VENTANA ALK IHC Companion Diagnostic
Development of a "Follow-on" CDx

Abigail McElhinny, PhD *October 8, 2015*

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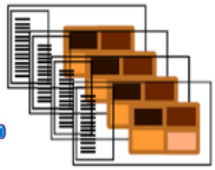
Companion Diagnostic Assay Systems

The system, not just the antibody, ensures correct selection of patients

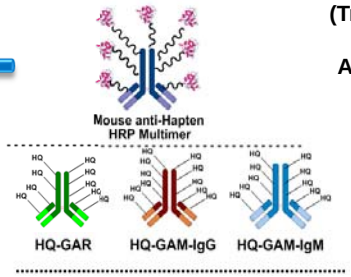
Automated Slide Stainer



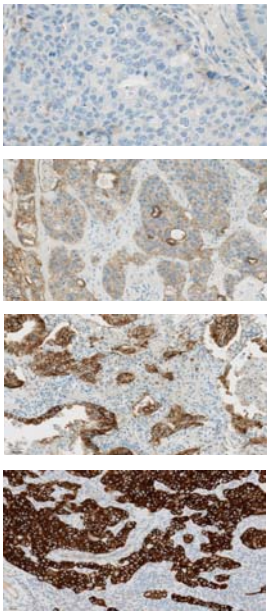
recommended tissue controls

Interpretation Guide (Training on Scoring Algorithm)

Primary Antibody



Sensitive Detection Chemistries



Patient sample results within dynamic range of the assay: Scoring Algorithm Development based on clinical outcome

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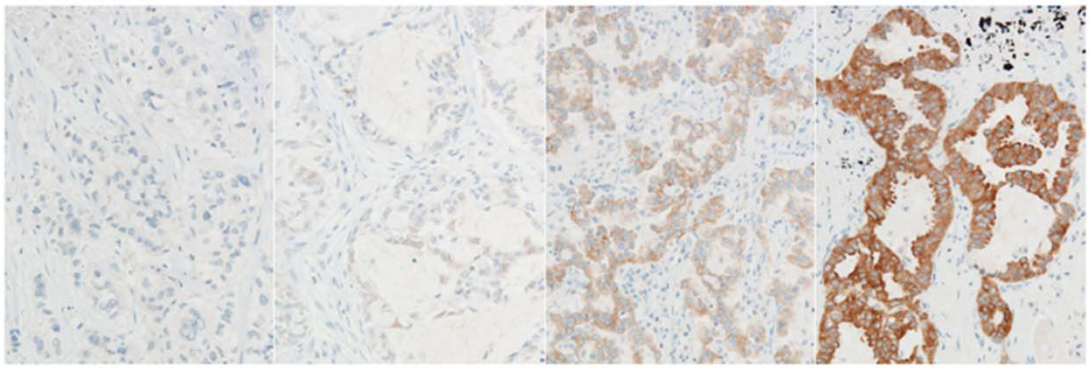
Feasibility testing revealed 100% concordance with FISH

ALK Immunohistochemistry for Non-small Cell Lung Cancer (NSCLC): ALK Protein Expression Levels



Negative | Weakly Positive | Moderately Positive | Strongly Positive

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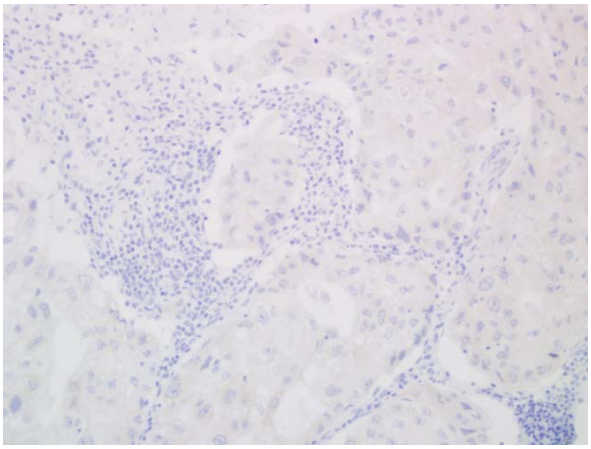
Clinical Benefit of VENTANA ALK (D5F3) IHC Assay

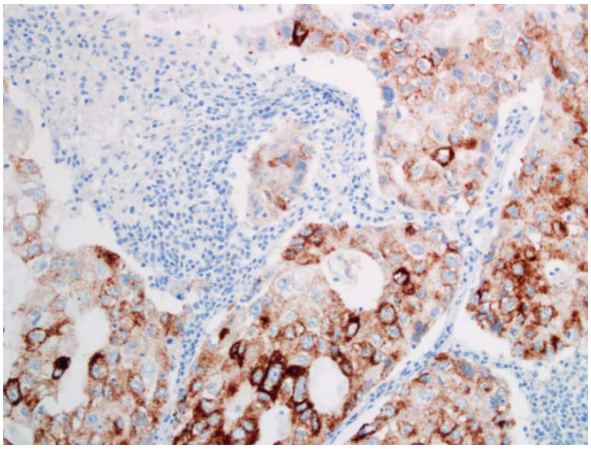
Potential reduction of false negatives in cases that are FISH positive

Traditional DAB ALK Staining



VENTANA ALK (D5F3) CDx Assay

OptiView DAB IHC Detection and OptiView Amplification



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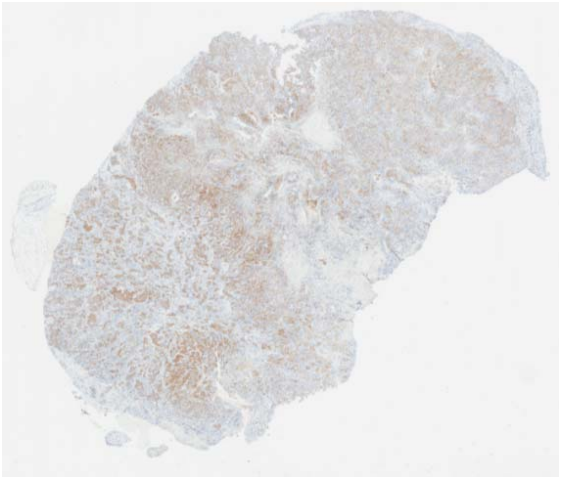
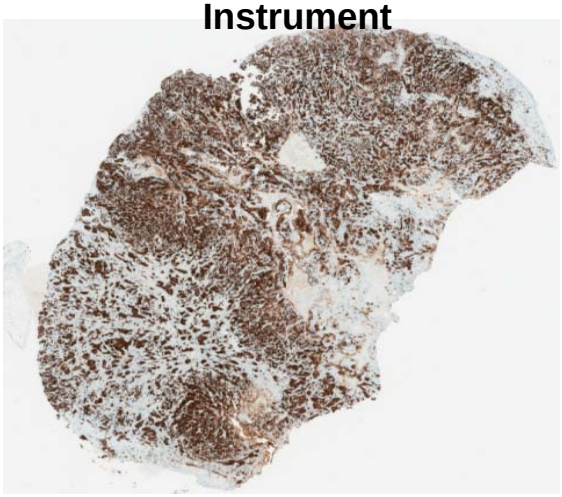
Clinical Benefit of VENTANA ALK (D5F3) CDx Assay

Potential reduction of false negatives in cases that are FISH positive

ALK D5F3 RUO Stained on Leica Bond Instrument



VENTANA ALK (D5F3) CDx Assay VENTANA BenchMark XT Instrument



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The Clinical Benefit of OptiView Amplification Kit:

*Enables Binary Scoring Algorithm for Determining ALK status (Positive or Negative)**

Positive for ALK	Negative for ALK
Presence of strong granular cytoplasmic staining in tumor cells (any percentage of positive tumor cells). Known staining elements should be **excluded**, including: ▪ *light cytoplasmic stippling in alveolar macrophages,* ▪ *cells of neural origin (nerve and ganglion cells),* ▪ *glandular epithelial staining, and* ▪ *cells within lymphocytic infiltrate.* Some background staining also may be observed within normal mucosa in NSCLC (including mucin) and in necrotic tumor areas, which also should be excluded from the clinical evaluation.	**Absence of strong granular cytoplasmic staining in tumor cells.**

*VENTANA ALK (D5F3) CDx Assay Package Insert, Ventana 2015

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First to Market CDx vs Follow-on CDx

- First to market test, is generally the enrollment assay for the pivotal trial of the a new therapy → direct link to outcome
- Follow-on tests should test "intent to treat" and "intent to test" population and show link to outcome
- Challenges:
  - Clinical samples with outcome not always available to test with follow-on CDx
  - For selection trial: Approved CDx negatives/Follow-on positives lack outcome data
  - "Gold standard" (approved) CDx may be less accurate than follow-on test → concordance alone not enough (third method or clinical data necessary to determine truth)

Challenge: regulators look for concordance to "Gold Standard" as well as clinical outcome link

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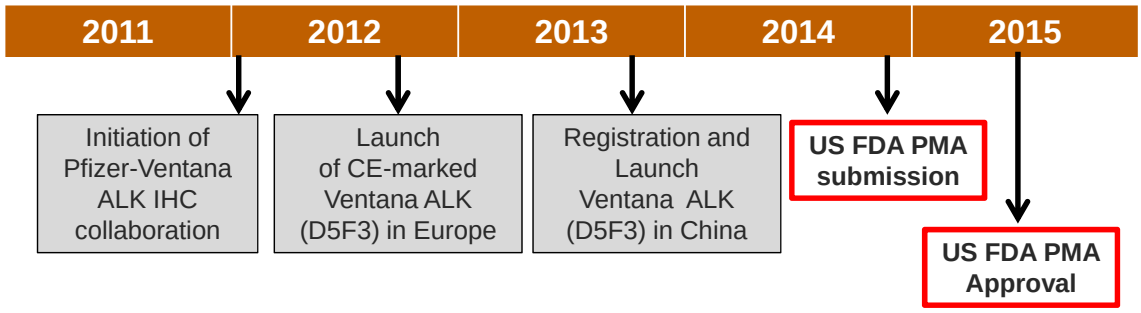
Regulatory Considerations

- US FDA approved VENTANA ALK (D5F3) CDx Assay in June 2015
- Submission based on:
  - Parallel testing of a subset of Ph3 patients
  - HR analysis of FISH+ patients that were also tested by IHC
  - HR simulation for the entire study population
  - Discordance analysis
- Allowed for availability of a clinically useful and much needed standardized ALK IHC test to patients in timely manner by not requiring prospective trial (agency understood medical value of test)

Challenges:

- Lack of outcome data on FISH-/IHC+ patients
- Some countries will not accept CDx based on concordance and patient population (e.g. Japan)

Successful Development of a CDx Requires Close Collaboration between the Diagnostic and Pharmaceutical Partners



Study A8081014

Cut slide stability prevented retrospective analysis of early A8081014 samples

VENTANA ALK (D5F3) CDx Assay Method Comparison Study

A8081014 IHC tested samples:
FISH+ N=179
FISH- N=754

Study A8081029

VENTANA ALK (D5F3) CDx Assay Method Comparison Study

A8081029 IHC tested samples:
FISH+ N=193
FISH- N=405

Concordance Data: A8081014 Study

A8081014: Phase 3, Randomized, Open-label Study Of The Efficacy And Safety Of Crizotinib Versus Pemetrexed/Cisplatin Or Pemetrexed/Carboplatin In Previously Untreated Patients With Non-squamous Carcinoma Of The Lung Harboring A Translocation Or Inversion Event Involving The Anaplastic Lymphoma Kinase (Alk) Gene Locus.

- enrollment by FISH
- parallel testing with Ventana ALK (D5F3) IHC assay for a subset of cases, as study was already ongoing
- FISH and IHC results obtained for 933 patients
- Outcome data available for FISH+ cases

Study 1014		ALK FISH Status		
		Pos	Neg	Total
ALK IHC Status	Pos	154	28	182
	Neg	25	726	751
	Total	179	754	933

Study 1014 Agreement Rates			
Rate	n/N	%	95% CI
PPA	154/179	86.0	80.2-90.4
NPA	726/754	96.3	94.7-97.4
OPA	880/933	94.3	92.6-95.6

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Concordance Data: A8081014 Study

High concordance between ALK (D5F3) IHC and Vysis FISH → small n for discordant analysis

Discordances are balanced between IHC^{+}/FISH^{-} and IHC^{-}/FISH^{+}

No Outcome Data supplemental data and pathologist analysis used to inform reason for discordance

Study 1014		ALK FISH St			greement Rates		
		Pos	Neg			%	95% CI
ALK IHC Status	Pos	154	28		79	86.0	80.2-90.4
	Neg	25	726	751	NPA	726/754	96.3	94.7-97.4
		179	754	933	OPA	880/933	94.3	92.6-95.6

Outcome Data Available

Small N for crizotinib arm

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Additional observations: Higher number of discordances close to FISH cut-off

- Disagreement between the two assays appears to be greatest for cases closest to the FISH diagnostic cutoff
- Concordance decreases when comparing patients with FISH scores within the initial FISH equivocal zone

	Patients With FISH Scores 10–50% Only			Patients With FISH Scores 10–25% Only		
Rate	n/N	%	95% CI [b]	n/N	%	95% CI [b]
PPA	30/48	62.5	48.4-74.8	14/28	50.0	32.6-67.4
NPA	17/18	94.4	74.2-99.0	17/18	94.4	74.2-99.0
OPA	47/66	71.2	59.4-80.7	31/46	67.4	53.0-79.1

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Additional observations: Higher number of discordances close to FISH cut-off

- Concordance increases when excluding patients with FISH scores within the initial FISH equivocal zone

Rate	Patients With FISH Scores 10–50% Excluded			Patients With FISH Scores 10–25% Excluded		
	n/N	%	95% CI [b]	n/N	%	95% CI [b]
PPA	124/131	94.7	89.4-97.4	140/151	92.7	87.4-95.9
NPA	709/736	96.3	94.7-97.5	709/736	96.3	94.7-97.5
OPA	833/867	96.1	94.6-97.2	849/887	95.7	94.2-96.9

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Available Outcome Data for Study 1014 FISH^{+}/IHC^{-} Patients

- 15/25 FISH^{+}/IHC^{-} patients enrolled in the Study 1014
  - 9 patients in chemotherapy arm
  - 6 patients in crizotinib treatment arm
- 2 of the 6 FISH^{+}/IHC^{-} patients in the crizotinib arm responded to treatment (FISH scores: 67% and 72%)
- 2 patients displaying disease progression had FISH scores of 15% and 17%
- 2 patients with stable disease had FISH scores of 15% and 18%
- Hypothesis: some FISH false-positive results may contribute to FISH^{+}/IHC^{-} discordance

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Assessment of FISH$^-$/IHC$^+$ Cases

- 28 FISH$^-$/IHC$^+$ patients, no outcome available
- IHC slides available for pathologist review (Ventana pathologist)
  - 14/28 cases (50%), displayed strong (3+) staining in more than 90% of tumor cells (entire tumor area was ALK-positive)
  - 12/28 cases (43%) exhibited heterogeneity of ALK staining
  - 26 /28 cases (92.9%) were unequivocally ALK-positive by the Ventana anti-ALK (D5F3) assay (strong (3+) staining observed)
- Hypothesis: errors in ALK FISH staining or interpretation of these cases may have contributed to number of discordances

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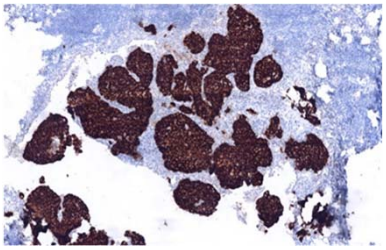
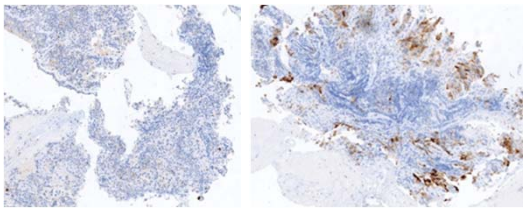
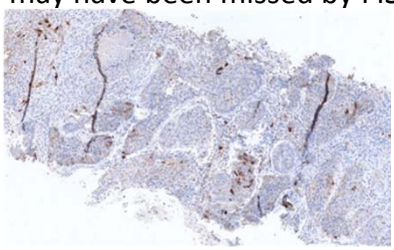
Various patterns of ALK IHC positivity seen in the IHC+/FISH– discordant cases



IHC+/FISH– case displaying strong ALK IHC staining in >90% of tumor cells

FISH-/IHC+ case displaying heterogeneous ALK IHC staining; note distinct tumor areas of ALK-positive staining (right image) and ALK-negative staining (left image), area of positivity may have been missed by FISH





IHC+/FISH– case displaying heterogeneous ALK IHC staining intensity; note positive and negative tumor throughout tissue specimen

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Summary of observations

- Disagreement between the two assays appears to be greatest for cases closest to the FISH diagnostic cutoff
- Concordance decreases when comparing patients with FISH scores within the initial FISH equivocal zone
- Concordance increases when excluding patients with FISH scores within the initial FISH equivocal zone
- Mean and median FISH scores were significantly lower for the discordant cases than for the overall concordance population
- 4/25 $FISH^{+}/IHC^{-}$ cases had FISH scores of 15-18% and did not respond

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What about outcome data from the study? Effective Patient Selection with Stand-Alone IHC

- The therapeutic benefit of crizotinib treatment was estimated for the hypothetical study in which the Ventana ALK IHC assay would have been used for enrollment
- Hazard Ratio (HR) almost identical for IHC+ trial and actual FISH+ trial
  - HR for ALK^{FISH+} Study 1014 cases identified as ALK^{IHC+} (i.e. FISH+/IHC+ cases) was 0.401 and the ALK^{FISH+} trial was 0.454

Observed HR for ALK^{FISH+} Patients		Estimated HR for ALK^{IHC+}|ALK^{FISH+} Patients		
HR	95% CI	HR	SE	95% CI
0.454	0.346–0.596	0.401	0.237	0.252–0.639

  - Standardized Ventana stand-alone IHC as effective as FISH in selecting patients who benefit from crizotinib
  - Slightly better point estimate for HR for FISH+/IHC+ compared to all FISH+ cases suggests there may be some false FISH positive results in FISH+ population, although sample size is small for definitive conclusion
  - Simulated outcome analysis on FISH-/IHC+ also supported overall conclusion that stand-alone IHC is effective in selecting patients for treatment for crizotinib

VENTANA ALK (D5F3) CDx Assay Comparison to FISH

*Potential benefits of IHC testing vs. FISH**

Feature	VENTANA ALK (D5F3)	FISH
Fully Automated	Yes	No
Use of Bright field Microscopy	Yes	No
Turn Around Time	3.5 hours	2-10 days
Scoring Algorithm	Any percent strongly positive tumor cells	Requires 50 tumor cells
Morphology	Can be visualized in context due to bright field microscopy	Cannot be viewed with fluorescent technology
Assay Failure Rate	2% failure rate	5-40% failure rate
Archivable Slides	Yes	No, signal fades
Workflow	Can be run in conjunction with other advanced staining tests	Must be performed in molecular laboratory or sent to reference center for testing
Cost	$$	$$$$

* - Zhou et al. Accurate and Economical Detection of ALK Positive Lung Adenocarcinoma with Semi quantitative Immunohistochemical Screening. PLoS ONE (2014)
- Ying et al. Diagnostic value of a novel fully automated immunochemistry assay for detection of ALK rearrangement in primary lung adenocarcinoma. Annals of Oncology (2013)
- Selinger et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. Modern Pathology (2013)
- Laffert et al. Multicenter ALK Testing in Non–Small-Cell Lung Cancer: Results of a Round Robin Test. Thoracic Oncology (2014)

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Doing now what patients need next