Using ALK IHC to identify potential responders to ALK inhibitors outside of NSCLC

[1]Joanne Xiu, [1]Zoran Gatalica, [1]Brian L. Abbott, [1]Sandeep K. Reddy, [2]Ari M. Vanderwalde, [3]Fadi S. Braiteh
[1]Caris Life Sciences, Phoenix, AZ; [2]West Cancer Center, University of Tennessee, Memphis, TN; [3]Comprehensive Cancer Centers of Nevada, University of Nevada School of Medicine, Las Vegas, NV

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Abstract

Background: In addition to anaplastic lymphomas and Non-Small Cell Lung Cancer (NSCLC), other solid malignancies can exhibit aberration in ALK expression potentially amenable to benefit from TKI ALK inhibitors. ALK expression by immunohistochemistry (IHC) has been extensively studied in NSCLC as a cost-effective and accurate screening tool for ALK rearrangement, and may identify patients (pts) with other mechanisms of ALK over-expression; however it has not been systematically studied outside of NSCLC.

Methods: To validate ALK IHC testing in a CLIA-certified lab, 86 cancer cases with documented ALK rearrangement by FISH (Abbott, Vysis break apart) results were tested by IHC using anti-ALK antibody D5F3 (Ventana) and evaluated by two pathologists (threshold: 3+ in > 1% of cells). 43 NSCLC and 43 tumors of 23 other cancer types were included. Follow-up with treating physicians on ALK positive (pos.) pts. was attempted. Additionally, a prospective dataset of 4678 routinely profiled tumors tested for ALK IHC was analyzed.

Results: In the validation dataset of 43 NSCLC, ALK IHC was concordant with FISH in 93% of cases (sensitivity = 90%, specificity = 95%). In non-NSCLC, all 4 ALK-FISH pos. tumors were also pos. for ALK IHC (sensitivity = 100%). Of FISH negative tumors, 39/43 were also negative by IHC (specificity = 90%). Of the 4 IHC+/FISH+ tumors, 1 was myxoid leiomyosarcoma of the uterus (Pt. A), 1 was inflammatory myofibroblastic tumor (IMT) (B), 1 was neuroendocrine tumor of the lung (C) and 1 was neuroblastoma (D). Of the 4 discordant (IHC +/FISH -) tumors, 1 was melanoma (E), 2 were serous ovarian cancers (F and G) and 1 was small cell cancer of the cervix (H). Follow-up was available for 5 IHC pos. pts. (A, B, F, G and H). A and B were treated with crizotinib: A deceased within 2 months and B has ongoing partial response to crizotinib for > 2 years to date. G planned to start on a phase 1b crizotinib trial, however, she was lost to follow up. F and H were not treated with ALK inhibitors. Prospective testing of ALK IHC on a large cohort of tumors (N =4678) from 38 cancer types are presented here.

Conclusions: The expanded study shows that cancer types including glioblastoma (19.9% of 121 samples), neuroendocrine tumors(12.6% of 103 samples), ovarian tumors (9.6% of 750 samples) and soft tissue tumors (9.2% out of 120 samples) are more likely to present with ALK positivity by IHC than others. In the broader set of non-NSCLC samples with concurrent testing, all FISH-positive patients were also positive by IHC, while 83% of FISH negative patients were also negative by IHC. These data show great potential for ALK IHC as a screening tool for ALK rearrangement detection in various cancer types.

Results (on an expanded prospective cohort):

Figure 1: Left: ALK FISH data from a total of 6867 tumors (6102 from NSCLC, 765 from non-NSCLC) and ALK IHC data from a total of 4678 tumors (540 NSCLC, 4138 non-NSCLC). Distribution frequencies are shown below. Not all samples received concurrent IHC and FISH testing; total N numbers in various cancer types with IHC or FISH tests are shown on the left. Stars indicate tumor types with concurrent positive IHC and FISH testing data available and shown in Figure 2. **Right:** ALK IHC expression frequencies in various cancer types when different cutoffs are used.

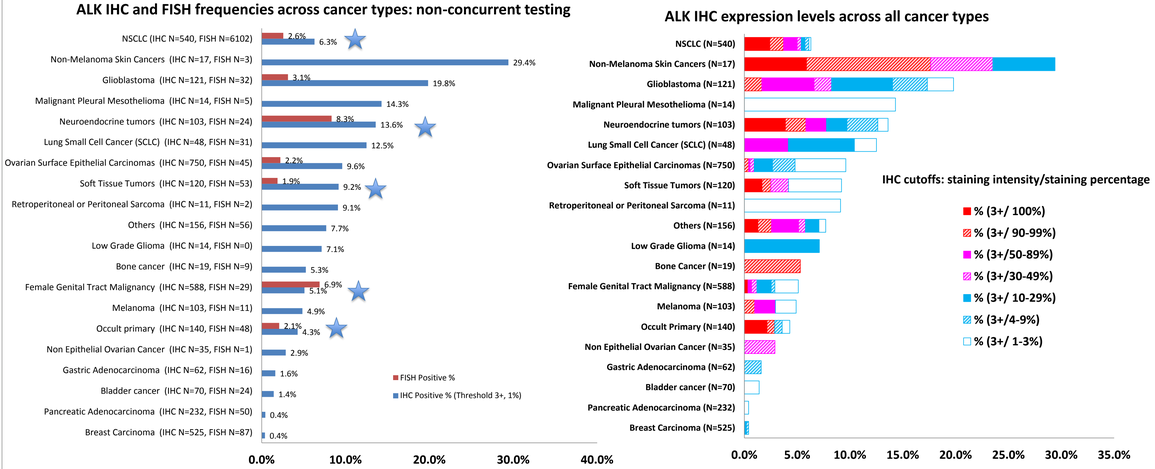
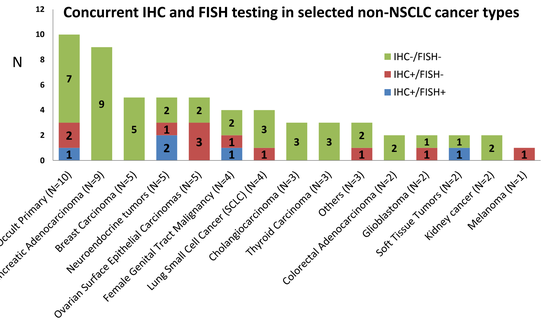


Table 1: cancer types with no ALK IHC or FISH positivity detected in our cohort.

Cancer type	IHC (N)	FISH (N)
Cholangiocarcinoma	75	13
Thyroid Carcinoma	13	11
Colorectal Adenocarcinoma	566	117
Kidney cancer	56	8
Prostatic Adenocarcinoma	51	11
Small Intestinal Malignancies	31	2
Esophageal and GEJ cancer	78	16
Gastrointestinal Stromal Tumors	11	4
Head and neck Squamous Carcinoma	47	22
Liver Hepatocellular Carcinoma	31	5
Lymphoma	16	16
Male Genital Tract Malignancy	11	1
Prostatic Adenocarcinoma	51	11
Small Intestinal Malignancies	31	2
Thymic Carcinoma	13	4
Thyroid Carcinoma	13	11
Uveal Melanoma	5	2
Kidney cancer	56	8

Figure 2: A subcohort of tumors had concurrent IHC and FISH tests. Shown are results seen in selected non-NSCLC cancer types. No case was FISH positive and IHC negative: in non-NSCLC, sensitivity=100%; specificity=83%. As shown on the right, there is a trend for IHC+/FISH+ tumors to have higher ALK expression level than IHC+/FISH- tumors, which should be validated in a larger cohort.

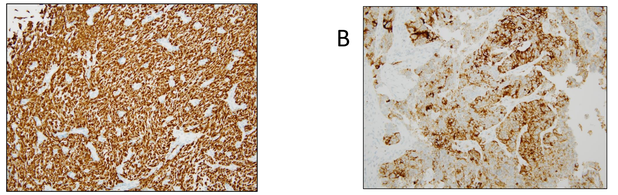


Cancer type	IHC+/FISH- N and staining details	FISH+/IHC+ N and staining details
Occult Primary	N=2 (3+/100%; 3+/100%)	N=1 (3+/90%)
Neuroendocrine	N=1 (3+/60%)	N=2 (both 3+/100%)
Ovarian	N=3 (3+/1%; 3+/1%; 3+/30%)	N=0
Female Genital	N=1 (3+/5%)	N=1 (3+/100%)
Small Cell Lung	N=1 (3+/10%)	N=0
Others	N=1 (3+/15%)	N=0
GBM	N=1 (3+/50%)	N=0
Sarcoma	N=0	N=1 (3+/100%)
Melanoma	N=1 (3+/70%)	N=0

Table 2: Patient treatments and outcomes

Patient	IHC	FISH	Cancer type	Gender	Age	Treated with ALK inhibitor?	Clinical course
A	3, 100%	Positive	myxoid leiomyosarcoma of the uterus	Female	64	Crizotinib	Deceased in two months
B	3, 100%	Positive	inflammatory myofibroblastic tumor	Female	76	Crizotinib	Prolonged partial response >2 years
F	3+, 20%	Negative	Ovarian cancer	Female	77	No	Not treated with ALKi
G	3+, 10%	Negative	Ovarian cancer	Female	76	Lost follow up	Lost follow up
H	3+, 90%	Negative	Small cell cancer of the cervix	Female	68	No	not treated with ALKi

Figure 3: Example ALK IHC (D5F3) images. A: inflammatory myofibroblastic tumor showing strong ALK IHC staining, 3+/100%; B: high grade serous ovarian tumor showing ALK IHC staining of 3+/20%



Conclusions

- Systematic IHC staining using the D5F3 antibody and 3+/ 1% cutoff revealed cancer types that express ALK protein at various levels, ranging from 20% in glioblastoma to less than 1% in pancreatic and breast cancer. In addition, cancer types including colorectal cancer didn't show ALK protein expression, despite a large cohort of tumors tested.
- In occult primary tumors, neuroendocrine, female genital tract and soft tissue tumors, concurrent FISH and IHC tests revealed ALK FISH-positivity seen in a sub-cohort of ALK IHC-positive tumors.
- Our results suggest the presence of ALK translocation in glioblastoma and ovarian cancer, however the small number of tumors with concurrent FISH/IHC failed to show concordance. These tumor types warrant further investigation in a larger cohort by concurrent testing.
- Overall, using FISH as the comparison, our study of 765 tumors from various non-NSCLC cancer types show the sensitivity and specificity of ALK IHC (D5F3) to be 100% and 83%.
- While the response to ALK-inhibitors in ALK IHC-positive but FISH-negative tumors are still being investigated, our results support the use of ALK IHC as a screening tool in non-NSCLC tumors to enrich for tumors positive for ALK-FISH.
- Based on the high probability of IHC+/FISH- , using a different (higher) threshold in non-NSCLC screening is worth further investigation.

References

- Mosse, et. al., (2013) "Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study.", Lancet Oncol 14(6):472-80
- Wynes, et. al., (2014) "An International Interpretation Study Using the ALK IHC Antibody D5F3 and a Sensitive Detection Kit Demonstrates High Concordance between ALK IHC and ALK FISH and between Evaluators" J Thorac Oncol.;9: 631–638)