The Development Of A Highly Sensitive Mouse Monoclonal Antibody For Screening ALK Rearrangements In Lung Cancers

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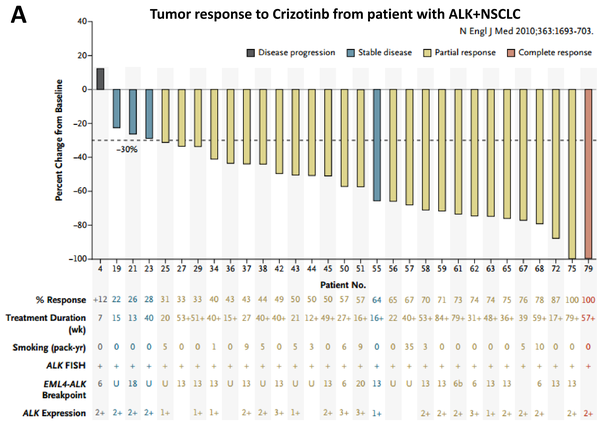
Abstract

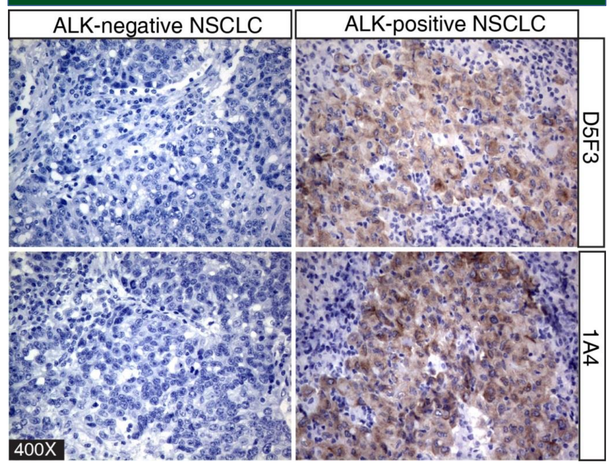
The anaplastic lymphoma kinase (ALK) rearrangements, mostly EML4-ALK fusion, occur in 3-7% of lung cancer patients and define a patient population that could respond to receptor tyrosine kinase inhibitor such as Crizotinib and Ceritinib [1]. Currently, ALK testing is mostly conducted by either fluorescence *in situ* hybridization (FISH) or polymerase chain reaction (PCR) methods, each of which has its own limitations. The detection of the ALK protein in lung cancer patient tissues by IHC was found to be difficult mostly due to the low abundance of its fusion product. To overcome this limitation, we have developed an ALK mouse monoclonal antibody (clone 1A4) that is more sensitive than a current rabbit ALK antibody, clone D5F3. Our initial analyses revealed that 1A4 can correctly identify all 5 EML4-ALK positive samples that were previously confirmed by qPCR tests, while it did not yield significant background on any of the 30 EML4-ALK negative lung cancer samples. By using a different IHC detection system, we further tested 1A4 on 17 ALK-positive and 10 ALK-negative lung cancer biopsy specimens that have been validated by either FISH or PCR previously. The IHC result of the antibody agreed 100% with the previous molecular diagnosis. The high concordance of the IHC results by the novel ALK antibody with other DNA/RNA-based detection methods suggested that 1A4 could be used routinely for screening ALK rearrangements among patients.

Background



The percentage of tumor response, treatment duration, and smoking history. Selected tumor characteristics are listed in the table below the graph, with each table entry corresponding to a patient in the graph above [2].

Comparison between 1A4 and D5F3

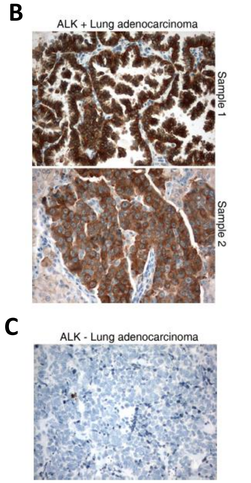


Sensitivity comparison between 1A4 and D5F3. The IHC staining results of D5F3 and 1A4 on ALK-positive NSCLC samples using a detection system from GBI-lab (PV9000).

1A4 staining and ALK FISH results

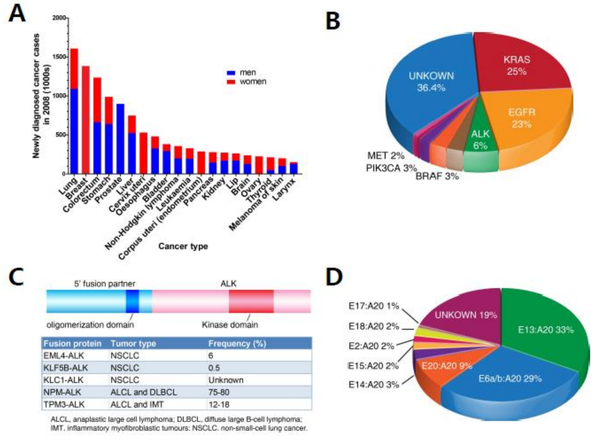
A

Patient ID	FISH	PCR	1A4 IHC	Comments
shi/312038-8	+	+	+	EML4-ALK
shi/289984-1	+	+	+	EML4-ALK
shi/309348-2	+	+	+	EML4-ALK
shi/239323-2	+	+ / -	+	EML4-ALK
shi/295879-1	+	/	+	
shi/290577-25	+	-	+	
shi/306208-6	+	-	+	
shi/305449-1	+	/	+	
347141-1 shi	+	/	+	
295025-17 shi	+	-	+	
shi/269666-1	-	+	+	EML4-ALK
shi/269093	failed	+ / -	+	EML4-ALK
shi/296428-2	failed	+	+	EML4-ALK
shi/271544-4	failed	+	+	EML4-ALK
shi/336960-1	-	+	+	EML4-ALK
Shi/339053-4	-	+	+	EML4-ALK
shi/339053-4	NA	-	-	
shi/347408-2	NA	-	-	
shi/348306-3	NA	-	-	
shi/348030-7	NA	-	-	
shi/349256-7	NA	-	-	
shi/348920-2	NA	-	-	
shi/348307-4	NA	-	-	
shi/343708-2	NA	-	-	
shi/332715-4	NA	-	-	
shi/340837-2	NA	-	-	
shi/341071-5	NA	-	-	



The 1A4 staining result correlates with the ALK FISH and qPCR results in the lung adenocarcinoma sample set from Beijing Chaoyang Hospital. (**A**), Summary of the ALK diagnosis by different techniques. (**B**), Representative 1A4 staining of the ALK+ lung adenocarcinoma. (**C**), Representative 1A4 staining of the ALK-negative lung adenocarcinoma.

Background

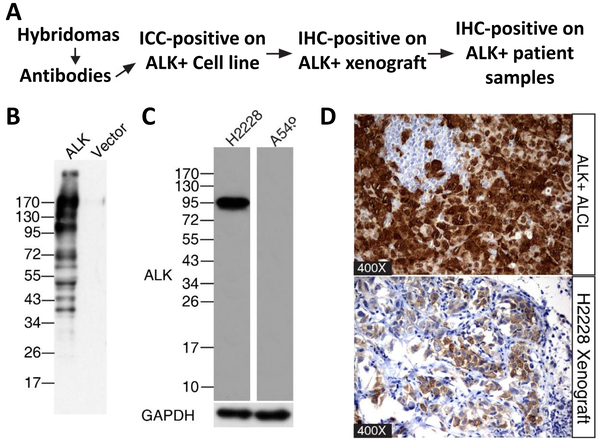


Fusion protein	Tumor type	Frequency (%)
EML4-ALK	NSCLC	6
KLF5B-ALK	NSCLC	0.5
KLC1-ALK	NSCLC	Unknown
NPM-ALK	ALCL and DLBCL	75-80
TPM3-ALK	ALCL and IMT	12-18

ALCL, anaplastic large cell lymphoma; DLBCL, diffuse large B-cell lymphoma; IMT, inflammatory myofibroblastic tumours; NSCLC, non-small-cell lung cancer.

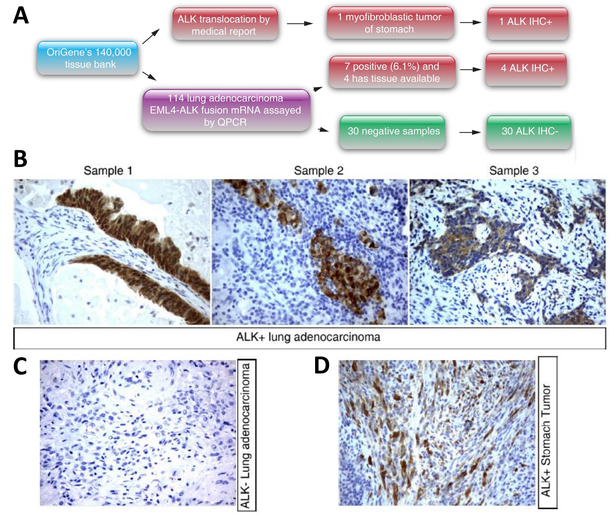
Lung cancer and different forms of ALK translocations. (**A**), Top 20 human cancers around the world. Lung cancer is the most common cancer type (WCRF, 2008). (**B**), Top oncogenic molecular mutations in lung adenocarcinomas. (**C**), Schema of ALK fusion protein resulted from ALK translocations and representative ALK-translocation events in lymphomas and lung cancer. (**D**), The frequency of different EML4-ALK translocation varieties reported in NSCLC.

Generating an ALK IHC antibody



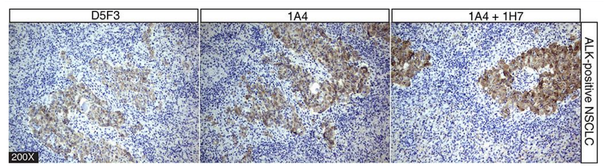
Generating a high-affinity ALK antibody for IHC application. (**A**), The hybridoma screening process to select antibodies that recognize ALK-fusion proteins in lung cancer cells [3]. (**B**), The ALK antibody, 1A4 clone, recognized full-length ALK protein that was transfected and expressed in 293T cells. (**C**), The ALK antibody, 1A4, recognized ALK-fusion protein in lung cancer cell line (H2228) at the dilution of 1:10,000. (**D**), The IHC staining of ALK-positive anaplastic large cell lymphoma (ALCL) tissues and paraffin embedded xenograft tissues derived from H2228 cells.

1A4 staining and ALK status



The correlation of 1A4 staining with ALK translocation determined by qPCR. (**A**), A scheme of screening ALK+ patients from OriGene's tissue bank and the summary of IHC validation results. (**B**), Representative pictures of 1A4 staining on ALK+ lung adenocarcinoma tissues. (**C**), Representative pictures of 1A4 staining on ALK negative lung adenocarcinoma. (**D**), 1A4 staining on myofibroblastic stomach tumor.

ALK antibody cocktails with higher sensitivity



The development of more sensitive ALK IHC antibodies by mixing several clones. The two-cocktail recipes were shown to have higher sensitivity than 1A4 did using a relatively insensitive detection system.

References

1. Shaw AT, *et al*. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. The lancet oncology, 2011, 12(11): 1004-1012.
2. Kwak EL, *et al*. Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. New England Journal of Medicine, 2010, 363(18): 1693-1703.
3. Ma D, *et al*. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. BMC Biotechnology, 2012, 12: 88