The analysis of *ALK* fusion variants in 4991 *EGFR/MET* mutation-negative non-squamous non small-cell lung carcinomas (NSCLCs)

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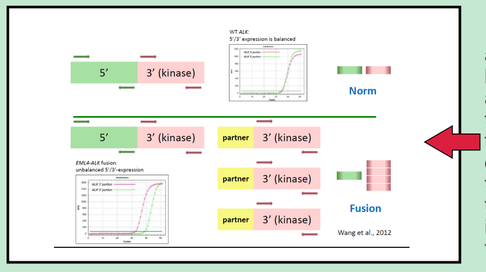
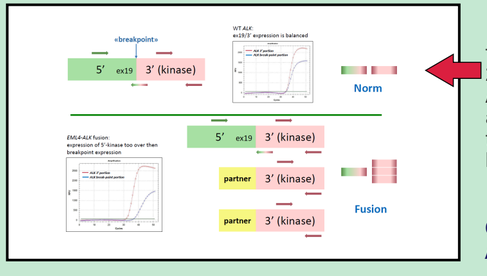
Background & Purpose

- IHC- and FISH-based methods of ALK testing do not identify variants of ALK fusions. NGS analysis is expensive, hence its use is still limited.
- ALK translocations result in increased transcription of the kinase portion of the gene, therefore PCR analysis for unbalanced 5'/3'-end expression is a cost-efficient tool for a comprehensive detection of all variants of ALK rearrangements.

Patients & Methods



- ALK-positive samples show a higher unbalance the 5 '/ 3' ratio between the portions of exon 9-10 and exon 22-23 (kinase domain) of the ALK gene expression compared to other NSCLC. The kinase domain of the **ALK** is translocated to a highly expressed partner, therefore the 3'-end of the transcript is overrepresented as compared to the 5'-end.

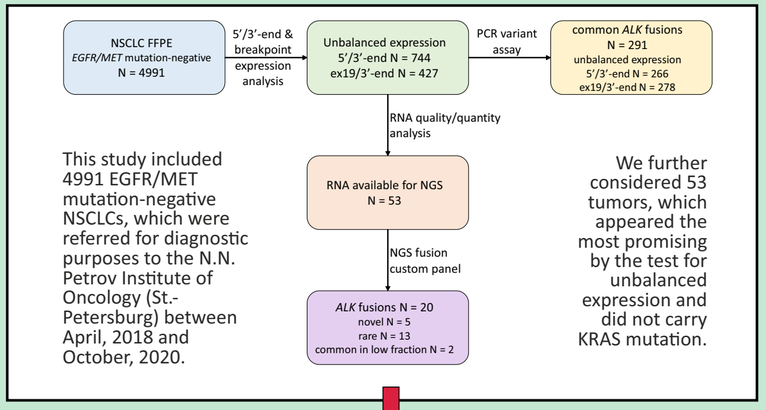


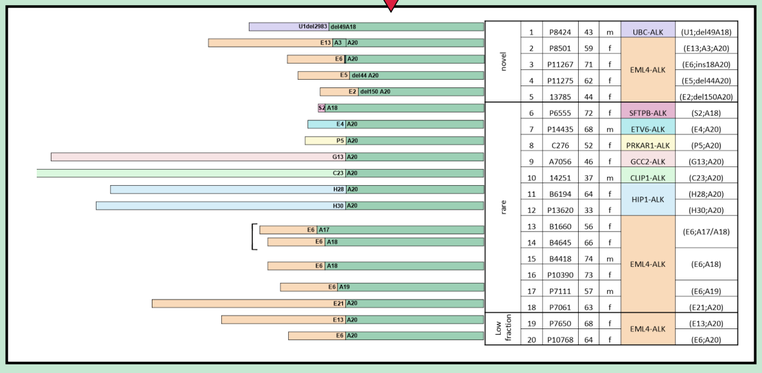
- We also used as a 5 'region a fragment corresponding to exon 19-20, the breakpoint of most frequent ALK rearrangements. But this analysis does not detect translocations whose breakpoint is located in front of exon 19.

Conflict-of-interest statement:
All authors have nothing to disclose

Results

744/4991 (14.9%) NSCLCs showed evidences for ALK unbalanced 5'/3'-end expression, although only 427 of them demonstrated a disbalance between the border of the rearrangement (exon 19) and the 3'-portion of the gene.





novel	1	P8424	43	m	UBC-ALK	(U1;del49A18)
	2	P8501	59	f	EML4-ALK	(E13;A3;A20)
	3	P11267	71	f		(E6;ins18A20)
	4	P11275	62	f		(E5;del44A20)
	5	13785	44	f		(E2;del150A20)
rare	6	P6555	72	f	SFTPB-ALK	(S2;A18)
	7	P14435	68	m	ETV6-ALK	(E4;A20)
	8	C276	52	f	PRKAR1-ALK	(P5;A20)
	9	A7056	46	f	GCC2-ALK	(G13;A20)
	10	14251	37	m	CLIP1-ALK	(C23;A20)
	11	B6194	64	f	HIP1-ALK	(H28;A20)
	12	P13620	33	f		(H30;A20)
	13	B1660	56	f	EML4-ALK	(E6;A17/A18)
	14	B4645	66	f		
	15	B4418	74	m		(E6;A18)
	16	P10390	73	f		
	17	P7111	57	m		(E6;A19)
	18	P7061	63	f		(E21;A20)
Low fraction	19	P7650	68	f	EML4-ALK	(E13;A20)
	20	P10768	64	f		(E6;A20)

Variant-specific PCR assay, which was designed to detect 20 the most common ALK fusions, revealed translocation in 291/4991 (5.9%) NSCLCs; 266/291 (91.4%) and 278/291 (95.5%) of these ALK-rearranged tumors demonstrated 5'/3' and ex19/3'unbalanced expression, respectively.

NGS revealed 20 instances of ALK translocations (5 novel (UBCex1-ALKex18, EML4ex13-ALKex3-ALKex20, EML4ex6-ins18bp-ALKex20 and EML4ex5del10-del44ALKex20) variants; 13 tumors with known ALK translocations; 2 common variants present in a low fraction of tumor cells).

In addition, we subjected to QIAseq RNAscan NGS analysis 32 young-onset NSCLCs, which were negative by PCR ALK/ROS1/RET translocation assays; no NSCLCs with ALK translocations were revealed, although a novel ACTB-ROS1 fusion was observed in a single case.

Conclusions

This study provides a framework for non-expensive and efficient detection of ALK fusions.

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