New York State Histotechnological Society
2018 Region I Annual Symposium

March 17, 2018

Saratoga Springs, NY

“Morphologic Proteomics - a new frontier or an old friend?”

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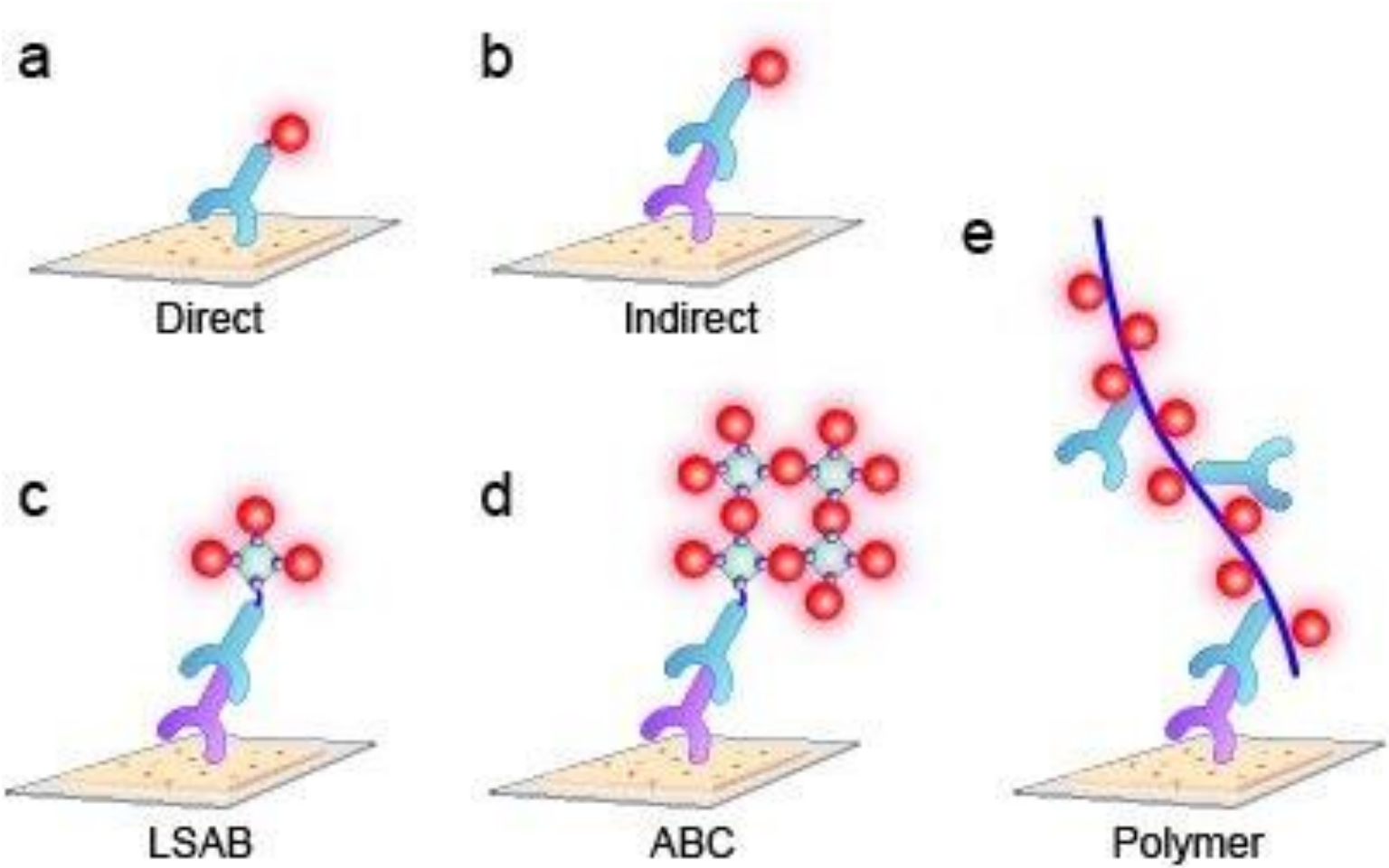
Immunohistochemistry



Is immunohistochemistry a stain?

No, it's a "Test"!

Dr. Clive R. Taylor

Predictive Biomarkers and Companion Diagnostics. The Future of Immunohistochemistry: "In Situ Proteomics," or Just a "Stain"?

Clive R. Taylor, MA, MD, DPhil, FRCPath, FRCP(Ir)

INTRODUCTION: DEFINITIONS

Predictive biomarkers are so named because the level of expression in a tumor may be of value in predicting the effectiveness of a particular "targeted" therapy for that specified tumor.

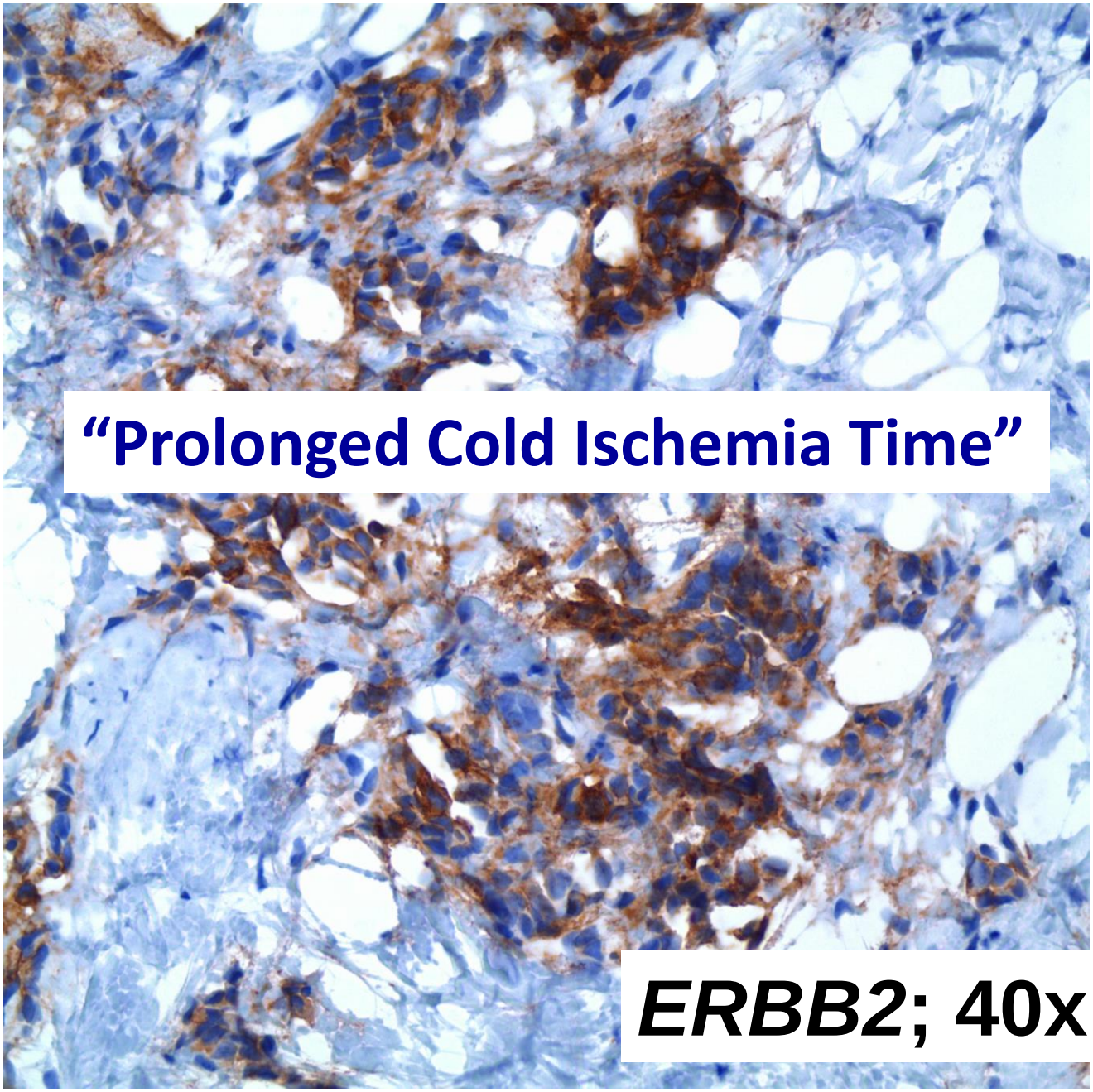
A "companion diagnostic" is a test or assay that detects a predictive biomarker, thereby allowing classification of patients (tumors) into responders and nonresponders, for the corresponding therapeutic agent.

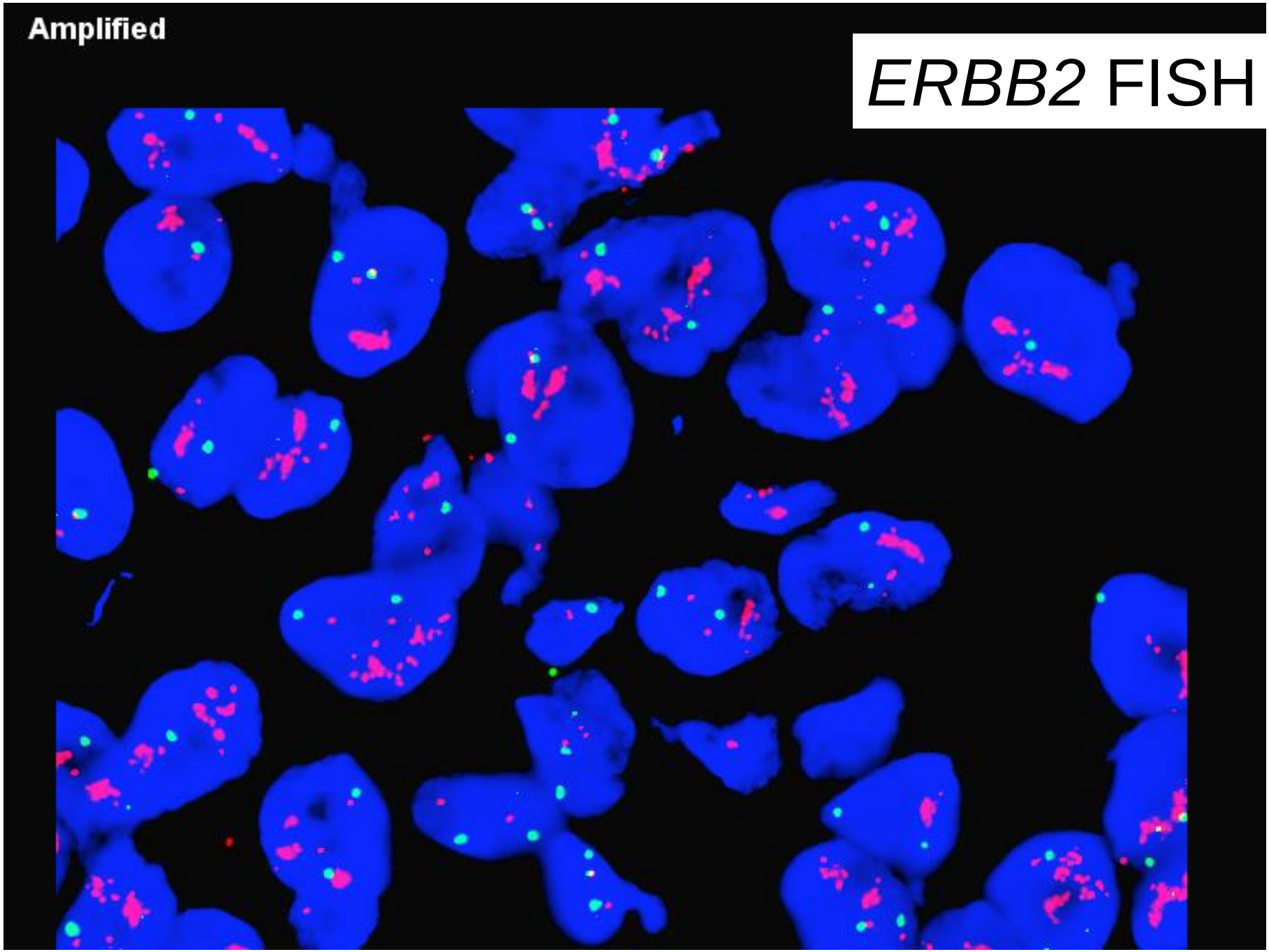
The terms "companion diagnostic," "predictive diagnostic," "precision diagnostic," "theranostic," and "advanced personalized diagnostic" are to a degree used synonymously, such that the context of use is important.[1,2] A companion diagnostic is defined in relation to a specified therapeutic agent and is approved by clinical trials that establish utility for classifying patients into responders and nonresponders for the drug in question. Companion diagnostics are classified as class III medical devices (in vitro diagnostic [IVD]) by the Food and Drug Administration (FDA), because the risk of the companion diagnostic equates to the risk of the drug that may be administered (solely) on the basis of a positive test.[3–6]

"IHC Assay Total Test Concept"

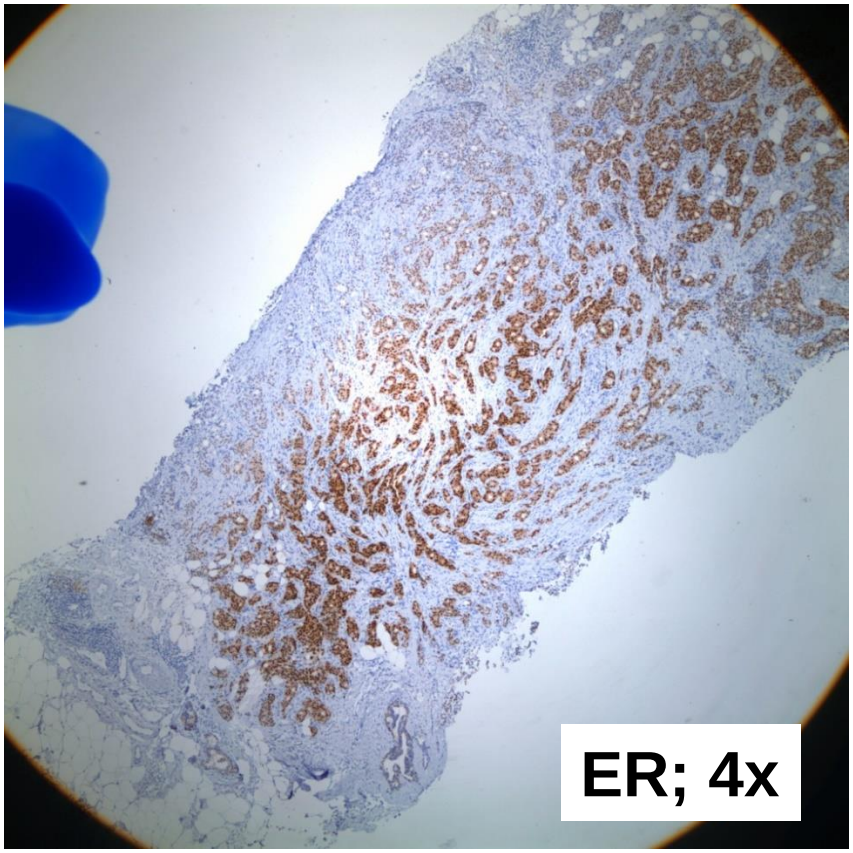
- **Pre-analytic**
  - Test selection
  - Specimen type, acquisition, transport time
  - Fixation: type and time
  - Tissue processing, type, and temperature
- **Analytic**
  - AR procedure
  - Protocol, control selection
  - Reagent validation
  - Technical staff training/certification
  - Laboratory certification
- **Post-analytic**
  - Control evaluation
  - Interpretation of results
  - Reporting of results
  - Pathologist, experience, and CME

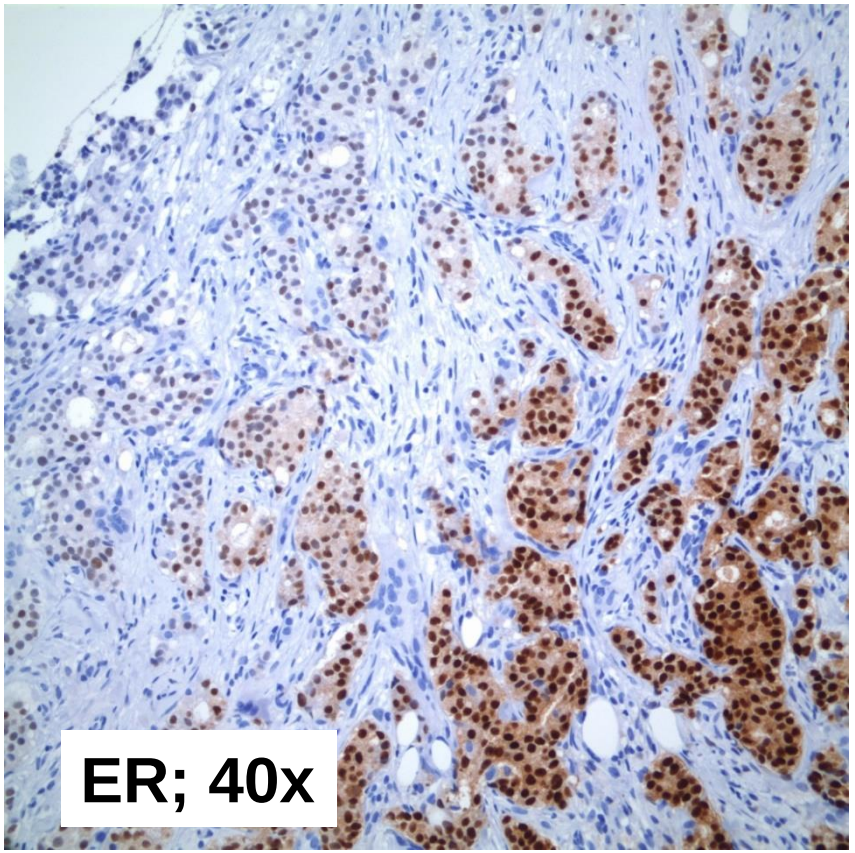
Dr. Clive Taylor - *Arch Pathol Lab Med* 2000;124;945-951


“Prolonged Cold Ischemia Time”
ERBB2; 40x

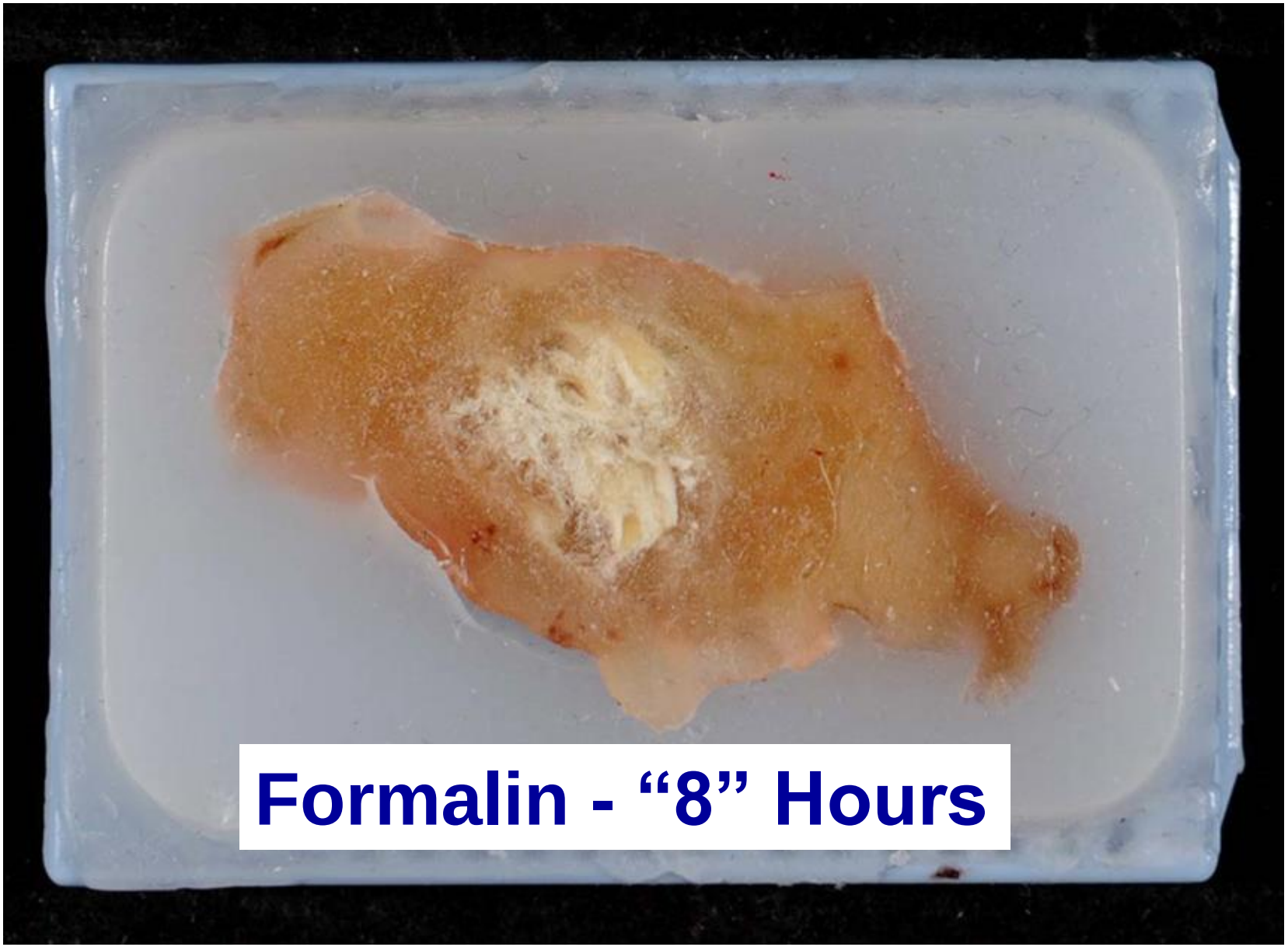

Amplified
ERBB2 FISH

Breast Needle Core Biopsy #1





"Edge Effect" Due To Drying


Formalin - “8” Hours

Case 1.

- 88 y.o. Male
- Right pleural effusion
- 1 ThinPrep, 2 smears, and a formalin-fixed, paraffin-embedded cell block
- “Positive for malignant cells (TTF-1 positive); favor metastatic lung adenocarcinoma”
- Reflex testing for EGFR, ALK, ROS1, and PD-L1

HARTFORD HOSPITAL LABORATORY

80 Seymour St., Hartford CT 06102-5037 CT REG HP-0254 (860) 696-8020 1-800-286-9800

Cytopathology Report

PATIENT NAME:	[redacted]	SPEC #:	**HN17-953**
MED. REC. #:	1003613907	SEX:	M
ACCOUNT #:	100043517156	DOB (AGE):	7/13/1928 (Age: 88)
DATE OBTAINED:	4/21/2017	LOCATION:	CB5
DATE RECEIVED:	4/24/2017	SUBMITTING MD:	[redacted]
DATE REPORTED:	4/25/2017 13:33	CC:	

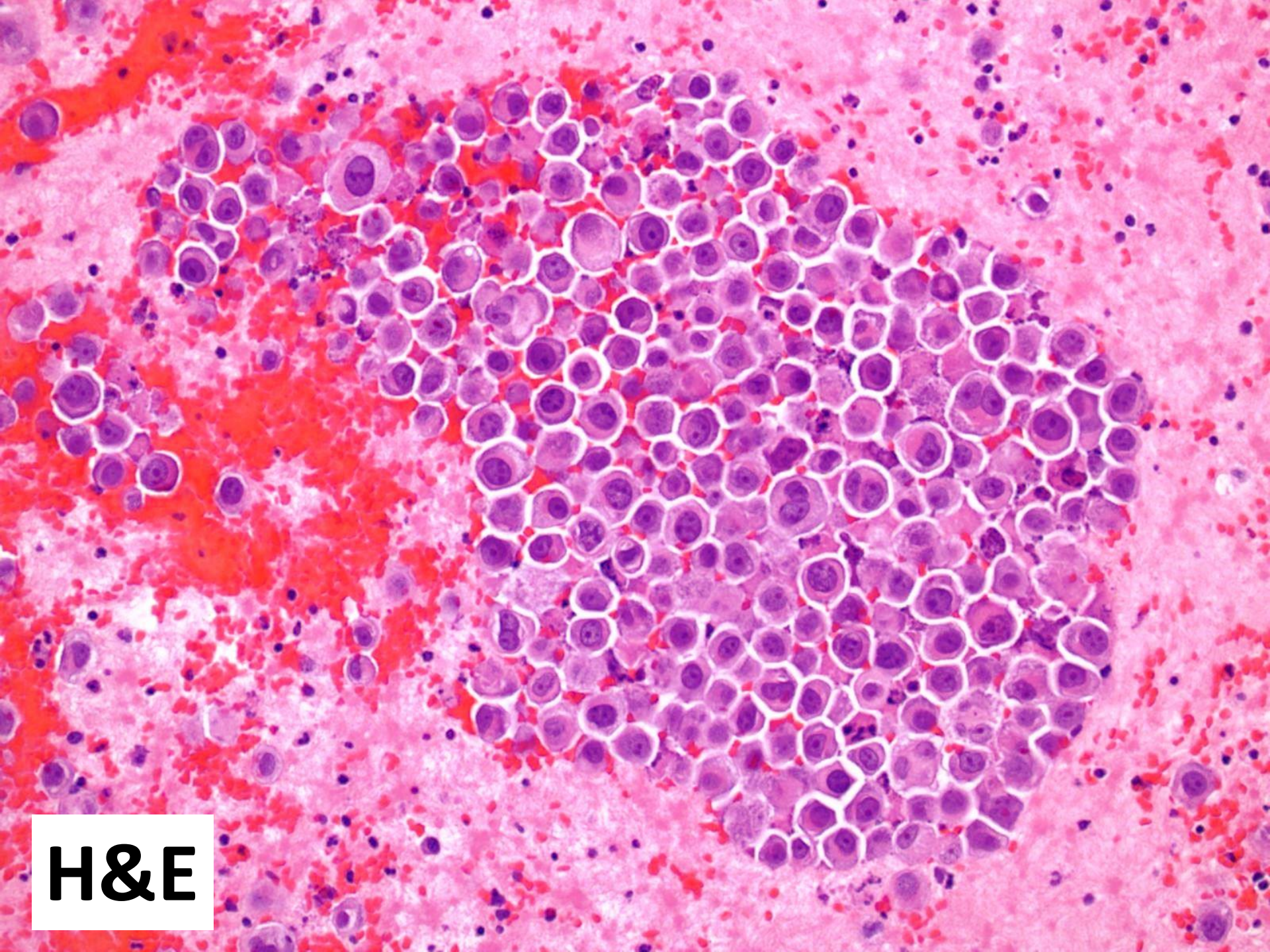
DIAGNOSIS

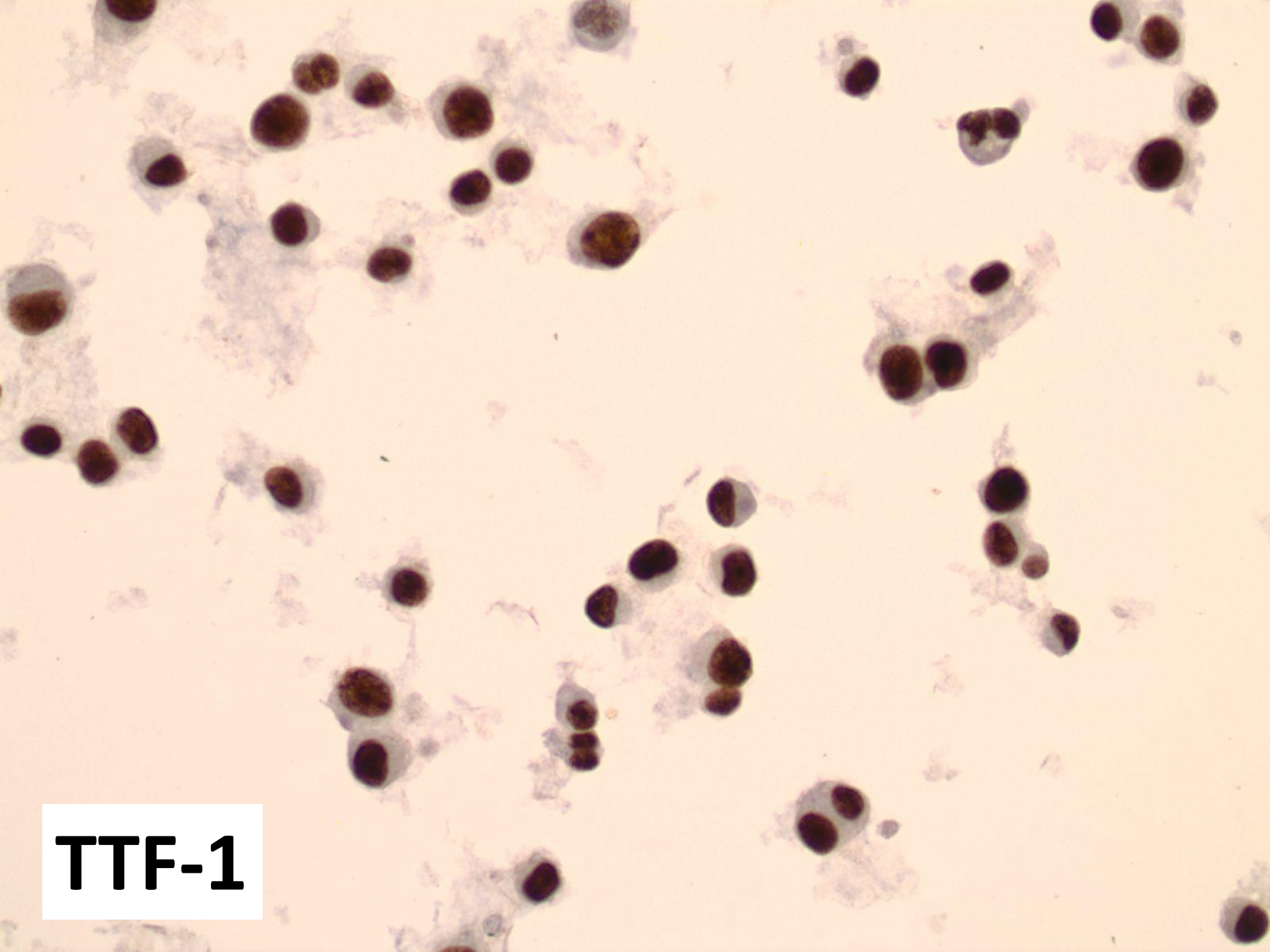
PLEURAL FLUID RIGHT AND CELL BLOCK 88305:

POSITIVE FOR MALIGNANT CELLS (TTF-1 POSITIVE), FAVOR METASTATIC LUNG ADENOCARCINOMA.
88342

Electronically Signed Out
[redacted]

Clinical Diagnosis and History:
RIGHT PLEURAL EFFUSION

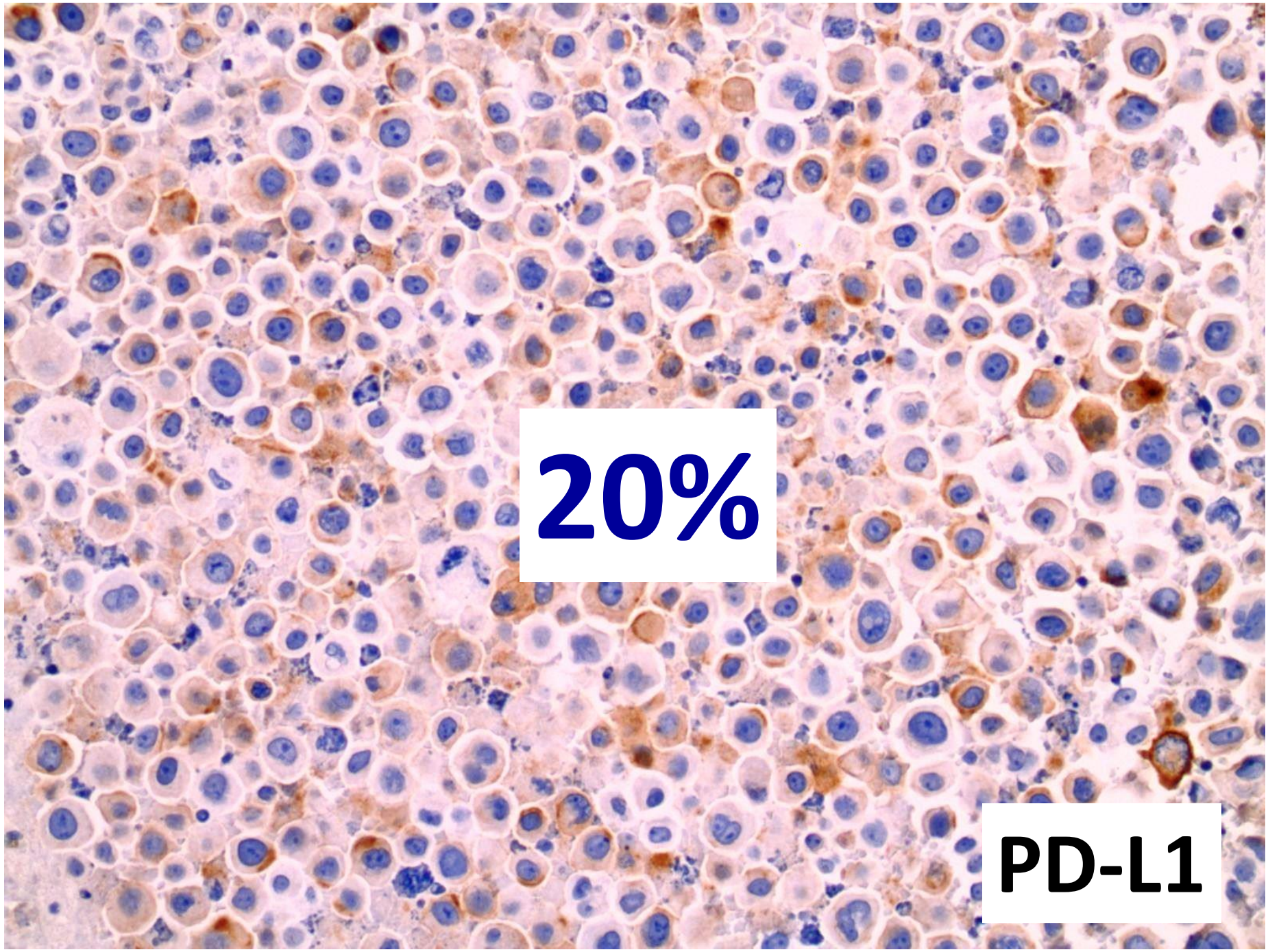

H&E


TTF-1

Ancillary Testing

- ALK (FISH) - Negative
- ROS1 (FISH) - Negative
- EGFR (PCR) - Negative


"PD-L1"
Engineered Immunotherapies
IMPROVE TARGETING


20%
PD-L1

Review of Clinical Next-Generation Sequencing

Sophia Yohe, MD; Bharat Thyagarajan, MD, PhD

• *Context.*—Next-generation sequencing (NGS) is a technology being used by many laboratories to test for inherited disorders and tumor mutations. This technology is new for many practicing pathologists, who may not be familiar with the uses, methodology, and limitations of NGS.

Objective.—To familiarize pathologists with several aspects of NGS, including current and expanding uses; methodology including wet bench aspects, bioinformatics, and interpretation; validation and proficiency; limitations; and issues related to the integration of NGS data into patient care.

Data Sources.—The review is based on peer-reviewed literature and personal experience using NGS in a clinical setting at a major academic center.

Conclusions.—The clinical applications of NGS will increase as the technology, bioinformatics, and resources evolve to address the limitations and improve quality of results. The challenge for clinical laboratories is to ensure testing is clinically relevant, cost-effective, and can be integrated into clinical care.

(*Arch Pathol Lab Med.* 2017;141:1544–1557; doi 10.5858/arpa.2016-0501-RA)

Next-generation sequencing (NGS) or massively parallel sequencing, a method of simultaneously sequencing millions of fragments of DNA (or complementary DNA), has been rapidly adopted in the clinical laboratory because of its ability to simultaneously analyze several genes or gene regions with a single test compared to traditional methods. As with any new technology, the use of NGS in the clinical laboratory has evolved and will continue to evolve over time. New applications for the technology continue to be developed, new bioinformatics and wet bench techniques are being developed to address current limitations and improve performance, and new knowledge regarding interpretation of gene variants is being accumulated. This organization in which NGS-related issues have been discussed and addressed.[6,7]

CURRENT AND EXPANDING USES OF NGS

Next-generation sequencing is an established test method for germline (inherited) and somatic (acquired mutations) genetic mutations in many clinical laboratories. For inherited diseases, testing for germline mutations may include targeted panel, whole exome, whole genome, or mitochondrial DNA sequencing.[8,9] Targeted panel testing, which varies between laboratories, is possible for a wide variety of inherited disorders such as immune deficiencies, bone

FOUNDATIONONE

Report Date
16 May 2017

Tumor Type
Uterus endometrial adenocarcinoma (NOS)

Date of Birth	19 March 1968	**Medical Facility**	Hartford Hospital		
Sex	Female	**Ordering Physician**	Brown, Amy K.	**Specimen Received**	03 May 2017
FMI Case #	TRF210810	**Additional Recipient**	Not Given	**Specimen Site**	Uterus
Medical Record #	1004486915	**Medical Facility ID #**	202744	**Date of Collection**	07 February 2017
Specimen ID	HS17-2276 A5	**Pathologist**	Not Provided	**Specimen Type**	Block

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS[1]

37 genomic findings

18 therapies associated with potential clinical benefit

0 therapies associated with lack of response

28 clinical trials

[1]Reduced sensitivity due to sample quality – See Appendix: Performance Specifications for details.

TUMOR TYPE: UTERUS ENDOMETRIAL ADENOCARCINOMA (NOS)

Genomic Alterations Identified[†]

ATM L2077I, R457*
FBXW7 R479Q
KIT P627L
NF1 R816*
PIK3CA R88Q, R992Q
PTEN R130Q, R233Q
ERRFI1 E416*, L246*
APC S2129L
ARID1A C2094*, R1989*, S1992*
ATRX G1071*
CARD11 E1035K
CTNNA1 R546*
CYLD E679*
EP300 D1399N
FAM123B R225I
FANCC R555Q
GNAQ R183*
LRP1B D2585Y
MSH6 E946*
NTRK1 A5T
PDGFRB E97*
PIK3R1 R642*
POLE A456P
PRKCI R480C
RAD50 E92*
RANBP2 R1203C
SF3B1 R425Q
SMARCA4 R1189Q
STAG2 E383*

Additional Findings[†]

For more comprehensive information please log on to the Interactive Cancer Explorer™
To set up your Interactive Cancer Explorer account, contact your sales representative or call 1-888-988-3639.

Electronically Signed by James Sah, M.D. | Jeffrey S. Ross, M.D., Medical Director | 16 May 2017
Foundation Medicine, Inc. / 1-888-988-3639

Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 / CLIA 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 / CLIA 22D2027561

FOUNDATIONONE

Report Date
16 May 2017

Tumor Type
Uterus endometrial adenocarcinoma (NOS)

Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
ATRX G1071*	None	None	None
CARD11 E103SK	None	None	None
CTNNA1 R546*	None	None	None
CYLD E679*	None	None	None
EP300 D1399N	None	None	None
FAM123B R225I	None	None	None
FANCC R555Q	None	None	None
GNAQ R183*	None	None	None
LRP1B D2585Y	None	None	None
Microsatellite status MSI-Unknown	None	None	None
MSH6 E946*	None	None	None

Case 2.

- 50 y.o. Female
- Bilateral salpingo-oophorectomy
- Bilateral ovarian clear cell carcinoma
- Please send to Foundation Medicine for “FoundationOne” testing

HARTFORD HEALTHCARE LABORATORIES, LLC, HARTFORD

80 Seymour St., Hartford CT 06102-5037 CT REG HP-0332 (860) 696-8020 1-800-286-9800

Surgical Pathology Report

PATIENT NAME:	[redacted]	SPEC #:	**HS16-16389**
MED. REC. #:	1001997886	SEX:	F
ACCOUNT #:	100038369717	DOB (AGE):	12/31/1965 (Age: 50)
DATE OBTAINED:	10/4/2016	LOCATION:	B8E
DATE RECEIVED:	10/4/2016	SUBMITTING MD:	[redacted]
DATE REPORTED:	10/7/2016 11:51	CC:	

DIAGNOSIS

A. RIGHT TUBE AND OVARY AND B. LEFT TUBE AND OVARY, BILATERAL SALPINGO-OOPHORECTOMY: BILATERAL OVARIAN CLEAR CELL CARCINOMA

Tumor type:	**Clear cell carcinoma**
Histologic grade:	**High grade**
Tumor size:	**29 cm (left ovary) and 7.5cm (right ovary)**
Tumor location:	**Bilateral ovaries**
Ovarian surface:	**Left ovarian capsule ruptured with tumor on the surface**
Residual ovary:	**Follicle cysts of right ovary**
Fallopian tube:	**Negative for atypia or malignancy**
Washings:	**Not done**
Other findings:	**Pleural effusion positive for adenocarcinoma (HN16-1838; see comment);**

FOUNDATIONONE

Patient Name [redacted]

Report Date
15 February 2017

Tumor Type
Ovary clear cell carcinoma

Date of Birth	31 December 1965	**Medical Facility**	Hartford Hospital		
Sex	Female	**Ordering Physician**	[redacted]	**Specimen Received**	02 February 2017
FMI Case #	TRF206001	**Additional Recipient**	Not Given	**Specimen Site**	Ovary
Medical Record #	1001997886	**Medical Facility ID #**	202744	**Date of Collection**	04 October 2016
Specimen ID	HS16-16389 B6	**Pathologist**	Not Provided	**Specimen Type**	Block

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

18 genomic findings

9 therapies associated with potential clinical benefit

1 therapy associated with lack of response

21 clinical trials

TUMOR TYPE: OVARY CLEAR CELL CARCINOMA

Genomic Alterations Identified†

ERBB2 L755S, V842I
ERBB3 V104M
PIK3CA R88Q
ARID1A E1958*
TP53 R283H
ATR L2139fs*1
CARD11 R423W
CHD4 R1421*, R390C, R975H
GNAS R201C
MSH6 R1035*
PPP2R1A R105Q, R183W
SMARCA4 R905C
SPEN A2251fs*102

Additional Findings†

Tumor Mutation Burden TMB-High; 36 Muts/Mb

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- 18 genomic findings
- 9 therapies associated with potential clinical benefit
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- 21 clinical trials

TUMOR TYPE: OVARY CLEAR CELL CARCINOMA

Genomic Alterations Identified†

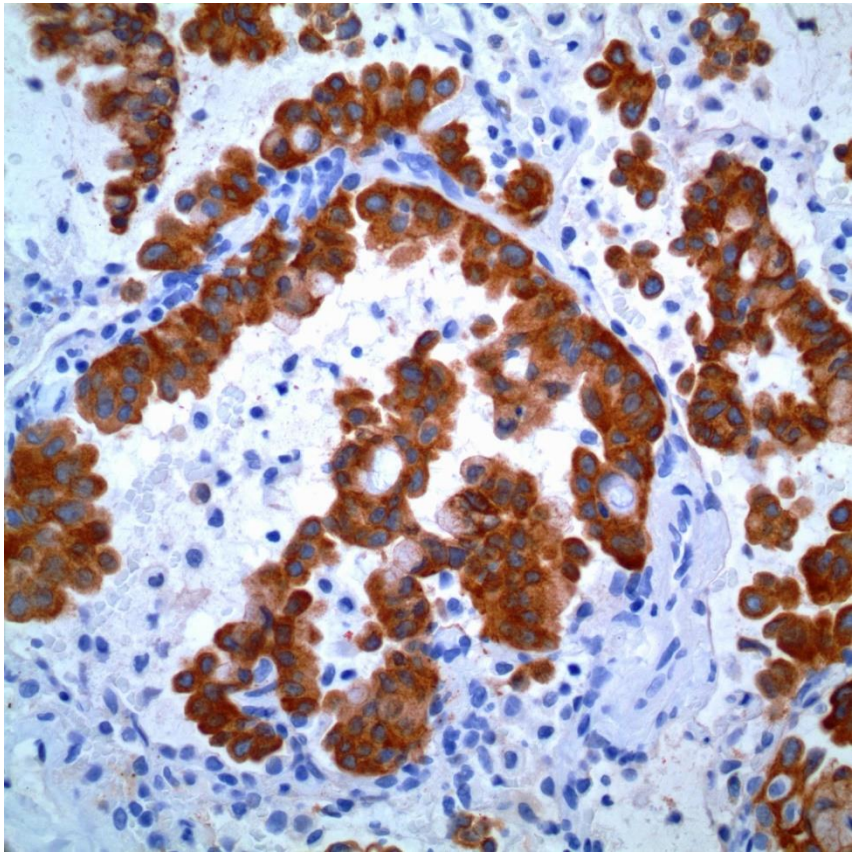
ERBB2 L755S, V842I
ERBB3 V104M
PIK3CA R88Q
ARID1A E1958*
TP53 R283H
ATR L2139fs*1
CARD11 R423W
CHD4 R1421*, R390C, R975H
GNAS R201C
MSH6 R1035*
PPP2R1A R105Q, R183W
SMARCA4 R905C
SPEN A2251fs*102

Additional Findings†

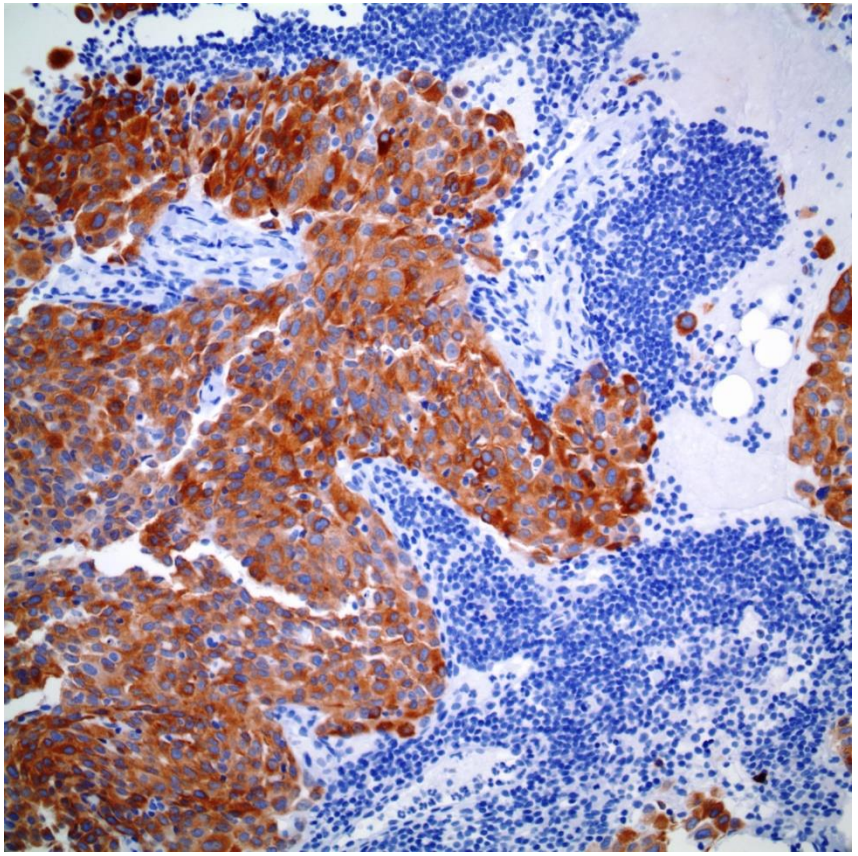
Tumor Mutation Burden TMB-High; 36 Muts/Mb

† For a complete list of the genes assayed and performance specifications,

IHC Detection of Genomic Alterations

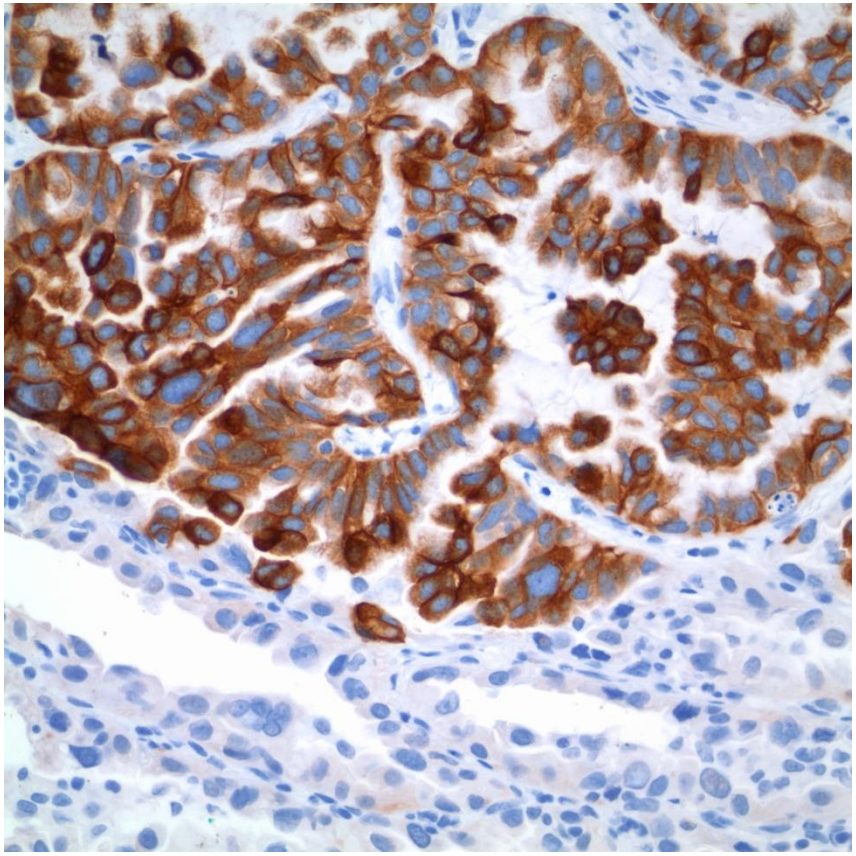


ALK

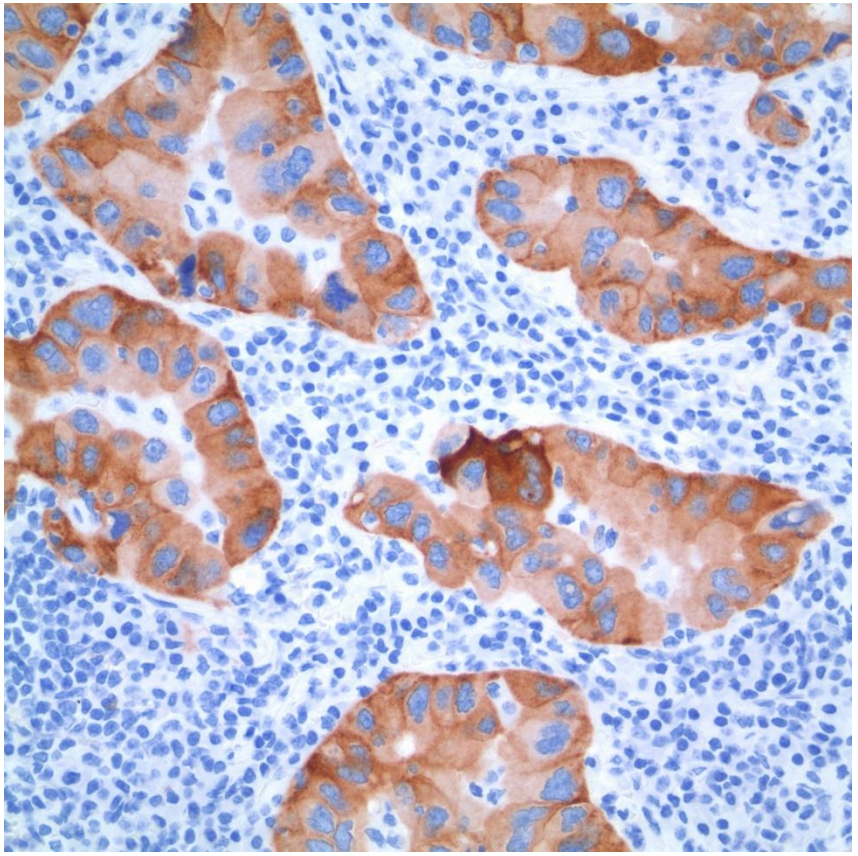


ROS1

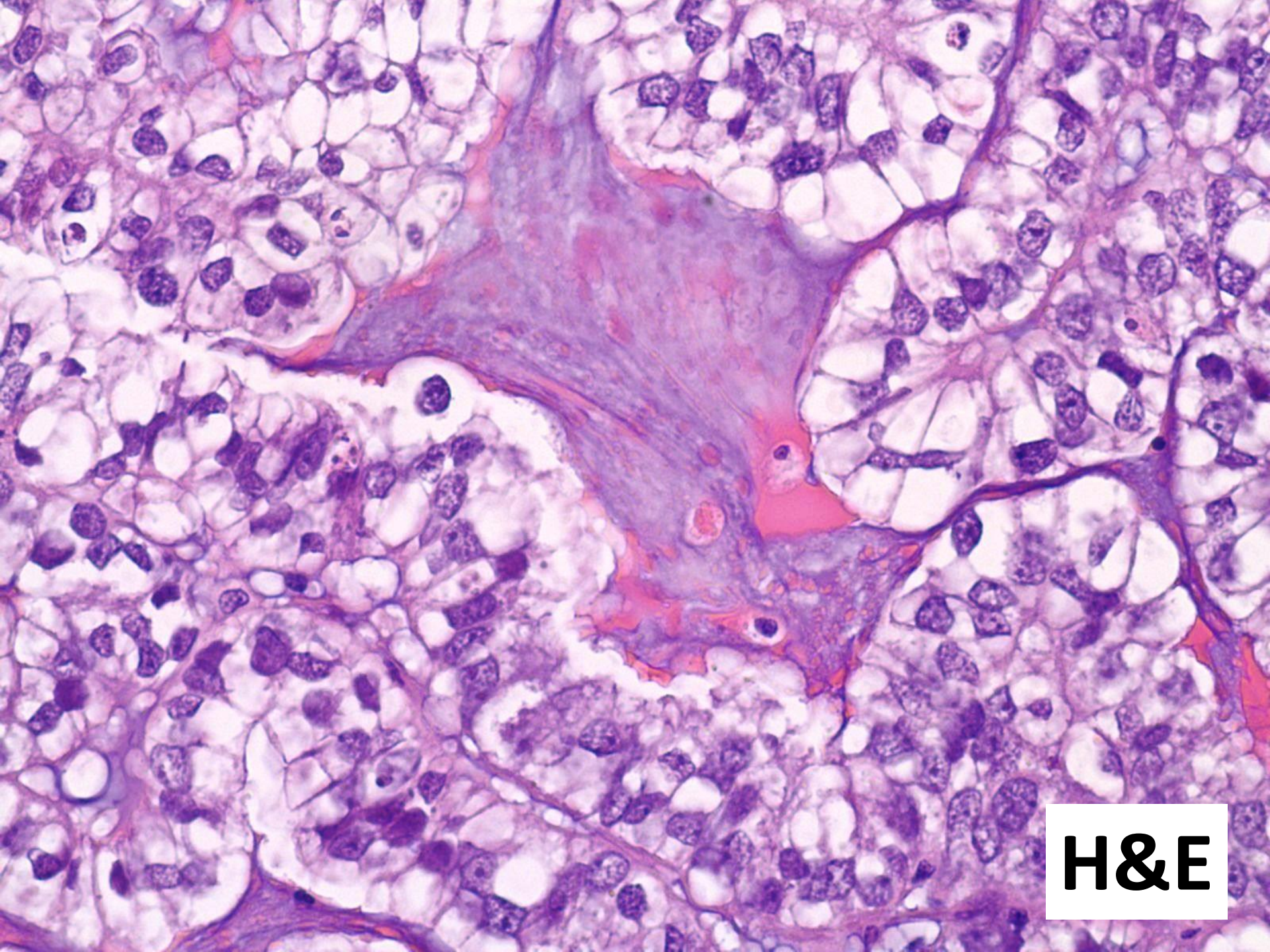
IHC Detection of Genomic Alterations



EGFR L858R



EGFR Exon 19 Del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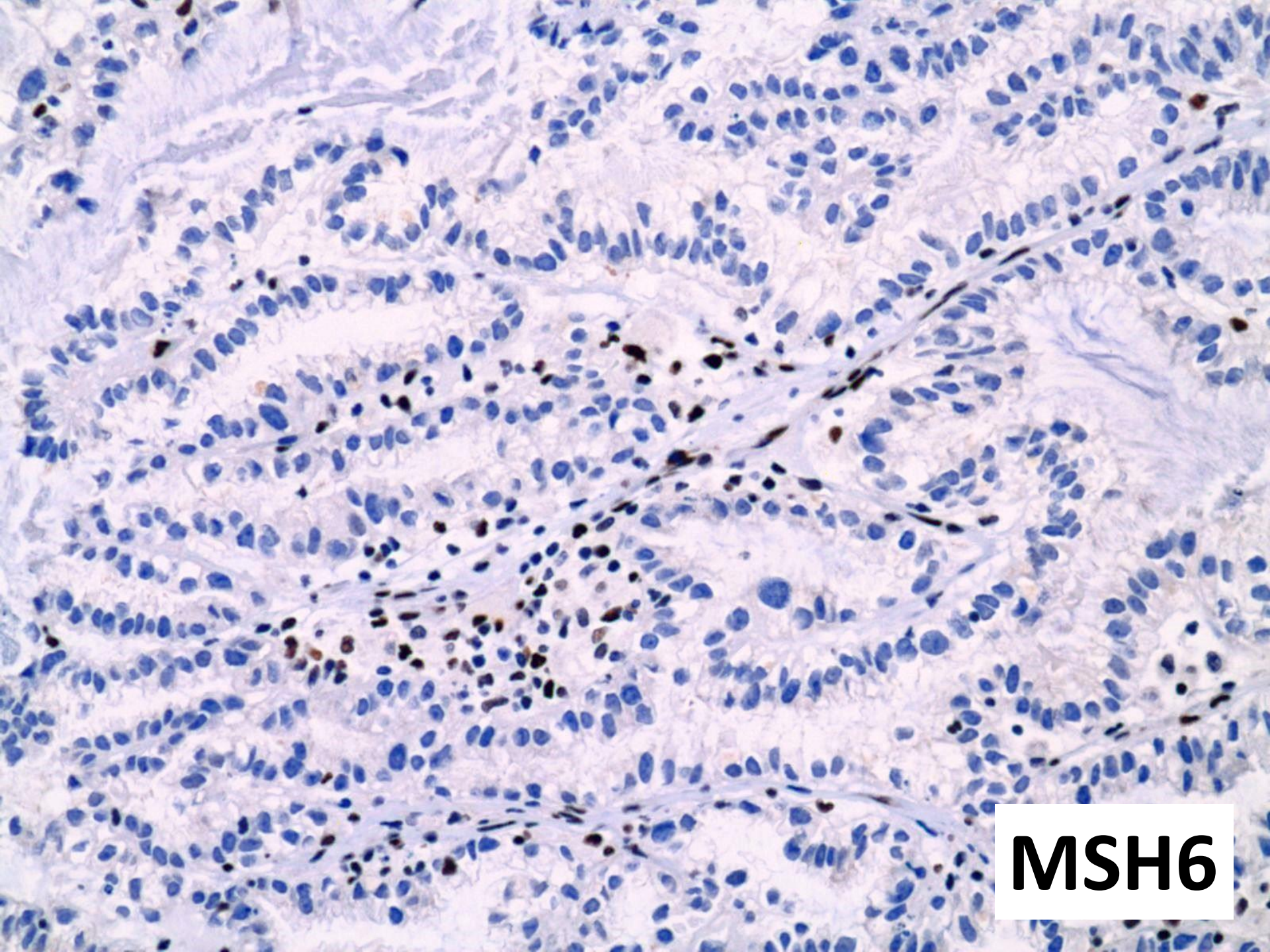
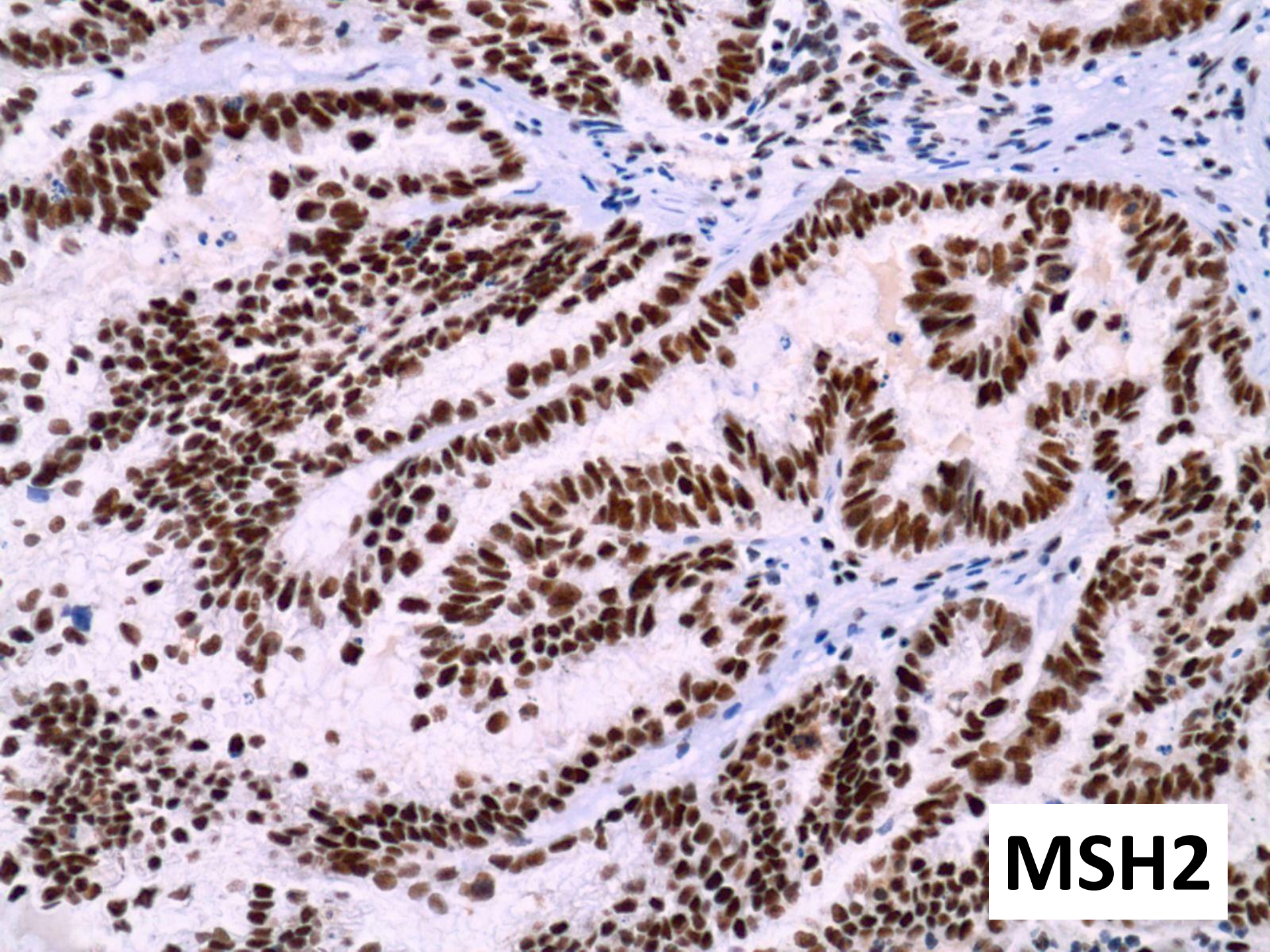

MSH6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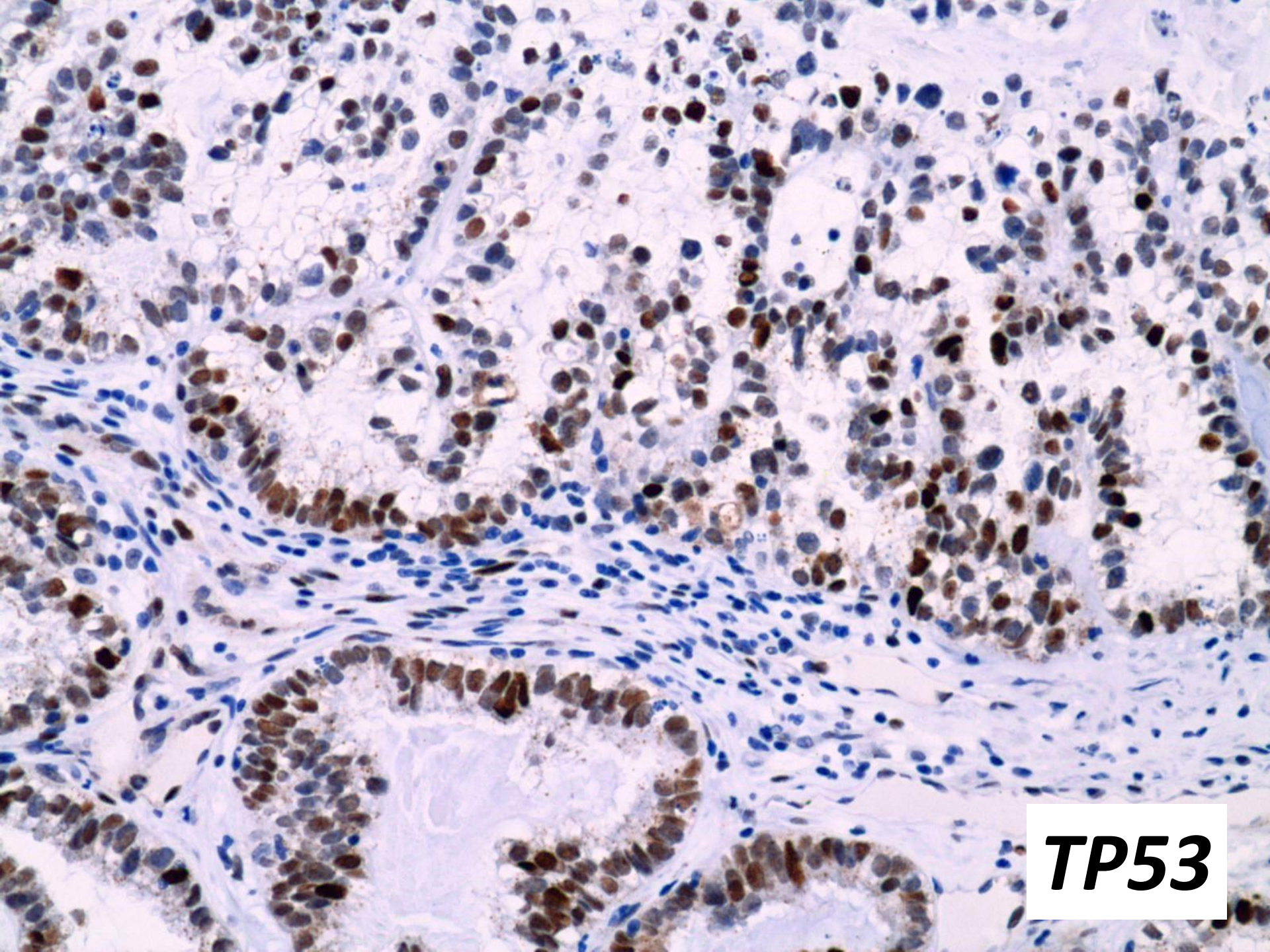
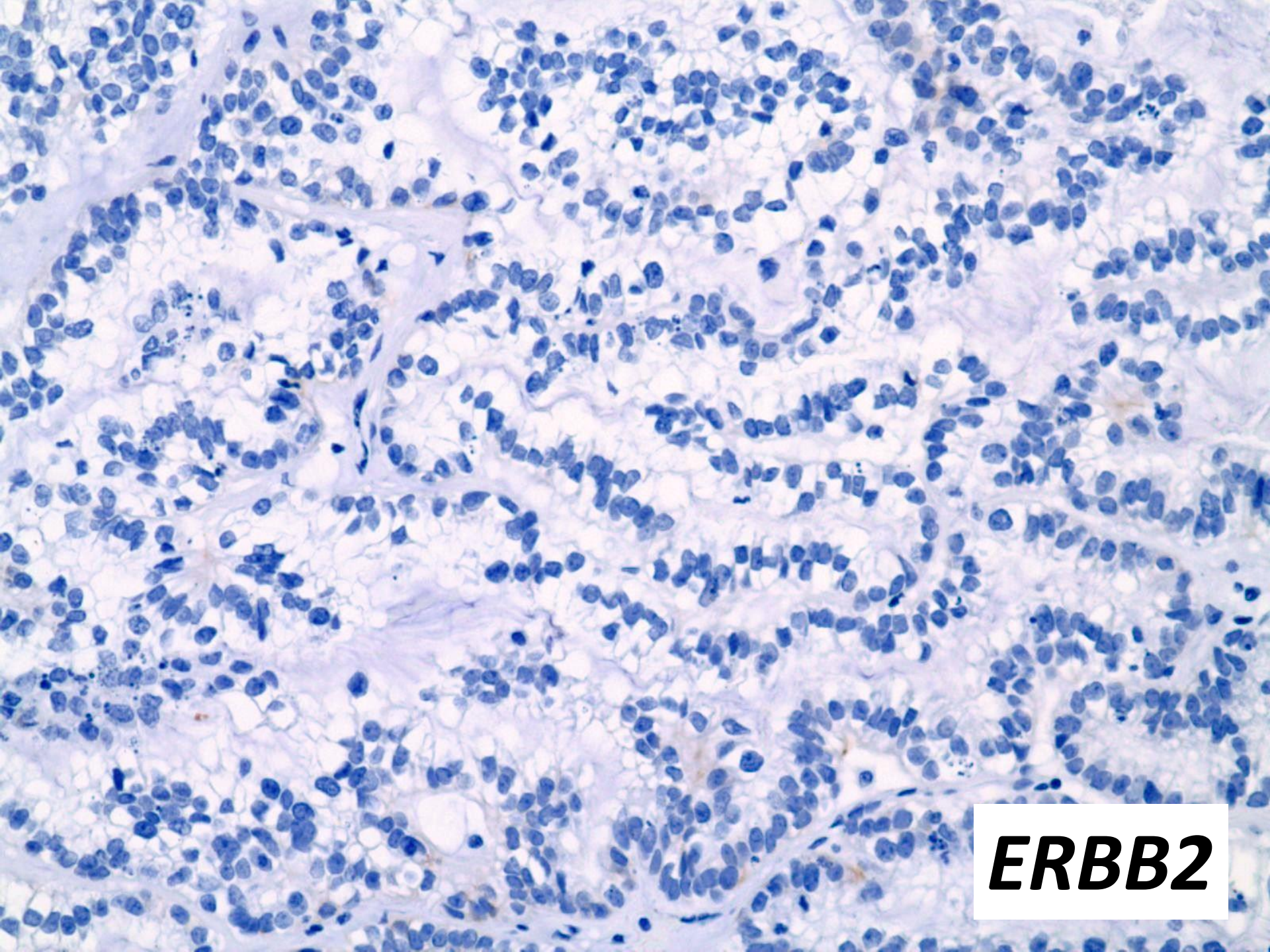
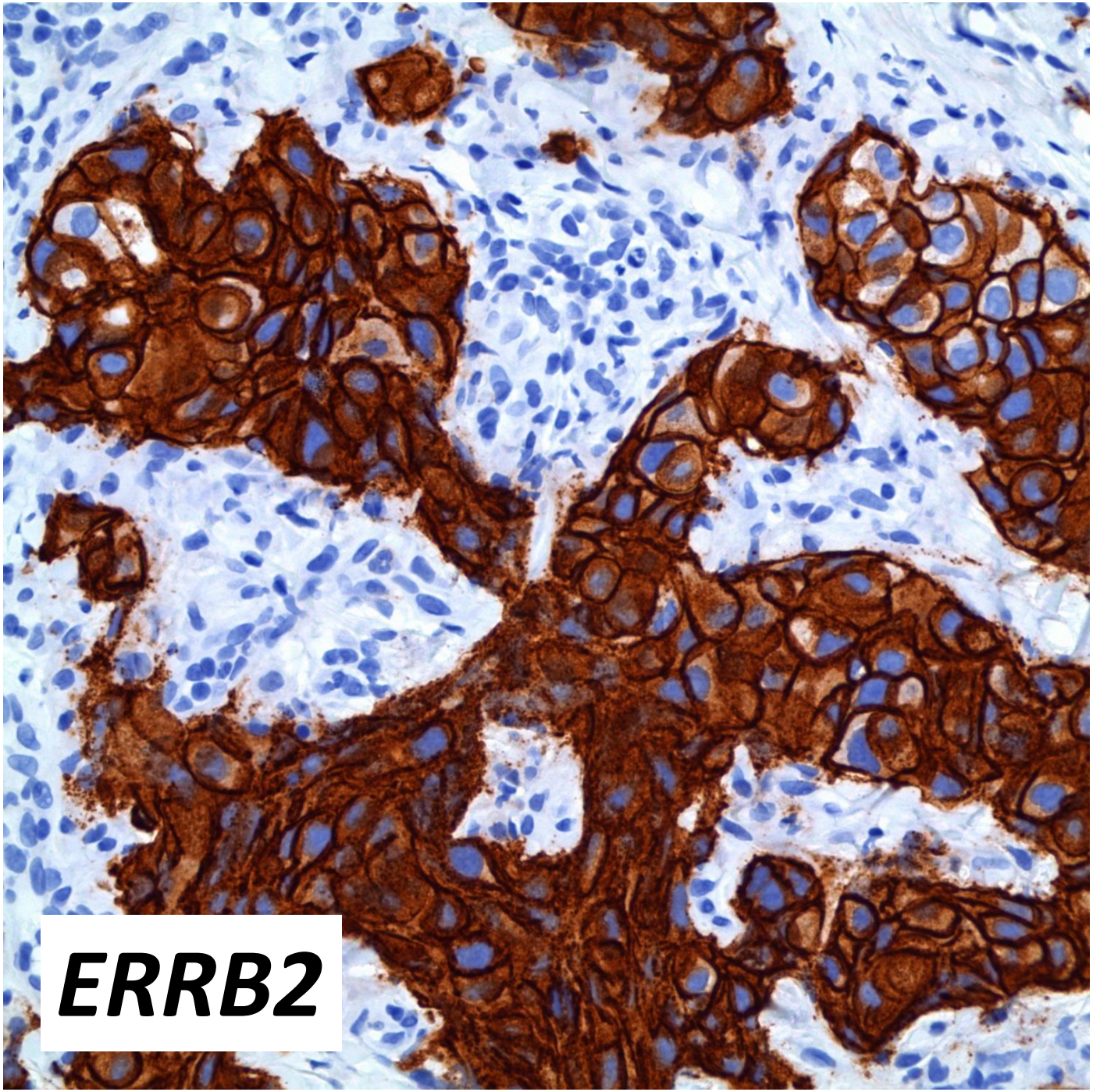

TP53


ERBB2


ERRB2

FOUNDATIONONE

Patient Name [redacted]

Report Date
15 February 2017

Tumor Type
Ovary clear cell carcinoma

GENOMIC ALTERATIONS

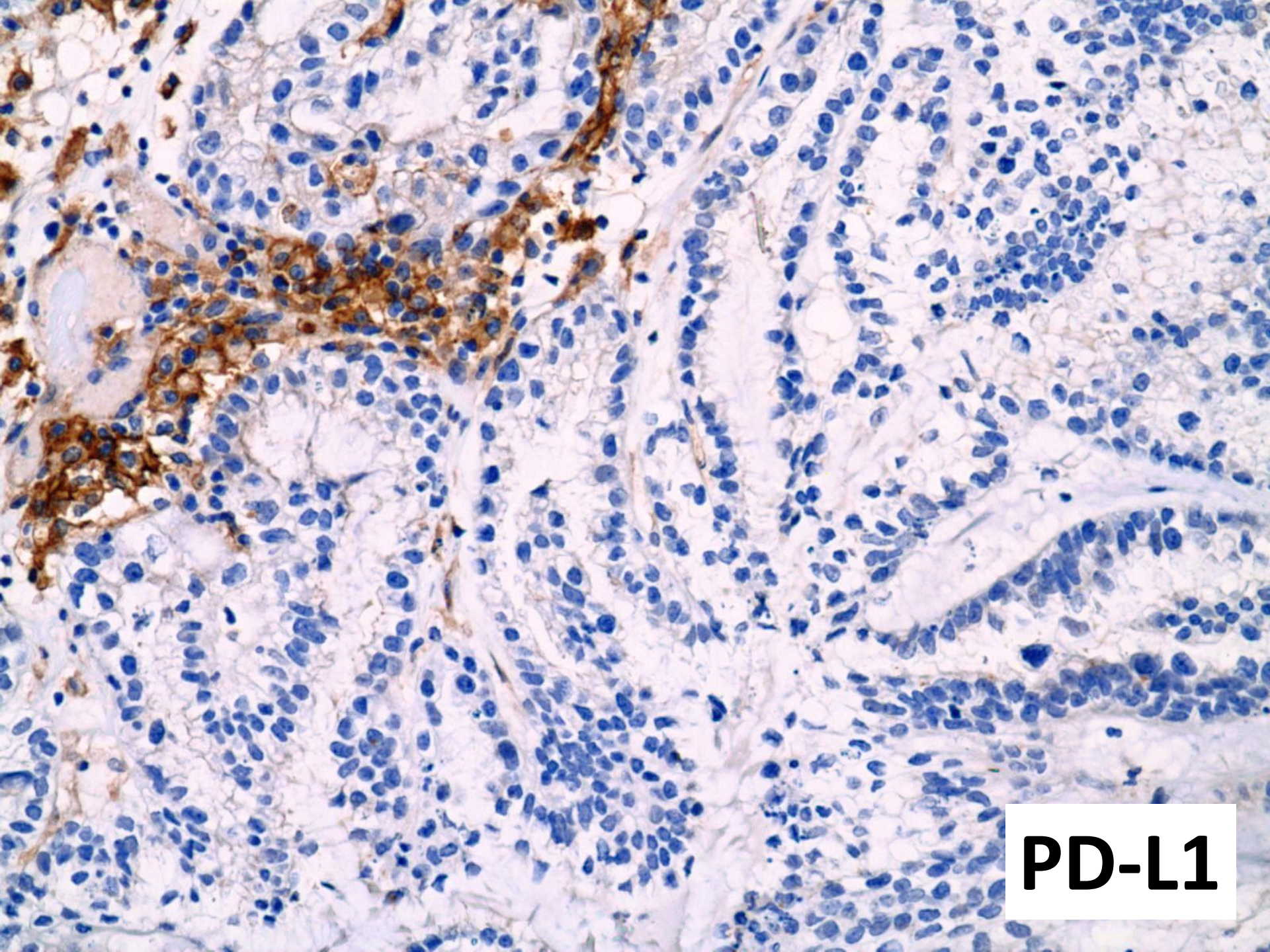
GENE ALTERATION

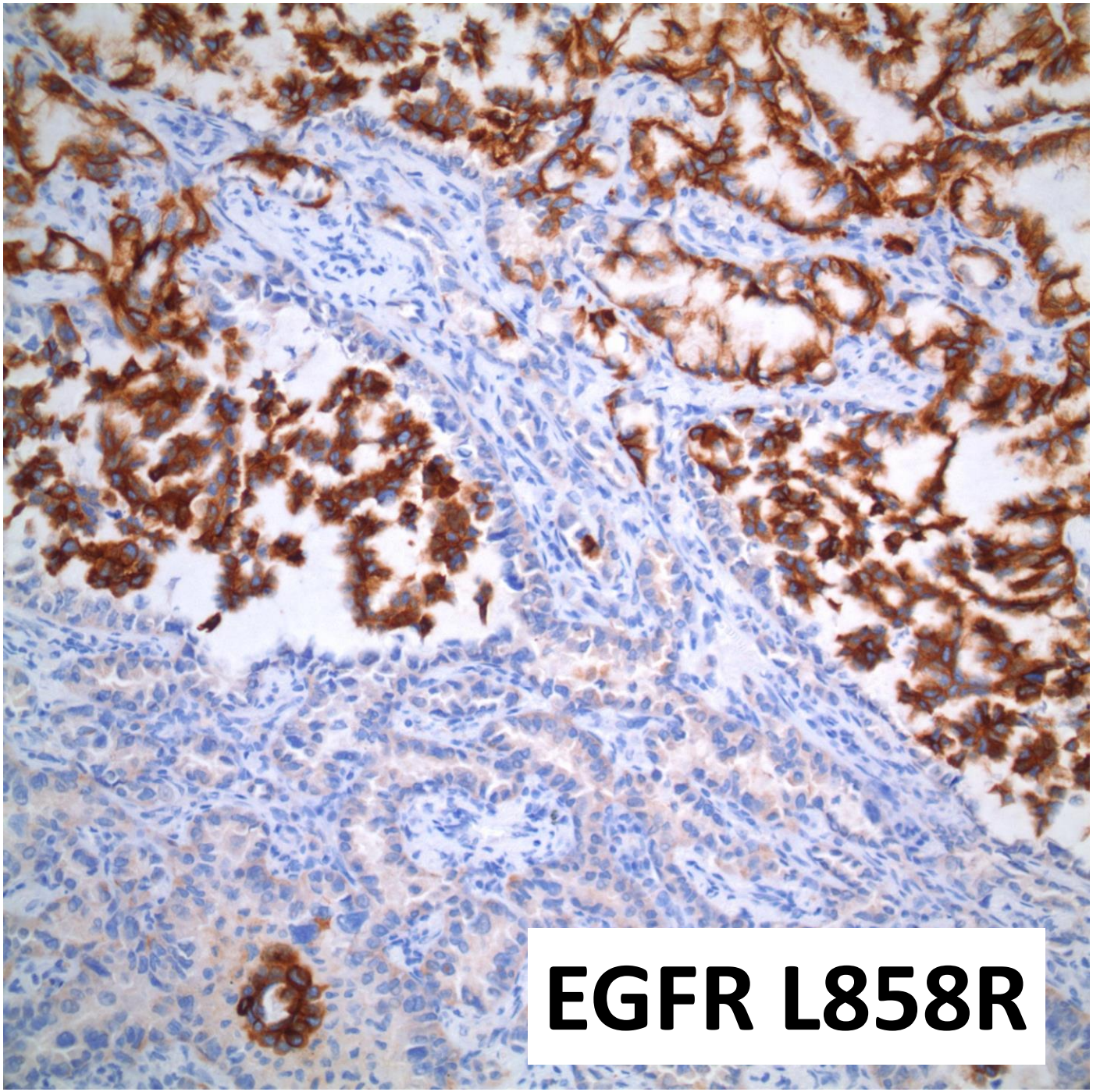
- ***ERBB2***
  L755S, V842I

INTERPRETATION

Gene and Alteration: ERBB2 (also known as HER2) encodes the receptor tyrosine kinase HER2, which is in the same family as EGFR. Amplification or overexpression of ERBB2 can lead to excessive proliferation and tumor formation[1]. ERBB2 L755S has been characterized as activating[2,3,4] and, based on clinical data[5,6](Wagle et al., 2014; ASCO Abstract 536) and preclinical support[4,6,7], may predict resistance to trastuzumab. On the basis of limited clinical evidence (Hoadley et al., AACR Abstract S3-06) and extensive preclinical evidence[2,3,6,7,8], this mutation may also confer resistance to lapatinib. However, preclinical studies suggest that L755S mutations are sensitive to irreversible EGFR/HER2 inhibitors such as afatinib[4,7] and neratinib[4,6,7,8], and patients with breast cancer and ERBB2 L755S achieved complete or partial responses to neratinib[9](; Hyman et al., 2016; AACR Abstract PD5-05). ERBB2 mutations such as also observed here have been shown to be activating[2,4,8,10,11,12,13,14,15,16]. Patients with other ERBB2 activating mutations have had clinical responses to regimens that include ERBB2 targeted therapies, including trastuzumab[17,18,19,20,21], pertuzumab[17,18], lapatinib[18,22,23], afatinib[20,24,25], neratinib[9,26,27], and dacomitinib[28].

Frequency and Prognosis: ERBB2 mutation has been observed in 5.7% (2/35) ovarian clear cell carcinomas (OCCC) (COSMIC, Feb 2017). ERBB2 amplification has been observed in 9.3% of pure OCCC and 3.8% of mixed OCCC[29]. In the scientific literature, ERBB2 amplification has been reported in 7-35% of ovarian carcinomas analyzed, with 19-29% of ovarian tumors reported to harbor HER2 protein


PD-L1


EGFR L858R

Next-gen immunohistochemistry

David L Rimm

The combination of mass spectroscopy with immunohistochemistry allows highly multiplexed, directly quantitative imaging of tissue samples for both basic and clinical research.

The term 'next generation' has most often been applied to DNA sequencing in comparisons of new technologies to Sanger sequencing. The advance from ~400 base pairs per run to millions of base pairs per run and from single-digit coverage to up to ~1,000× coverage represents a technological increase in orders of magnitude. With the reports in this journal[1] and in *Nature Medicine*[2] of mass spectroscopy coupled with imaging, we see the first true glimpse of next-gen immunohistochemistry (IHC). Traditional IHC has allowed assessment of a single protein's expression in a binary manner using chromogens such as DAB[3] or, at rastering laser ablation to vaporize the antibodies (and tissue) at 1-micrometer resolution, followed by detection in a CyTOF instrument that measures the number and mass of metals in each micrometer. Then these are spatially reassigned using sophisticated programming to create a two-dimensional image that can be made to look similar to a routine IHC or QIF image. In contrast, Angelo *et al.*[2] use a rasterizing oxygen duoplasmatron primary ion beam. The beam sweeps the IHC specimen, and lanthanide adducts of the bound antibodies are liberated as secondary ions. Then the secondary ions are analyzed via a magnetic

trates the importance of the two-dimensional information that is lost when tumors are ground up and analyzed by expression profiling or other tissue-disruptive methods.

Angelo *et al.*[2], using the method they call multiplexed ion beam imaging (MIBI) rigorously validate their technology against both mass cytometry and quantitative IHC. They illustrate the production of images that look similar to IHC or QIF images but have quantitative multiplexed information. A critical hurdle for multiplexing is to prove that the multiplexed sample performs identically to the sample in which the reagents are measured one at a time. This was shown for both methods, but Angelo *et al.* also show equivalence of their MIBI method with US Food and Drug Administration–cleared methods currently used in the clinic for measurement of estrogen receptor in breast cancer. Moving toward the same goal as the Bodenmiller group, Angelo *et al.* also illustrate the power of multiplexing when it is combined with spatial information.

As MSIHC is technically similar to con-

Mass spectrometry

From Wikipedia, the free encyclopedia

Mass spectrometry (**MS**) is an analytical technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio. In simpler terms, a mass spectrum measures the masses within a sample. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.

A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds.

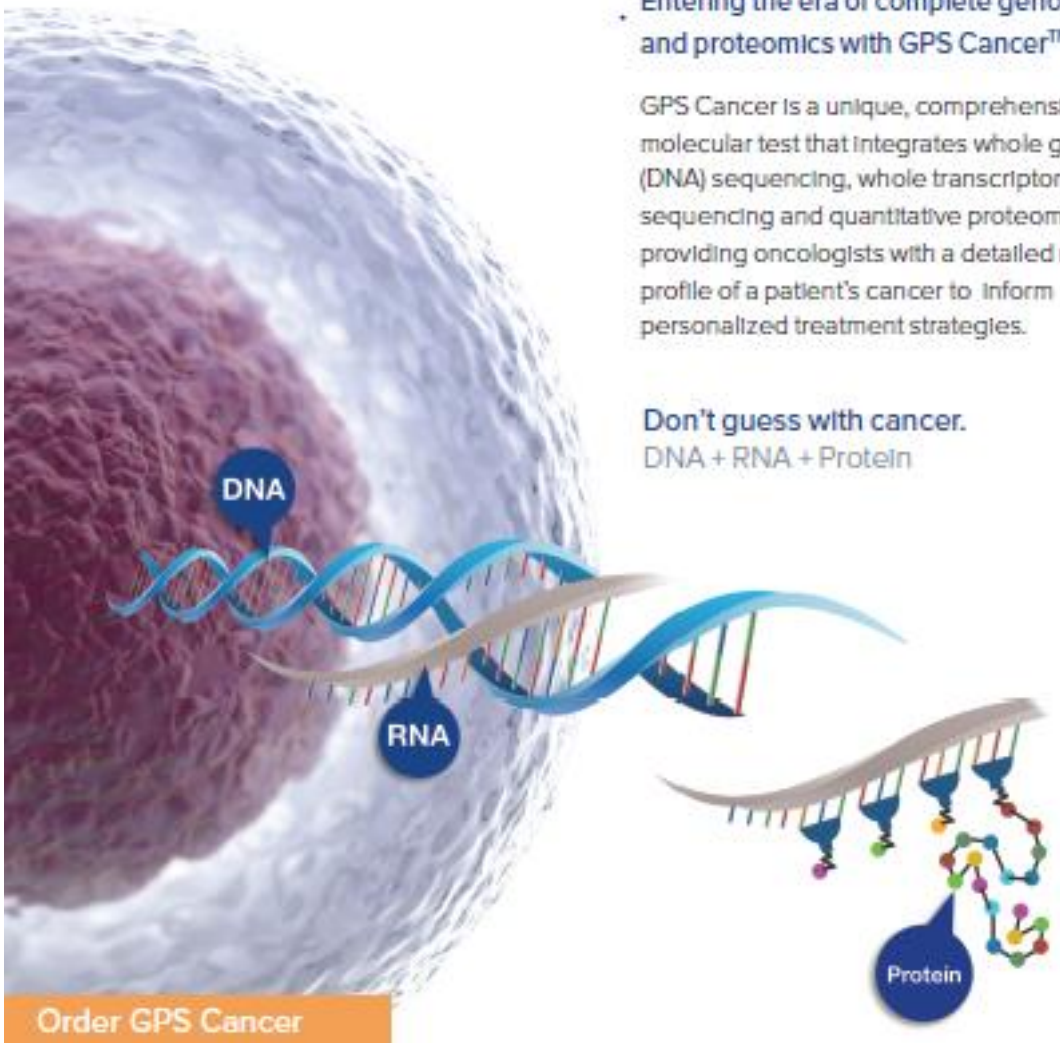
In a typical MS procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons. This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection.[1] The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern.


Thermo
LTQ ORBITRAP XL
FINNIGAN LTQ
Thermo

GPS Cancer

NantHealth

What information do you need to make more informed treatment decisions for your patients?



Entering the era of complete genomics and proteomics with GPS Cancer™

GPS Cancer is a unique, comprehensive molecular test that integrates whole genome (DNA) sequencing, whole transcriptome (RNA) sequencing and quantitative proteomics, providing oncologists with a detailed molecular profile of a patient's cancer to inform personalized treatment strategies.

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DNA + RNA + Protein

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GPS Cancer®

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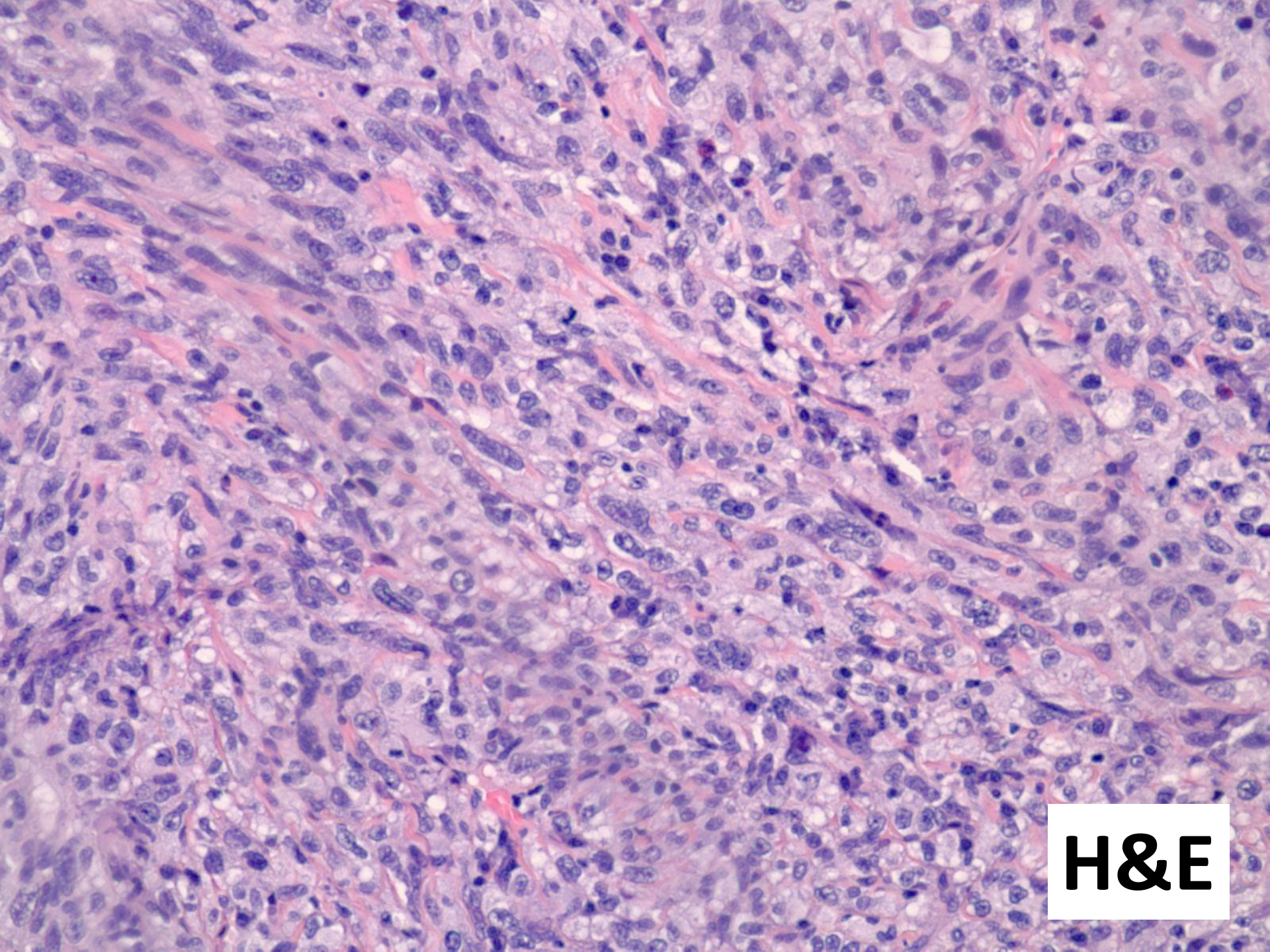
Protein expression biomarkers

Protein expression analysis performed by targeted mass spectrometry

Marker		Description
ALK Protein	★	Expression of mutant ALK protein can drive uncontrolled proliferation of tumor cells. Tumors expressing mutant ALK protein have been shown to positively respond to ALK targeted treatments. Shaw AT, et al. N Engl JMed. 2013 Jun 20; 368(25):2385-94.
AR Protein	▲	Agents that disrupt androgen receptor (AR) binding of androgens are approved for prostate cancer, and many are currently in clinical trials for breast cancer. McGhan LJ, et al. Ann Surg Oncol. 2014 Feb;21(2):361-7.
AXL Protein	■	Targeted inhibition of the AXL kinase shows tumor growth inhibiting activity and AXL specific inhibitors are currently in clinical trials. Linger RMA, et al. Expert Opin Ther Targets.2010; 14(10):1073-1090.
EGFR Protein	★	Tumors overexpressing the EGFR protein are reported to have an improved response to anti-EGFR therapies. Pirker, R et al. Lancet Oncol. 2012; 13: 33-42. Nabholtz JM et al. Ann Oncol. 2014 Aug;25(8):1570-7.
ERCC1 Protein	●	Expression of the ERCC1 protein, which is involved in DNA repair, can cause resistance to platinum-based therapies. Olaussen KA, et al. N Engl J Med. 2006 Sep 7; 355(10):983-991. Bepler G, et al. J Clin Oncol. 2013 Jul 1; 31(19):2404-2412.
FGFR1 Protein FGFR2 Protein FGFR3 Protein FGFR4 Protein	■	The FGFR family is overexpressed in a variety of cancers. Drugs targeting FGFR proteins are currently in clinical trials. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 10:116-29, 2010. Hallinan N, Finn S, Cuffe S, et al. Targeting the fibroblast growth factor receptor family in cancer. Cancer Treat Rev 46:51-62, 2016.
FRα Protein	▲	The folate receptor alpha (FRα) protein plays a critical role in folate uptake. Therefore FRα is a potential biomarker of tumor response to anti-folate chemotherapy. Hartmann, LC et al. Int J Cancer. 2007; 121(5): 938-42. Shia, J et al. Hum Pathol. 2008; 39(4): 498-505.
hENT1 Protein	▲	Nucleoside analogs such as gemcitabine depend on hENT1 for effective delivery into the cell. Multiple studies have shown that expression of hENT1 result in better overall survival in gemcitabine treated patients. Farrell, JJ et al. Gastroenterology. 2009; 136(1): 187-95. Spratlin, JL et al. Cancers (Basel). 2010; 2: 2044-2054.
HER2 Protein	★	Overexpression of the HER2 protein can cause unregulated cell proliferation. There are many approved targeted therapies for HER2. De Laurentiis, M et al. Ann Oncol. 2005 May;16 Suppl 4:iv7-13.

Case 3.

- 58 y.o. Female
- Lung CA
- Lung, RUL, wedge resection and completion lobectomy
- Poorly-differentiated adenocarcinoma, solid pattern; TTF-1(+), Napsin-A(-), CK5(-), p40(-)
- EGFR(-), ALK(-)
- Specimen sent to NantHealth for GPS testing


H&E

Likely Benefit

Treatment Agent	Associated Biomarker	Patient Result (amol/µg)
MET Targeted Clinical Trial	MET Protein	404
Atezolizumab, Nivolumab, Pembrolizumab	PDL1 Protein	212
Gemcitabine	hENT1 Protein	245
Irinotecan, Topotecan	TOPO1 Protein	1745
Carboplatin, Cisplatin, Oxaliplatin	ERCC1 Protein	ND
AXL Targeted Clinical Trial	AXL Protein	242

Unlikely Benefit

Treatment Agent	Associated Biomarker	Patient Result (amol/µg)
Temozolomide	MGMT Protein	347
Docetaxel, *nab*-paclitaxel, Paclitaxel	TUBB3 Protein	2280

GPS Cancer™

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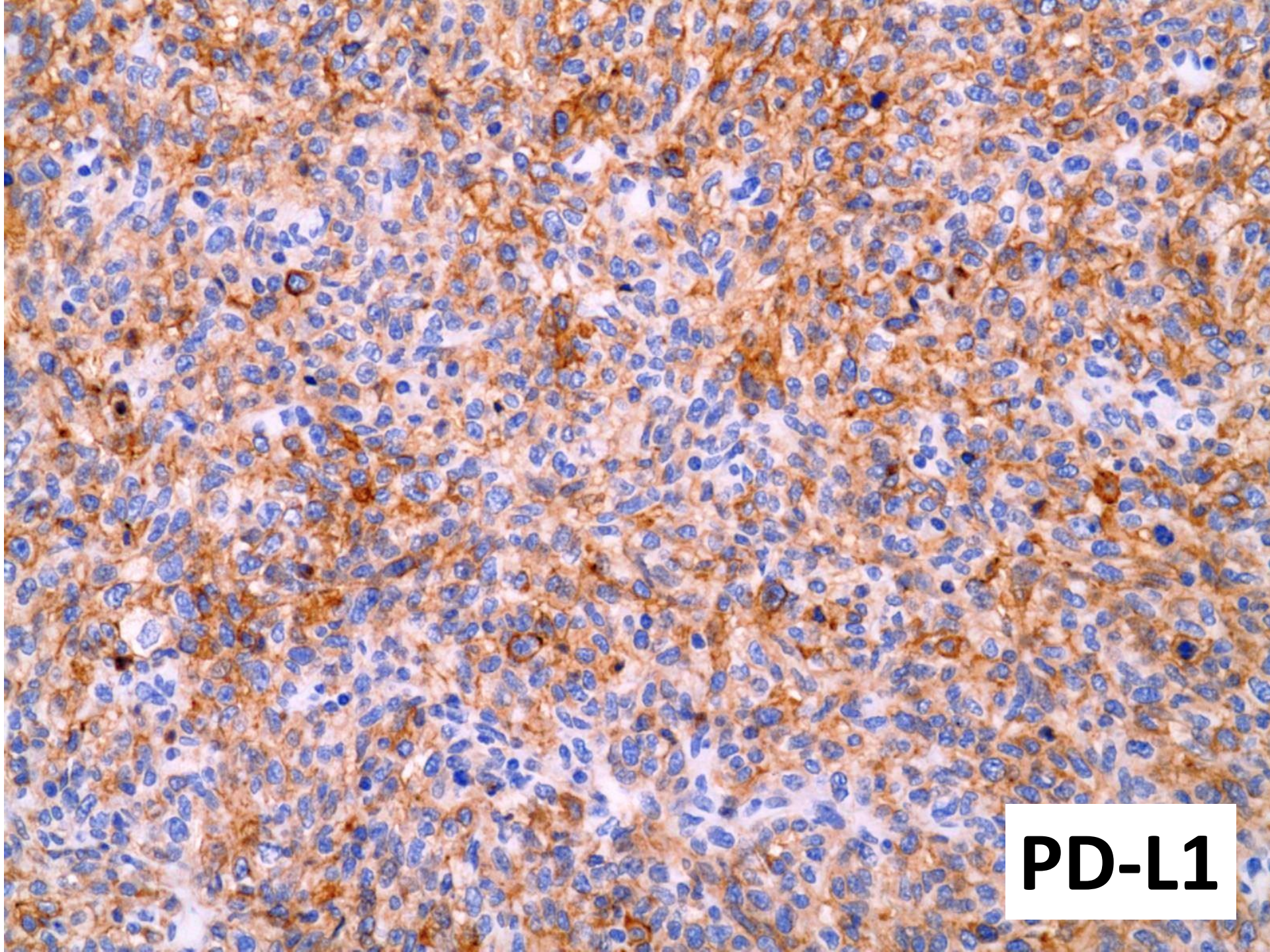
Quantitative Proteomics Report

Patient name:	[redacted]	Requisition #:	2662
Sex:	Female	Date received:	06-Feb-2017
Date of birth:	14-Feb-1960	Date reported:	15-Feb-2017
Medical record #:	Not Provided	Physician:	[redacted]
Incoming specimen ID:	S16-5172-F8	Physician institution:	
Specimen UID:	CG0236	Pathology institution:	Hartford Hospital - Pathology
Specimen source:	Lung, right upper lobe		
Diagnosis code:	C34.11, Malignant neoplasm of upper lobe, right bronchus or lung		

Significant Results:

- ***The expression level of PDL1 protein represents a significant finding.***

Pathology comments:
The specimen was adequate for evaluation and tumor cells were collected using a pathologist-directed laser microdissection system. This is part 1 of the GPS Cancer Report (quantitative proteomic analysis). Part 2 of the report covering genomic analysis is pending.


PD-L1

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Proteomics - Lung Differentiation Markers

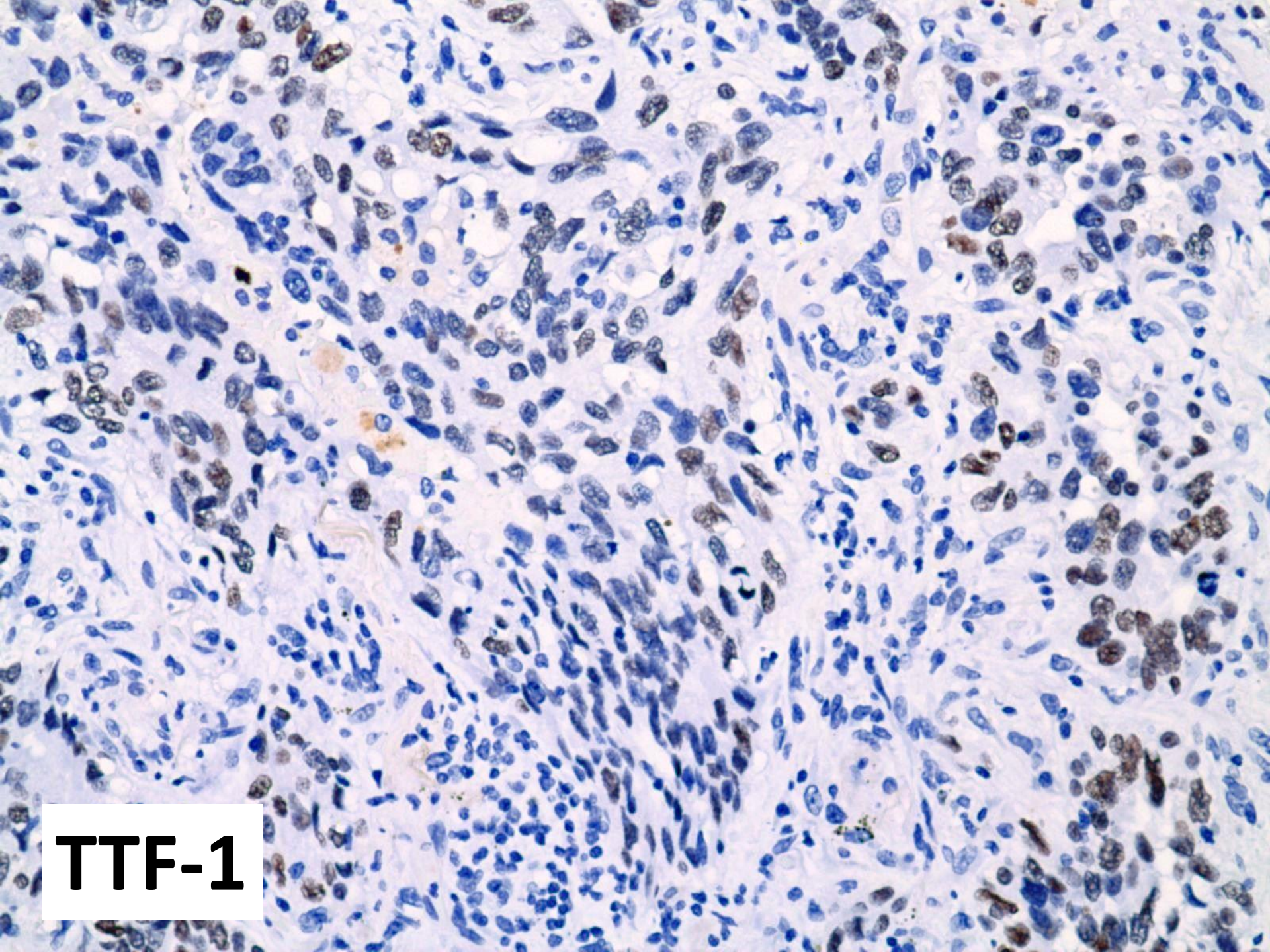
Classification of Origin	Clinical Marker	Patient Results (amol/µg)	Evidence Cutoff (amol/µg)
Adenocarcinoma (ACC)	CK-7	2945	> 14000
	TTF-1	740	> 125
Squamous Cell Carcinoma (SCC)	CK-5	2620	> 15500
	TP63	ND	> 125

Assessment of the Lung Differentiation Protein Markers suggests **the patient's sample is of Adenocarcinoma origin.**

Proteomics - Prognostic Markers

Implication	Clinical Marker	Patient Result (amol/µg)	Evidence Cutoff
Unlikely KRAS Amplification	KRAS	ND	1670[8]

8 - KRAS levels above 1670 amol/µg are correlated with KRAS gene amplification (as determined by FISH analysis) in gastroesophageal cancer and are prognostic of poor outcome (see References section).


TTF-1

The Oncologist®

Lung Cancer

Fluorescence In Situ Hybridization, Immunohistochemistry, and Next-Generation Sequencing for Detection of EML4-ALK Rearrangement in Lung Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. EML4-ALK • Non-small cell lung cancer • Fluorescence in situ hybridization • Immunohistochemistry • Next-generation sequencing

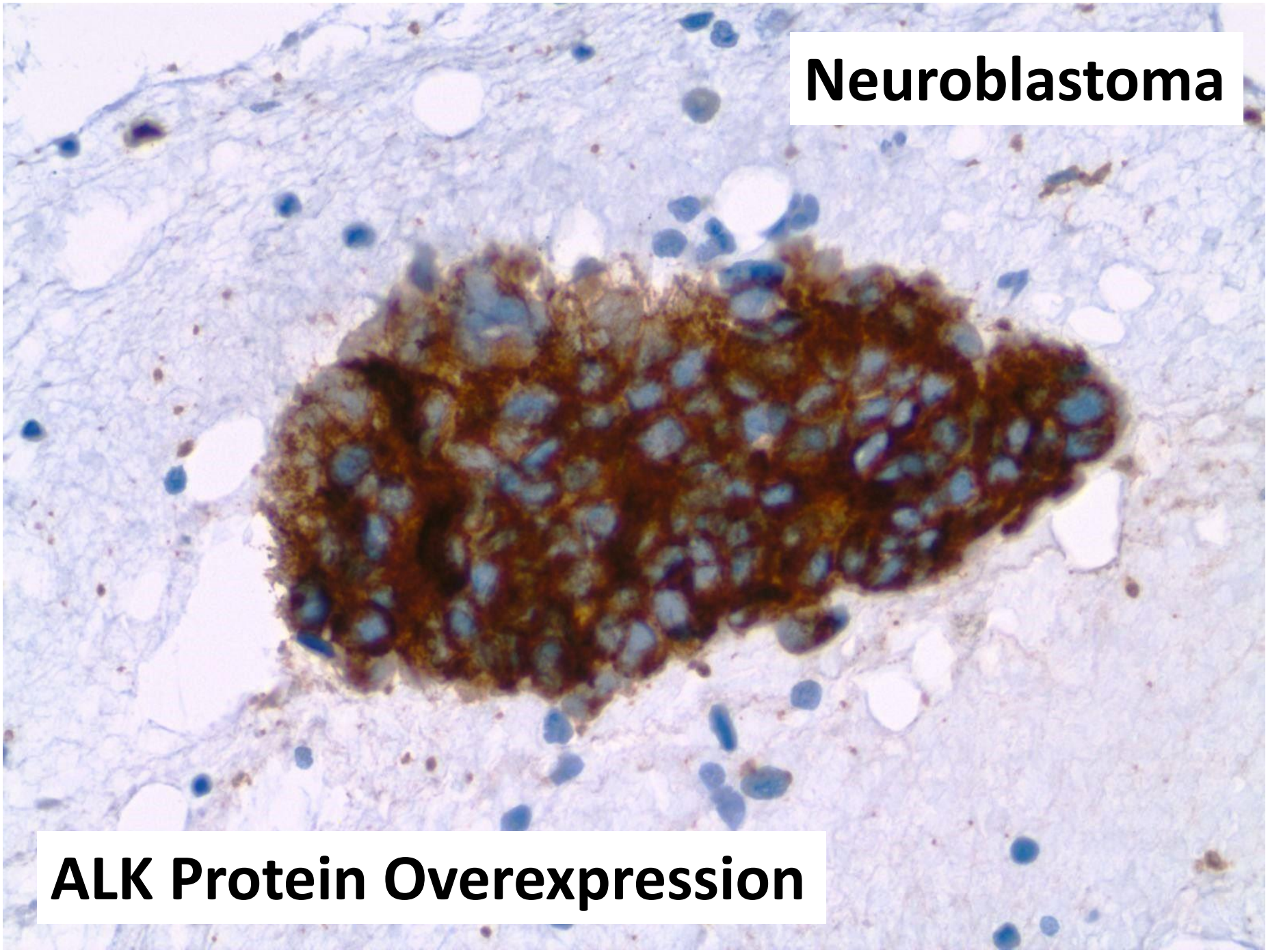
Abstract

Background. The U.S. Food and Drug Administration-approved method for detecting EML4-ALK rearrangement is fluorescence in situ hybridization (FISH); however, data supporting the use of immunohistochemistry (IHC) for that purpose are accumulating. Previous studies that compared FISH and IHC considered FISH the gold standard, but none compared data with the results of next-generation sequencing (NGS) analysis.

Materials and Methods. We studied FISH and IHC (D5F3 antibody) systematically for EML4-ALK rearrangement in 51 lung adenocarcinoma patients, followed by NGS in case of discordance.

Results. Of 51 patients, 4 were positive with FISH (7.8%), and 8 were positive with IHC (15.7%). Three were positive with both. NGS confirmed that four of the five patients who were positive with IHC and negative with FISH were positive for ALK. Two were treated by crizotinib, with progression-free survival of 18 and 6 months. Considering NGS as the most accurate test, the sensitivity and specificity were 42.9% and 97.7%, respectively, for FISH and 100% and 97.7%, respectively, for IHC.

Conclusion. The FISH-based method of detecting EML4-ALK rearrangement in lung cancer may miss a significant number of patients who could benefit from targeted ALK therapy. Screening for EML4-ALK rearrangement by IHC should be strongly considered, and NGS is recommended in borderline cases. Two patients who were negative with FISH and positive with IHC were treated with crizotinib and responded to therapy. ***The Oncologist*** 2015;20:1–7


Neuroblastoma
ALK Protein Overexpression

FISH, ALK PARAFFIN

RESULT: NEGATIVE
ALK GENE RE-ARRANGEMENT WAS NOT DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH), IN THE TUMOR CELLS
nuc ish(ALKx2-3)[100/106]

Results-Comments
FISH Probes: Dual Color ALK Break-apart Probe **ALK (2p23)**
Nuclei Scored: 106

The ALK break-apart probe (Kreatech, Amsterdam, Netherlands) was applied to FFPE sections of the provided specimen. At least 106 nuclei were examined and a signal pattern consistent with ALK gene rearrangement was detected in 6/106 (5.7%) of nuclei examined. This finding is within the normal reference range for this probe (<15%).

The clinical significance of low level ALK gene re-arrangement (<15%) and multiple ALK copy numbers (possibly representing ALK amplification or chromosome 2 polysomy) are not known. ALK re-arrangement in lung carcinoma is associated with specific translocations (e.g. EML4-ALK) and enhanced responsiveness to anti-ALK1 targeted therapies (XALKORI® (crizotinib)).

These results and interpretations are made within the limits of sample collection, methodology and our current knowledge. Results should be correlated by the referring physician with respect to the ongoing clinical situation of the patient.

Background: The anaplastic lymphoma kinase (ALK) gene is implicated in the oncogenesis of both hematopoietic and nonhematopoietic malignancies. The ALK gene is rearranged in ~3-5% of Non-small Cell Lung Cancer (NCSLC) patients. The ALK gene rearrangement is typically caused by either an interstitial deletion or an inversion in the short arm of chromosome 2 (2p) that results in EML4- ALK gene fusion, however, other fusion partners have been described. In NSCLC, detection of ALK gene rearrangement is unlikely in EGFR or KRAS mutant tumors (and vice versa). Higher rates of ALK gene rearrangements were found in non-smoking patients, distinct tumor histology and advanced clinical stage.

Nat Rev Genet. 2013 Jan;14(1):35-48. doi: 10.1038/nrg3356. Epub 2012 Dec 4.

Next-generation proteomics: towards an integrative view of proteome dynamics.

Altelaar AF[1], Munoz J, Heck AJ.

Author information

Abstract

Next-generation sequencing allows the analysis of genomes, including those representing disease states. However, the causes of most disorders are multifactorial, and systems-level approaches, including the analysis of proteomes, are required for a more comprehensive understanding. The proteome is extremely multifaceted owing to splicing and protein modifications, and this is further amplified by the interconnectivity of proteins into complexes and signalling networks that are highly divergent in time and space. Proteome analysis heavily relies on mass spectrometry (MS). MS-based proteomics is starting to mature and to deliver through a combination of developments in instrumentation, sample preparation and computational analysis. Here we describe this emerging next generation of proteomics and highlight recent applications.