Results: In the validation dataset of 43 NSCLC, ALK IHC was concordant with FISH in 39/43 cases (sensitivity = 90%, specificity = 90%). Of the 4 FISH-negative tumors, 1 was myxoid leiomyosarcoma of the uterus (Pt. A), 1 was inflammatory myofibroblastic tumor (IMT) (Pt. B), 1 was neuroendocrine tumor of the lung (Pt. C) and 1 was neuroblastoma (Pt. D). Of the 44 discordant cases, 2 were ovarian cancers (F and G) and 1 was small cell cancer of the lung (Pt. H). Follow-up was available for 5 IHC pos. pts. (A, B, F, G and H) and A was treated with crizotinib for > 2 years and B has ongoing partial response to crizotinib for > 2 years to date. G planned to start on a phase Ib crizotinib trial, however, she was lost to follow up. H and M were not treated with ALKi. Prospective testing of ALK IHC on a large cohort of tumors (N=4678) 38 cancer types are presented here. Table 1: cancer types with no ALK IHC or FISH data from our study.

Conclusions: The expanded study shows that cancer types including glioblastoma (19.8% of 121 samples), mesothelioma (22.6% of 103 samples), ovarian tumors (9.6% of 750 samples) and soft tissue tumors (3.3% 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: 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 are presented in Table 1. ALK IHC+/FISH+ tumors were also positive by IHC in ~4% of NSCLC samples, sensitivity=100%; specificity=83%. As shown on the right, there is a trend of ALK IHC expression levels across all cancer types

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.

Table 2: Patient treatments and outcomes

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Conclusions: Systematic IHC staining using the D5F3 antibody and 3+1/2+ cutoff revealed cancer types that express ALK protein at various levels, ranging from 20% in glioblastoma to less than 2% 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 gynecological and soft tissue tumors, concurrent FISH and IHC tests revealed ALK FISH positivity seen in a sub-cohort of ALK IHC positive tumors. Our finding of 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 tumors 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 FISH as ALK IHC positive. Our study’s results on 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 worth further investigation.

References