AACR22 Abstract 1254

Actionable Fusions Detected by RNA-seq Co-occur with PD-L1 Expression and Driver Mutations in Solid Tumors Patients

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Background

Incorporating gene fusions into a comprehensive profile is critical not only because very effective therapies targeting oncogenic fusion proteins exist but they may also negate the response to therapy of an actionable SNV and InDel mutations. We examined the co-occurrence of actionable gene fusions detected by RNA-seq with actionable SNVs/InDels and with the immunotherapy response biomarker, PD-L1.

Methods

In 2021, 5341 FFPE samples were analyzed by our clinical laboratory using a novel, clinical grade hybridization-based RNA sequencing assay that detects the expressed chimeric transcripts resulting from gene fusions (Figs. 1, 2) with 95.9% sensitivity and 100% specificity.. DNA mutations (SNV/Indels) were detected with a clinical grade NGS assay and PD-L1 protein expression was determined by IHC using an appropriate LDT or FDA approved assays (SP42 or 22C3 antibodies). De-identified data were analyzed following an approved IRB protocol.

Results

Of the 5341 patients tested for gene fusions only 0.7% were profiled with a comprehensive fusion detection panel for 250 clinically relevant fusion genes. Conversely, 67% of all patients were tested only for NTRK gene fusions. The prevalence of the following most relevant fusions was: ALK=2.31% (in particular EML4-ALK), NTRKs=0.85%, RET=0.92% and ROS1=0.80% all mostly detected in lung cancers and FGFR2=5.60% in cholangiocarcinoma and PAX-FOXO in rhabdomyosarcomas. Among NTRK fusions, NTRK3 was detected in 52.9% of the positive cases; in particular, dominant fusions are ETV6-NTRK3 (26%) and EML4-NTRK3 (20%). In the 163 fusion positive cases, 37 cases had available DNA mutation testing and PD-L1 expression testing. These patients presented 24 different actionable fusions, including ALK (2), FGFR1/2 (5), NTRK 1/2/3 (10), NRG1 (3), RET (3) and ROS1 (3) fusions. Interestingly, while 73% (27/37) of those tumors were PD-L1 positive, similar to the 75% found on the fusion negative samples, PD-L1 was positive in 88% (15/17) of the lung samples with pathogenic mutations from this subset. This was strikingly higher than the 45% (192/424) found in the fusion negative cohort. Finally, excluding TP53 mutations, NTRK fusions frequently co-occurred with RNF43, FBXW7, TERT promoter, and ARID1A pathogenic mutations. FGFR fusions co-occurred with BAP1, PBRM1 or KRAS mutations, which correlates with the fact that both FGFR2 fusions and swi/snf alterations are enriched in intrahepatic cholangiocarcinoma, where the FGFR fusions were detected. RET fusions co-occurred with ARID1A and TERT. ROS1 fusions co-occurred with SMARCA4 and KRAS pathogenic mutations simultaneously. NRG1 fusions co-occurred with ARID1A and FBXW7 mutations. EML4-ALK fusions co-occurred with FGFR2 and KMT2D variants of unknown significance.

Conclusion

Actionable cancer driving gene fusions were detected by RNA-sequencing and co-occur with other biomarkers that can guide selection of therapies, such as immune checkpoint inhibitors (ICI) and olaparib. The data suggests that gene fusion testing is an important addition on the genomic profiling for therapy selection in solid tumors.

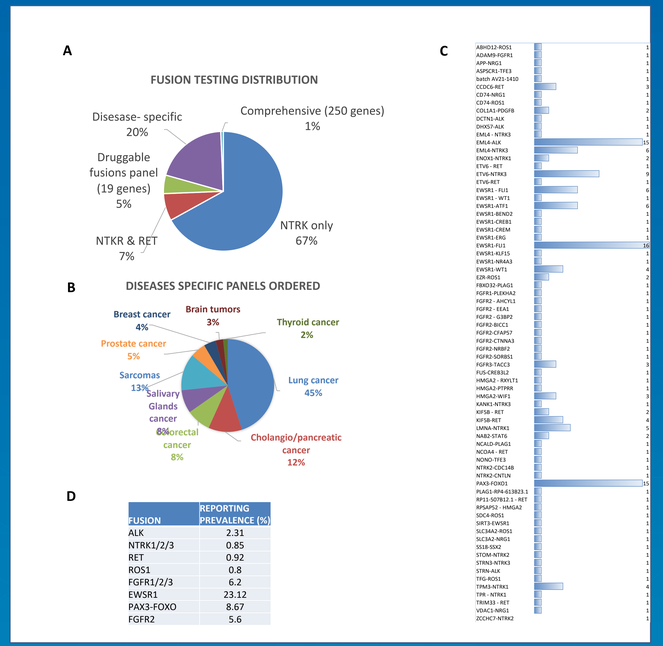
Highlights

- Gene fusions testing in clinical practice was mainly ordered for select fusion genes
- Therapeutically actionable fusions often co-occur with other driver mutations
- Therapeutically actionable fusions are co-detected with immunosuppressive phenotype
- Gene fusions and driving mutations might jointly drive tumors biology

Solid Tumor Relevant Gene Fusions and Aberrant Transcripts

250 clinically relevant fusion genes									
ABL1	C11orf95	CREB1	ESPL1	HERPUD1	MAST2	NOTCH2	PML	SET	TFE3
ACSL3	CAMTA1	CREB3L1	ETV1	HEY1	MEAF6	NPM1	POU5F1	SLC34A2	TFG
ACTB	CANT1	CREB3L2	ETV4	HIP1	MET	NR4A3	PPARG	SLC45A3	THADA
ACTG1	CARS	CREBBP	ETV5	HMGA2	MET e14 *	NRG1	PRCC	SND1	THRAP3
AFDN	CARS1	CRTC1	ETV6	HMGN2P46	MKRN1	NTRK1	PRKACA	SNURF	TMPRSS2
AFF1	CBFA2T3	CRTC3	EWSR1	HNRNPA2B1	MLLT1	NTRK2	PRKAR1A	SRF	TP63
AFF3	CCDC170	CSF1	EZR	IL2RB	MLLT10	NTRK3	PRKD1	SRGAP3	TPM3
AFF4	CCDC6	CTLA4	FAM131B	IRF4	MLLT11	NUP214	PRKD2	SS18	TPM4
AHRR	CCNB3	CTNNB1	FEV	ITK	MLLT3	NUP98	PRKD3	SSX1	TPR
AKAP9	CCND1	CTNNBL1	FGFR1	JAK2	MN1	NUTM1	PTPRK	SSX2	TRIM24
AKT3	CCND2	DDIT3	FGFR2	JAZF1	MPRIP	NUTM2A	RAD51B	SSX4B	UBTF
ALK	CCND3	DDX3X	FGFR3	KAT6A	MRTFB	NUTM2B	RAF1	STAT6	USP6
AR	CD274	DDX5	FGFR4	KDM5A	MSH2	OMD	RANBP2	STIL	VT1IA
ARv7*	CD74	DHH	FLI1	KIAA1549	MYB	PAN3	RARA	STRN	WDFY2
ARv9*	CDH11	DNAJB1	FLT3	KIF5B	MYBL1	PATZ1	RASGEF1A	SUZ12	WIF1
ARID1A	CDK4	DUX4	FOXO1	KIT	MYC	PAX3	RET	SYK	WT1
ASPSCR1	CDK6	EGFR	FOXP1	KLK2	MYH9	PAX7	RHEBL1	TACC3	WWTR1
ATF1	CDKN2D	EGFRvIII*	FRK	KMT2A	MYLK	PAX8	ROS1	TAF15	YAP1
ATIC	CHCHD7	ELK4	FUS	KNL1	NAB2	PBX1	RPS6KC1	TAL1	YWHAE
AXL	CIC	ELL	GLI1	KRAS	NCOA1	PCM1	RSPO3	TBL1XR1	ZNF444
BCOR	CIITA	EML4	GLIS1	LIFR	NCOA2	PDGFB	RUNX1	TCF12	
BCR	CLTC	EPC1	GLIS2	LMNA	NCOA4	PDGFRA	RUNX1T1	TCF3	
BRAF	CNBP	EPS15	GLIS3	LPP	NDRG1	PDGFRB	SDC4	TCF7L2	
BRCA1	COA5	ERBB2	GNAS	MAML1	NFATC2	PHF1	SEC31A	TEAD1	
BRCA2	COL1A1	ERG	GOPC	MAML2	NFIB	PIK3CA	SEPTIN6	TEAD2	
BRD4	COL1A2	ESR1	HAS2	MAST1	NOTCH1	PLAG1	SEPTIN9	TEAD3	

1. The fusion panel targets fusions involving 250 clinically relevant fusion genes relevant to most frequent cancers, including breast, colorectal, lung, lymphoma, pancreatic, prostate, salivary gland, sarcomas, and thyroid cancers. Fusion genes are included on NCCN and WHO guidelines, published clinical studies, and the 120 most frequent curated fusions in solid tumors from COSMIC (v91) providing >90% prevalence per fusion pair.

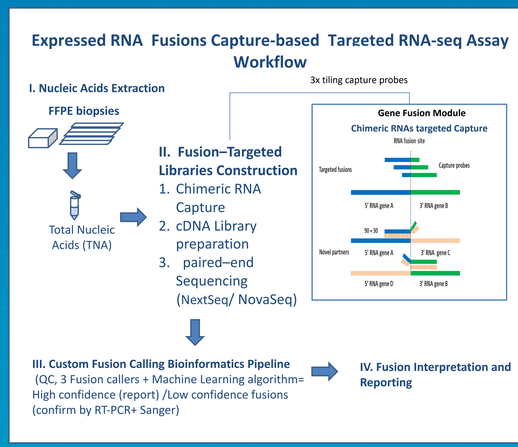


FUSION	REPORTING PREVALENCE (%)
ALK	2.31
NTRK1/2/3	0.85
RET	0.92
ROS1	0.8
FGFR1/2/3	6.2
EWSR1	23.12
PAX3-FOXO	8.67
FGFR2	5.6

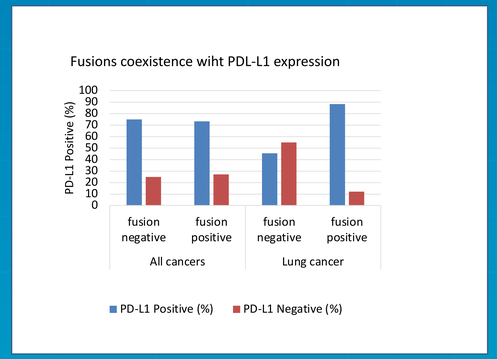
3. Physicians ordered fusions tests for 5341 cases. (A) All tested cases. (B) Distribution of cases tested for disease specific fusions. (C) Fusions from 163 positive reported cases. (D) Prevalence of select common fusions reported across all cancers tested.

case	Fusion detected (NGS)	PD-L1 (IHC) Test Result
1	STOM-NTRK2	EXPRESSED
2	MET Exon 14 Deletion	EXPRESSED
3	KIF5B-RET	EXPRESSED
4	SLC3A2-NRG1	EXPRESSED
5	NTRK2-CDC14B	EXPRESSED
6	EML4-ALK	EXPRESSED
7	SDC4-ROS1	HIGH EXPRESSION
8	TPM3-NTRK1	HIGH EXPRESSION
9	FGFR2-AHCYL1	HIGH EXPRESSION
10	KIF5B-RET	HIGH EXPRESSION
11	MET Exon 14 Skipping	HIGH EXPRESSION
12	CCDC6-RET	HIGH EXPRESSION
13	EML4-NTRK3	HIGH EXPRESSION
14	EML4-NTRK3	HIGH EXPRESSION
15	MET Exon 14 Deletion	HIGH EXPRESSION
16	EML4-NTRK3	HIGH EXPRESSION
17	FGFR2-CTNNA3	HIGH EXPRESSION
18	NTRK2-CNTLN	HIGH EXPRESSION
19	MET Exon 14 Skipping	HIGH EXPRESSION
20	MET Exon 14 Skipping	HIGH EXPRESSION
21	EML4-ALK	HIGH EXPRESSION
22	APP-NRG1	HIGH EXPRESSION
23	ADAM9-FGFR1	HIGH EXPRESSION
24	FGFR2-NRBF2	HIGH EXPRESSION
25	LMNA-NTRK1	HIGH EXPRESSION
26	EML4-NTRK3	HIGH EXPRESSION
27	MET Exon 14 Skipping	HIGH EXPRESSION

4. PD-L1 expression in Fusion Positive samples



2: A new Solid Tumor Gene Fusion RNA-Seq assay workflow. Starting with FFPE total nucleic acid extraction, the assay detects only expressed fusions through RNA fusion sequence- targeted, hybridization capture-based, stranded pair-ended RNA-sequencing on Novaseq instruments. Key step resides on custom based design of capture baits mapping to the chimeric RNA sequence` as deduced from chromosomal breakpoints in annotated private and public databases.



5. Co-existence of oncogenic fusions and Immunotherapy biomarker PD-L1.

NTRK fusions	Co-occurring mutations
STOM-NTRK2	TP53
TPM3-NTRK1	RNF43
TPM3-NTRK1	TP53
TPM3-NTRK1	FBXW7
EML4-NTRK3	RNF43
EML4-NTRK3	ARID1A
EML4-NTRK3	TERT
NTRK2-CNTLN	TP53
NTRK2-CNTLN	NFE2L2
NTRK2-CNTLN	PTEN
LMNA-NTRK1	KMT2D
LMNA-NTRK1	TP53
LMNA-NTRK1	TP53
LMNA-NTRK1	RNF43
LMNA-NTRK1	FBXW7
LMNA-NTRK1	POLE
LMNA-NTRK1	ARID1A
LMNA-NTRK1	ARID1A
NTRK2-CDC14B	FBXW7
NTRK2-CDC14B	TP53
NTRK2-CDC14B	TP53

ALK fusions	Co-occurring mutations
EML4-ALK	TP53
EML4-ALK	KMT2D
EML4-ALK	FGFR2

RET fusions	Co-occurring mutations
KIF5B-RET	ARID1A
CCDC6-RET	TP53
CCDC6-RET	TERT
KIF5B-RET	TP53

ROS1 fusion	Co-occurring mutations
ABHD12-ROS1	SMARCA4
ABHD12-ROS1	TP53
ABHD12-ROS1	KRAS
SDC4-ROS1	TP53

NGR1 fusions	Co-occurring mutations
SLC3A2-NRG1	TP53
VDAC1-NRG1	TP53
VDAC1-NRG1	TP53
VDAC1-NRG1	FBXW7
APP-NRG1	ARID1A

FGFR fusions	co-occuring mutations
FGFR2-AHCYL1	BAP1
FGFR3-TACC3	TP53
FGFR2-CTNNA3	BAP1
ADAM9-FGFR1	KRAS
FGFR2-NRBF2	BAP1
FGFR2-EEA1	PBRM1
FGFR2-EEA1	BAP1

6. Co-occurring driving mutations found on cases with select therapeutically actionable fusions