

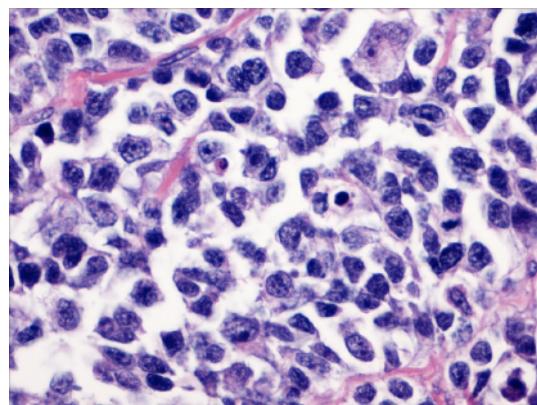
# "Next Generation Immunohistochemistry"

A Window Onto The Molecular Biology of Tumors

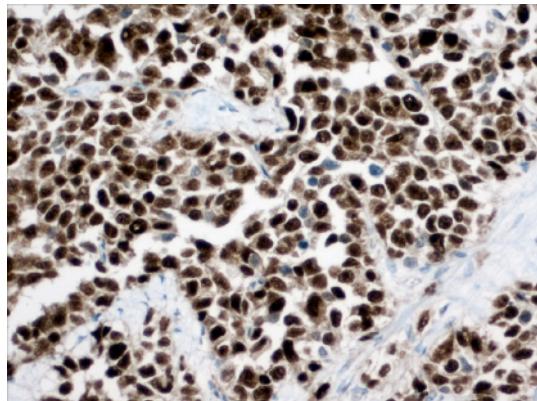
**Allen M. Gown, M.D.**

Medical Director and Chief Pathologist  
PhenoPath Laboratories  
Seattle, Washington  
Clinical Professor of Pathology,  
University of British Columbia

# Hematoxylin & Eosin



## Immuno histo chemistry



## Immunohistochemistry

The application of antibodies with predefined specificities to tissue coupled with the use of detection systems permitting visualization of the target

## Albert Coons

American pathologist and immunologist  
1912-78



THE DEMONSTRATION OF PNEUMOCOCCAL ANTIGEN IN TISSUES BY THE USE OF FLUORESCENT ANTIBODY<sup>1</sup>

ALBERT H. COONS,<sup>2</sup> HUGH J. CREECH, R. NORMAN JONES AND ERNST BERLINER

From the Department of Bacteriology and Immunology, Harvard Medical School and School of Public Health, the Department of Anatomy, Harvard Medical School, Boston, and the Chemical Laboratory, Harvard University, Cambridge, Mass.

Received for publication July 13, 1942

*J. clin. Path.*, 1974, **27**, 14-20

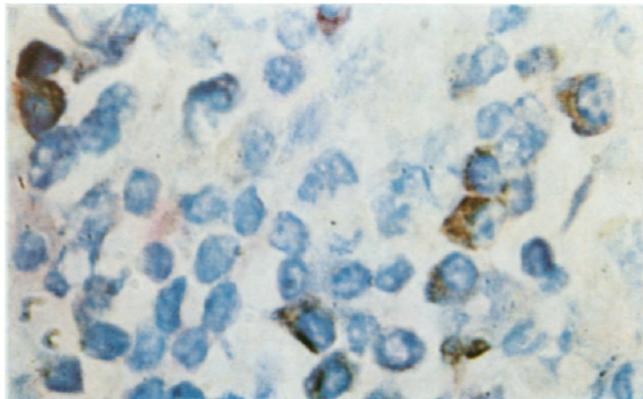
The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase-labelled antibody

C. R. TAYLOR AND J. BURNS

From the Department of Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford

- Routine, paraffin embedded tissues
- Endogenous peroxidase revealed with alpha-naphthol-pyronin (reddish purple)
- Antibody-conjugated peroxidase demonstrated using diaminobenzidine (DAB; brown)

**Rabbit anti-gamma heavy chains followed by swine anti-rabbit conjugated to horseradish peroxidase**



Taylor CR and Burns J., J Clin Pathol 27:14-20, 1974

**Taylor CR and Kledzik G  
Hum Pathol 12:590-6, 1981**

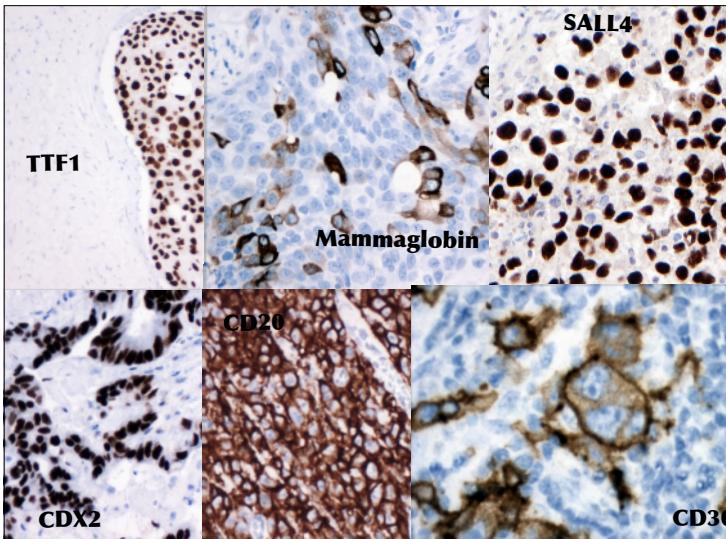
**The application of 'immunostains' provides an independent method of cell identification against which traditional subjective morphologic criteria may be compared: histopathology may thereby be transformed from something of an art to more of a science.**

## **Cell Type Analysis Has Driven IHC Development**

- Immunohistochemistry can identify cell type with greater certainty than H&E-based morphologic patterns
- Most of tumor classification based upon cell type (e.g., squamous cell carcinoma, neuroendocrine carcinoma, acinar cell tumor, etc.)
- Cell (tumor) type is a surrogate for predicting the behavior of tumor

## **Cell Type Analysis Has Driven IHC Development**

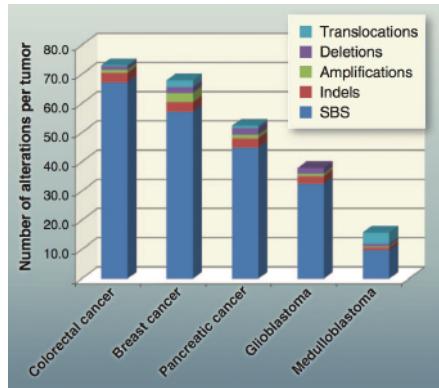
<i>Marker</i>	<i>Normal Tissues</i>	<i>Tumor</i>
TTF-1	Lung epithelium	Lung adenocarcinoma
CDX-2	Colorectal epithelium	Colorectal adenocarcinoma
SALL4	Germ cells	Germ cell tumor
Mammaglobin	Breast epithelium	Breast carcinoma
CD20	B cells	B cell lymphoma
CD30	Activation antigen	Hodgkins lymphoma
Insulin	Beta cells of pancreatic islet	Insulinoma



**Next Generation Immunohistochemistry**  
**PROVIDING A WINDOW ONTO THE MOLECULAR ALTERATIONS UNDERLYING CANCERS AND THUS IDENTIFYING APPROPRIATE THERAPIES**

## Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shabin Zhou, Luis A. Diaz Jr., Kenneth W. Kinzler\*



## Examples of Targeted Therapies for Tumors

Tumor	Gene/Protein	Drug	Companion Test
Breast CA	ER	Tamoxifen	ER IHC
Breast CA	HER2	Trastuzumab	HER2 IHC, HER FISH
GIST	Mutated c-kit	Imatinib	Molecular testing for c-kit mutations
CLL	CD20	Rituximab	CD20 IHC
Various	VEGF	Bevacizumab	NONE
Lung CA	Mutated EGFR	Gefitinib	Molecular testing for EGFR mutations
Lung CA	ALK	Crizotinib	ALK FISH
Melanoma	BRAF V600E	Vemurafenib	Molecular testing for BRAF mutations

## Major Genetic Alterations in Cancer

**Mutation**

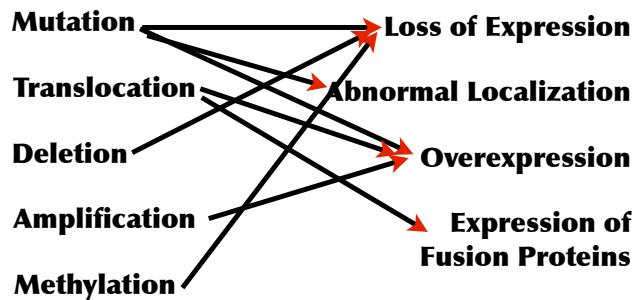
**Translocation**

**Deletion**

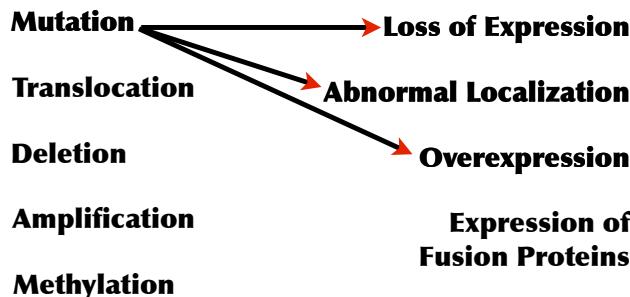
**Amplification**

**Methylation**

## Major Genetic Alterations in Cancer



## Major Genetic Alterations in Cancer



## Identifying Mutations by Immunohistochemistry

- Mutant protein (e.g., BRAF, IDH1, EGFR)
- Abnormal localization (e.g.,  $\beta$ -catenin)
- Abnormal accumulation (e.g., p53)
- Loss of expression (e.g., MMR, INI-1/SMARCB1)

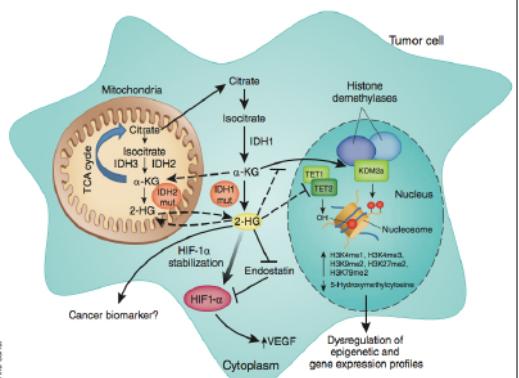
## Detection of Mutant Proteins by Immunohistochemistry

- Isocitrate dehydrogenase (IDH)-1 R132H (histidine replacing arginine) mutation
- BRAF V600E (glutamic acid replacing valine) in melanoma, thyroid, others - target of vemurafenib

- Warburg hypothesis that altered cellular respiration is origin of cancer

- IDH1 is enzyme integral to cell respiration
- Mutation causes decrease in alpha-ketoglutarate (KG) and increase in 2-hydroxyglutarate (2-HG)
- Oncometabolite driven epigenetic aberrations

## IDH1 and Cancer



RESEARCH ARTICLE

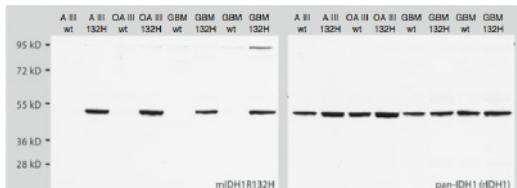
*Brain Pathol* 20:245-54, 2010

### Characterization of R132H Mutation-specific IDH1 Antibody Binding in Brain Tumors

David Capper<sup>1</sup>; Susanne Weißert<sup>1</sup>; Jörg Balss<sup>2</sup>; Antje Habel<sup>1</sup>; Jochen Meyer<sup>2</sup>; Diana Jäger<sup>1</sup>; Ulrike Ackermann<sup>2</sup>; Claudia Tessmer<sup>2</sup>; Andrey Korshunov<sup>2</sup>; Hanswalter Zentgraf<sup>2</sup>; Christian Hartmann<sup>1,2</sup>; Andreas von Deimling<sup>1,2</sup>

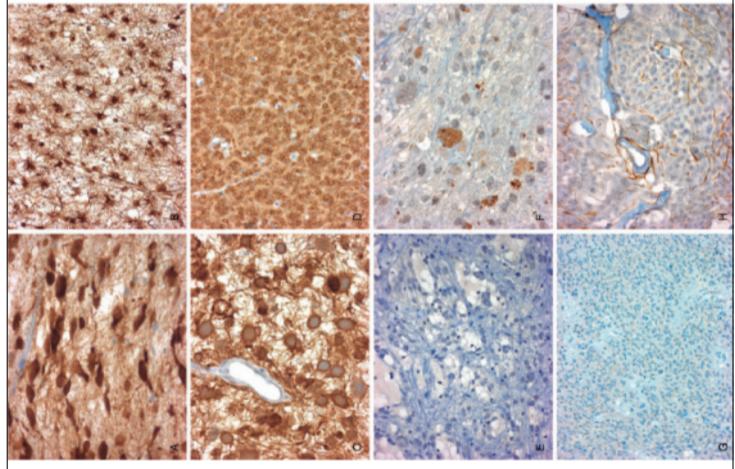
<sup>1</sup> Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, <sup>2</sup> Clinical Cooperation Unit Neuropathology G380, and <sup>3</sup> Monoclonal Antibody Unit, German Cancer Research Center, Heidelberg, Germany.

- Generated to 13 amino acid peptide coupled to KLH (containing R132H)
- R132H mutation constitutes >90% of IDH1 mutations seen in gliomas

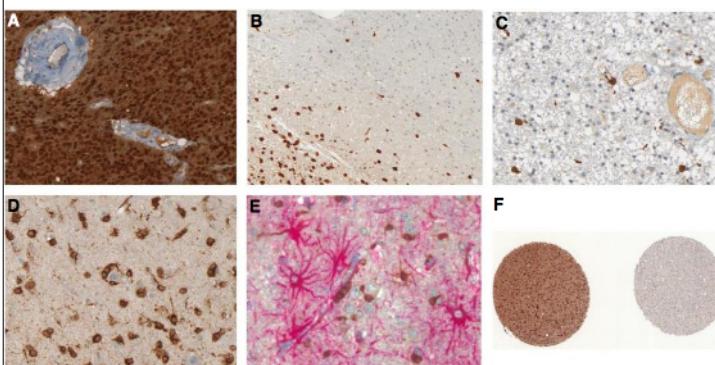


## Mutant Specific IDH1 Antibody

Capper D et al, *Brain Pathol* 20:245-54, 2010



## IDH1 with R132H mutation



- Generated to synthetic peptide
- Does not cross react with wild type IDH1

## IHC v. Sequencing

*Brain Tumor Pathol* 2010; 28:111-123

DOI: 10.1007/s00018-011-0052-7

ORIGINAL ARTICLE

Detection of IDH1 mutation in human gliomas: comparison of immunohistochemistry and sequencing

Shigeo Takata - Wei Tian - Masahide Matsuda - Tetsuya Yamamoto - Eiji Ishikawa - Mika Kato Kaneko - Kentaro Tomizaki - Yukiharu Kato - Akira Matsumoto

### Using IMab-1, identifying R132H mutation

N = 49 Gliomas

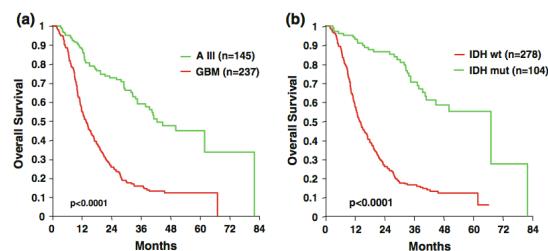
Sequencing
9

IHC
12

**Patients with *IDH1* wild type anaplastic astrocytomas exhibit worse prognosis than *IDH1*-mutated glioblastomas, and *IDH1* mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas**

Christian Hartmann · Bettina Hentschel · Wolfgang Wick · David Capper · Jörg Felsberg ·  
Matthias Simon · Manfred Westphal · Gabriele Schackert · Richard Meyermann ·  
Torsten Pietsch · Guido Reifenberger · Michael Weller · Markus Loeffler · Andreas von Deimling

- IDH status is more prognostic for overall survival than standard histologic criteria
- By sequencing as well as IHC, with IHC identifying cases initially missed by sequencing
- IHC missed two cases positive by sequencing



## Grade v. *IDH1* mutation status

# IDH1 Mutation in Gliomas

## *IDH1* Mutations in Diffusely Infiltrating Astrocytomas

Grade Specificity, Association With Protein Expression, and Clinical Relevance

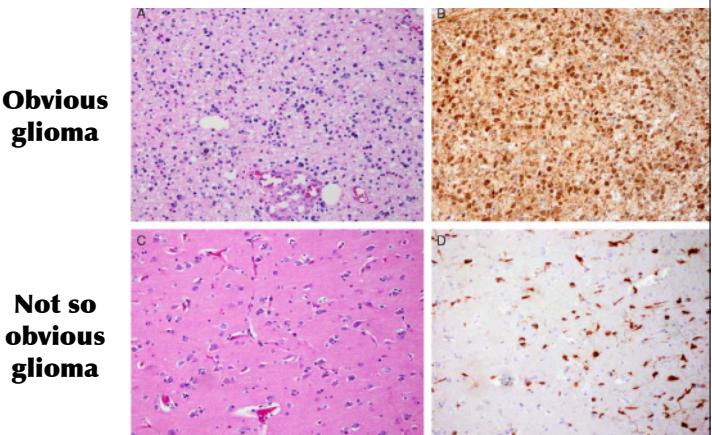
Balaram Thota, MSc,<sup>1</sup> Sudhanshu K. Shukla, MSc,<sup>2</sup> Mallavarapu R. Srividya, MSc,<sup>1</sup> Shivayogi D. Shwetha, MSc,<sup>1</sup> Arimappan Ariwaragan, MS, MCh,<sup>3</sup> Kandavel Thennarasu, PhD,<sup>4</sup> Yasha T. Chickabasaviah, MD,<sup>1</sup> Alangar S. Hegde, MCh, PhD,<sup>5</sup> Bangalore A. Chandramouli, MCh,<sup>3</sup> Kumarel Somasundaram, PhD,<sup>2</sup> and Vani Santosh, MD<sup>1</sup>

	Diffuse Astrocytoma Grade II	Anaplastic Astrocytoma Grade III	Glioblastoma Multiiforme Grade IV
IDH1 Mutant	<b>12</b> <b>(100%)</b>	<b>13</b> <b>(92.9%)</b>	<b>6</b> <b>(12.5%)</b>
IDH1 Wild type	<b>0</b> <b>(0%)</b>	<b>1</b> <b>(7.1%)</b>	<b>42</b> <b>(87.5%)</b>

Am J Clin Pathol 12:38:177-84, 2012

## Gliosis Versus Glioma?: Don't Grade Until You Know

Marie Rivera-Zengotita, MD and Anthony T. Yachnis, MD

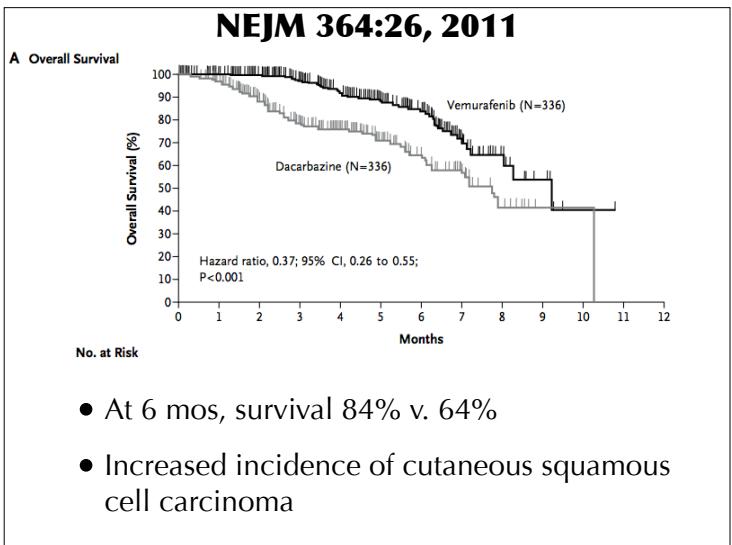


## Chapman PB et al., NEJM 364:26, 2011

### Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation

Paul B. Chapman, M.D., Axel Hauschild, M.D., Caroline Robert, M.D., Ph.D., John B. Haanen, M.D., Paolo Ascierto, M.D., James Larkin, M.D., Reinhard Dummer, M.D., Claus Garbe, M.D., Alessandro Testori, M.D., Michele Maio, M.D., David Hogg, M.D., Paul Lorigan, M.D., Celeste Lebbe, M.D., Thomas Jouary, M.D., Dirk Schadendorf, M.D., Antoni Ribas, M.D., Steven J. O'Day, M.D., Jeffrey A. Sosman, M.D., John M. Kirkwood, M.D., Alexander M.M. Eggermont, M.D., Ph.D., Brigitte Dreno, M.D., Ph.D., Keith Nolop, M.D., Jiang Li, Ph.D., Betty Nelson, M.A., Jeannie Hou, M.D., Richard J. Lee, M.D., Keith T. Flaherty, M.D., and Grant A. McArthur, M.B., B.S., Ph.D., for the BRIM-3 Study Group\*

- 675 patients with previously untreated metastatic melanoma with BRAF V600E mutation



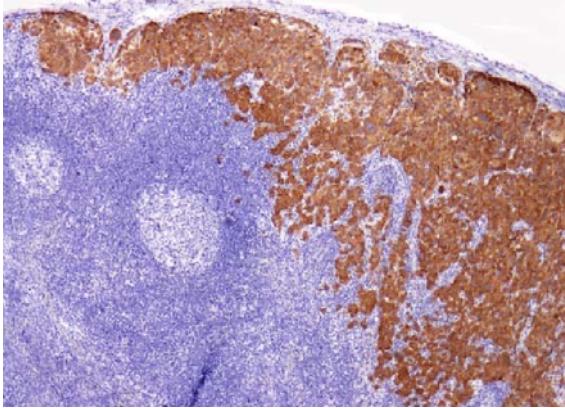
- At 6 mos, survival 84% v. 64%
- Increased incidence of cutaneous squamous cell carcinoma

## Immunohistochemical Analysis of BRAF<sup>V600E</sup> Expression of Primary and Metastatic Melanoma and Comparison With Mutation Status and Melanocyte Differentiation Antigens of Metastatic Lesions

Klaus J. Busam, MD,\* Cyrus Hedvat, MD,\* Melissa Pulitzer, MD,\* Andreas von Deimling, MD,†‡ and Achim A. Jungbluth, MD§

- Monoclonal antibody VE1
- N = 44 metastatic melanomas with known BRAF V600E status
- 0/22 BRAF V600E-negative tumors positive
- 22/22 BRAF V600E-positive tumors positive (16 strongly and homogeneously)

**Busam KJ, Am J Surg Pathol 37:413-20, 2013**



## Diagnostic value of immunohistochemistry for the detection of the BRAF<sup>V600E</sup> mutation in primary lung adenocarcinoma Caucasian patients

M. Ilio<sup>1,2,3,4</sup>, E. Long<sup>1,3</sup>, V. Hofman<sup>1,3,4</sup>, B. Dadon<sup>2</sup>, C. H. Marquette<sup>1,5</sup>, J. Mouroux<sup>1,6</sup>, J. M. Vignaud<sup>7</sup>, H. Begueret<sup>8</sup>, J. P. Merlio<sup>8</sup>, D. Capper<sup>9,10</sup>, A. von Deimling<sup>9,10</sup>, J. F. Emile<sup>11,12</sup> & P. Hofman<sup>1,2,3,4</sup>

<sup>1</sup>Inserm U1081/CNRS UMR7294, Team 3, University of Nice Sophia Antipolis, Institute for Research on Cancer and Aging in Nice (IRCAN), Nice, France; <sup>2</sup>Hôpital Sainte-Présentine; <sup>3</sup>Laboratory of Clinical and Experimental Pathology, Pasteur Hospital, Nice; <sup>4</sup>Cancer Research Association (ARC) Laboratory Team, Villefranche Department of Pathology; <sup>5</sup>Pneumology, Hôpital Sainte-Présentine, Nice; <sup>6</sup>Laboratory of Pathology, Centre Hospitalier, Nancy; <sup>7</sup>Laboratory of Pathology, Haut-Lévêque Hospital, Pessac, France; <sup>8</sup>Department of Neurosurgery, Ruprecht-Karls-University Heidelberg, Heidelberg; <sup>9</sup>Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg; <sup>10</sup>Department of Pathology, Andreieff Hospital, Paris; <sup>11</sup>G4A, Université de Versailles Sartirouges, France

**Ann Oncol**  
24:742-8,  
2013

## Detection of the BRAF<sup>V600E</sup> mutation in serous ovarian tumors: a comparative analysis of immunohistochemistry with a mutation-specific monoclonal antibody and allele-specific PCR

Hans Bösmüller MD<sup>a,b,\*</sup>, Anna Fischer<sup>b</sup>, Deborah L. Pham<sup>b</sup>, Tanja Fehm<sup>c</sup>, David Capper<sup>d,e</sup>, Andreas von Deimling<sup>d,e</sup>, Irina Bonzheim<sup>b</sup>, Annette Staebler<sup>b,1</sup>, Falko Fend<sup>b,1</sup>

<sup>a</sup>Department of Pathology, Krankenhaus Barnherzige Schwestern Linz, Austria  
<sup>b</sup>Institute of Pathology, Tübingen, Germany  
<sup>c</sup>University Women's Hospital, University Hospital Tübingen, Eberhard Karls-University Tübingen, Tübingen, Germany  
<sup>d</sup>Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany  
<sup>e</sup>Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany

**Am J Surg Pathol**  
36:844-50,  
2012

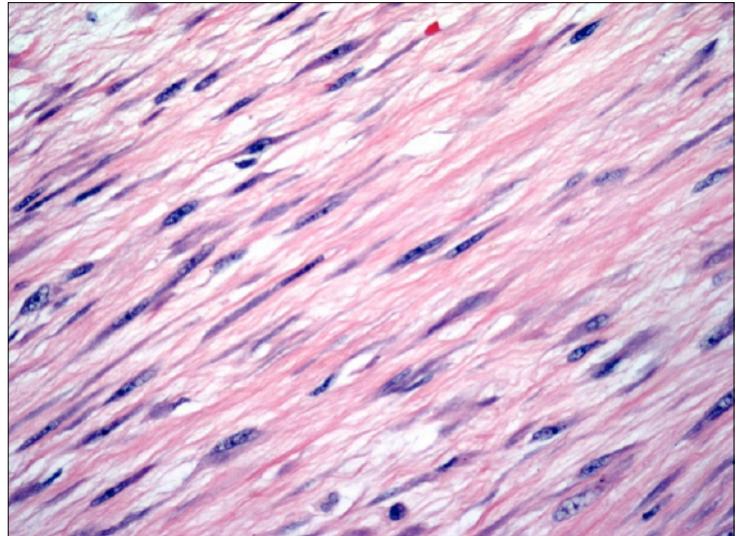
## Immunohistochemical Detection of the BRAF V600E-mutated Protein in Papillary Thyroid Carcinoma

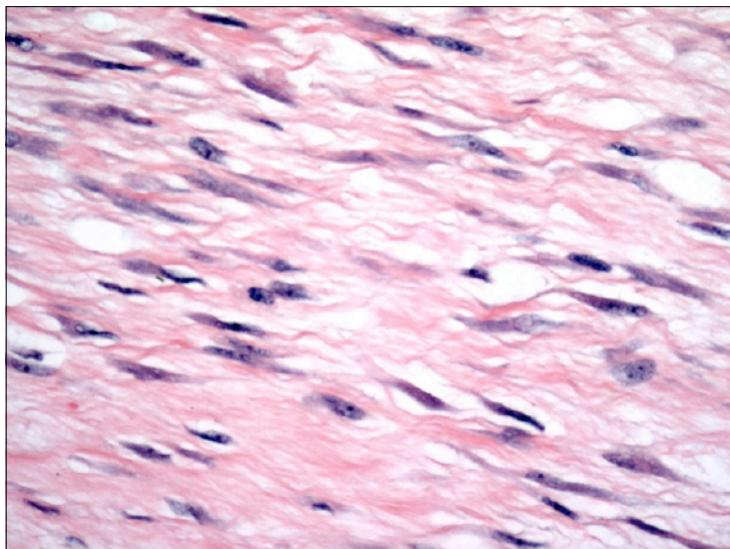
Oskar Koperek, MD,\* Christoph Kornauth, MD,\* David Capper, MD,†‡  
Anna Sophie Berghoff, MD,§ Reza Asari, MD,|| Bruno Niederle, MD,||  
Andreas von Deimling, MD,†‡ Peter Birner, MD, MSc,\* and Matthias Preusser, MD§

## Identifying Mutations by Immunohistochemistry

- Mutant protein (e.g., BRAF, IDH1, EGFR)
- Abnormal localization (e.g.,  $\beta$ -catenin)
- Abnormal accumulation (e.g., p53)
- Loss of expression (e.g., MMR, INI-1/ SMARCB1)

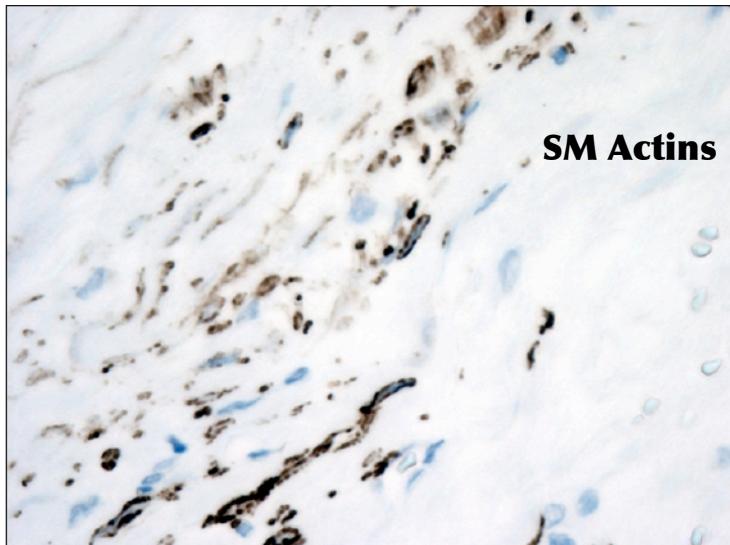
**49 year old male presents with 5 cm mesenteric mass 2 years after partial gastrectomy for GIST**





## DIFFERENTIAL DIAGNOSES

- Recurrent gastrointestinal stromal tumor
- Other (myofibroblastic process? desmoid?)



**S100**



**Cytokeratin** **Negative**

**CD34** **Negative**

**CD117** **Negative**

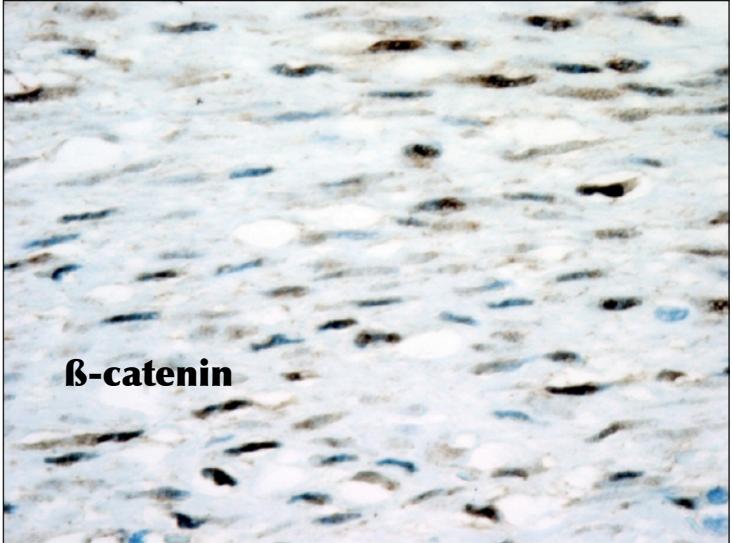
**SM Actins** **Positive**

**Desmin** **Negative**

**S100** **Negative**

**$\beta$ -catenin** **Nuclear**

**$\beta$ -catenin**



## Mesenteric Fibromatosis

- Aggressive fibromatosis, desmoid tumor
- All ages
- Associated with Gardner syndrome
- Abdominal and extra-abdominal (shoulder, chest wall, back)
- Deep-seated, poorly circumscribed
- Most present with asymptomatic abdominal mass

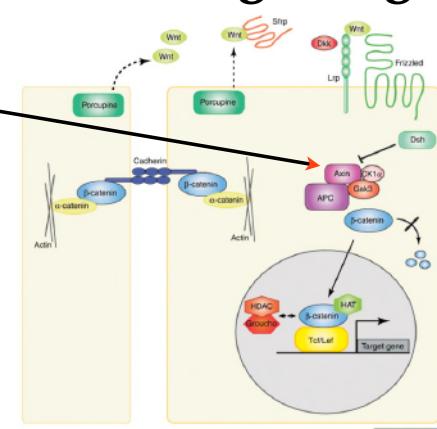
## Mesenteric Fibromatosis

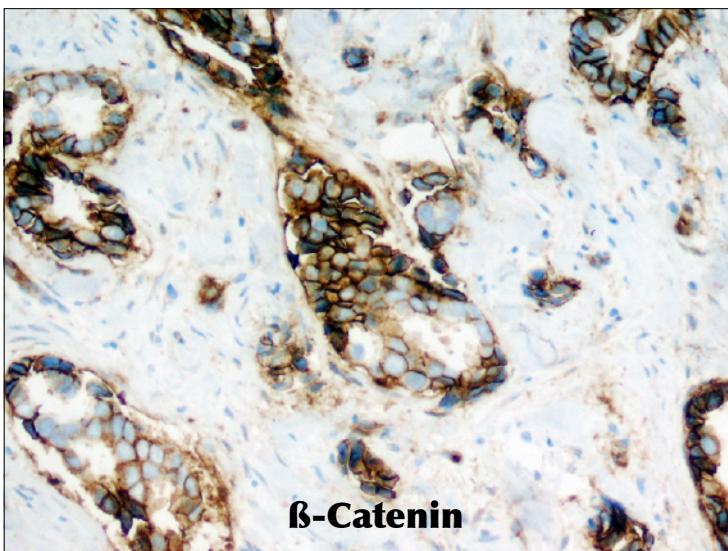
- Elongated, slender spindle shaped cells of uniform appearance
- Varily prominent vessels, some perivascular edema
- Cells usually arranged in sweeping bundles
- Collagen hyalinization and myxoid change variably present

## $\beta$ -Catenin: Role in Cell Adhesion and Signaling

Axin and APC are negative regulators of Wnt signalling cascade. Axin and APC phosphorylate  $\beta$ -catenin on APC binding sites, thereby degrading and inactivating the protein.

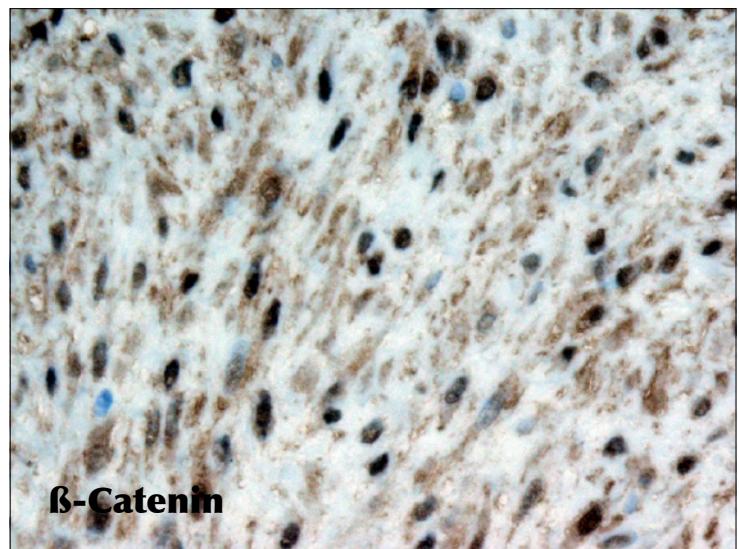
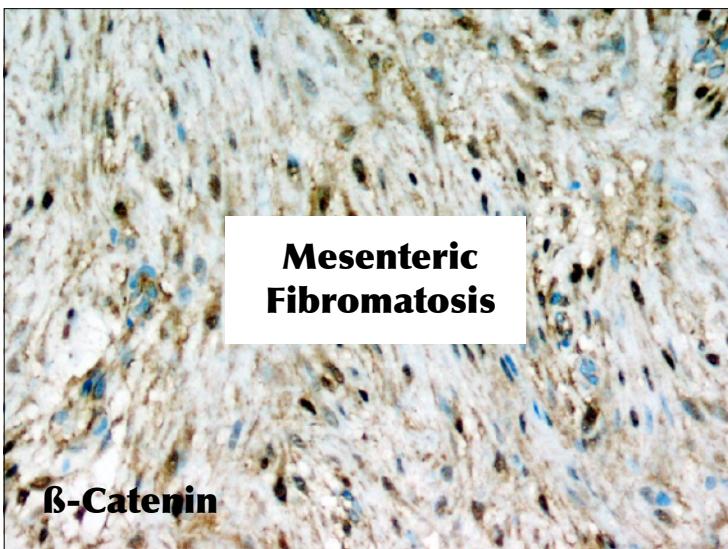
Regulation of  $\beta$ -catenin critical to APC's tumor suppressor effect





## β-Catenin and Fibromatoses

- Montgomery et al, AJSP, 2002
- Fibromatoses have mutation in APC/ β-catenin pathway
- Abnormal nuclear accumulation of β-catenin protein
- Studied expression by IHC in mesenteric fibromatosis, GIST, and sclerosing mesenteritis



## Is Nuclear β-catenin Expression Found in Other Tumors?

Nuclear beta-catenin in mesenchymal tumors

Tony L Ng<sup>1</sup>, Allen M Gown<sup>2</sup>, Todd S Barry<sup>2</sup>, Maggie CU Cheang<sup>1</sup>, Andy KW Chan<sup>1</sup>, Dmitry A Turbin<sup>1</sup>, Forrest D Hsu<sup>1</sup>, Robert B West<sup>3</sup> and Torsten O Nielsen<sup>1</sup>

<sup>1</sup>Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, British Columbia, Canada;

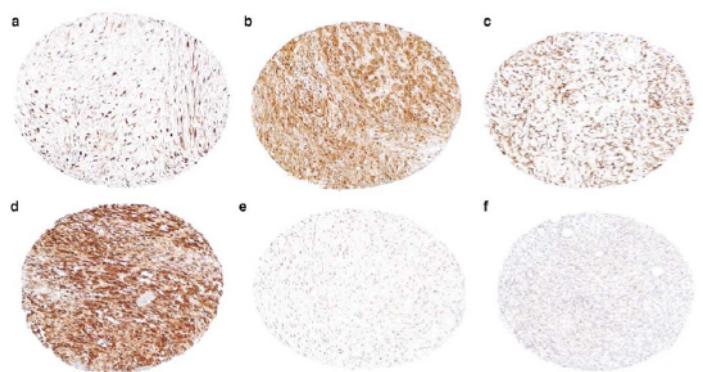
<sup>2</sup>PhenoPath Laboratories, Seattle, Washington, USA and <sup>3</sup>Department of Pathology, Stanford University Medical Center, Stanford, CA, USA

Modern Pathology 18:68-74, 2005

**Ng TL et al., Modern Pathology 18:68-74, 2005**

- Tissue microarray based study
- N = 549
- 4 separate microarrays
- Wide spectrum of soft tissue tumors represented

**Ng TL et al., Modern Pathology 18:68-74, 2005**



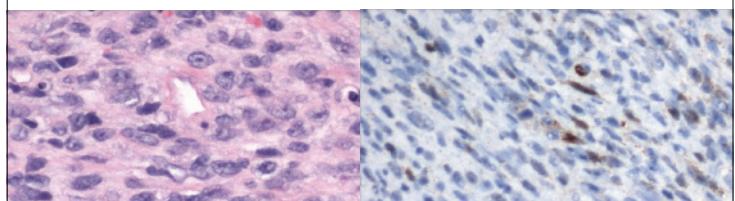
**Ng TL et al., Modern Pathology 18:68-74, 2005**

### **Tumors POSITIVE for high level nuclear β-catenin expression**

- Desmoid type fibromatosis (71%)
- Solitary fibrous tumor (40%)
- Endometrial stromal sarcoma (40%)
- Synovial sarcoma (28%)

### **Why does synovial sarcoma have nuclear β-catenin?**

- 'Downstream' effect of t(X;18)(p11.2;q11.2) translocation
- SYT-SSX2 fusion protein plays direct role in recruiting β-catenin to nucleus



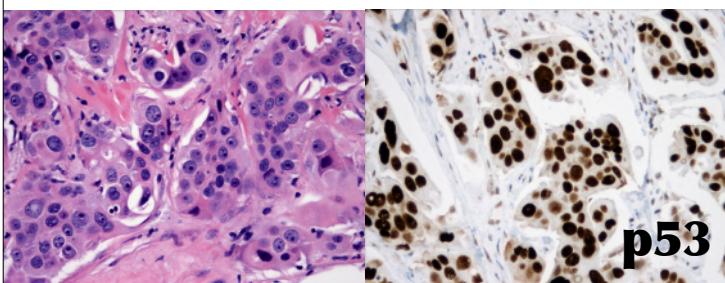
### **Abnormal localization of β-catenin to nucleus**

- May be mutation of β-catenin or adenoma polyposis coli (APC) genes
- APC mutations more common in setting of familial adenomatous polyposis
- β-catenin mutations more common in sporadic aggressive fibromatosis
- **Demonstrates that mutation of one gene may result in abnormal localization of another gene product**

### **Identifying Mutations by Immunohistochemistry**

- Mutant protein (e.g., BRAF, IDH1)
- Abnormal localization (e.g., β-catenin)
- **Abnormal accumulation (e.g., p53)**
- Loss of expression (e.g., MMR, INI-1/SMARCB1)

# Identifying Mutated p53 in Human Tumors



## p53 Immunohistochemistry

- Rapid
- Inexpensive
- Widely available
- Surrogate marker for mutational p53 status?

J Pathol 222:191-8, 2010

### The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas

Martin Köbel,<sup>1</sup> Alexander Reuss,<sup>2</sup> Andreas du Bois,<sup>3</sup> Stefan Kommoß,<sup>3</sup> Friedrich Kommoß,<sup>3</sup> Dongxia Gao,<sup>4</sup> Steve E Kalloger,<sup>5</sup> David G Huntsman<sup>6</sup> and C Blake Gilks<sup>1\*</sup>

<sup>1</sup> Department of Pathology and Laboratory Medicine, Calgary Laboratory Services/Alberta Health Services and University of Calgary, Canada

<sup>2</sup> Coordinating Center for Clinical Trials (KKS), University Marburg (AGO-OVAR Statistical Centre), Germany

<sup>3</sup> Arbeitsgemeinschaft Gynäkologische Onkologie Studiengruppe (AGO-OVAR), Germany

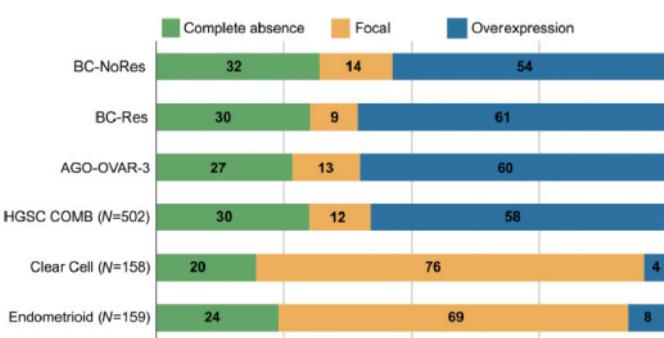
<sup>4</sup> Genetic Pathology Evaluation Centre of the Prostate Research Centre, Department of Pathology, Vancouver General Hospital and British Columbia Cancer Agency, Vancouver, BC, Canada

- DO-7 anti-p53 monoclonal antibody (cross reacts with wild type and mutant)
- Scored in three bins: complete absence of expression, focal expression, overexpression (>50%)
- Outcome in two different cohorts

## p53 and Ovarian Cancer

- Missense mutations would be predicted to correlate with nuclear overexpression
- Approximately one-third of TP53 mutations are null (nonsense, frameshift, splice site mutations) probably resulting in complete absence of protein expression
- Deletions would also predict to result in complete absence of protein expression
- Might expect three immunostaining patterns

## p53 and Ovarian Cancer



Kobel M et al., J Pathol 222:191-8, 2010

## p53 and Ovarian Cancer

Kobel M et al., J Pathol 222:191-8, 2010

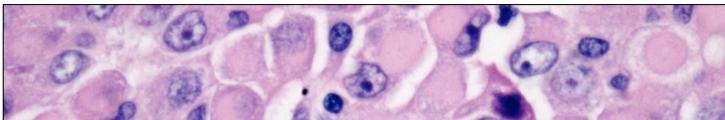
- Pelvic high grade serous ovarian cancers show either complete loss or overexpression in 88% of cases
- p53 overexpression associated with reduced risk of recurrence
- Complete absence of expression associated with unfavorable outcome
- **Suggests functional differences underlying overexpression v. absence of expression**

## Identifying Mutations by Immunohistochemistry

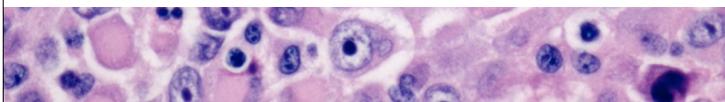
- Mutant protein (e.g., BRAF, IDH1)
- Abnormal localization (e.g.,  $\beta$ -catenin)
- Abnormal accumulation (e.g., p53)
- Loss of expression (e.g., MMR, INI-1/SMARCB1)

## Examples of Mutations Leading to Loss of Protein Expression

INI-1/SMARCB1	Rhabdoid tumors (and others)	
Mismatch Repair (MLH1, MSH2, MSH6, PMS2)	Colorectal adenocarcinoma	
E-cadherin	Lobular breast cancer	
Succinic dehydrogenase	Subset of gastrointestinal stromal tumors	
PTEN	Endometrial, breast cancer	



## Rhabdoid Tumors and Loss of INI1/SMARCB1 Gene Expression

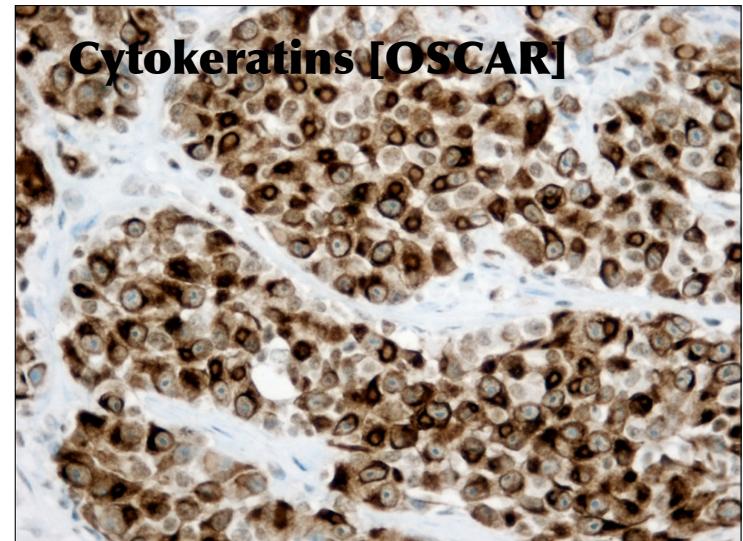


## Rhabdoid Tumor

- Controversial entity since description as variant of Wilms tumor by Beckwith and Palmer (1978)
- Most frequent in kidney of infants and children
- Has been described as primary tumor in liver, soft tissue, mediastinum, pancreas, GI tract, uterus, skin, bladder, etc.
- In CNS, called atypical teratoid/rhabdoid tumor

## Rhabdoid Tumor

- Usually show cytokeratin and vimentin co-expression (to inclusions)
- No other consistent findings
- Descriptions of focal expression of synaptophysin, neurofilament, desmin, muscle actins, S-100, CD57, CD99, WT-1



## INI1/SMARCB1 and Rhabdoid Tumors

- Deletions of 22q11.22
- Loss of expression of INI1 (hSNF5, SMARCB1, BAF47), candidate tumor suppressor gene
- CNS tumors have high frequency of monosomy 22
- Extra-renal tumors have high incidence of homozygous deletion
- 20% of tumors show no (apparent) alterations in INI1

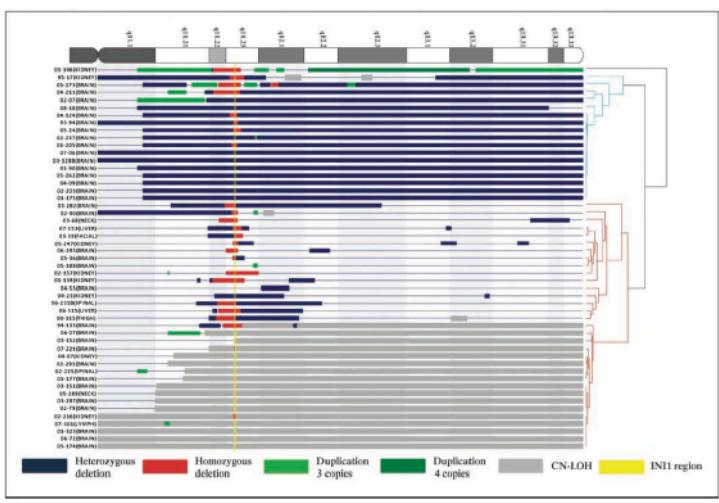
Clin Cancer Res 15:1923-30, 2009

**Genomic Analysis Using High-Density Single Nucleotide Polymorphism-Based Oligonucleotide Arrays and Multiplex Ligation-Dependent Probe Amplification Provides a Comprehensive Analysis of *INI1/SMARCB1* in Malignant Rhabdoid Tumors**

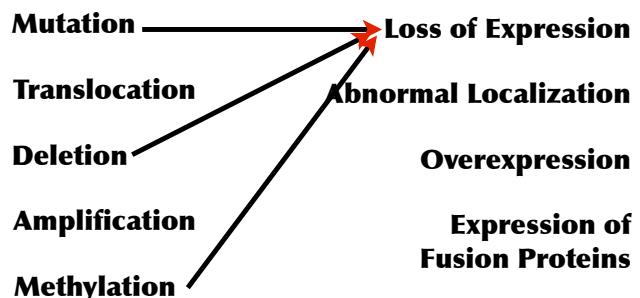
Eric M. Jackson,<sup>1</sup> Angela J. Sievert,<sup>2,3</sup> Xiaowu Gai,<sup>5</sup> Hakon Hakonarson,<sup>2,4</sup> Alexander R. Judkins,<sup>6</sup> Laura Tooke,<sup>4</sup> Juan Carlos Perin,<sup>5</sup> Hongbo Xie,<sup>5</sup> Tamim H. Shaikh,<sup>2,4</sup> and Jaclyn A. Biegel<sup>2,4</sup>

- 51 rhabdoid tumors
- INI1 (SMARCB1) inactivation shown to be via variety of mechanisms, including deletions, mutations, and loss of heterozygosity
- Two (both) inactivating events identified in virtually all

Clin Cancer Res 15:1923-30, 2009



## Major Genetic Alterations in Cancer



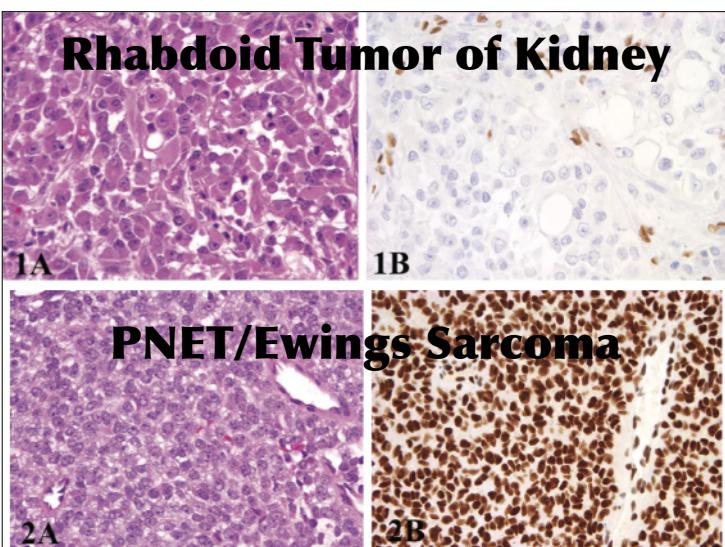
Am J Surg Pathol 28:1485-91, 2004

Immunohistochemical Analysis of hSNF5/INI1 Distinguishes Renal and Extra-renal Malignant Rhabdoid Tumors From Other Pediatric Soft Tissue Tumors

Andrew C. Hoot, MD,\* Pierre Russo, MD,\* Alexander R. Judkins, MD,\* Elizabeth J. Perlman, MD,† and Jaclyn A. Biegel, PhD†

- 27 Rhabdoid tumors with molecular analysis
- 17 Rhabdoid tumors without molecular analysis
- Examined INI1 protein by IHC and compared with PNET/ES, DSRCT, ARMS, ES

## Rhabdoid Tumor of Kidney



# Conclusions

- All rhabdoid tumors showed INI1/SMARCB1 loss by IHC, regardless of mechanism (deletion, mutation)
- Those rhabdoid tumors showing loss of INI1/SMARCB1 loss but no mutation or deletion may have other pathway(s) leading to loss of expression
- INI1/SMARCB1 immunostaining integrates the molecular changes in rhabdoid tumors and is the method of choice of identifying these tumors and distinguishing them from other tumors

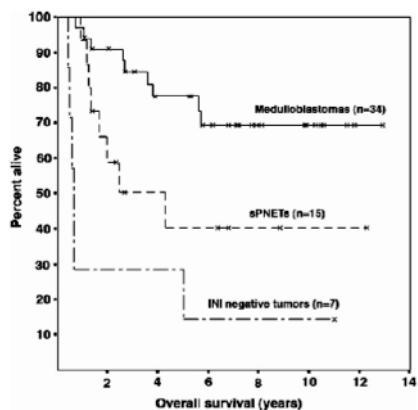
**Am J Surg Pathol 30:1462-8, 2006**

Immunohistochemical Analysis of INI1 Protein in Malignant Pediatric CNS Tumors: Lack of INI1 in Atypical Teratoid/Rhabdoid Tumors and in a Fraction of Primitive Neuroectodermal Tumors without Rhabdoid Phenotype

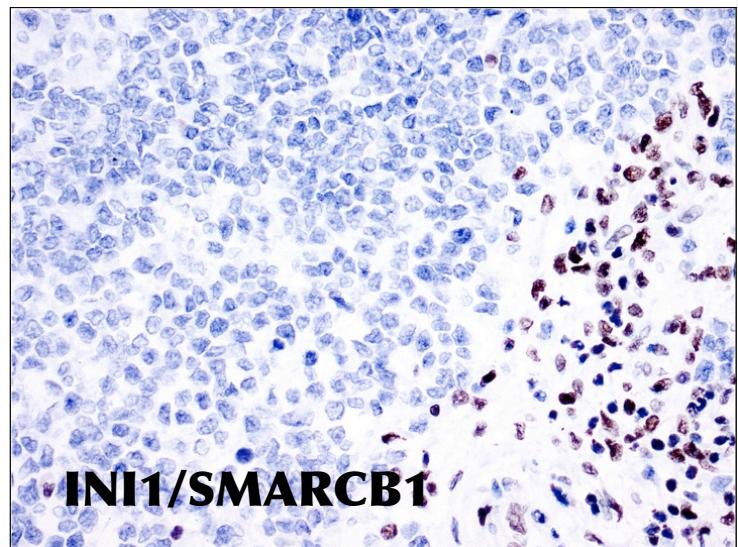
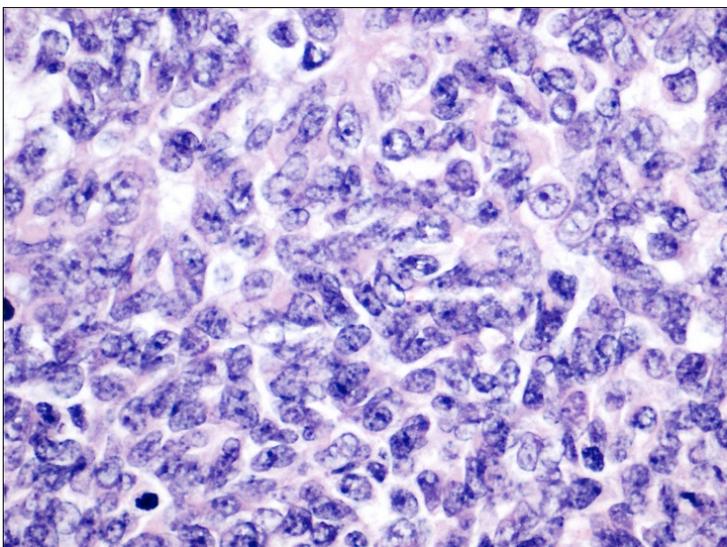
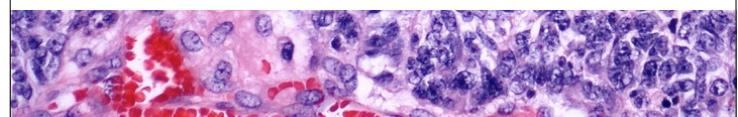
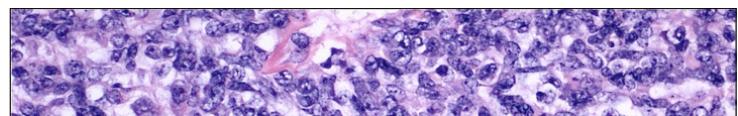
Christine Haberler, MD,\* Ute Laggner, MD,\* Irene Slavc, MD,† Thomas Czech, MD,‡  
Inge M. Ambros, MD,§ Peter F. Ambros, PhD,§ Herbert Budka, MD,\*  
and Johannes A. Hainfellner, MD\*

- 289 malignant pediatric CNS tumors
- IHC documented loss of INI1 in all cases of AT/RT
- Some medulloblastomas and sPNETs showed loss of INI1

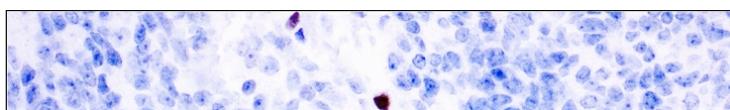
**Haberler C et al., Am J Surg Pathol  
30:1462-8, 2006**



**Posterior Fossa Tumor  
4 year old female**



**INI1/SMARCB1**



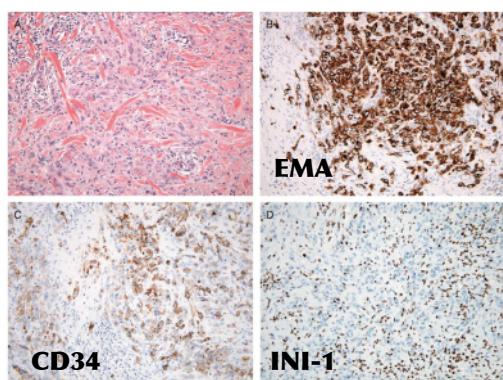
## Atypical Teratoid/ Rhabdoid Tumor, Confirmed by INI1 Loss



**Am J Surg Pathol 33:542-50, 2009**

Loss of INI1 Expression is Characteristic of Both Conventional and Proximal-type Epithelioid Sarcoma

Jason L. Hornick, MD, PhD, Paola Dal Cin, PhD, and Christopher D.M. Fletcher, MD, FRCPath



## INI1/SMARCB1 and Pediatric CNS Tumors

- IHC preferred to FISH, microsatellite analysis, and mutational analysis
- IHC for INI1 protein should be performed on all embryonal pediatric CNS tumors

**Mod Pathol 21:647-52, 2008**

**Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior**

Jason X Cheng, Maria Tretiakova, Can Gong, Saptarshi Mandal, Thomas Krausz and Jerome B Taxy

Department of Pathology, University of Chicago, Chicago, IL, USA

- Rare, highly aggressive primary renal tumor
- Typically affects young patients with sickle cell trait or disease
- Rhabdoid features in some cases
- Relationship to renal rhabdoid tumors?

**Am J Surg Pathol 32:1168-74, 2008**

SMARCB1/INI1 Protein Expression in Round Cell Soft Tissue Sarcomas Associated With Chromosomal Translocations Involving EWS: A Special Reference to SMARCB1/INI1 Negative Variant Extraskeletal Myxoid Chondrosarcoma

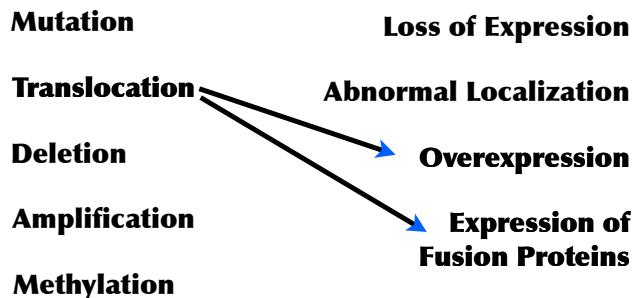
Kenichi Kohashi, <sup>1</sup>TABLE 1. BAF47 Expression in Sarcomas Associated With Chromosomal Translocations Involving EWS  
Sadafumi Tamai, <sup>1</sup>Tomomi Tomoaki

Diagnosis	No. Cases	Fusion Gene Positive Cases	BAF47 Negative Cases
EWS/PNET	52	EWS-FL11 (+)	7 0
		EWS-ERG (+)	1 0
		Not detected	1 0
		Not done	43 0
Extraskeletal myxoid chondrosarcoma	24	EWS-CHN (+)	6 0
		TAF2N-CHN (+)	1 0
Clear cell sarcoma of soft tissue	14	Not detected	7 4
		Not done	10 0
		EWS-ATFI (+)	5 0
Desmoplastic small round cell tumor	2	Not detected	2 0
		Not done	7 0
		EWS-WT1 (+)	2 0
Myxoid/round cell liposarcoma	1	EWS-CHOP (+)	1 0

## Loss of INI-1 Expression

- Renal and extra-renal rhabdoid tumors
- Atypical teratoid/rhabdoid tumors
- Epithelioid sarcoma
- Medullary renal cell carcinoma
- Extraskeletal myxoid chondrosarcoma
- Epithelioid MPNST
- Myoepithelial carcinoma

# Major Genetic Alterations in Cancer

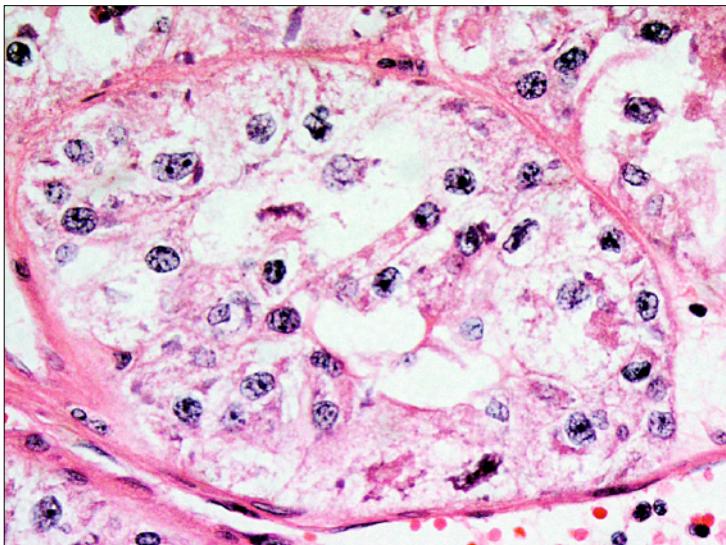
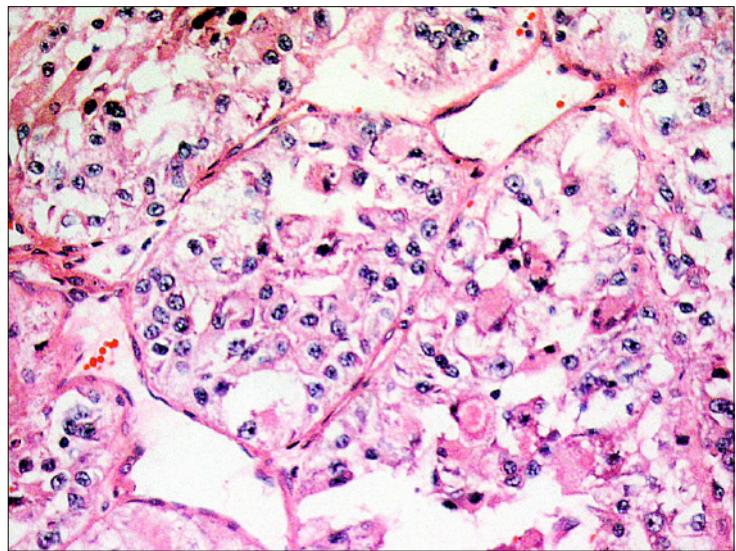


## Examples of Chromosomal Translocations Identifiable by NG-IHC

Tumor	Translocation	Fusion Generated	NG-IHC Target
PNET/ES	t(11;22)(q24;q12)	EWSR1-FLI1	FLI1
ALCL	t(2;5)(p23;q35)	NPM-ALK	ALK
ASPS	der(17)t(X;17)(p11;q25)	ASPL-TFE3	TFE3
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SYT-SSX1	TLE-1
DSRCT	t(11;22)(q11;q12)	EWSR1-WT1	WT-1
AML	t(8;21)(q22;q22)	AML1-ETO	AML1-ETO
Lung cancer	Chromosome 2 inversion	EML4-ALK	ALK

## Alveolar Soft Part Sarcoma

- Rare tumor in young patients with poor prognosis
- First coined and described by Christopherson, 1952
- Characteristic histology: organoid nests, pseudoalveolar pattern
- Can show clear cell change, PAS+ crystalline inclusions



**der(17)t(X;17)(p11;q25) of ASPS and generation of ASPL-TFE3 fusion protein**

**ASPL (Chr 17)**

N

C

N

C

**TFE3 (X Chr)**

### **der(17)t(X;17)(p11;q25) of ASPS and generation of ASPL-TFE3 fusion protein**

**ASPL (Chr 17)**

N  
N

**TFE3 (X Chr)**

C  
C

### **der(17)t(X;17)(p11;q25) of ASPS and generation of ASPL-TFE3 fusion protein**

**ASPL (Chr 17)**

N  
N

**TFE3 (X Chr)**

C  
C

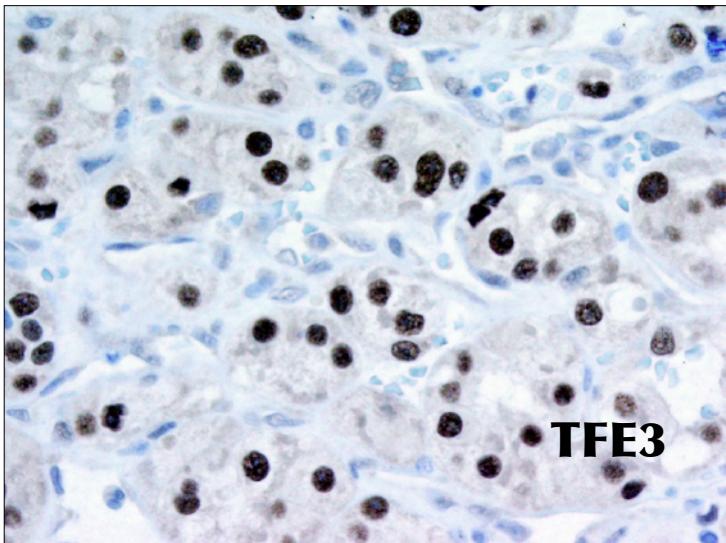
### **der(17)t(X;17)(p11;q25) of ASPS and generation of ASPL-TFE3 fusion protein**

N  
N  
**ASPL - TFE3**

C  
C

### **Composite ASPL-TFE3 Protein**

- ASPL promotor constitutively expressed and fusion protein highly expressed
- TFE3 (in family of basic helix loop helix leucine zipper nuclear transcription factors) binds to DNA



### **TFE3 Expression Is Also Found in Some RCCs**

*Group of renal cell carcinomas distinguished by chromosomal translocations with breakpoints involving the TFE3 gene on Xp11.2*

**Table 1** Cancers with Xp11 translocation/TFE3 gene fusions

Fusion	Tumour	Age range, years	Translocation
<i>ASPL-TFE3</i>	ASPS	5–40	der(17)(X;17)(p11.2q25)
<i>ASPL-TFE3</i>	RCC	2–68	t(X;17)(p11.2;q25)
<i>PRCC-TFE3</i>	RCC	2–70	t(X;1)(p11.2;q21)
<i>PSF-TFE3</i>	RCC	3–68	t(X;1)(p11.2;q34)
<i>NonO-TFE3</i>	RCC	39	inv(X)(p11.2;q12)
<i>CLTC-TFE3</i>	RCC	14	t(X;17)(p11.2;q23)
<i>Unknown</i>	RCC	32	t(X;3)(p11.2;q23)
<i>Unknown</i>	RCC	77	t(X;10)(p11.2;q23)

ASPS, alveolar soft part sarcoma; RCC, renal cell carcinoma.

Ross H and Argani P. *Pathology* 42:369–73, 2010

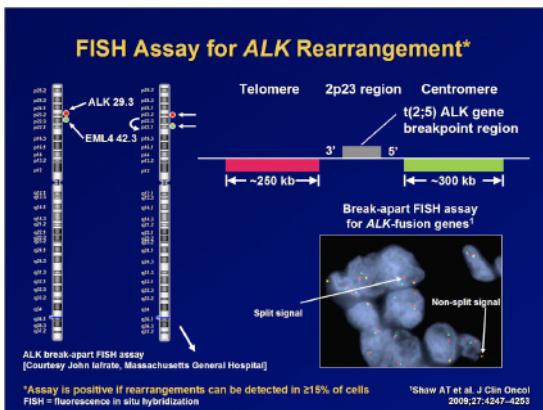
## Lessons Learned from IHC of Alveolar Soft Part Sarcoma

- There is no “cell of origin” of tumors
- Some tumors differentiate towards recognized cell types, but other tumors arise as a consequence of specific chromosomal alterations not related to cell type (e.g., synovial sarcoma, ASPS, PNET/ES, etc.)
- Ability to detect translocations opens windows onto other related tumors (eg., translocation renal cell carcinomas, PEComas)

## Detection of Translocated ALK Protein by Immunohistochemistry

- ALK activation in subset of nonsmall cell lung CAs
- Results from fusion of ALK with EML4 via translocation on chromosome 21
- Confers sensitivity to crizotinib

## ALK Rearrangement in ~5% of Lung Cancers



J Thorac Oncol 8:45-51, 2013

Immunohistochemistry is a Reliable Screening Tool for Identification of ALK Rearrangement in Non-Small-Cell Lung Carcinoma and is Antibody Dependent

Chris M.J. Conklin, MD,\* Kenneth J. Craddock, MD,† Cherry Have, BSc, MLT,† Janessa Laskin, MD,‡ Christian Couture, MD,§ and Diana N. Ionescu, MD,‡

- Tested several different anti-ALK antibodies
- Compared result with gold standard, i.e., presence of translocation involving ALK using FISH

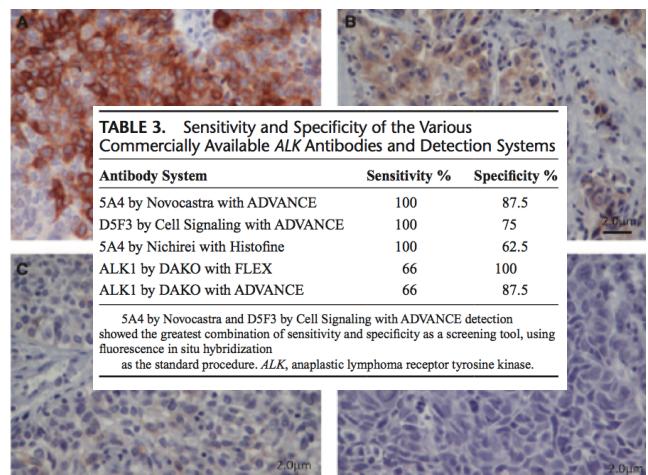
J Thorac Oncol 8:45-51, 2013

Immunohistochemistry is a Reliable Screening Tool for Identification of ALK Rearrangement in Non-Small-Cell Lung Carcinoma and is Antibody Dependent

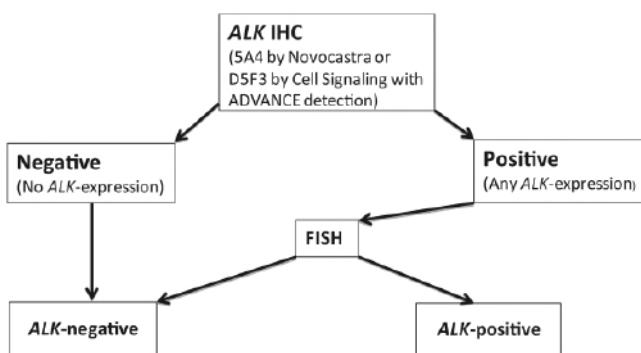
Chris M.J. Conklin, MD,\* Kenneth J. Craddock, MD,† Cherry Have, BSc, MLT,† Janessa Laskin, MD,‡ Christian Couture, MD,§ and Diana N. Ionescu, MD,‡

- N = 377 lung tumors
- Compared 5A4, D5F3, ALK1

Conklin et al., J Thorac Oncol 8:45-51, 2013



## How to Integrate IHC and FISH



Conklin et al., J Thorac Oncol 8:45-51, 2013

## Comparison of NG-IHC v. FISH for Detection of ALK

Paper	N (IHC+FISH)	Antibody	Sensitivity	Specificity
Mino-Kenudson et al 2010	<b>153</b>	<b>D5F3</b>	<b>100%</b>	<b>99%</b>
Paik et al, 2011	<b>735</b>	<b>5A4</b>	<b>100%</b>	<b>100%</b>
Martinez 2012	<b>79</b>	<b>D5F3</b>	<b>83%</b>	<b>100%</b>
McLeer-Florin 2012	<b>200</b>	<b>5A4</b>	<b>100%</b>	<b>98.3%</b>
Conklin et al 2013	<b>377</b>	<b>5A4</b>	<b>100%</b>	<b>87.5%</b>
Scholl et al 2013	<b>186</b>	<b>5A4</b>	<b>93%</b>	<b>100%</b>

J Thorac Oncol 8:255-6, 2013

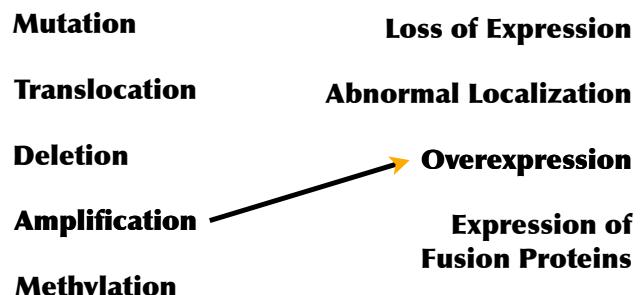
EDITORIAL

### Optimal Detection of ALK Rearranged Lung Adenocarcinomas

Niki Karachalios, MD,\* and Rafael Rosell, MD, PhD\*†

- FISH is sensitive and specific but not infallible
- ALK IHC improves detection of ALK rearrangements when used together with FISH

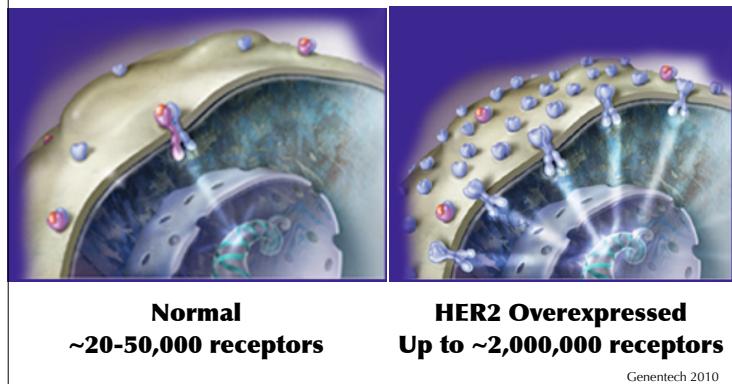
## Major Genetic Alterations in Cancer



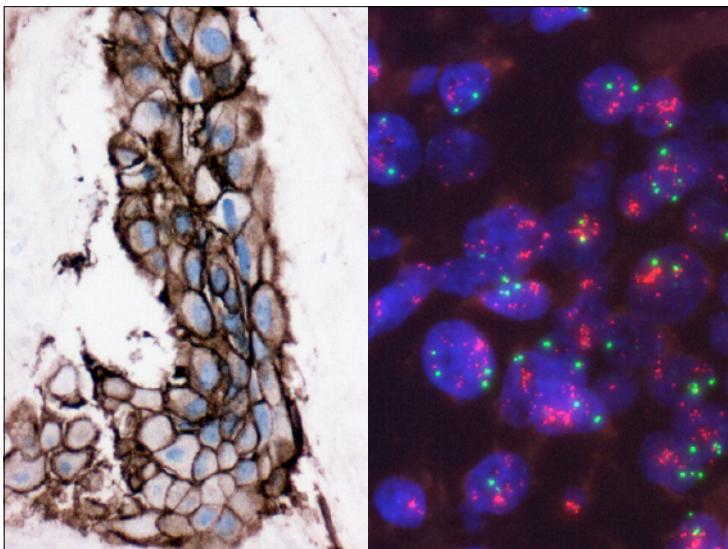
### Examples of Amplified Proteins Identifiable by NG-IHC

Tumor	Protein amplified
Breast cancer	<b>HER2</b>
Liposarcoma	<b>MDM-2, CDK4</b>
Lymphoma	<b>bcl-2</b>
Lung cancer	<b>EGFR</b>

## HER2 Overexpression in Breast Cancer



Genentech 2010



# FISH v. IHC

**“...There is no gold standard at present; no assay currently available is perfectly accurate to identify all patients expected to benefit or not from anti-HER2 therapy.”**

*From 2007 ASCO-CAP Guidelines*

## Data Presented At SABCS, December 2012

N = 9,022 breast cancer cases, 2008-2012

	Negative (0, 1+)	Positive (3+)
Non-amplified	<b>3903 (98.6%)</b>	<b>13</b>
Amplified	<b>57</b>	<b>450 (97.2%)</b>
TOTALS	<b>3960</b>	<b>463</b>

## Next Generation Immunohistochemistry

1

- NG-IHC can be used to identify molecular alterations that characterize selected malignancies
- NG-IHC acts as a surrogate for molecular studies, and is less expensive and time consuming and, in some cases, can provide more information

## Next Generation Immunohistochemistry

2

- NG-IHC can integrate different genotypic changes which result in the same phenotypic changes
- NG-IHC can thus expand and better define categories of disease



## What does the Future of Pathology Look Like?

- The paradigm shift to molecular based classification of tumors will continue and will accelerate
- Molecular oncidiagnostics will play an increasingly large role in tumor analysis

## Morphology (H&E)

↑  
Immunophenotype  
(Cell Type)

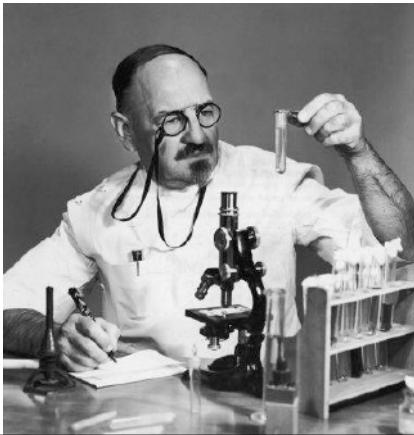
Molecular Alteration

↓  
Immunophenotype  
(Next Gen IHC)

## From Morphology And Back

	Anaplastic large cell lymphoma	Rhabdoid Tumor	MSI Type Colorectal CAs	Lobular Breast CA
IHC-Cell type	CD30	Vimentin and CK + inclusions	Maybe loss of CK20 and CDX2	"Lobular" cell, CK pattern
Genetic	t(2;5)	INI1/SMARCB1 mutation	Mutations of MMR genes	Molecular switching off of E-cad
New IHC	ALK	INI1/SMARCB1 loss	Loss of MMRs	Loss of E-cadherin
New related entities	Small cell monomorphic variant	Chondrosarcoma, epithelioid sarcoma	MSI Type Colorectal CAs with no special histologic features	"Pseudo-intraductal" carcinoma

## The Role of the Pathologist (Then)



## The New Paradigm of Pathology

Patients

Oncologists

BioPharma  
Targeted  
Therapies

Published  
Literature  
Pathology,  
Oncology



## The Changing Role of the Pathologist

"As more drugs that target specific components of signal-transduction pathways become available and as we increase our knowledge of the complexity of these signalling networks, ***the burden of selecting the correct drug combinations for each individual cancer patient will ultimately shift to the pathologist***, who must identify the underlying defect in each tumor."

Shaw RJ and Cantley LC. Nature 441:424-30, 2006

## Thank you for your attention

Questions?

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