMD-2-2-200	65%	77	M	carcinoma	pT3	pN0	cM0	IIB
MMD-2-2-201	40%	68	M	adenocarcinoma	pT2a	pN2	cM0	III A
MMD-2-2-202	35%	74	M	keratinizing squamous cell carcinoma	pT4	pN1	cM0	III A
MMD-2-2-203	30%	55	M	adenocarcinoma	pT3	pN2	cM0	III A
MMD-2-2-204	20%	69	M	keratinizing squamous cell carcinoma	pT2a	pN1	cM0	IIA
MMD-2-2-205	40%	66	F	pleomorphic carcinoma	pT2a	pN0	cM0	IB
MMD-2-2-206	20%	73	M	adenocarcinoma with acinar type	pT2a	pN1	cM0	IIA
MMD-2-2-207	30%	48	M	keratinizing squamous cell carcinoma	pT3	pN1	cM0	III A
MMD-2-2-208	45%	61	F	adenocarcinoma - mixed type	pT2a	pN2	cM0	III A
MMD-2-2-209	20%	77	M	keratinizing squamous cell carcinoma	pT2b	pN0	cM0	IIA
MMD-2-2-210	30%	61	M	moderately differentiated adenocarcinoma	T2a	N0	M0	IB
MMD-2-2-211	30%	76	M	squamous cell carcinoma	T2b	N0	M0	IIA
MMD-2-2-212	20%	73	M	moderately differentiated squamous cell carcinoma	T1a	N0	M0	IA
MMD-2-2-213	35%	82	M	adenocarcinoma - mixed type	T1b	N0	M0	IA

Materials and Methods

FFPE samples from 20 NSCLC patients (Indivumed, GmbH) were analyzed in the study (Table 2). An RNA sample containing an ROS1 fusion was used as the positive control, and total RNA extract from FFPE normal lung tissue was used as the negative control (Table 3). In addition, FFPE controls containing TPM3-NTRK1, NCOA4-RET, CCDC6-RET were also used to evaluate the ability of the panel to detect fusions (Table 3). Total nucleic acid (TNA) was extracted from the FFPE sections using the Agencourt® FORMAPURE® kit (Beckman Coulter). 100 - 200 ng of TNA from each sample was used for library preparation. The barcoded libraries from 13 or 14 samples were pooled and sequenced on an Illumina MiSeq using a v2 flow cell. The FASTA files from the MiSeq were then analyzed by the ArcherDX data analysis pipeline (v3.3.0) to determine the presence of gene fusions with driver genes including ALK, FGFR3, MET, NTRK1, NTRK3, NTRK3, PPARG, RET, and ROS1. The detected fusions in clinical samples were further confirmed by Sanger sequencing and FISH.

Table 3. Fresh or FFPE-cell line samples used in the study.

Sample type	Sample name	Known fusions	Supplier
RNA	Postive Control	SLC34A2-ROS1 fusion	ArcherDX
RNA	Negative Control	Negative	
FFPE mouse Xenograft	MMD-2-6-039	TPM3-NTRK1	MolecularMD
	MMD-2-6-040	TPM3-NTRK1	
	MMD-2-6-041	NCOA4-RET	
	MMD-2-6-042	CCDC6-RET	
	MMD-2-6-043	CCDC6-RET	

Results

Total Nucleic Acid Extraction and cDNA QC

Total Nucleic Acid (TNA) was extracted from a 10-micron FFPE section (or equivalent) of each sample. The concentration of TNA was measured by Qubit. For all of the clinical FFPE samples, one 10 micron section was sufficient to yield more than the required 200 ng TNA for library preparation (Table 4). Although three of the twenty (15%) FFPE samples failed the initial sample quality metrics (highlighted in yellow in Table 4), libraries were prepared for all 20 NCSLC FFPE samples and sequenced on MiSeq.

Table 4. Summary of TNA extraction and cDNA QC

Sample Name	TNA Yield (ng)	cDNA qPCR Ct	Qualified for Assay
Postive Control	NA	23.3	YES
Negative Control	NA	23.9	YES
MMD-2-6-039	3181.5	29.5	YES
MMD-2-6-040	5145	27.3	YES
MMD-2-6-041	3416	25.4	YES
MMD-2-6-042	5320	26.7	YES
MMD-2-6-043	5460	24.7	YES
MMD-2-2-194	577	31.9	NO
MMD-2-2-195	902	28.2	YES
MMD-2-2-196	1440	28.0	YES
MMD-2-2-197	2568	28.8	YES
MMD-2-2-198	764	29.5	YES
MMD-2-2-199	2076	30.3	NO
MMD-2-2-200	618	26.0	YES
MMD-2-2-201	649	26.4	YES
MMD-2-2-202	1656	28.2	YES
MMD-2-2-203	276	28.5	YES
MMD-2-2-204	702	27.4	YES
MMD-2-2-205	1028	25.6	YES
MMD-2-2-206	1236	28.2	YES
MMD-2-2-207	631	26.3	YES
MMD-2-2-208	720	27.3	YES
MMD-2-2-209	721	27.5	YES
MMD-2-2-210	563	31.7	NO
MMD-2-2-211	505	29.4	YES
MMD-2-2-212	782	29.6	YES
MMD-2-2-213	1884	28.7	YES

QC Metrics of Sequencing Runs

13 or 14 libraries were sequenced on a standard v2 flow cell on MiSeq, with a target of one million reads per library. The actual median total TNA reads was 921k, while the total TNA reads per sample ranged from 620K to 112K. In addition, the total RNA reads per sample ranged from 120k to 720K, while the percentage of Read on Target was above 99% for all the samples tested (Figure 1).

The unique start sites per GSP2 or per GSP2 control, however, varied significantly among these samples, indicative of varied amount of unique amplifiable molecules in these samples (Figure 2). Three samples with unique start sites per GSP2 control less than five, did not pass fusion QC (indicated by yellow arrows in Figure 2). Among these three samples, two also did not pass the initial cDNA QC (Table 4). One sample that did pass the initial cDNA QC, failed the fusion QC. Overall, the success rate for the 20 clinical samples tested with this panel was 85%.

Results continued

QC Metrics of Sequencing Runs

Figure 1. Total Reads and Read-on-Target

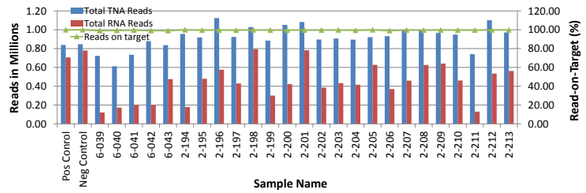
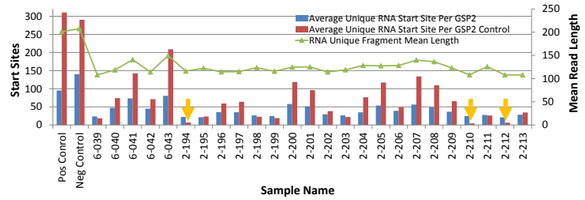


Figure 2. Unique start sites per GSP2 and RNA mean read Length



Fusions Detected in Fresh or FFPE Cell Line Samples

The known fusions, including SLC34A2-ROS1, TMP3-NTRK1, and CCDC6-RET were successfully detected in the positive RNA and FFPE cell line samples (Table 5). The NCOA4:exon9-RET:exon12 fusion in sample MMD-2-6-041, however, was not detected due to misalignment of 5' region of NCOA4 exon 9 to an intron region.

Table 5. Summary of TNA extraction and cDNA QC

Sample Name	Reads	% Reads	Unique start site	Unique start site%	Annotation 1	Annotation 2	Breakpoint 1	Breakpoint 2
Pos Conrol	21803	98.6	638	87.8	SLC34A2:exon:4[1]	ROS1:exon:32[2]	chr4(+):25665952	chr6(-):117650609
Pos Conrol	334	5.8	127	13.3	SLC34A2:exon:4[1]	ROS1:exon:32[2]	chr4(+):25665952	chr6(-):117645578
Pos Conrol	163	14.2	65	17.4	SLC34A2:exon:4[1]	ROS1:exon:33[2]	chr4(+):25665952	chr6(-):117647577
Pos Conrol	28	100	22	100	SLC34A2:exon:4[1]	ROS1:exon:35[2]	chr4(+):25665952	chr6(-):117642557
Pos Conrol	10	0.1	6	1.2	SLC34A2:exon:4[1]	ROS1:exon:36[2]	chr4(+):25665952	chr6(-):117641193
MMD-2-6-039	365	55.5	49	44.5	TPM3:exon:7[3]	NTRK1:exon:10[5]	chr1(-):154142876	chr1(+):156844363
MMD-2-6-039	74	11.2	14	12.7	TPM3:exon:9[4]	NTRK1:exon:10[5]	chr1(-):154141781	chr1(+):156844363
MMD-2-6-040	1075	52.7	173	49.4	TPM3:exon:7[3]	NTRK1:exon:10[5]	chr1(-):154142876	chr1(+):156844363
MMD-2-6-040	205	10	64	18.3	TPM3:exon:9[4]	NTRK1:exon:10[5]	chr1(-):154141781	chr1(+):156844363
MMD-2-6-042	777	100	58	100	CCDC6:exon:8[6]	RET:exon:12[7]	chr10(-):61554231	chr10(+):43612032
MMD-2-6-043	3395	100	232	100	CCDC6:exon:8[6]	RET:exon:1[7]	chr10(-):61554231	chr10(+):43612032

[1]:SLC34A2, NM_006424; [2]:ROS1, NM_002944; [3]:TPM3, NM_153649; [4]:TPM3, NM_152263; [5]:NTRK1, NM_002529; [6]:CCDC6, NM_005436; [7]:RET, NM_020975.

Fusion detection in NSCLC FFPE samples

Among the 20 NSCLC samples, one sample was found to harbor a novel, in-frame fusion between exon 19 of MPRIP and exon 12 of RET (Figure 3). Using the RNA portion of the TNA extracted, cDNA was synthesized using an independent method, and amplified by PCR with primers targeting exon 19 of MPRIP and exon 12 of RET (Figure 4). The PCR product was sequenced by Sanger. The results confirmed the presence of an RNA transcript spanning exon 19 of MPRIP and exon 12 of RET (Figure 4). Furthermore, FISH was performed with the RET (10q11.2) break-apart probe, and confirmed the rearrangement involving the RET region of chromosome 10 (Figure 5). The novel MPRIP-RET fusion would retain most of coil-coil domain of MPRIP, and an intact kinase domain (exons 12-18) of RET, would presumably be oncogenic. The structure of the predicted MPRIP-RET fusion protein was also similar to those RET fusion proteins identified previously , including KIF5B-, CCDC6-, NCOA4-, TRIM33-, CUX1-, and KIAA1468-RET fusions (Figure 6, and Ref.1).

Conclusions

- The success rate of the analysis in the FFPE sample set tested with the Archer™ FusionPlex™ Lung Thyroid Panel is 85%.
- A novel MPRIP-RET in-frame gene fusion was identified in a clinical NSCLC FFPE sample, and confirmed by Sanger and FISH assays. The clinical significance and prevalence of such RET fusion are yet to be established.
- The AMP chemistry-based, Archer™ FusionPlex™ Lung Thyroid Panel can accurately detect gene fusions from both known and unknown partner and driver genes. The success of analysis with the AMP chemistry is directly related to RNA quality.

References

1. Takashi Kohno, Takashi Nakaoku, Koji Tsuta, Katsuya Tsuchihara, Shingo Matsumoto, Kiyotaka Yoh, and Koichi Goto (2015) Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. Transl Lung Cancer Res. 4: 156–164.

Results continued

Fusion detection in NSCLC FFPE samples

Figure 3. MPRIP exon19-RET exon12 in-frame fusion detected in MMD-2-2-203

Reads	% Reads	Unique start site	Unique start site%	Annotation 1	Annotation 2	Breakpoint 1	Breakpoint 2
460	50.1	67	51.1	MPRIP:exon:19:NM_201274	RET:exon:12	chr17(+):17078726	chr10(+):43612032

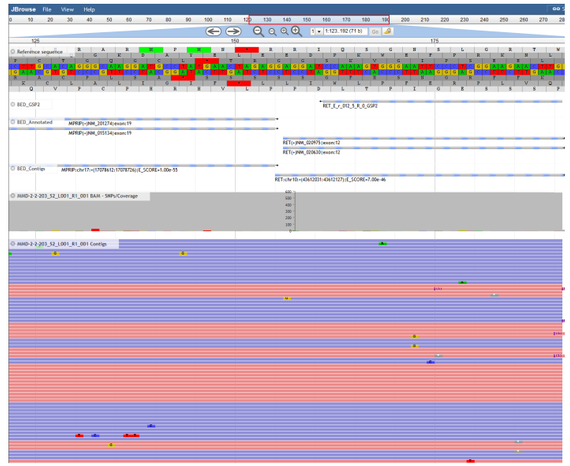


Figure 4. Confirmation of MPRIP-RET fusion by Sanger

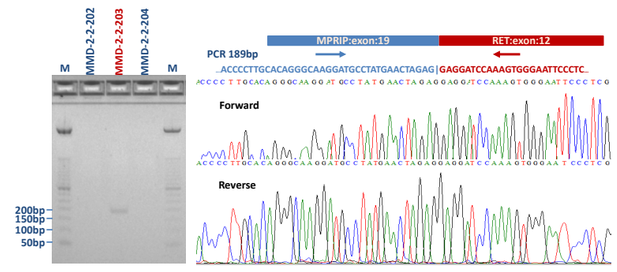


Figure 5. Confirmation of MPRIP-RET fusion by FISH.
83/100 (83%) interphase cells scored had a signal pattern consistent with rearrangement involving the RET region of chromosome 10.

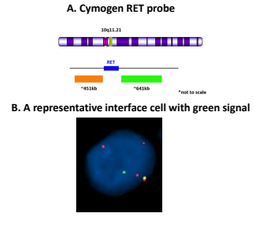
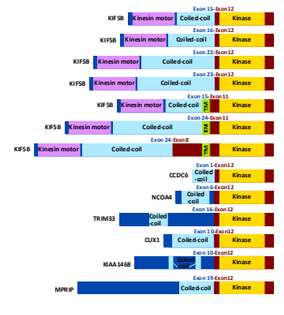


Figure 6. Structure of RET fusion proteins identified (adapted partially from Ref. 1)



For Further Information

Please contact BD@molecularmd.com
or visit www.molecularmd.com.

MolecularMD

Scan the QR code with your mobile device to download a copy of the poster and learn more.