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Supplemental Appendix for:
ALK-rearranged non-small cell lung cancer in 2020. Real-world triumphs in an era of multi-generation ALK-inhibitor sequencing informed by drug resistance profiling
Malinda Itchins et al.

Appendix 1.

Next Generation Sequencing (NGS) Assays Utilized in Case Reports

a) **RESOLUTION** BIOSCIENCE **Resolution ctDx Lung™ Assay**

Resolution Bioscience ctDx-Lung Panel (n = 21)*

SNV/Indel		Fusions	CNV	Suppressors
AKT1	MET	ALK	ALK	TP53
ALK	PIK3CA	FGFR3	EGFR	
BRAF	RET	NRTK1	FGFR1	
EGFR	ROS1	RET	HER2 (ERBB2)	
FGFR2		ROS1	JAK2	
HER2 (ERBB2)			MET	
KRAS			MYC	
NRAS			PD-L1 (CD274)	
MAP2K1			RICTOR	

* ctDx circulating tumor diagnostic; SNV single nucleotide variant; CNV copy number variation.

ALK exons 21-29 covered. Of the reported ALK resistance mutations covered, K1062M and R1113Q not covered as occur outside the ALK tyrosine kinase domain.

Coverage of Canonical Mutation Sites via Resolution ctDx Lung™ Assay:

This table presents a representative but not definitive list of mutations detectable by this assay. For each, unique read coverage for this specimen (median across the span of the locus) is reported. Detected mutation(s) are indicated in bold.

Mutation	Median Unique Reads Total (+/-)	Mutation	Median Unique Reads Total (+/-)
AKT1 E17K	5,993 (2,964 / 3,029)	KRAS G12A/D/V/C/R/S	2,936 (697 / 2,239)
ALK 1151Tins	5,287 (3,783 / 1,504)	KRAS G13C/R/S/A/D	2,943 (663 / 2,280)
ALK C1156Y	4,464 (2,509 / 1,955)	KRAS Q61K/L/R/H	4,528 (2,106 / 2,422)
ALK D1203N	7,532 (6,145 / 1,387)	MAP2K1 D67N	5,769 (2,939 / 2,830)
ALK E1210K	7,163 (5,260 / 1,903)	MAP2K1 K57N	9,003 (4,795 / 4,208)
ALK F1174L	9,019 (2,501 / 6,518)	MAP2K1 Q56P	9,097 (4,801 / 4,296)
ALK F1245C	6,869 (4,950 / 1,919)	NRAS G12C/R/S/A/D	4,021 (1,654 / 2,367)
ALK G1123S	6,326 (3,487 / 2,839)	NRAS Q61K/L/R/H	4,450 (2,401 / 2,049)
ALK G1202R	7,667 (2,930 / 4,737)	PIK3CA C420	3,463 (1,707 / 1,756)
ALK G1269A/S	7,664 (6,056 / 1,608)	PIK3CA E110	4,007 (1,041 / 2,966)
ALK I1171H/N/S/T	5,816 (2,715 / 3,101)	PIK3CA E542K	5,544 (2,888 / 2,656)
ALK L1152R	5,146 (3,678 / 1,468)	PIK3CA E543	5,540 (2,889 / 2,651)
ALK L1196M	9,297 (4,061 / 5,236)	PIK3CA E545K/Q	5,535 (2,918 / 2,617)
ALK L1198F	8,911 (3,694 / 5,217)	PIK3CA E726	6,257 (2,433 / 3,824)
ALK R1275Q	6,599 (4,876 / 1,723)	PIK3CA H1047L/R	4,929 (3,165 / 1,764)
ALK S1206Y	7,400 (5,758 / 1,642)	PIK3CA K111	3,956 (1,057 / 2,899)
ALK V1180L	8,593 (5,431 / 3,162)	PIK3CA M1043	5,260 (3,026 / 2,234)
BRAF G466V	4,124 (2,509 / 1,615)	PIK3CA N345	3,201 (927 / 2,274)
BRAF G469A/L	3,926 (2,208 / 1,718)	PIK3CA Q546	5,477 (2,930 / 2,547)
BRAF L597V	4,583 (2,675 / 1,908)	PIK3CA R88	4,015 (2,853 / 1,162)
BRAF V600E	4,655 (2,576 / 2,079)	PIK3CA R93	3,967 (3,168 / 799)
BRAF Y472C	3,521 (1,814 / 1,707)	RET M918T	6,633 (5,297 / 1,336)
EGFR C797S	7,051 (5,058 / 1,993)	ROS1 D2033N	5,071 (3,589 / 1,482)
EGFR D770_N771insG	6,588 (3,630 / 2,958)	ROS1 G2032R	5,649 (1,437 / 4,212)
EGFR E746_A750del	6,634 (1,969 / 4,665)	ROS1 G2101A	3,534 (808 / 2,726)
EGFR G719A/C/S	5,628 (2,283 / 3,345)	ROS1 L2026M	6,577 (2,381 / 4,196)
EGFR K745_A750del	6,688 (1,885 / 4,803)	ROS1 L2155S	5,509 (3,969 / 1,540)
EGFR L858R	5,961 (3,150 / 2,811)	ROS1 S1986Y/F	4,957 (1,799 / 3,158)
EGFR L861Q	5,981 (3,359 / 2,622)	TP53 C242fs*5	6,576 (1,079 / 5,497)
EGFR T790M	7,222 (3,921 / 3,301)	TP53 R175H	6,075 (3,833 / 2,242)
ERBB2 M774_A775insAYVM	7,001 (4,664 / 2,337)	TP53 R248Q	6,744 (670 / 6,074)
FGFR3 S249C	5,049 (2,013 / 3,036)	TP53 R273H	6,491 (3,607 / 2,884)
		TP53 S90fs*33	5,385 (4,513 / 872)

b) Peter MacCallum Cancer Centre (PMCC), Melbourne, Victoria, Australia Customized In-House Somatic Panel Mutation Assay. Accredited for Compliance with NPAAC Standards and ISO 15189

Targeted amplicon next generation sequencing analysis of tumor tissue to screen for mutations in selected exons of 19 cancer-related genes; AKT1, ALK, BRAF, CDKN2A, EGFR, ERBB2, FGFR2, FGFR3, KIT, KRAS, MAP2K1, MET, NRAS, PDGFRA, PIK3CA, PTEN, RAC1, RNF43, TP53. ALK exon 20 and 23-25 hotspots are covered. The assay does not detect copy number changes or structural rearrangements such as fusions.

c)

https://www.foundationmedicine.com/genomic-testing/foundation-one-cdx

GENES ASSAYED IN FOUNDATIONONE

FoundationOne is designed to include all genes known to be somatically altered in human solid tumors that are validated targets for therapy, either approved or in clinical trials, and/or that are unambiguous drivers of oncogenesis based on current knowledge. The current assay interrogates 315 genes as well as introns of 28 genes involved in rearrangements. The assay will be updated periodically to reflect new knowledge about cancer biology.

DNA Gene List: Entire Coding Sequence for the Detection of Base Substitutions, Insertion/Deletions, and Copy Number Alterations

ABL1	*ABL2*	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AMER1 (FAM123B)*	*APC*	*AR*
ARAF	*ARFRP1*	*ARID1A*	*ARID1B*	*ARID2*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*
BCORL1	*BLM*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTK*	*C11orf30 (EMSY)*
CARD11	*CBFB*	*CBL*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CD274*	*CD79A*	*CD79B*
CDC73	*CDH1*	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*
CDKN2C	*CEBPA*	*CHD2*	*CHD4*	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CRLF2*
CSF1R	*CTCF*	*CTNNA1*	*CTNNB1*	*CUL3*	*CYLD*	*DAXX*	*DDR2*	*DICER1*	*DNMT3A*
DOT1L	*EGFR*	*EP300*	*EPHA3*	*EPHA5*	*EPHA7*	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*
ERG	*ERRFI1*	*ESR1*	*EZH2*	*FAM46C*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*
FANCG	*FANCL*	*FAS*	*FAT1*	*FBXW7*	*FGF10*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FH*	*FLCN*	*FLT1*	*FLT3*
FLT4	*FOXL2*	*FOXP1*	*FRS2*	*FUBP1*	*GABRA6*	*GATA1*	*GATA2*	*GATA3*	*GATA4*
GATA6	*GID4 (C17orf39)*	*GLI1*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GPR124*	*GRIN2A*	*GRM3*
GSK3B	*H3F3A*	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*HSP90AA1*	*IDH1*	*IDH2*	*IGF1R*
IGF2	*IKBKE*	*IKZF1*	*IL7R*	*INHBA*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*
JAK2	*JAK3*	*JUN*	*KAT6A (MYST3)*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*
KIT	*KLHL6*	*KMT2A (MLL)*	*KMT2C (MLL3)*	*KMT2D (MLL2)*	*KRAS*	*LMO1*	*LRP1B*	*LYN*	*LZTR1*
MAGI2	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*
MEN1	*MET*	*MITF*	*MLH1*	*MPL*	*MRE11A*	*MSH2*	*MSH6*	*MTOR*	*MUTYH*
MYC	*MYCL (MYCL1)*	*MYCN*	*MYD88*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*
NOTCH2	*NOTCH3*	*NPM1*	*NRAS*	*NSD1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUP93*	*PAK3*
PALB2	*PARK2*	*PAX5*	*PBRM1*	*PDCD1LG2*	*PDGFRA*	*PDGFRB*	*PDK1*	*PIK3C2B*	*PIK3CA*
PIK3CB	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PLCG2*	*PMS2*	*POLD1*	*POLE*	*PPP2R1A*	*PRDM1*
PREX2	*PRKAR1A*	*PRKCI*	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTPN11*	*QKI*	*RAC1*
RAD50	*RAD51*	*RAF1*	*RANBP2*	*RARA*	*RB1*	*RBM10*	*RET*	*RICTOR*	*RNF43*
ROS1	*RPTOR*	*RUNX1*	*RUNX1T1*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*	*SF3B1*
SLIT2	*SMAD2*	*SMAD3*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*	*SOX10*
SOX2	*SOX9*	*SPEN*	*SPOP*	*SPTA1*	*SRC*	*STAG2*	*STAT3*	*STAT4*	*STK11*
SUFU	*SYK*	*TAF1*	*TBX3*	*TERC*	*TERT (promoter only)*	*TET2*	*TGFBR2*	*TNFAIP3*	*TNFRSF14*
TOP1	*TOP2A*	*TP53*	*TSC1*	*TSC2*	*TSHR*	*U2AF1*	*VEGFA*	*VHL*	*WISP3*
WT1	*XPO1*	*ZBTB2*	*ZNF217*	*ZNF703*					

DNA Gene List: For the Detection of Select Rearrangements

ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*EGFR*	*ETV1*	*ETV4*
ETV5	*ETV6*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*MSH2*	*MYB*	*MYC*	*NOTCH2*
NTRK1	*NTRK2*	*PDGFRA*	*RAF1*	*RARA*	*RET*	*ROS1*	*TMPRSS2*		

Additional Assays: For the Detection of Select Cancer Biomarkers

Microsatellite status

Tumor Mutational Burden

d) St Vincent's Pathology, Melbourne, Victoria, Australia Targeted DNA NGS and RNA Fusion Panels

Solid Tumor Cancer Targeted Panel. Cancer Gene Mutation Panel Version 2 - Targeted Mutation Detection by NGS of DNA via the Ampliseq panel, Thermo Fisher.

This 50 gene hotspot cancer panel targets a total of 2,855 variants: ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZF2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, and VHL.

For ALK the following are the following coordinates of the amplicons in exons 22, 23 and 25 are covered:

Exon 25: 29432655 – 29432735
Exon 23: 29443573 – 29443702
Exon 22: 29445209 – 29445320

Some of the detected variants may not have been validated on formalin fixed paraffin embedded clinical samples and therefore have not been fully validated to the current NPAAC requirements. Therefore NATA/RCPA accreditation does not cover the performance of these particular variants.

the DNA and RNA panels utilized are Ion Ampliseq panels from ThermoFisher. These assays are NATA accredited and are performed on the NGS Personal Genome Machine (PGM)

The RNA fusion NGS Panel detects transcripts from 37 ALK, 9 RET, 15 ROS1, and 11 NTRK1 fusion variants.

Gene	Exons	Type		Gene	Exons	Type
EML4-ALK	(E2A20)	Fusion		CD74-ROS1	(C6R32)	Fusion
EML4-ALK	(E2A20)	Fusion		CD74-ROS1	(C6R34)	Fusion
EML4-ALK	(E2A20)	Fusion		EZR-ROS1	(E10R34)	Fusion
EML4-ALK	(E2A20)	Fusion		GOPC-ROS1	(G8R35)	Fusion
EML4-ALK	(E6A20)	Fusion		GOPC-ROS1	(G4R36)	Fusion
EML4-ALK	(E6A19)	Fusion		LRIG3-ROS1	(L16R35)	Fusion
EML4-ALK	(E6A20)	Fusion		SDC4-ROS1	(S4R32)	Fusion
EML4-ALK	(E6A20)	Fusion		SDC4-ROS1	(S4R34)	Fusion
EML4-ALK	(E6A20)	Fusion		SDC4-ROS1	(S2R32)	Fusion
EML4-ALK	(E6A20)	Fusion		SDC4-ROS1	(S2R34)	Fusion
EML4-ALK	(E13A20)	Fusion		SLC34A2-ROS1	(S4R34)	Fusion
EML4-ALK	(E13A20)	Fusion		SLC34A2-ROS1	(S4R32)	Fusion
EML4-ALK	(E13A20)	Fusion		SLC34A2-ROS1	(S13R34)	Fusion
EML4-ALK	(E13A20)	Fusion		SLC34A2-ROS1	(S13R32)	Fusion
EML4-ALK	(E14A20)	Fusion		TPM3-ROS1	(T8R35)	Fusion
EML4-ALK	(E14A20)	Fusion		CCDC6-RET	(C1R12)	Fusion
EML4-ALK	(E14A20)	Fusion		CUX1-RET	(C10R12)	Fusion
EML4-ALK	(E15A20)	Fusion		KIF5B-RET	(K24R8)	Fusion
EML4-ALK	(E17A20)	Fusion		KIF5B-RET	(K24R11)	Fusion

EML4-ALK	(E17A20)	Fusion		KIF5B-RET	(K23R12)	Fusion
EML4-ALK	(E17A20)	Fusion		KIF5B-RET	(K22R12)	Fusion
EML4-ALK	(E17A20)	Fusion		KIF5B-RET	(K16R12)	Fusion
EML4-ALK	(E18A20)	Fusion		KIF5B-RET	(K15R12)	Fusion
EML4-ALK	(E18A20)	Fusion		KIF5B-RET	(K15R11)	Fusion
EML4-ALK	(E20A20)	Fusion		CD74-NTRK1	(C3N13)	Fusion
EML4-ALK	(E20A20)	Fusion		CEL-NTRK1	(C7N7)	Fusion
EML4-ALK	(E20A20)	Fusion		IRF2BP2-NTRK1	(I1N10)	Fusion
EML4-ALK	(E20A20)	Fusion		MPRIP-NTRK1	(M14N13)	Fusion
HIP1-ALK	(H28A20)	Fusion		MPRIP-NTRK1	(M18N13)	Fusion
HIP1-ALK	(H21A20)	Fusion		MPRIP-NTRK1	(M21N13)	Fusion
KIF5B-ALK	(K24A20)	Fusion		NFASC-NTRK1	(N18N10)	Fusion
KIF5B-ALK	(K17A20)	Fusion		NTRK1-DYNC2H1	(N17D86)	Fusion
KIF5B-ALK	(K15A20)	Fusion		SQSTM1-NTRK1	(S5N10)	Fusion
KIF5B-ALK	(K15A20)	Fusion		SSBP2-NTRK1	(S12N12)	Fusion
KLC1-ALK	(K9A20)	Fusion		TFG-NTRK1	(T5N10)	Fusion
TPR-ALK	(T15A20)	Fusion				