

Histopathology and molecular pathology of cancer

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National Institute of Oncology
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Budapest, 9/17/19.



Pathology

- EU
 - Autopsy
 - **Surgical pathology/histopathology**
 - Cytopathology
 - Molecular pathology
- US
 - AP – Anatomic pathology
 - Autopsy
 - **Surgical pathology/histopathology**
 - Cytopathology
 - CP – Clinical pathology
 - Chemistry
 - Microbiology
 - Transfusion
 - Molecular pathology

2

ANTHONY A. GAL



PLATE 1. (A) The many interactions of pathology with clinical and basic sciences. (B) The many interactions of surgical pathology with clinical and research disciplines. (C) Microscope. Front cover from *Common objects of the microscope*, by Reverend J. G. Wood, 1864. (D) Petals, pollen, seeds, and starch. Color plate III from *Common objects of the microscope*, by Reverend J. G. Wood, 1864. (E) Histochemical stains. Bottles of histochemical stains currently used in a modern surgical pathology laboratory. (F) Leaves of logwood. Hematoxylin is derived from the heartwood of the logwood tree (*Haematoxylum campechianum*). (Reprinted from Dan Slean, <http://www.albion.edu/biol/slean/haemcapg.htm>)

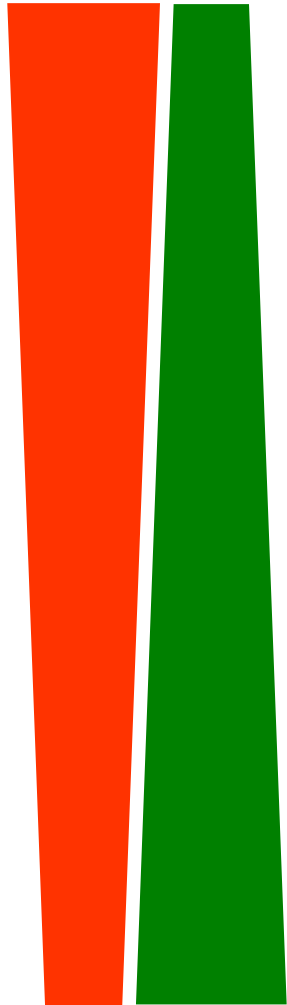
Advances in Anatomic Pathology, Vol. 8, No. 1, January, 2001

Role of oncologic pathology

- **The pathologic diagnosis is morphologically based**
 - Ancillary studies: IHC/MP
- **Aim: to provide appropriate information to the clinician treating the patient**
 - Treatment design and effectiveness (*predictive markers*)
 - Surgery
 - Radiation therapy
 - Drugs
 - Traditional chemotherapy
 - *Targeted therapy*
 - *Prognostic markers*: **TNM Stage**

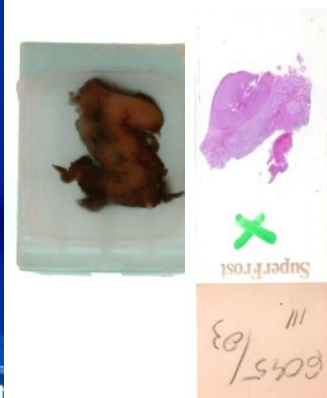
Diagnostic methods

Dx



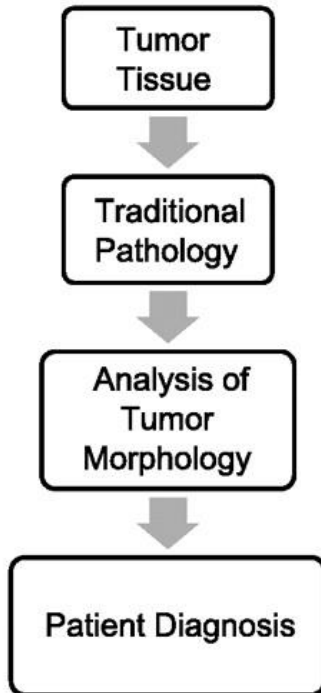
Staging

- **Preoperative**
 - Cytology
 - Biopsy (Histology – Surgical Pathology)
- **Intraoperative**
 - Frozen Section (FS)
 - Cytology
 - Provide fresh tissue for additional/ancillary studies
- **Postoperative**
 - Resected specimens' histological examination
 - Ancillary studies (IHC, MP)
- **Post mortem**
 - Autopsy

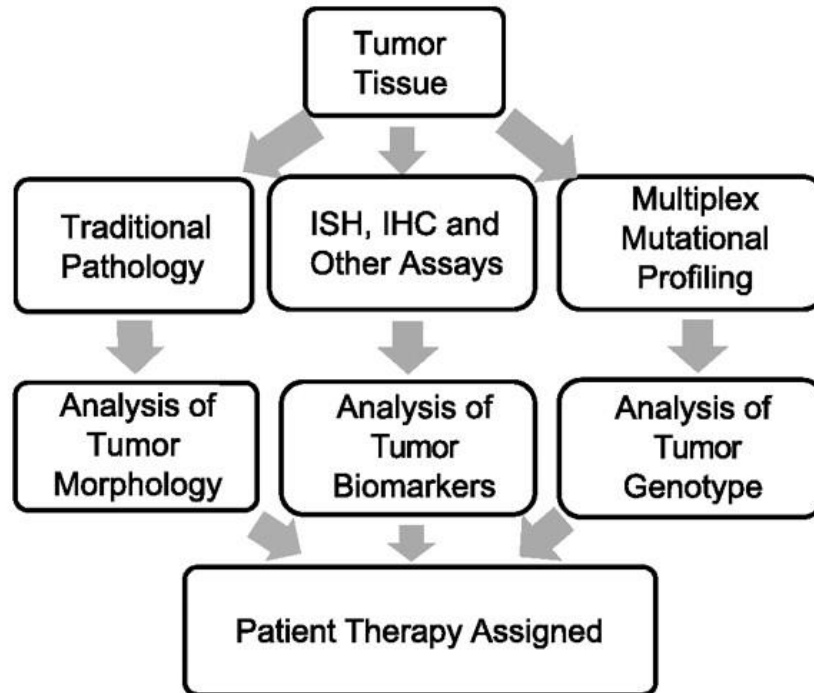


Role of pathology in oncology

A Traditional Tumor Analysis



B Integrated Molecular Analysis

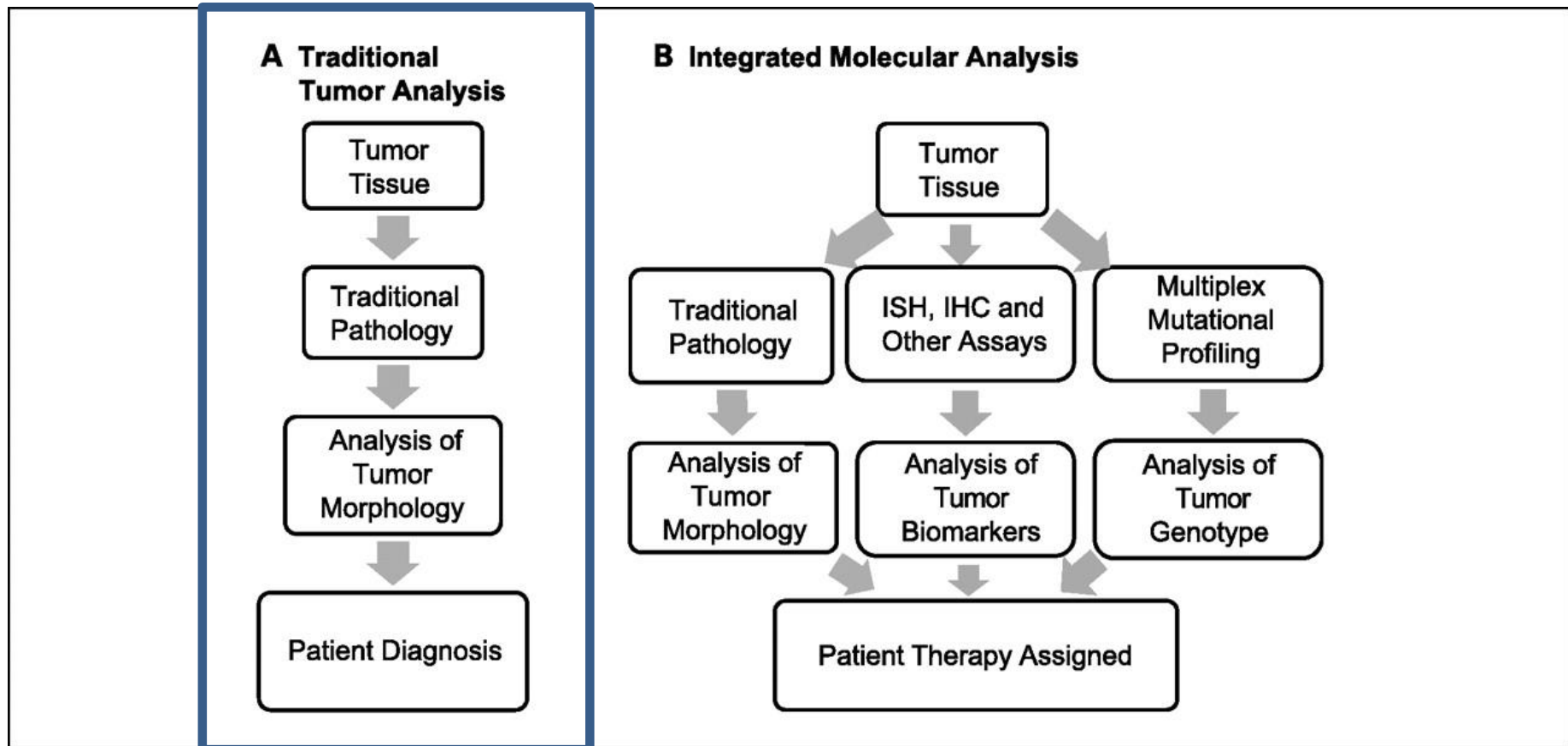


Pao W et al. Clin Cancer Res 2009;15:5317-5322

General process of pathological laboratory examinations

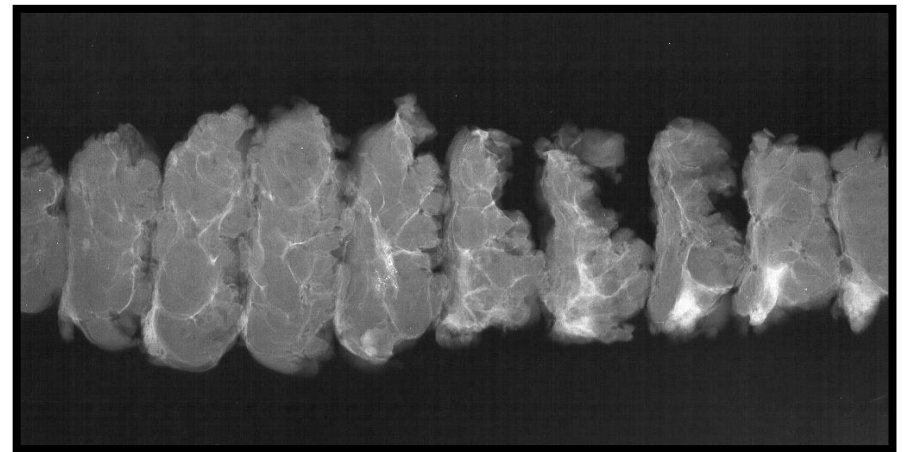
1. Specimen arrives, fixation
2. Macroscopic examination
3. Embedding
4. Sectioning, HE staining
5. Medical review
6. Immunohistochemistry
7. Preparing histological report
8. Molecular pathology
 - 8/1. Sample selection
 - 8/2. DNA isolation
 - 8/3. PCR, sequencing, FISH
 - 8/4. Preparing molecular pathological report

Role of pathology in oncology

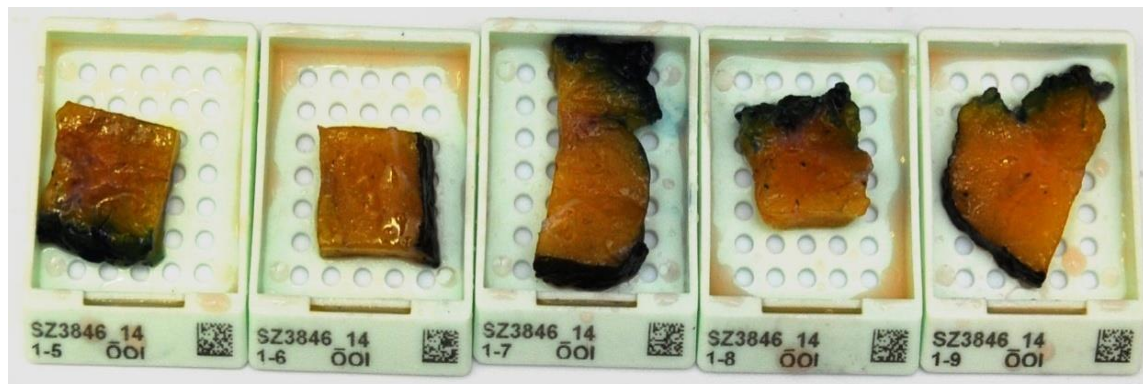
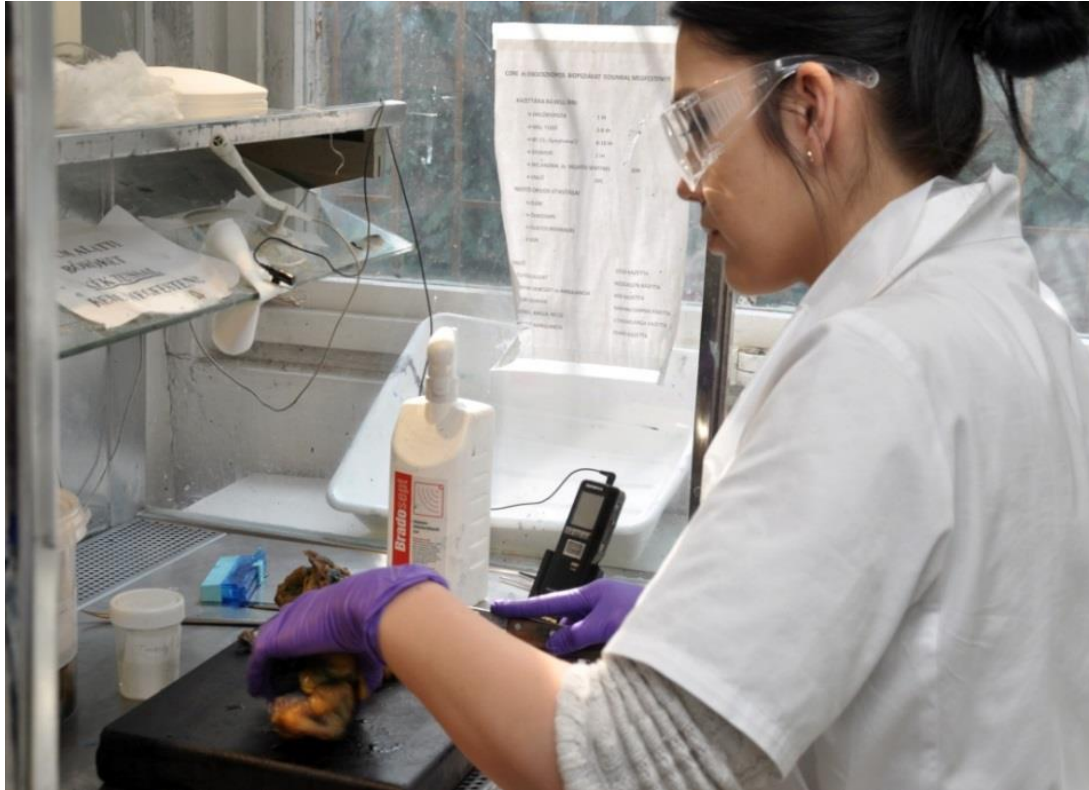


Pao W et al. Clin Cancer Res 2009;15:5317-5322

Macroscopic examination



Macroscopic examination

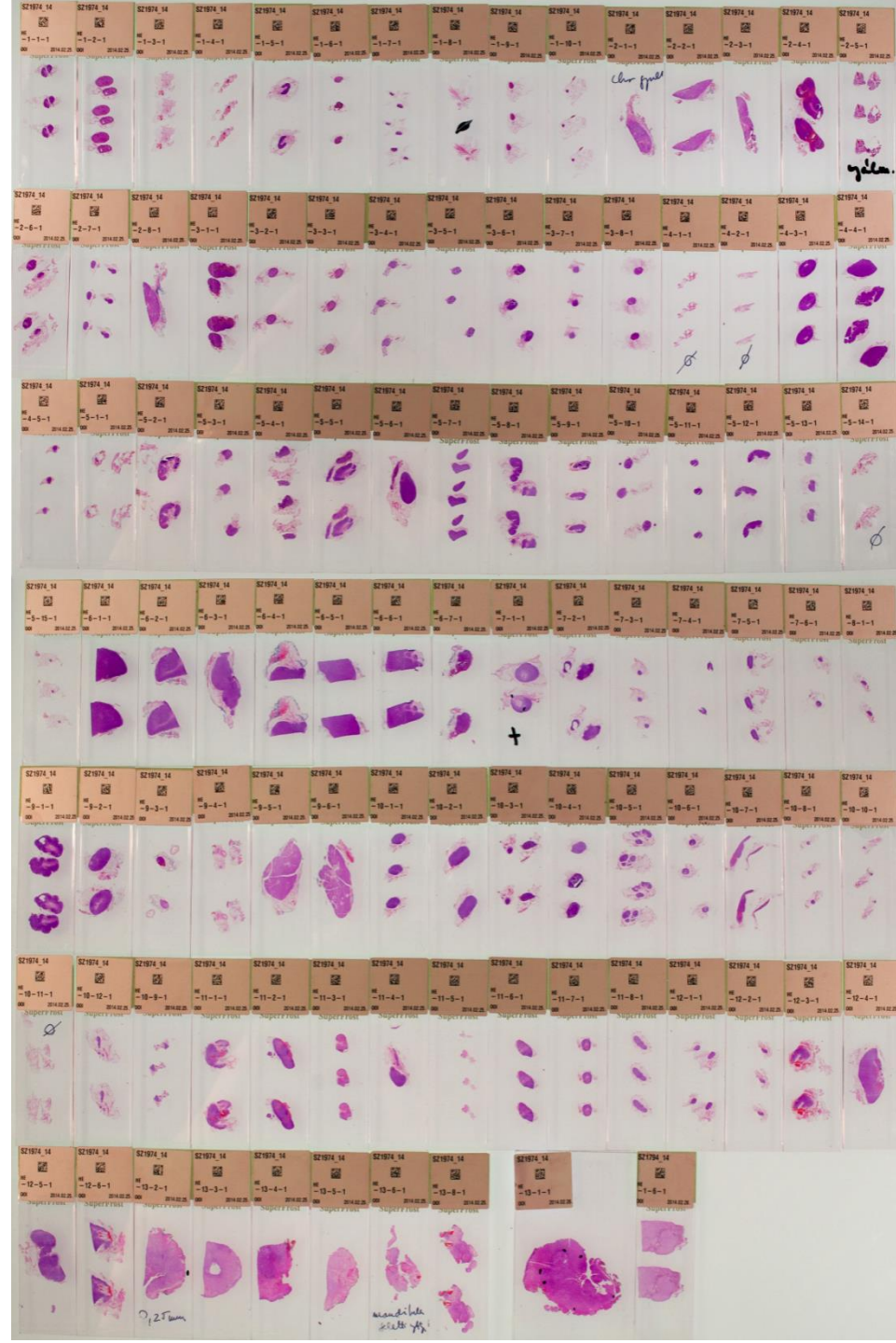


Cutting sections of the blocks (FFPE)





HE staining



„Traditional” histologic parameters

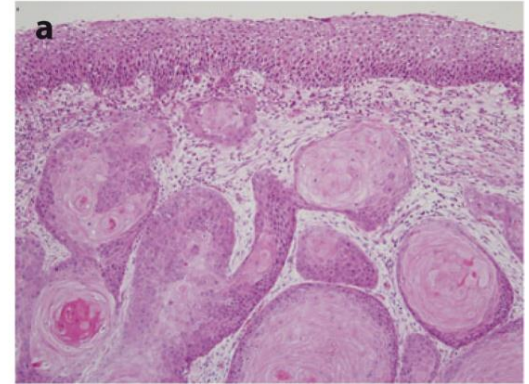
- **Diagnosis: What is the tissue/cell of origin, what is the phenotype?**
 - Dignity: Benign or malignant
 - Basic malignant tumor categories
 - Carcinoma
 - Sarcoma
 - Lymphoma
 - Melanoma
 - Germ cell
- **Tumor size**
- **Histological grade**
 - Differentiation – specialized function
 - How similar is to the cell/tissue of origin
 - Well proved grading systems
 - Breast: Nottingham Prognostic Index
 - Prostate: Gleason’s score
- **Vascular invasion**
- **Perineural invasion**
- **Margins**
- **Lymph node status**
- **pTNM: sum of the most important parameters**



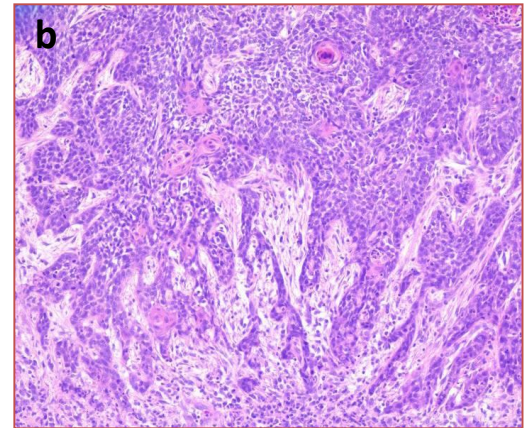
Squamous cell carcinoma, types

Head and neck

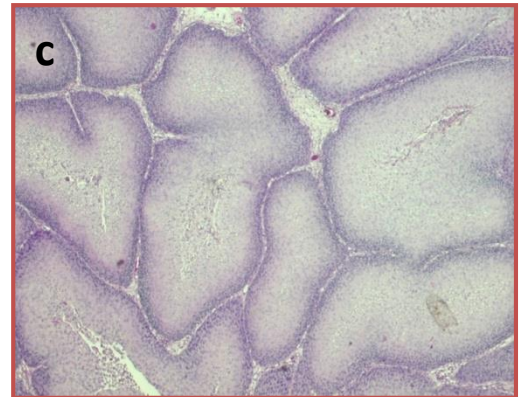
Conventional, NOS (a)



Basaloid (b)

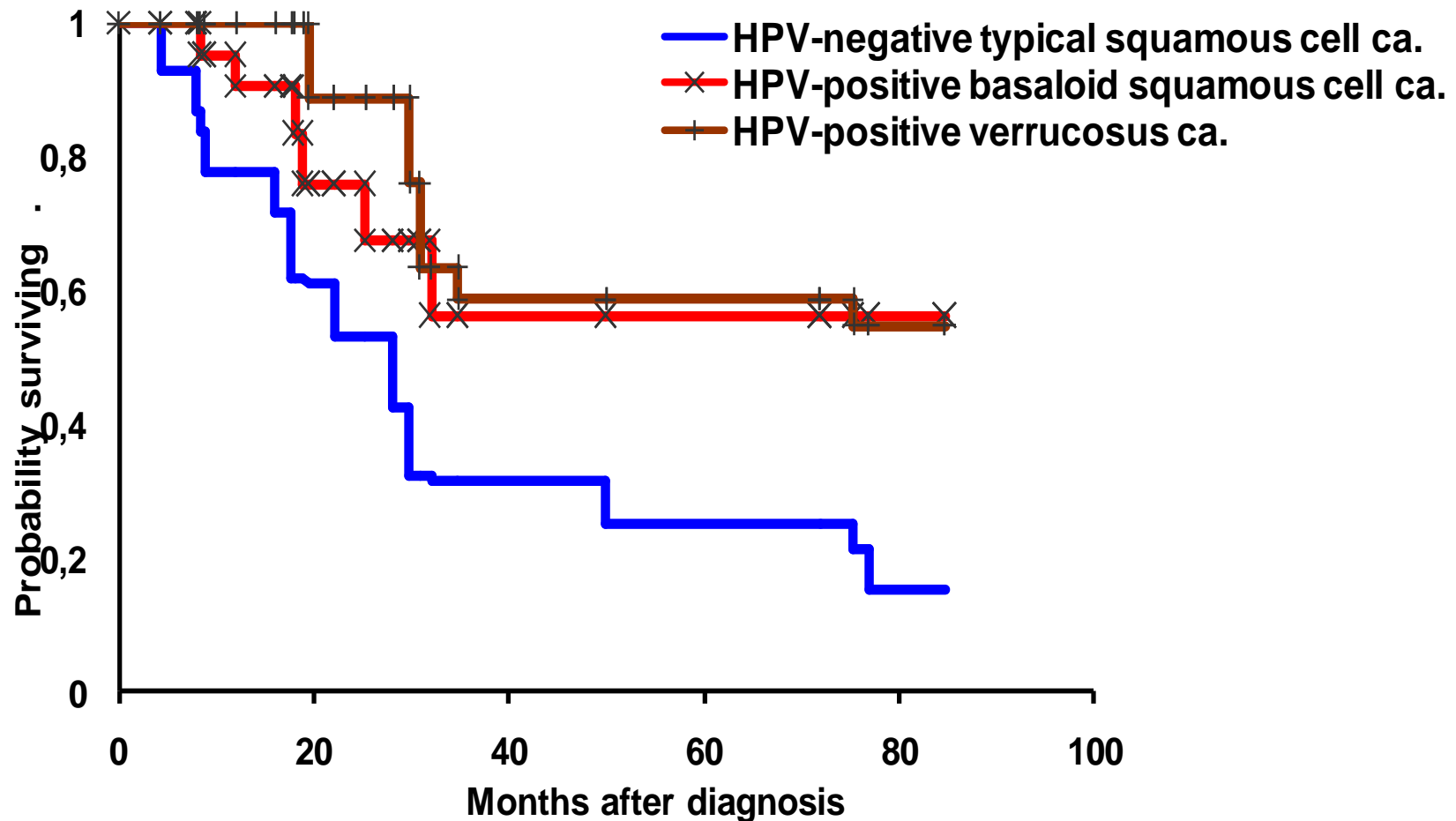


Verrucous (c)



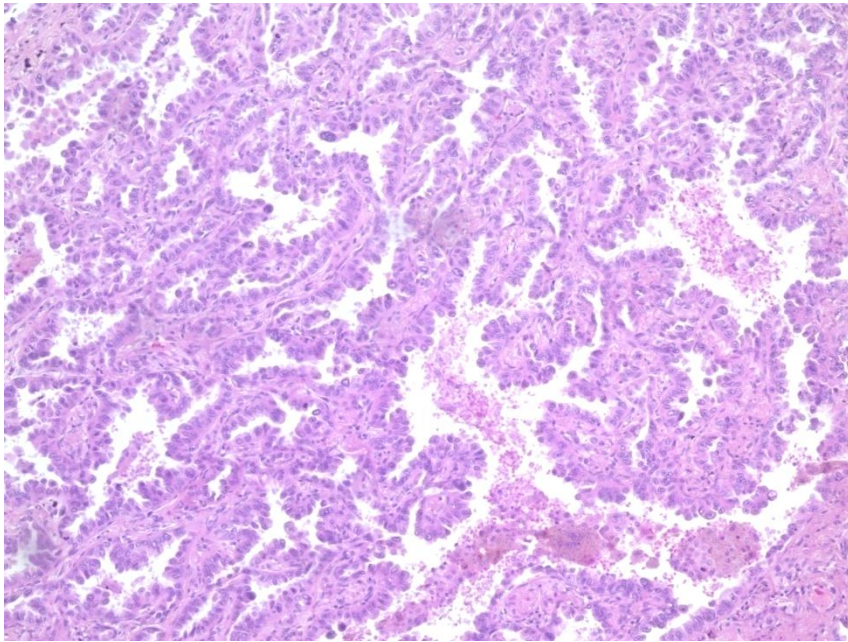
Survival and Histology/HPV status

n=136

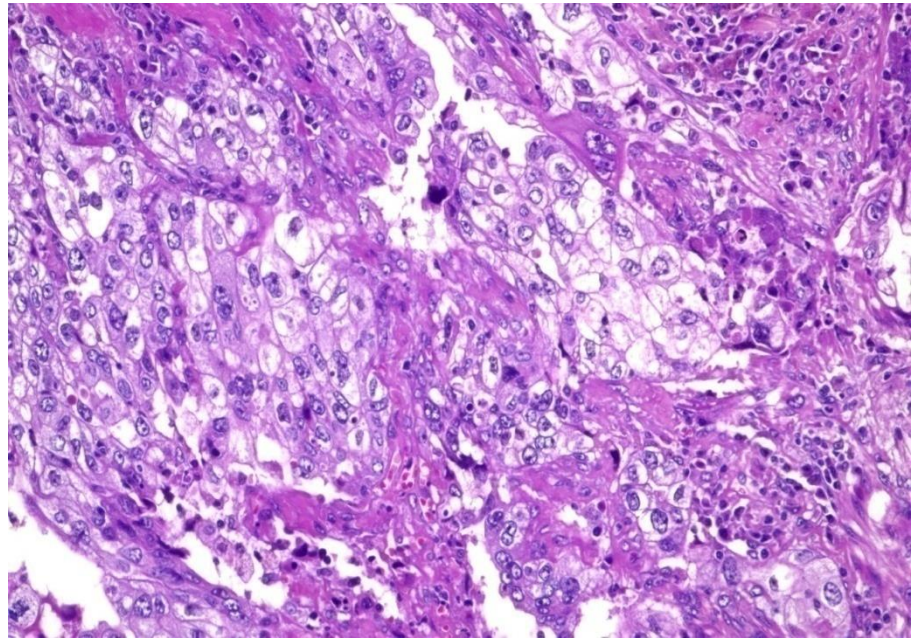


Differentiation – Histological grade

Lung adenocarcinoma



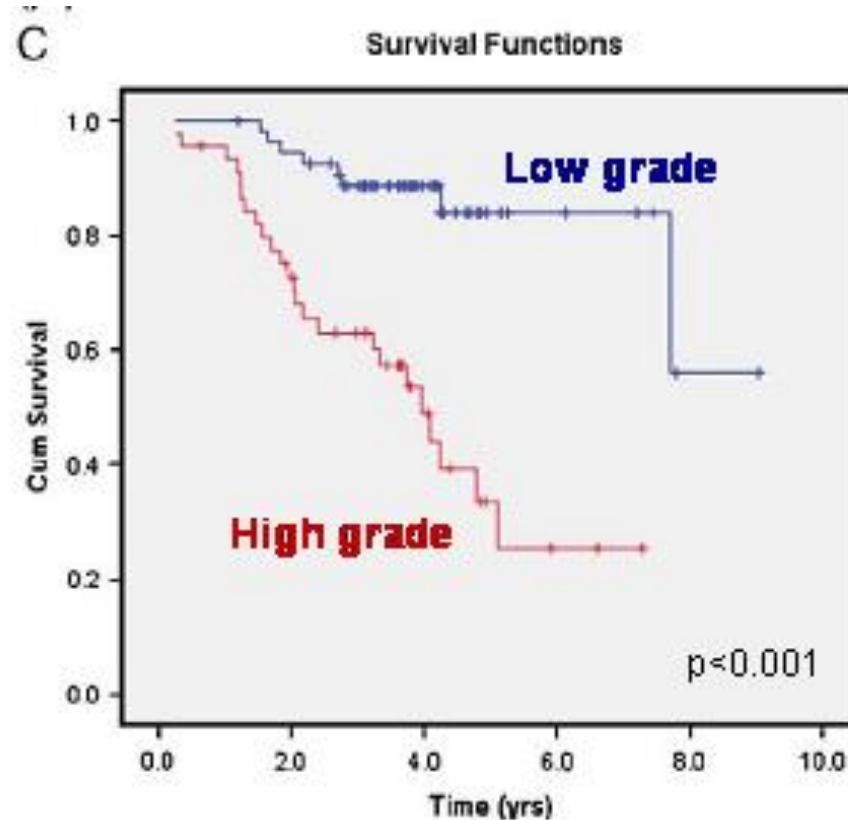
Low grade



High grade

100 resected primary lung adenocarcinoma

Survival and grade

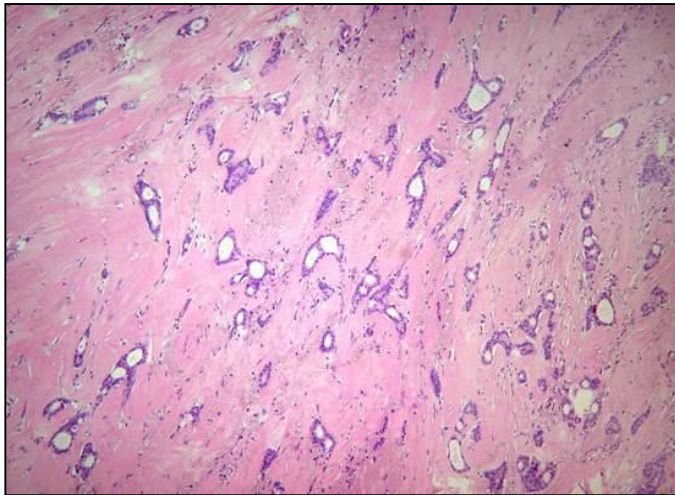


Histological grade

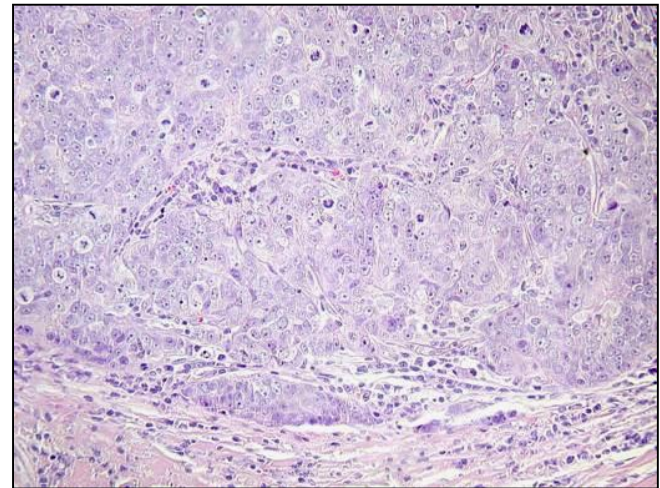
Breast cancer

Tumor grade 1-3:

Tubule formation + Nuclear grade + Mitotic count



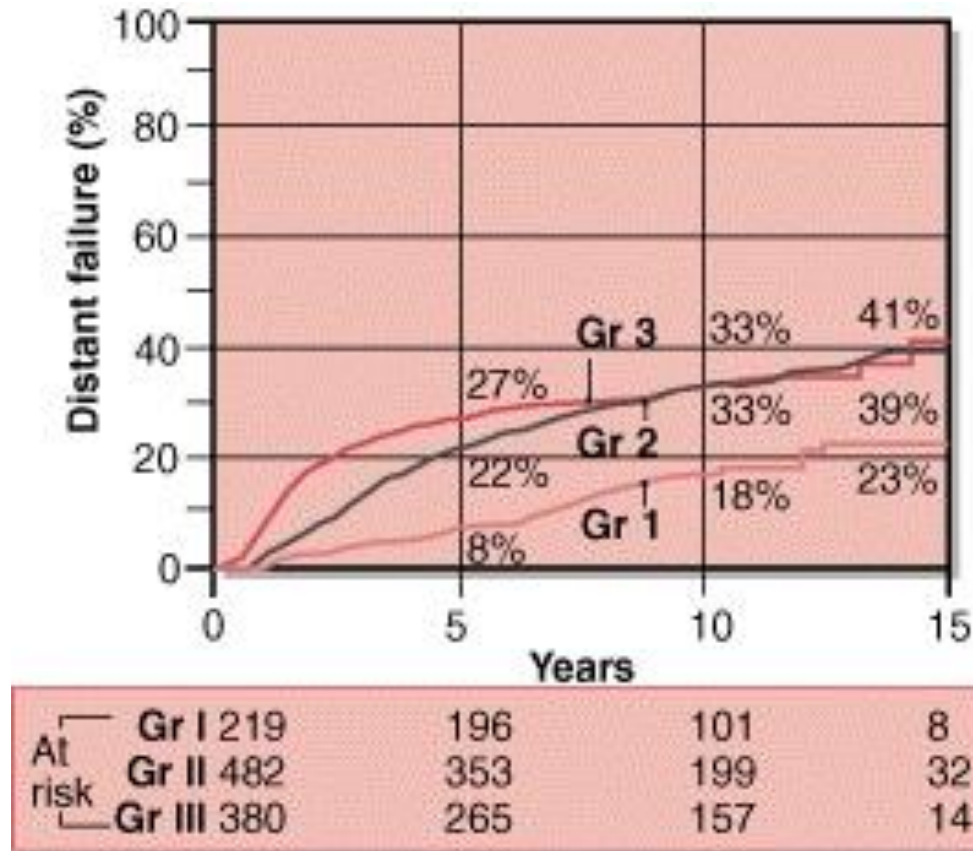
Grade I (well differentiated)



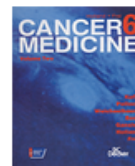
Grade III (poorly differentiated)

Histological grade and distant failure in breast cancer

n=1081



From: [Role of the Surgical Pathologist in the Diagnosis and Management of the Cancer Patient](#)



Holland-Frei Cancer Medicine. 6th edition.
Kufe DW, Pollock RE, Weichselbaum RR, et al., editors.
Hamilton (ON): [BC Decker](#); 2003.

[Copyright](#) © 2003, BC Decker Inc.

NCBI Bookshelf. A service of the National Library of Medicine. National Institutes of Health

Nottingham Prognostic Index

Size+Grade +LN status

Nottingham Prognostic Index: $(0,2 \times 0,9) + 1 + 1 = 2,18$

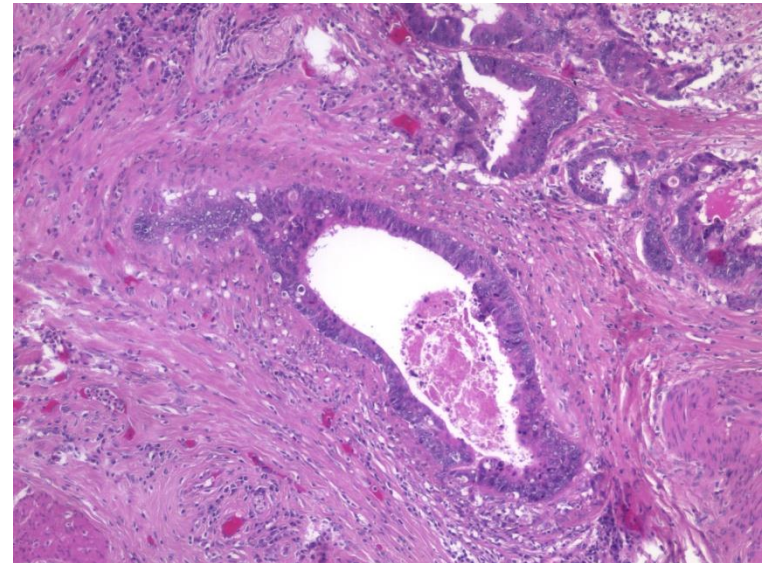
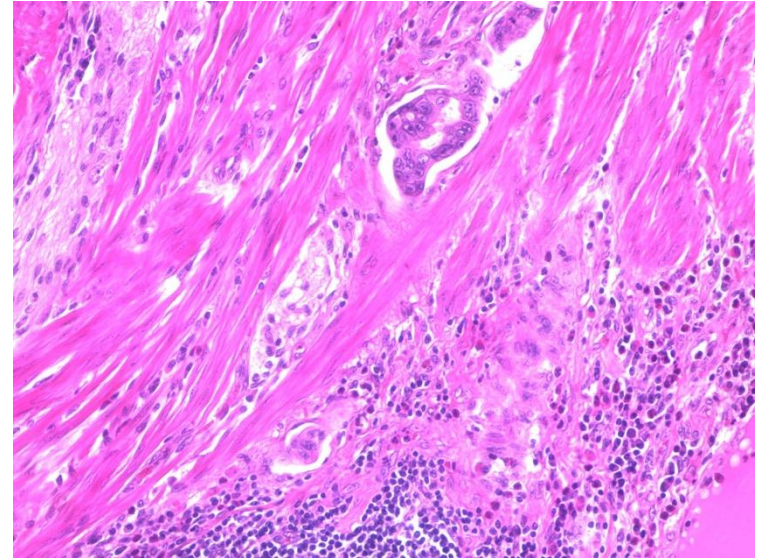
Excellent prognostic group

Prognostic value of NPI

Group	NPI	10 yr survival
Excellent	2.02-2.4	96%
Good	2.41-3.4	93%
Moderate 1	3.41-4.4	81%
Moderate 2	4.41-5.4	74%
Poor	5.41-6.4	55%
Very poor	6.41-6.8	38%

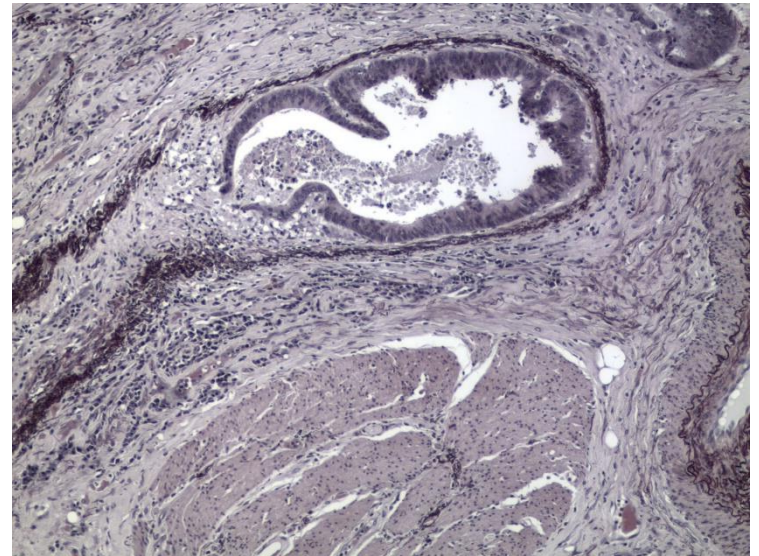
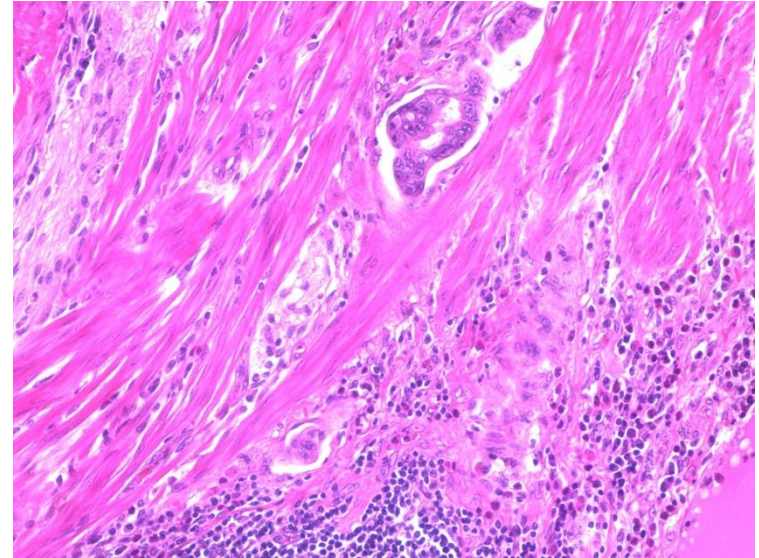
Vascular invasion in CRC

- **Lymphovascular invasion**
 - Associated with local lymph node metastasis
- **Venous invasion**
 - 11%-89,5% prevalence
 - Associated with tumor recurrence (mainly through hematogenous metastasis) and decreased survival
 - **May be missed on HE**



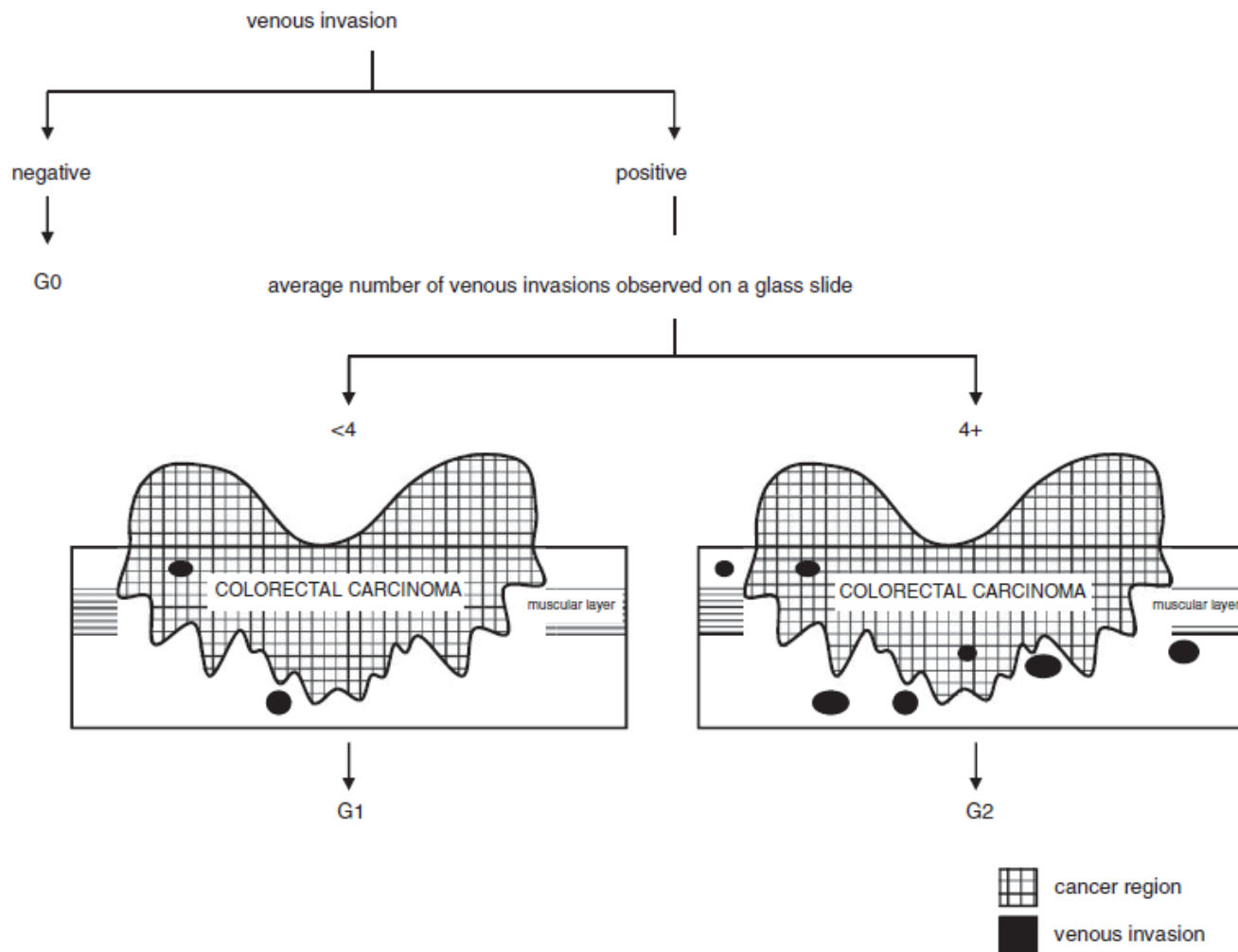
Vascular invasion in CRC

- **Lymphovascular invasion**
 - Associated with local lymph node metastasis
- **Venous invasion**
 - 11%-89,5% prevalence
 - Associated with tumor recurrence (mainly through hematogenous metastasis) and decreased survival
 - May be missed on HE
 - **Elastic stain** (orcein, van Gieson)



Venous invasion as prognostic factor in CRC

n=229 pT3, pT4 CRC



Venous invasion as prognostic factor in CRC

n=191 pT3 CRC

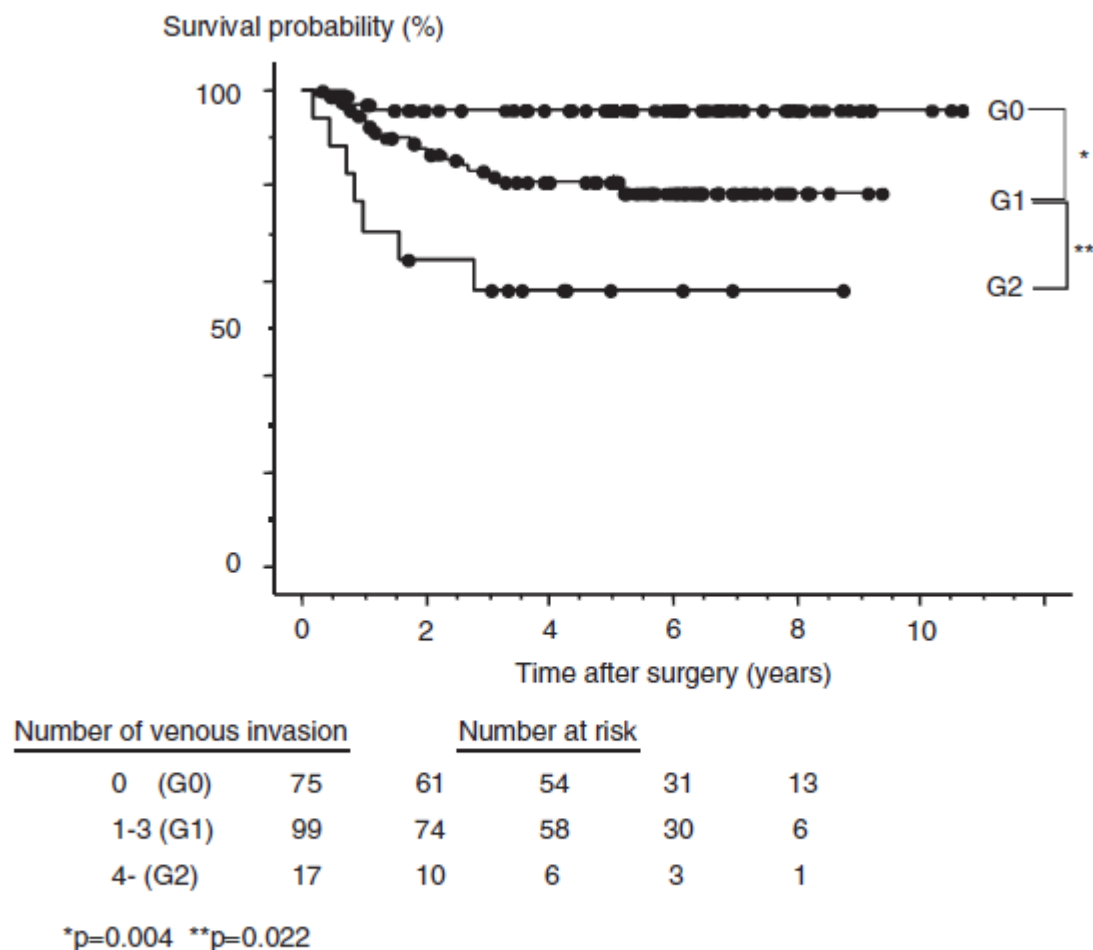
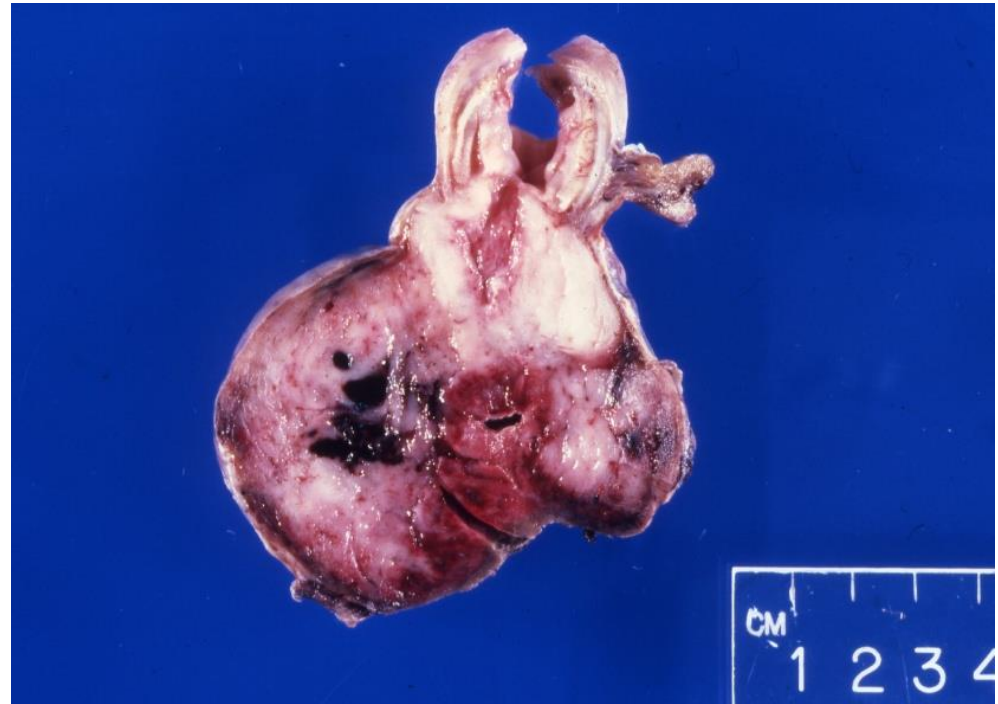


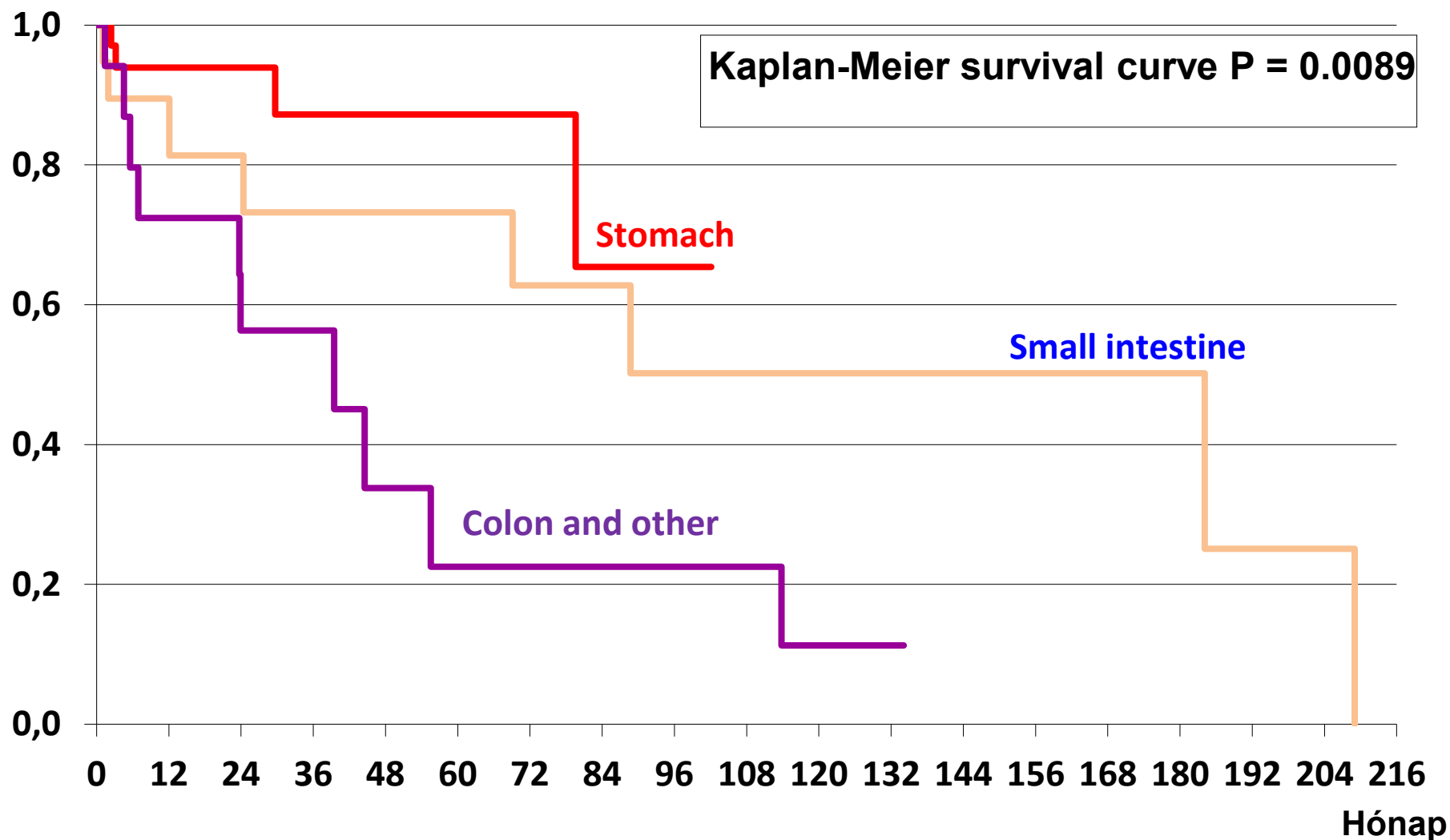
FIGURE 4. Kaplan-Meier estimates of survival (the pT3 group).

Gastrointestinal stromal tumor (GIST)

- Clinico-pathological prognostic factors
 - Localization
 - Size
 - Mitotic count



GIST, localization and survival. No = 76





SURGICAL PATHOLOGY
CRITERIA

Diagnostic Criteria

Multiple/Hereditary/Pediatric

Supplemental Studies

Molecular Genetic Studies

→ Grading/Staging/Report

Differential Diagnosis

Clinical

Bibliography

Printable Version

GENERAL LINKS

Surgical Pathology Criteria
Home

Diseases and Disorders By
Section

Keyword Search

Abbreviations

Stanford Pathology
Department

To Submit a Specimen for Review:

For Physicians

For Patients

Gastrointestinal Stromal Tumor (GIST)

Grading / Staging / Report

Grading

Risk for Metastasis/Progressive Disease

	Stomach	Duodenum	Jejunum & Ileum	Rectum
≤5 mits/50 hpf				
≤2cm	0 none	0 none	0 none	0 none
>2cm ≤5cm	very low	low	low	low
>5cm ≤10cm	low	high	moderate	high
>10cm	moderate	high	high	high
>5 mits/50 hpf				
≤2cm	few cases	no cases	few cases	high
>2cm ≤5cm	moderate	high	high	high
>5cm ≤10cm	high	high	high	high
>10cm	high	high	high	high

Large intestine tumors are rare, risk appears similar to jejunum&ileum

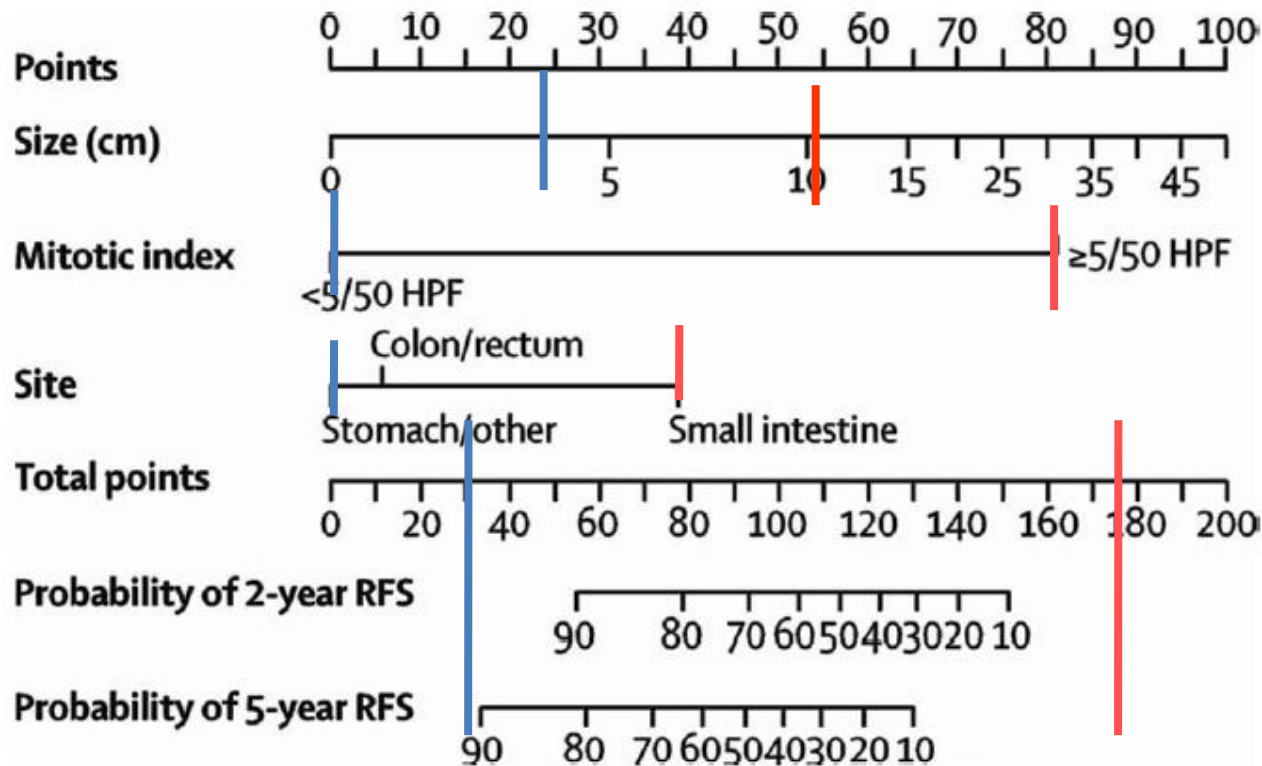
Esophageal tumors are too rare to develop criteria

With wide field microscope view (5mmsq), count 25 fields with same cutoff of 5 as above

Based on Miettinen and Lasota 2006

Progressive Disease or Death Risk Groups

Group	Approximate Progression Incidence
0 None	0
Very low	<2%
Low	<5%
Moderate	10-30%
High	>50%



4 cm GIST in
stomach,
MI<5/50 HPF



5 year recurrence free survival: 90%

Figure 4.

Nomogram predicting 2 and 5-year recurrence-free survival in patients with resected primary GIST. Points are assigned based on tumor size, mitotic index, and site by drawing an upward vertical line to the "Points" bar. Based on the sum of the points generated, a downward vertical line is drawn from the "Total Points" line to calculate 2 and 5-year RFS. From Gold JS, Gonen M, Gutierrez A, et al. Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localized primary gastrointestinal stromal tumor: a retrospective analysis. *Lancet Oncol* 2009; 10:1045–1052; with permission.

10 cm GIST in
small intestine,
MI>5/50 HPF



5 year recurrence free survival: <10%

„Traditional” histologic parameters

- **Diagnosis: What is the tissue/cell of origin, what is the phenotype?**
 - Dignity: Benign or malignant
 - Basic malignant tumor categories
 - Carcinoma
 - Sarcoma
 - Lymphoma
 - Melanoma
 - Germ cell
- **Tumor size**
- **Histological grade**
 - Differentiation – specialized function
 - How similar is to the cell/tissue of origin
 - Well proved grading systems
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- **Perineural invasion**
- **Margins**
- **Lymph node status**
- **pTNM: sum of the most important parameters**



TNM

- Components: T, N, M.
 - Tumor (mostly size, but there other parameters, e.g. visceral pleural invasion in lung cancer, local/serosal invasion in large bowel carcinoma)
 - Lymph node status (mostly number of involved nodes, but exceptions, e.g. in lung cancer location of nodes)
 - Distant metastasis
- Categories: T1a, ...; N0, ...; M1a, ...
- Descriptors: Parameters that define categories



Patient Name:		Client:	Baystate Medical Center	Accession #:	
Med. Rec. #:		Location:	PAHLD	Date Taken:	5/24/2000
DOB:	(Age: 78)	Billing #:		Received:	5/24/2000
Gender:	M			Signed Out:	5/29/2000
Physician(s):					

Final Diagnosis

PARTS 1 THROUGH 8:

LUNG, LEFT UPPER LOBE; LOBECTOMY:

- SQUAMOUS CELL CARCINOMA:

A. TUMOR SIZE: 6.5 CM X 3.2 CM X 4.8 CM.

B. HISTOLOGIC GRADE: T1.

C. EXTENT OF PRIMARY TUMOR:

- TUMOR INVADERS VISCERAL PLEURA.
- TUMOR IS 1 CM FROM BRONCHIAL MARGIN OF EXCISION.

- MARGINS OF EXCISION:

A. BRONCHIAL MARGIN OF RESECTION: NEGATIVE FOR TUMOR.
B. VASCULAR MARGIN OF EXCISION: NEGATIVE FOR TUMOR.

- LYMPH NODES (HILAR):

A. NUMBER EXAMINED: 11.

B. NUMBER POSITIVE: 3.

C. COMMENT: Two of the nodes are positive as a result of direct extension of tumor into matted nodes.

- (PARTS 1-3,5-8): LYMPH NODES (MEDIASTINAL L6, L5, L4, L9, L8, L10, L11):

A. NUMBER EXAMINED: 7.

B. NUMBER POSITIVE: 0.

- ADDITIONAL TUMOR FEATURES:

A. LYMPHATIC VESSEL INVASION IN LUNG: NOT IDENTIFIED.

B. BLOOD VESSEL INVASION IN LUNG: IDENTIFIED IN A LARGE PULMONARY ARTERY BRANCH (BLOCK 4-7).

C. COMMENTS:

- LYMPHOPLASMATIC RESPONSE IS PRESENT, MILD.
- STROMAL RESPONSE IS PRESENT, MODERATE.
- TUMOR NECROSIS IS PRESENT, EXTENSIVE.
- TUMOR INVADERS A SEGMENTAL BRONCHUS.

- NON NEOPLASTIC LUNG TISSUE:

A. ASSOCIATED OBSTRUCTIVE PNEUMONIA: EXTENDING TO HILAR REGION, ACUTE AND ORGANIZING.

B. OTHER FINDINGS:

- EMPHYSEMA.
- PLEURA FIBROSIS WITH FUSION OF VISCERAL AND PARIETAL PLEURAE.
- SUBPLEURAL FIBROSIS.

- ADDITIONAL TISSUE STUDIES: ELASTIC TISSUE STAIN DEMONSTRATES TUMOR PENETRATION OF VISCERAL PLEURA.

- pTN STAGE:

A. PRIMARY TUMOR: pT2.

B. REGIONAL LYMPH NODES: pN1.

Squamous cell carcinoma

6,5 cm

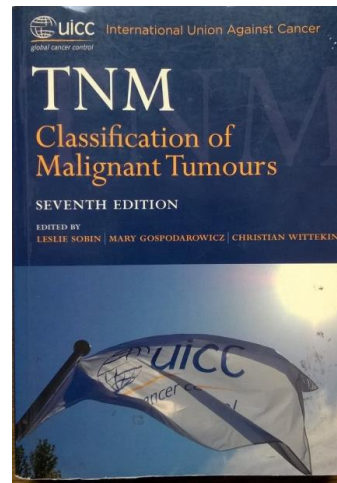
pT2N1

Electronically Signed Out

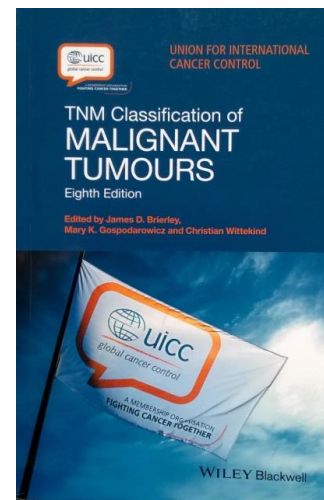
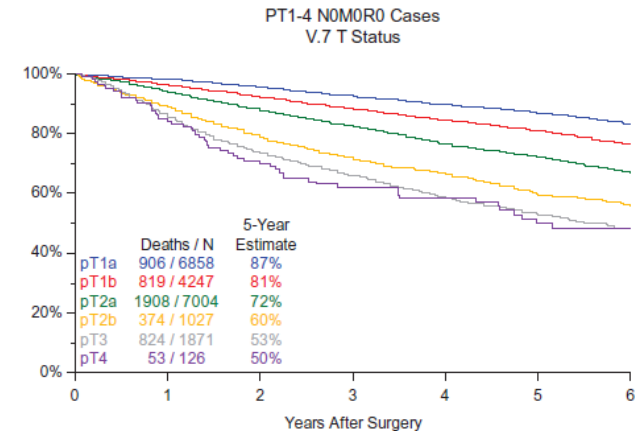
TNM v5,v6, v7 and v8

6,5 cm-es lung carcinoma

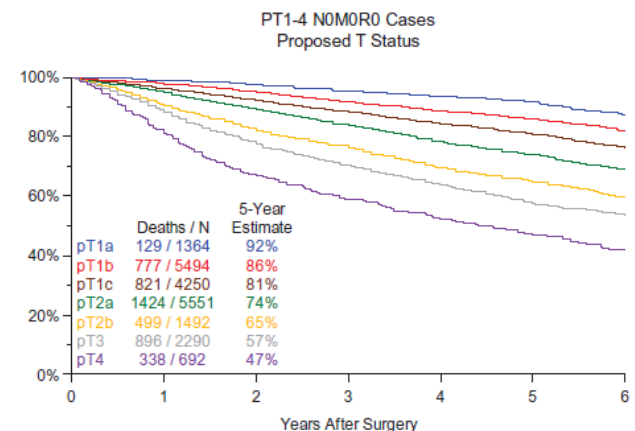
- UICC 5th ed. 1997 _ pT2
- UICC 6th ed. 2002 _ pT2
- UICC 7th ed. 2009 _ pT2b



A 7th Edition T Categories



Proposed T Categories



- UICC 8th ed. 2017 _ pT3



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Gender:	M			Signed Out:	5/29/2000
Physician(s):					

Final Diagnosis

- PARTS 1 THROUGH 8:**
- LUNG, LEFT UPPER LOBE; LOBECTOMY:
 - SQUAMOUS CELL CARCINOMA:
 - A. TUMOR SIZE: 6.5 CM X 3.2 CM X 4.8 CM.
 - B. HISTOLOGIC GRADE: T3.
 - C. EXTENT OF PRIMARY TUMOR:
 - TUMOR INVASES VISCERAL PLEURA.
 - TUMOR IS 1 CM FROM BRONCHIAL MARGIN OF EXCISION.
 - MARGINS OF EXCISION:
 - A. BRONCHIAL MARGIN OF RESECTION: NEGATIVE FOR TUMOR.
 - B. VASCULAR MARGIN OF EXCISION: NEGATIVE FOR TUMOR.
 - LYMPH NODES (HILAR):
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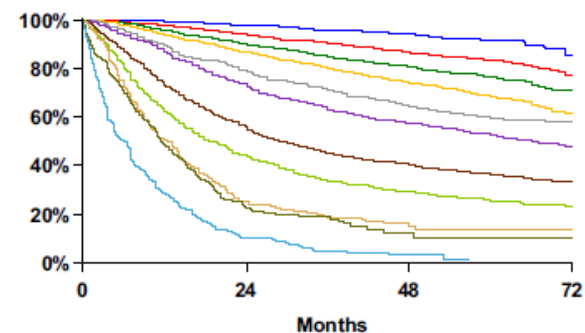
pT3N1

Electronically Signed Out

Stage groups and survival, lung cancer

TNM v8

	N0	N1	N2	N3	M1a any N	M1b any N	M1c any N
T1a	IA1	IIB	IIIA	IIIB	IVA	IVA	IVB
T1b	IA2	IIB	IIIA	IIIB	IVA	IVA	IVB
T1c	IA3	IIB	IIIA	IIIB	IVA	IVA	IVB
T2a	IB	IIB	IIIA	IIIB	IVA	IVA	IVB
T2b	IIA	IIB	IIIA	IIIB	IVA	IVA	IVB
T3	IIB	IIIA	IIIB	IIIC	IVA	IVA	IVB
T4	IIIA	IIIA	IIIB	IIIC	IVA	IVA	IVB



Proposed	Events / N	MST	24 Month	60 Month
IA1	68 / 781	NR	97%	92%
IA2	505 / 3105	NR	94%	83%
IA3	546 / 2417	NR	90%	77%
IB	560 / 1928	NR	87%	68%
IIA	215 / 585	NR	79%	60%
IIB	605 / 1453	66.0	72%	53%
IIIA	2052 / 3200	29.3	55%	36%
IIIB	1551 / 2140	19.0	44%	26%
IIIC	831 / 986	12.6	24%	13%
IVA	336 / 484	11.5	23%	10%
IVB	328 / 398	6.0	10%	0%

Stage and therapy in NSCLC

Stage	T	N	M	Surgery	Irrad.	Chemo.
I/A1,2,3	T1a,b,c	N0	M0	+	- (vagy: + !)	-
I/B	T2a	N0	M0	+	- (vagy: + !)	-
II/A	T2b	N0	M0	+	- (vagy: + !)	+
II/B	T1a-2b	N1	M0	+	-	+
	T3	N0	M0	+	+ / -	+
III/A	T1a-2b	N2	M0	+	+	+
	T3	N1	M0	+ / -	+ / -	+
	T4	N0 – N1	M0	+ / -	+ / -	+
III/B	T1a-2b	N3	M0	-	+	+
	T3-4	N2	M0	+ / -	+	+
III/C	T3-4	N3	M0	-	+	+
IV/A	T1- 4	N1- 3	M1a,b	-	+ / -	+
IV/B	T1-4	N1-3	M1c	-	-	+

Types of histological/surgical pathology report

- (a) descriptive (narrative),
- (b) standardised (synoptic, form-based),
- (c) structured (electronic, machine-readable XML – extensible markup language).

CRC slides and narrative report

1993



Elkezesi IDO: 11.25.
Anyag (localisatio): colon

WHO kód: 29000, 29070
Fagyasztásos szövettani vizsgálat, dg.:

13 cm hosszágú colon részlet. Középén 5 cm legnagyobb Ø-jű felhányt szélű kifelé nyúló kifelé nyúló van. Az egyik resztios vonaltól 3, a másiktól 3,5 cm-re kezdődik az elváltozás. A metszlapon a bél fal kissé megvastagodott, úgytűnik tumorosan infiltrált. A környező zsírszövetben 4 db borsnyi és kisebb nyirokcsomó van.. A vastagbéllel összefüggő 15x20 cm nagyságú csipleszt részletben gócos elváltozás nincs.

Szövettani leírás: 1993.11.29./CSL

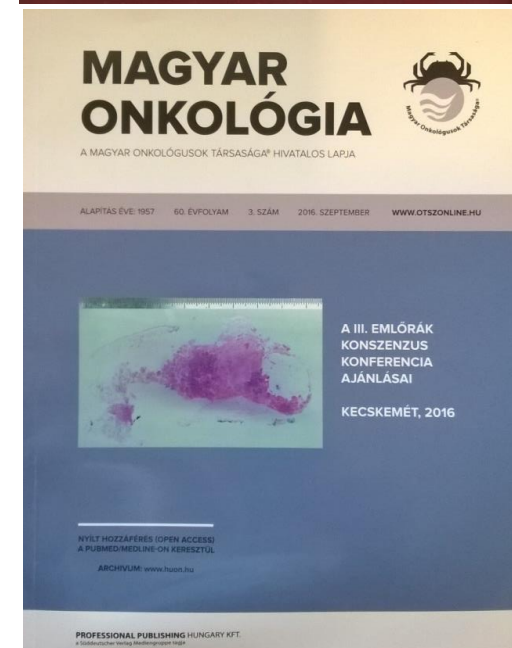
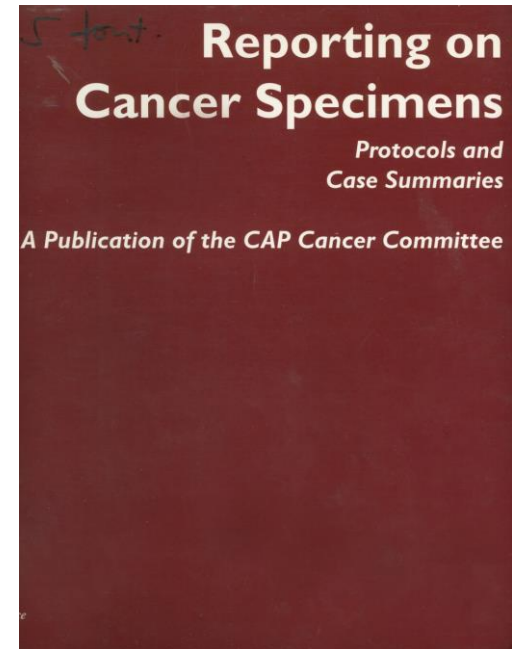
A preparátumban a vastagbél szöveti szerkezete csak a széli részen ismerhető fel. A preparátum közepén a lumenbe domborodó és a bél falat teljesen infiltráló daganat látható. A tumor változatos alakú és nagyságú mirigy-szerű lumeneket, helyenként cribriform mintázatot alkot. A daganatsejtek az atypia, polymorphia minden jelét mutató, de viszonylag jól differenciált magas, illetve köbös, hengerhám jellegű sejtek duzzadt magvakkal, melyek prominens nucleolust tartalmaznak, és köztük számos osztódó alak előfordul. Nyák secretio csak elvétve látható. A stromában közepes fokú lymphocytás, plasmasejtes infiltratio van. A vizsgált nyirokcsomókban daganatszövet nincs.

Dg.: Adenocarcinoma tubulare coli Duker B, Grade I.

Reporting on cancer specimens

Standardized reporting

- Guidelines
 - College of American Pathologists (www.cap.org)
 - Association of the Directors of Anatomic Pathology (www.adasp.org)
 - Royal College of Pathologists (www.rcpath.org)
 - Ackerman's Surgical Pathology Book - MSKCC (Elsevier, 10th Ed. 2011)
 - Stanford School of Medicine (www.surgpathcriteria.stanford.edu)
 - NIO
 - 3rd Breast Cancer Consensus Conference, Kecskemét 2016
- The report should include those parameters the are
 - *necessary to determine the stage of the disease*
 - *required to make therapeutic decisions*
 - *of prognostic and/or predictive significance*



CRC Guideline

NIO

Resection type

Tumor type

Histological grade

Tumor localization

Pre-existing polyp

Tumor size

Local invasion

Serosal surface

Vascular invasion

Perineural invasion

Tumor budding

Surgical margins

Polyps distant from the carcinoma

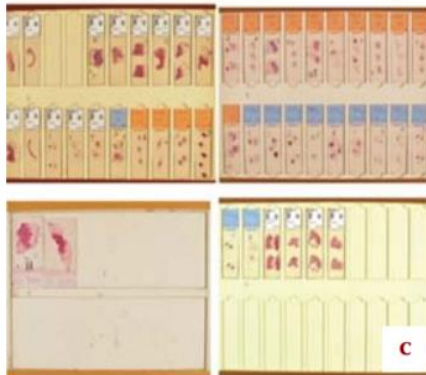
Lymph node status

pTNM stage

Modified Astler-Coller stage

Other/notes

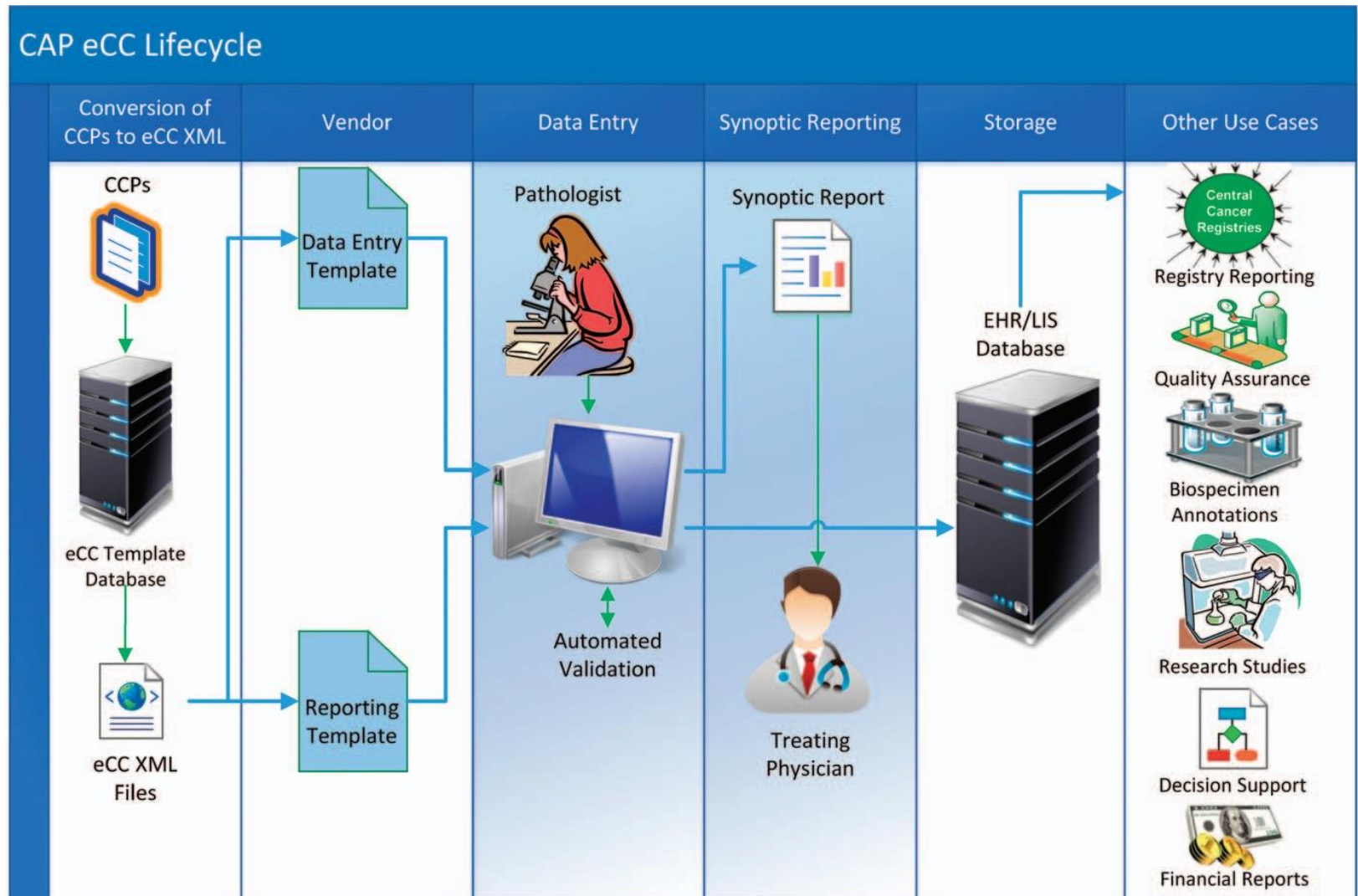
Pathological processing of a rectal adenocarcinoma resection surgical specimen and standardised report.



Rectal resection
Adenocarcinoma
Histological grade: poorly differentiated
Tumour localisation: upper third of the rectum
Macroscopic appearance: ulcerative infiltrative
Pre-existing polyps: not identified
Tumour size:
-largest diameter: 7 cm
-maximum tumour thickness: 2.5 cm
Local invasion: the tumour infiltrates the non-peritonealized perirectal fat tissue and the subserosal fat tissue
Serosal surface: infiltrated
Tumour perforation: present
Vascular invasion (lymph and blood vessel): present
Perineural invasion: present
Expressed tumour budding: present
Surgical margins: completely tumour-free. The tumour is located at a 1.2 mm depth from the nearest circumferential margin. Lateral (aboral and oral) margins as specified by macroscopic data
Non-neoplastic colonic mucosa: without any significant histopathological abnormalities
Polyps distant from the carcinoma: not identifiable
Lymph node status (the number of metastatic lymph nodes compared to the number of total examined lymph nodes): 18/29
-largest metastasis: 1.5 cm
-rupture of the tumour capsule: present
Two tumourous nodules present in the perirectal fat tissue, suggesting blood vessel invasion or tumour deposit
Serosal metastasis present
pTNM status: pT4a N2b M1b

d

Ecc-electronic cancer checklist



Pathologists are “Diagnostic Oncologists”

- Anatomical pathologists are society’s diagnostic oncologists
- Activities cut across the entire cancer care continuum from prevention/screening to diagnosis to prognosis/prediction to disease monitoring
- At least 60% of the average pathologist’s time relates to cancer related activities
- In Canada more pathologist FTEs are devoted to the cancer system than medical oncologists or radiation oncologists
- Information collected by pathologists is utilized by downstream users (including oncologists, cancer registrars, system planners, etc) to impact patient outcomes

CenterNews

SEPTEMBER 2012

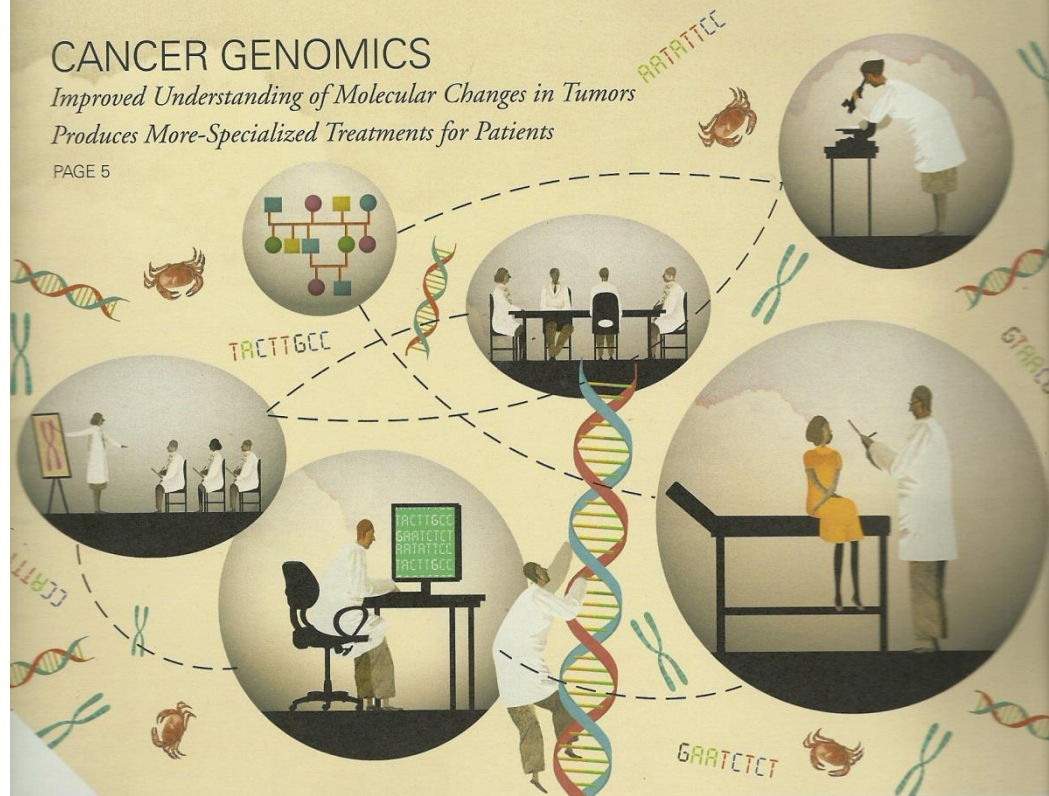
MEMORIAL SLOAN-KETTERING CANCER CENTER

CANCER GENOMICS

Improved Understanding of Molecular Changes in Tumors

Produces More-Specialized Treatments for Patients

PAGE 5



PLUS ALEXANDER RUDENSKY APPOINTED IMMUNOLOGY PROGRAM CHAIR 2 MSKCC CELEBRATES NATIONAL CANCER SURVIVORS DAY 2 NIKOLA PAVLETICH ELECTED TO THE NATIONAL ACADEMY OF SCIENCES 11 PHYSICIAN-SCIENTIST SCOTT ARMSTRONG JOINS MSKCC 15 EXPERIMENTAL THERAPEUTICS CENTER MARKS TEN-YEAR ANNIVERSARY 20



2



2



11



15



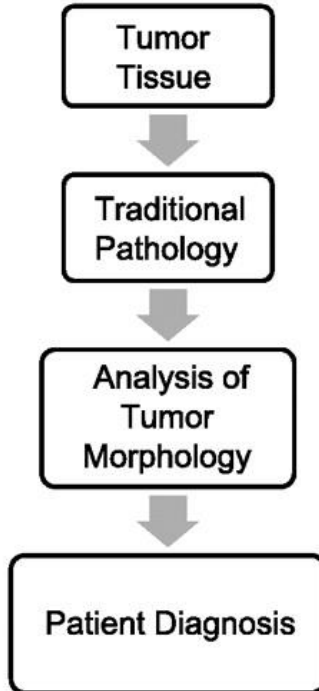
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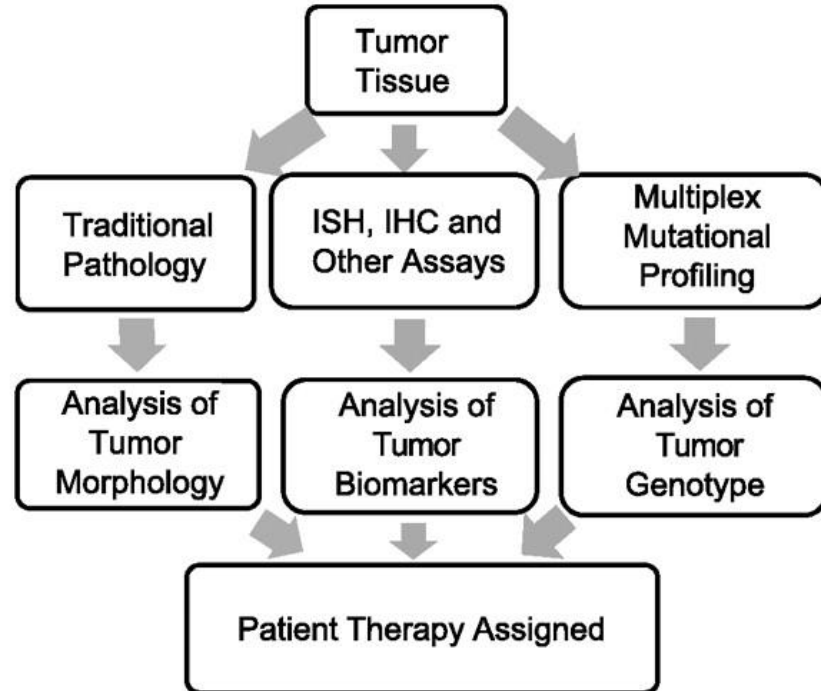
Memorial Sloan-Kettering
Cancer Center

Role of pathology in oncology

A Traditional Tumor Analysis



B Integrated Molecular Analysis



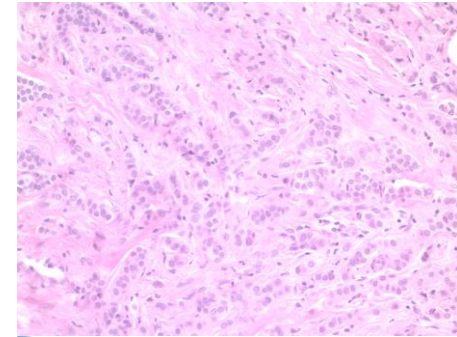
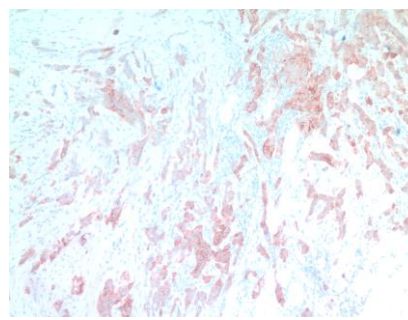
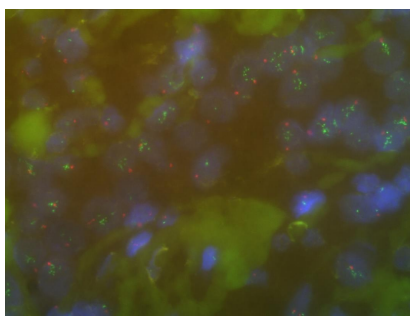
Pao W et al. Clin Cancer Res 2009;15:5317-5322

Practical use of molecular pathology

- 1. Ancillary study to support/make the diagnosis**
- 2. Define genetic abnormalities that are associated with prognosis or are predictive for effectiveness of therapy**
 - Targeted therapy**

Diagnostic methods in molecular pathology

- Immunohistochemistry
 - Detection of proteins
- In situ hybridization
 - Longer DNA sequences, translocations, amplification
- PCR-based methods
 - Smaller abnormalities, mutations of DNA
- Sequencing
- NGS – next generation sequencing
 - Sensitive method, simultaneous testing of several genes from the samples of multiple patients.



Gene 1
Gene 2
Gene 3
Gene 4
Gene 5
Gene ...
Gene n

RNA



Protein 1
Protein 2
Protein 3
Protein 4
Protein 5
Protein ...
Protein n

Morphology



PCR
ISH
Sequencing

Gene-expression
RT-PCR

Immunohistochemistry

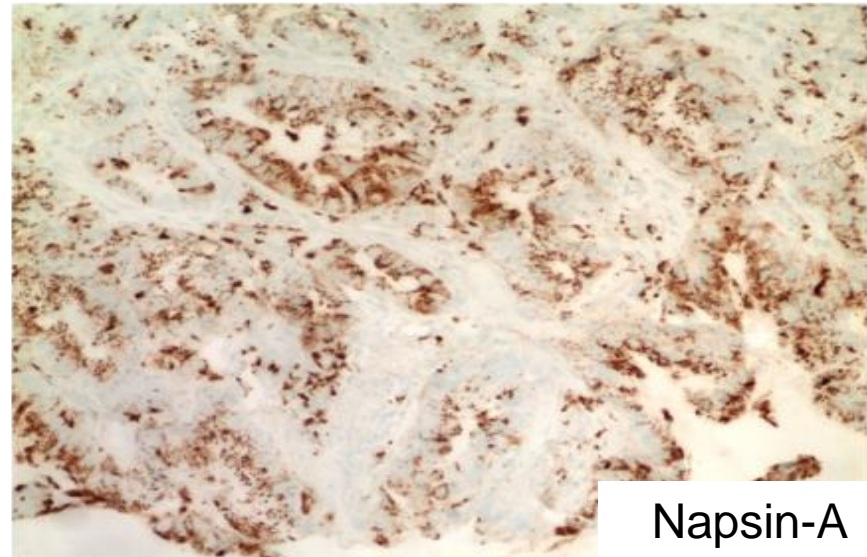
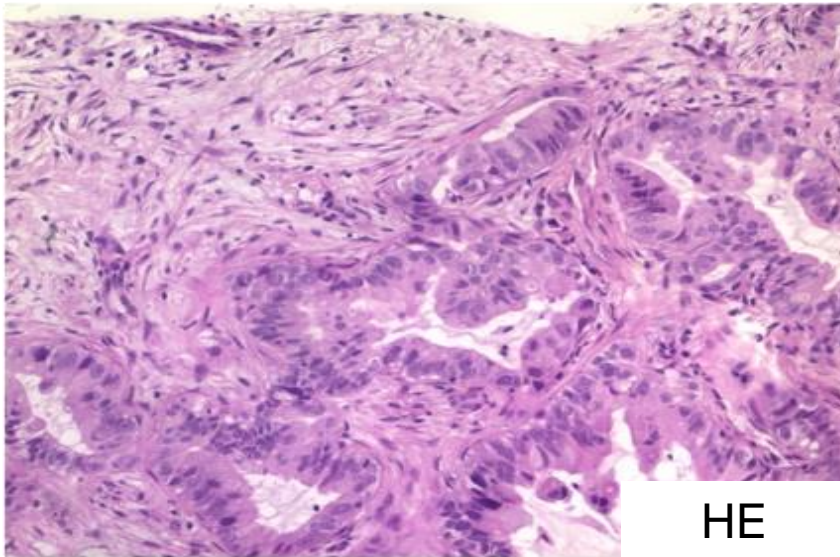
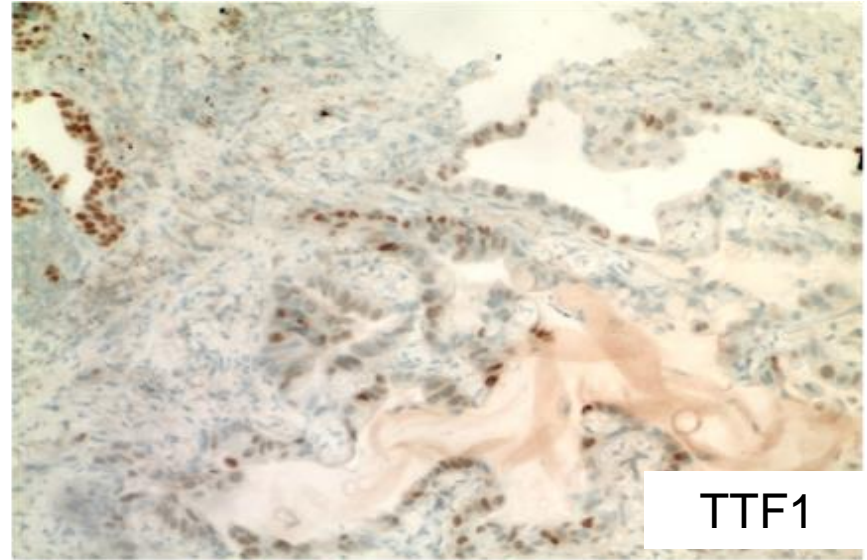
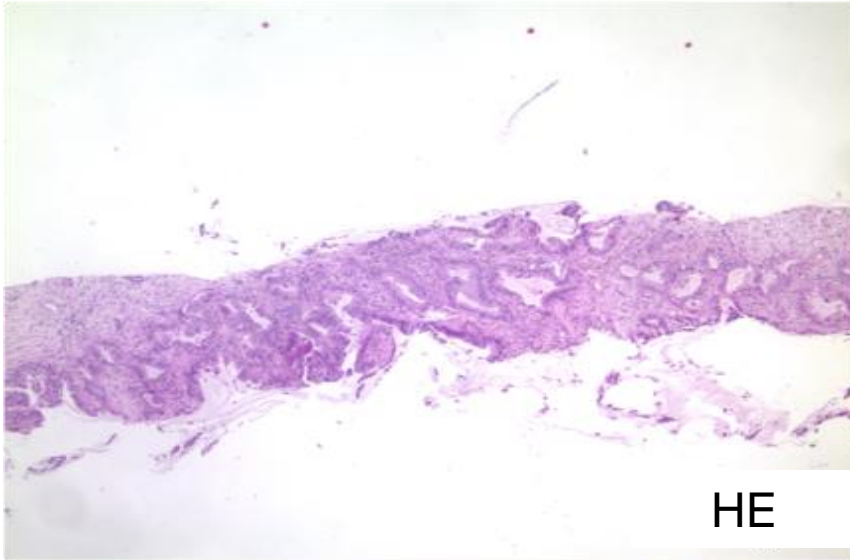


Molecular methods

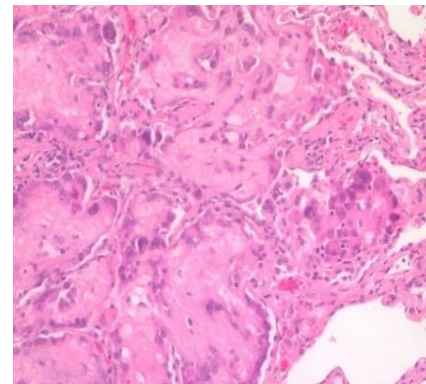
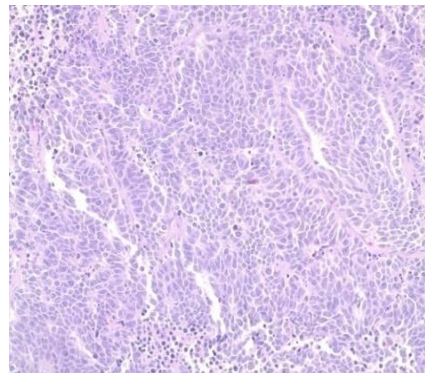
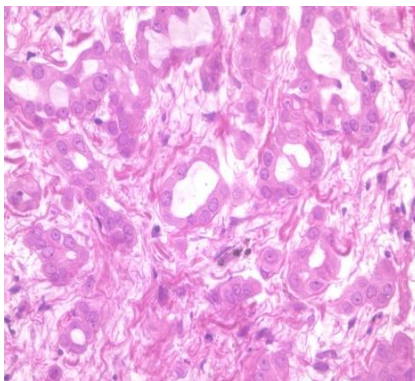
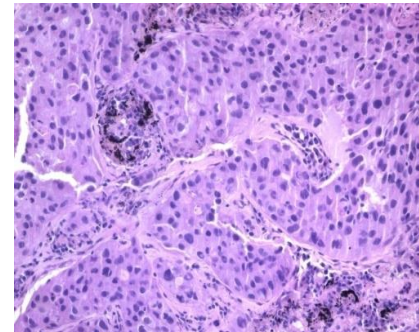
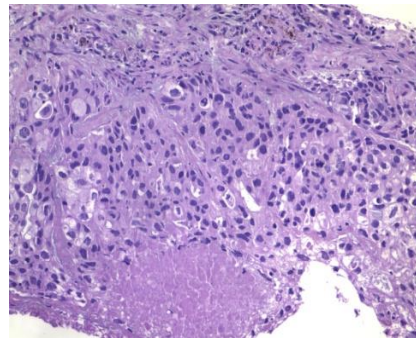
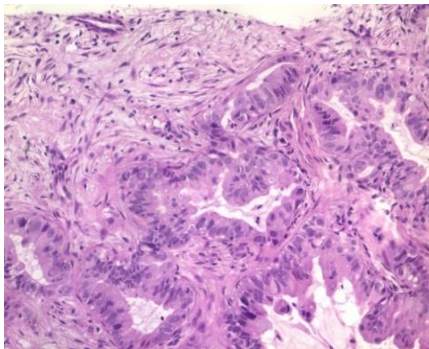
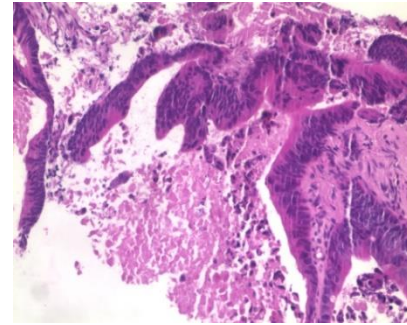
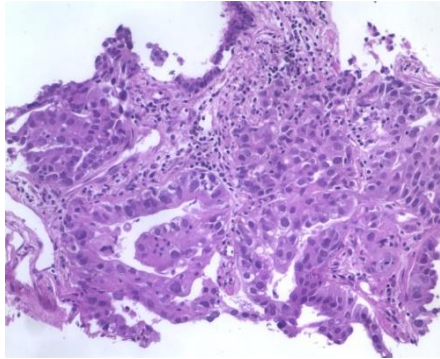
Practical use of molecular pathology

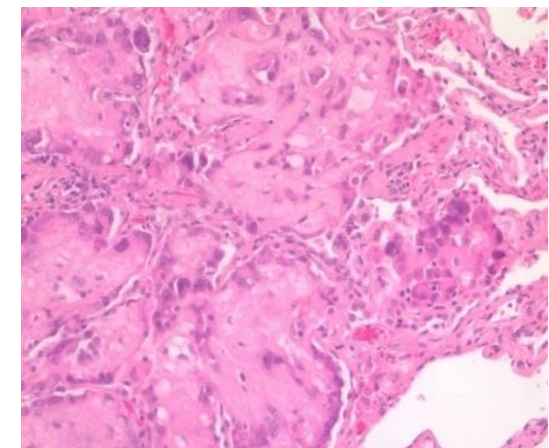
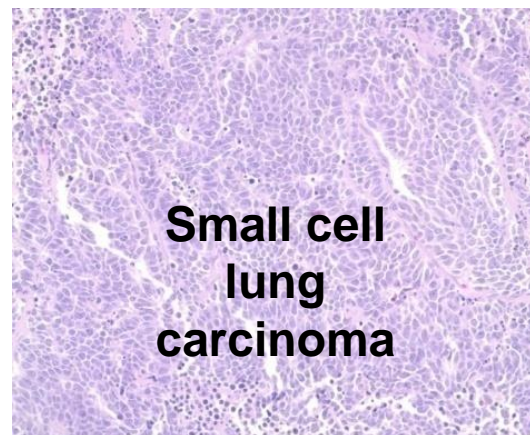
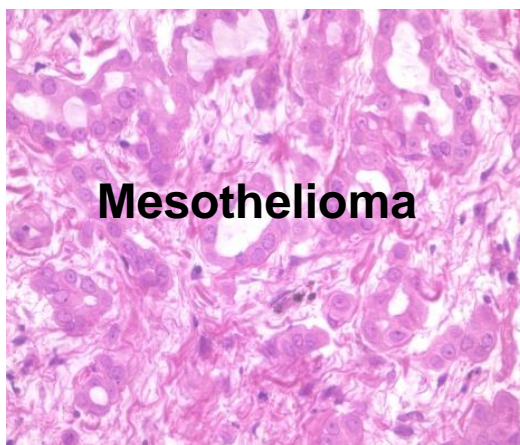
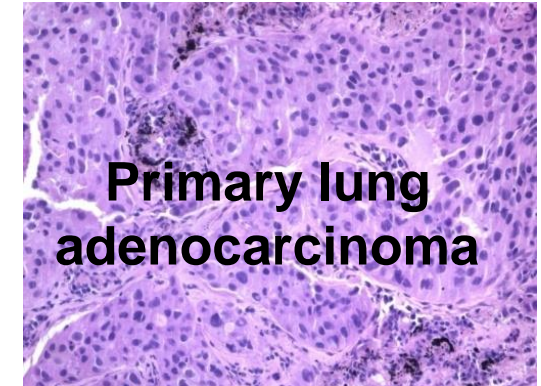
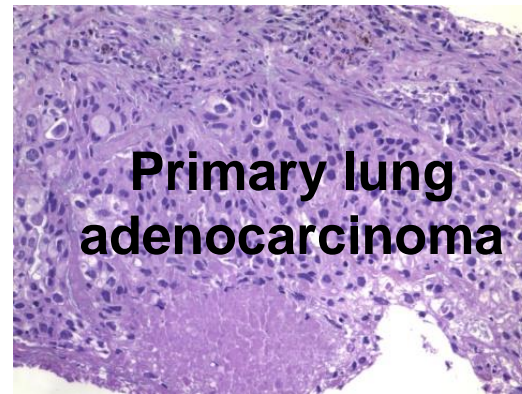
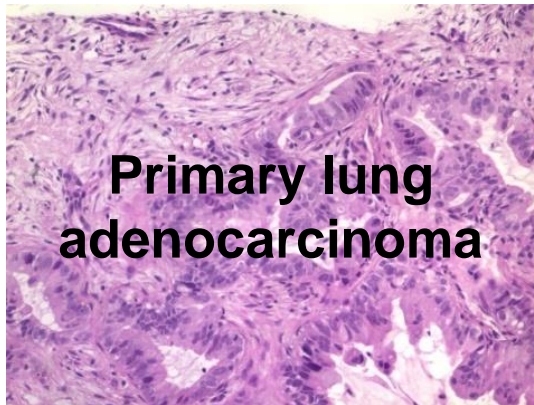
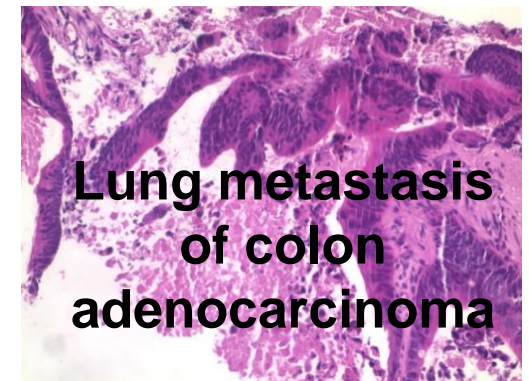
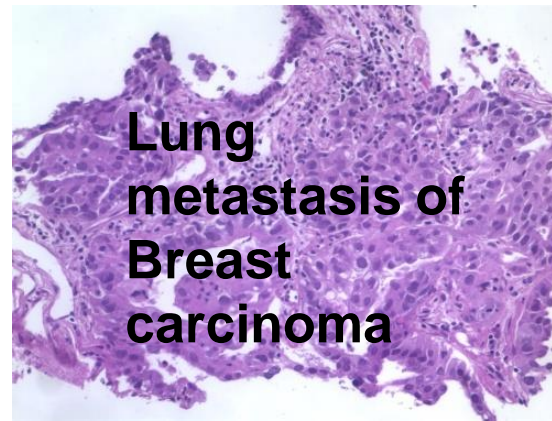
- **Ancillary study to support/make the diagnosis**
 - Detection of protein/genetic abnormalities specific for a tumor type
 - Immunohistochemistry – tumor type specific protein expression
 - Breast cancer: ER, GATA3
 - Colon adenocarcinoma: CDX2
 - Lung adenocarcinoma: TTF1, Napsin-A
 - In situ hybridization, RT-PCR, sequencing – tumor type specific genetic abnormalities
 - Translocations in sarcomas and lymphomas
 - » Ewing sarcoma: t(11;22) → EWS-FLI1
 - » Synovial sarcoma: t(X;18) → SYT-SSX1
 - » Epithelioid hemangioendothelioma: t(1;3) → WWTR1-CAMTA1

Lung adenocarcinoma core biopsy

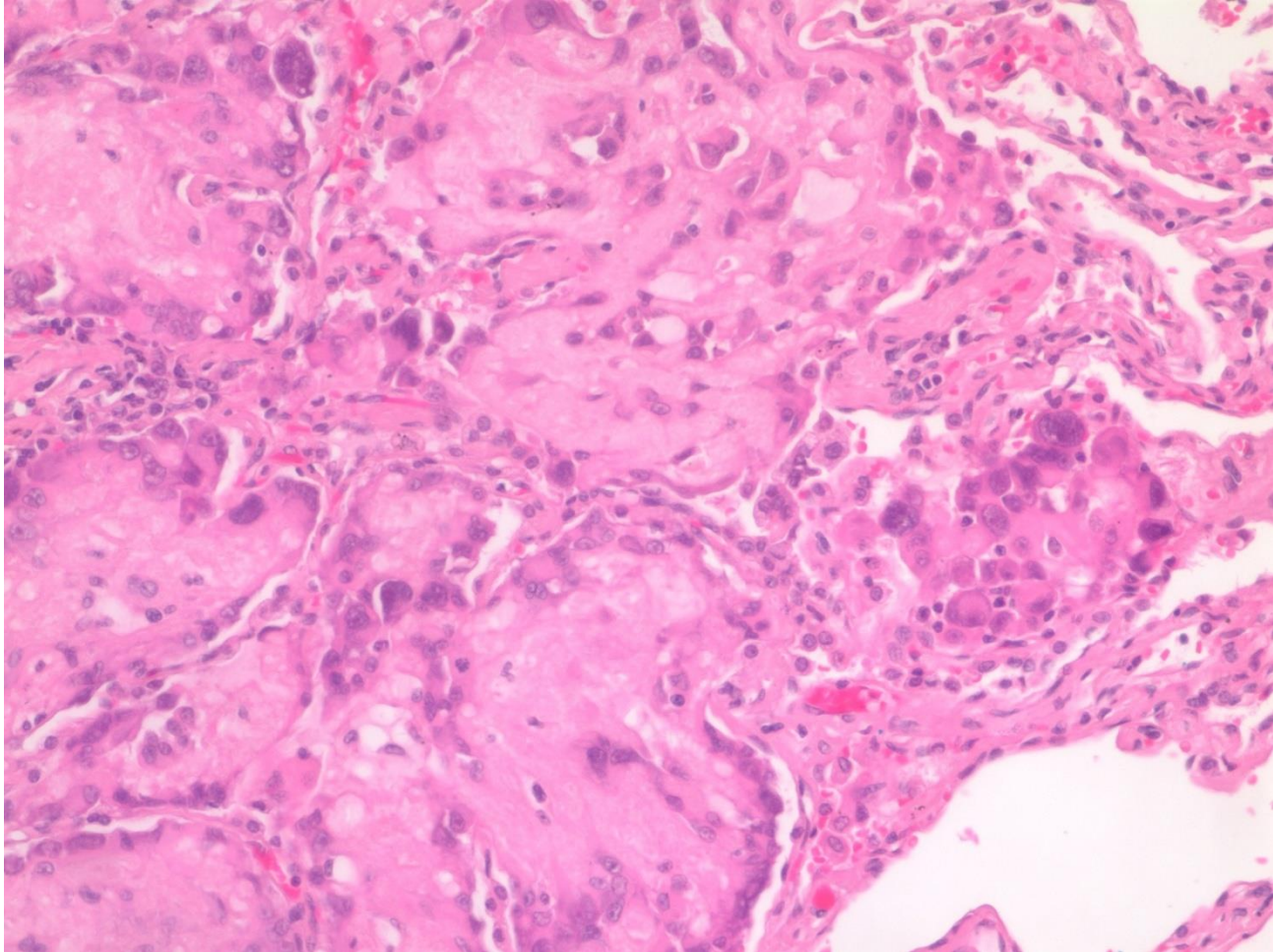


Lung tumors, HE

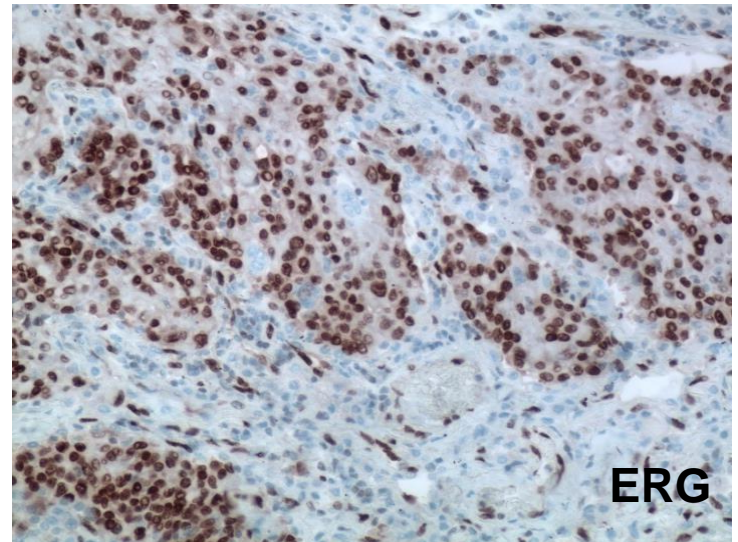
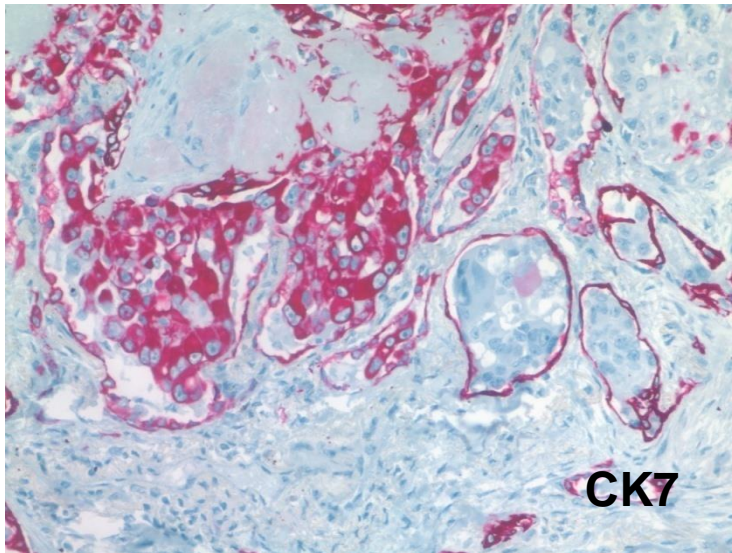
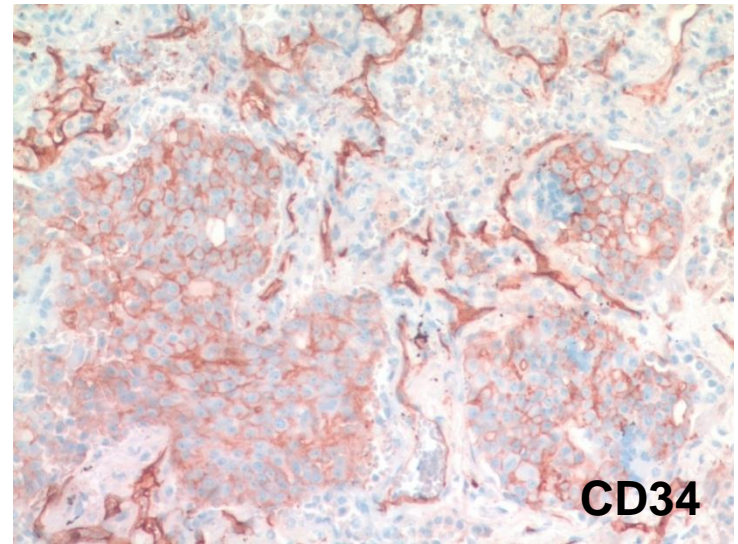
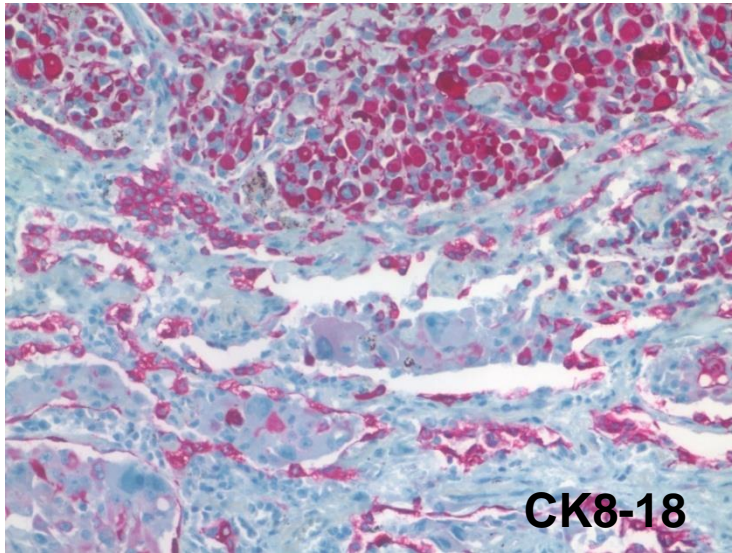




Immunohistochemistry in rare lung tumors



Immunohistochemistry



Epithelial markers

Vascular markers

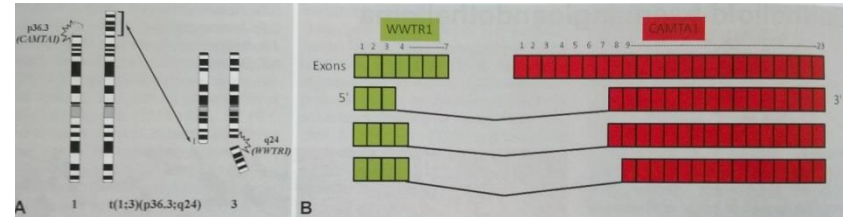
Epithelioid hemangioendothelioma

- Malignant vascular tumor, with relatively indolent behavior
- Soft tissue, bone, skin, lung and other parenchymal organs
- *Rare tumor*, wide age range, various anatomical locations, multifocality
 - *Differential diagnosis is wide*

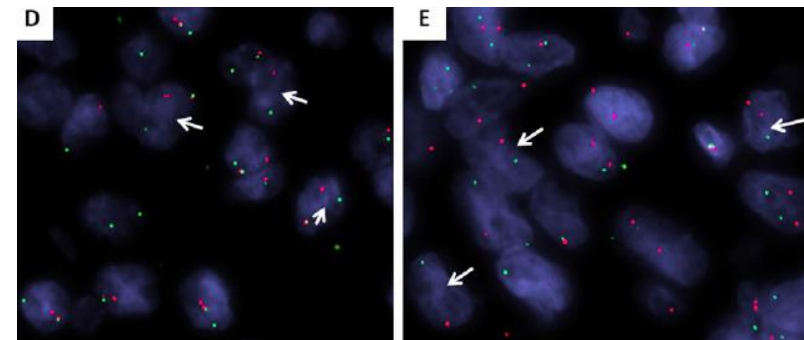
Epithelioid hemangioendothelioma

Fusion genes

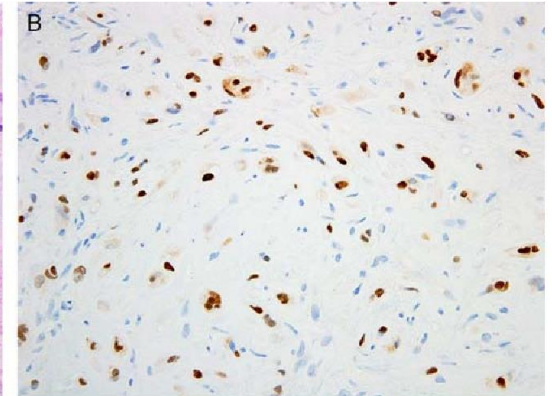
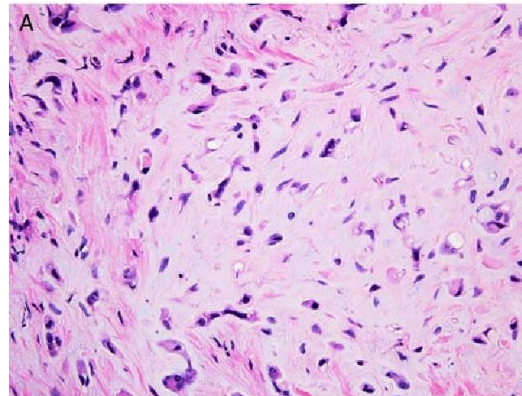
- 1. t(1;3) → **WWTR1-CAMTA1** (majority)
 - WWTR1 (TAZ): transcription co-activator
 - CAMTA1: calmodulin-binding transcription activator
- 2. t(11;X) → **YAP1-TFE3** (very rare)
 - YAP1: transcription co-activator
 - TFE3: transcription factor
- Detection:
 - RT-PCR
 - **FISH**
 - **Immunohistochemistry**



WHO 2015



AJSP.2015;39:132-139



Practical use of molecular pathology

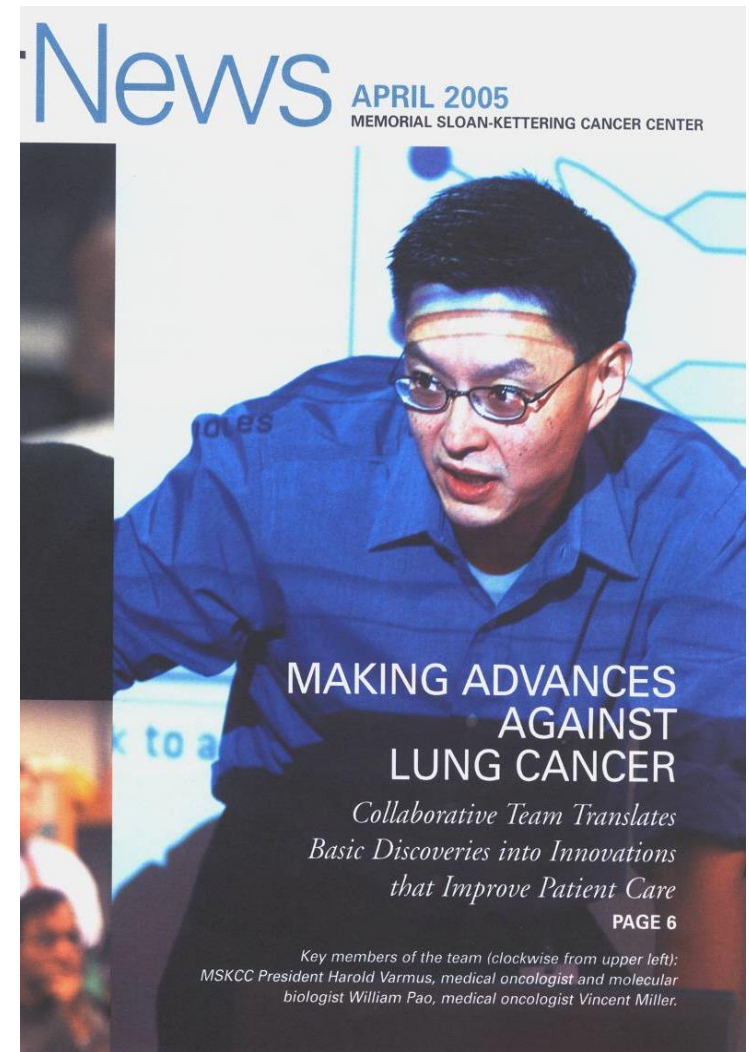
Define genetic abnormalities that are associated with prognosis or are predictive for effectiveness of therapy

- Targeted therapy**

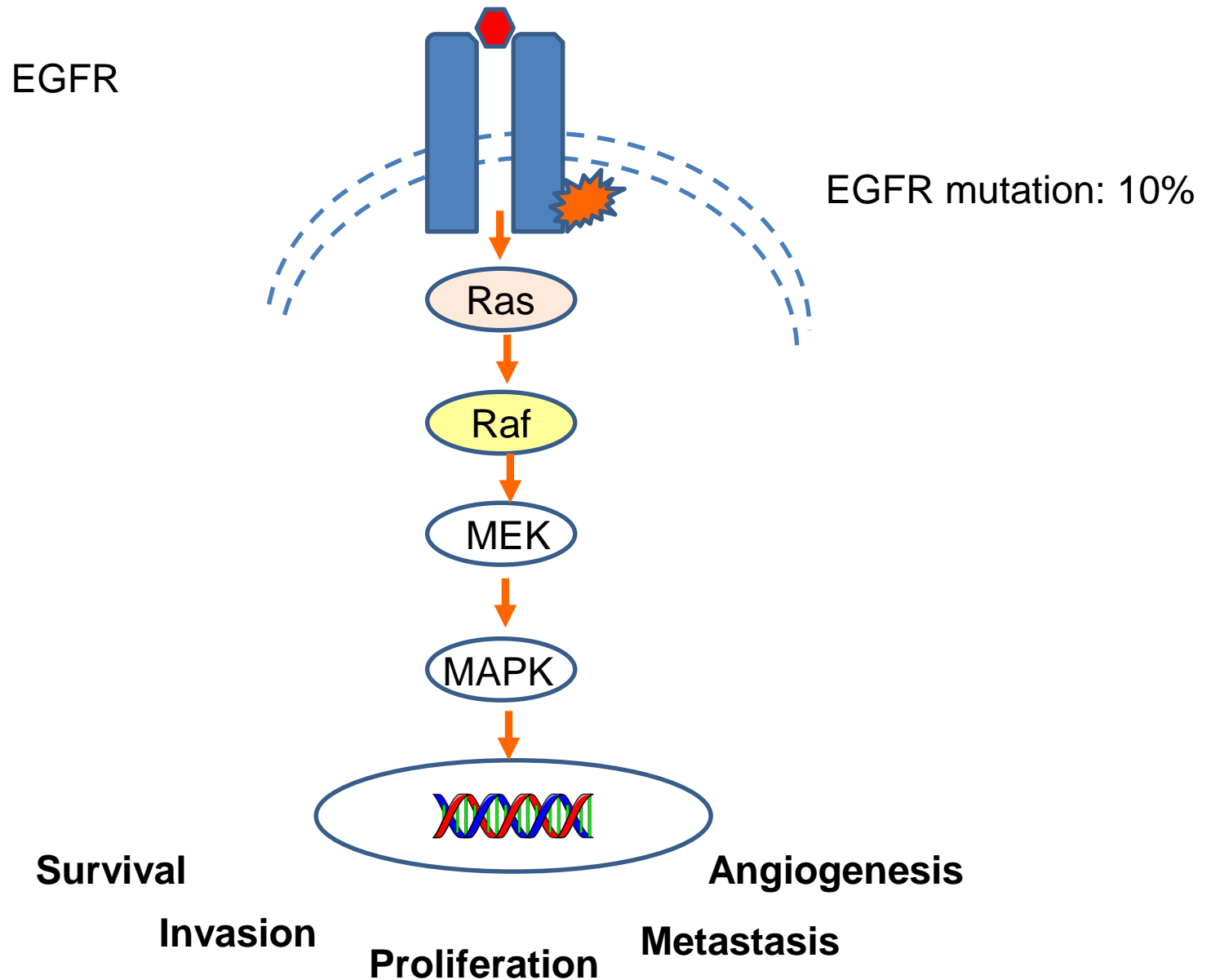
Tumour type	Gene	Abnormality	Drug/ indication
Lung adenocarcinoma	EGFR	mutation	EGFR TKI sensitivity
	RAS	mutation	EGFR TKI resistance
	ALK	translocation	Crizotinib sensitivity
	ROS1	translocation	Crizotinib sensitivity
Colon adenocarcinoma	KRAS	mutation	Anti-EGFR resistance
	NRAS	mutation	Anti-EGFR resistance
	BRAF	mutation	Negative prognostic factor
Melanoma	BRAF	mutation	Vemurafenib sensitivity
Breast carcinoma	ERBB2 (HER2)	amplification	Trastuzumab, Lapatinib sensitivity

Epidermal growth factor receptor (EGFR) gene mutations in lung adenocarcinomas

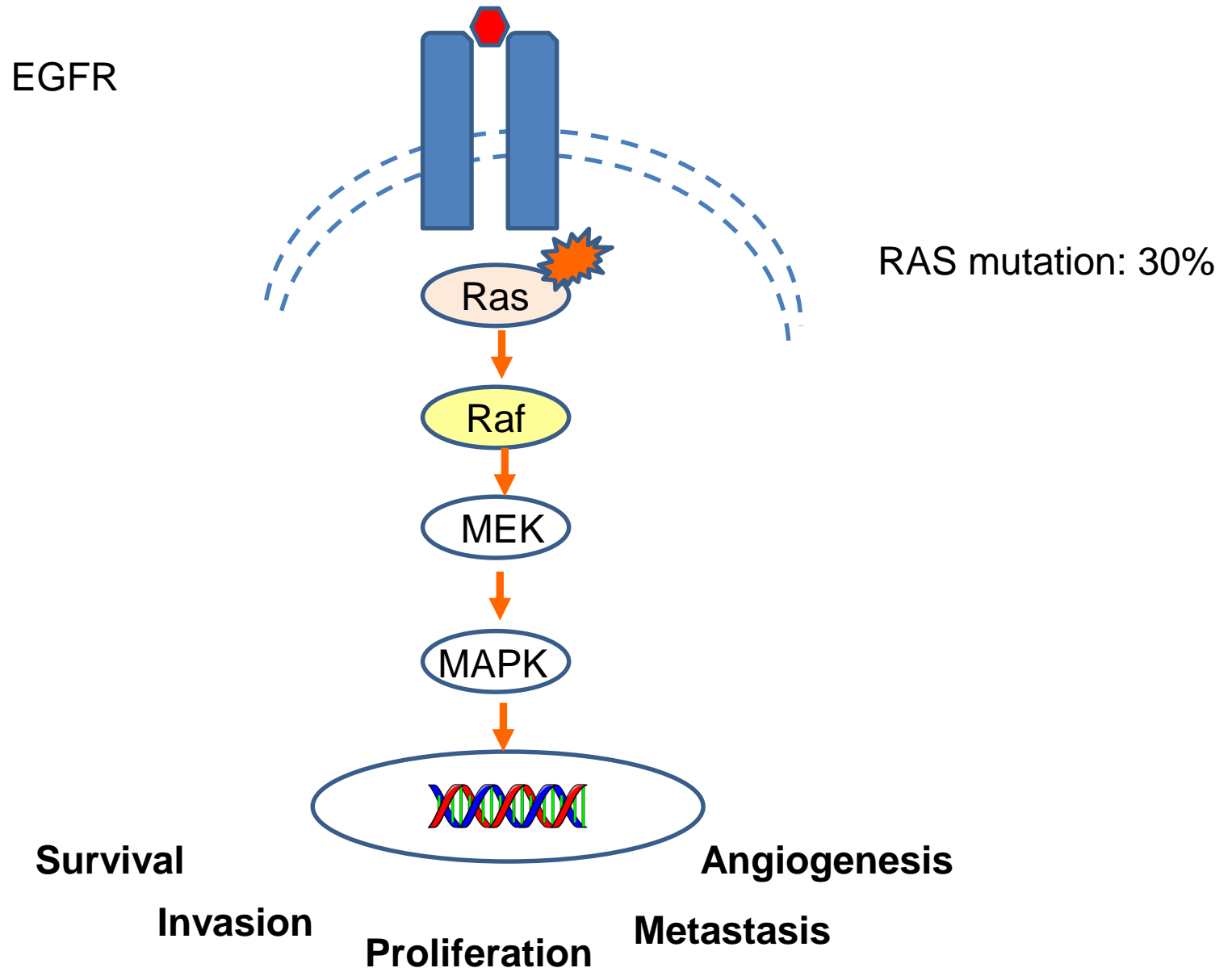
- Lynch, Paez, *Pao* - 2004
 - Somatic mutations of EGFR gene in exons 18-21-ben in lung NSCLC correlate with response to EGFR tirosine kinase inhibitor (TKI) therapy(erlotinib, gefitinb)
 - Female
 - Adenocarcinoma
 - Non-smokers



EGFR signaling pathway alteration in lung adenocarcinoma

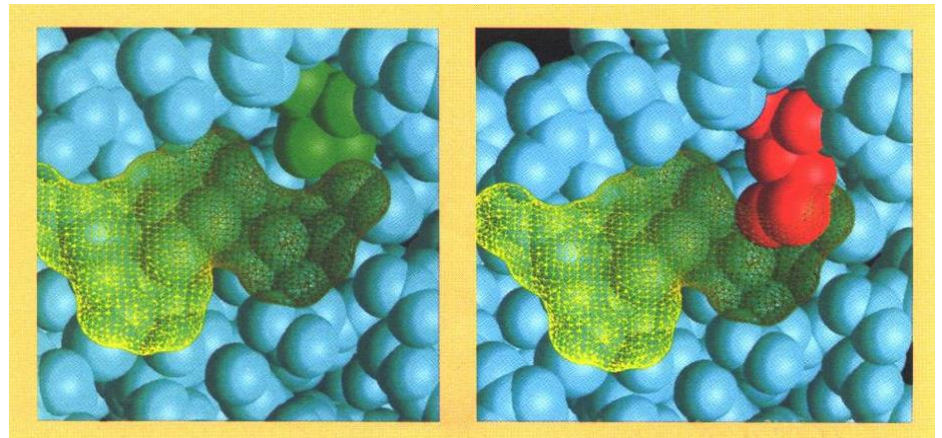


EGFR signaling pathway alteration in lung adenocarcinoma



EGFR mutations in lung adenocarcinoma

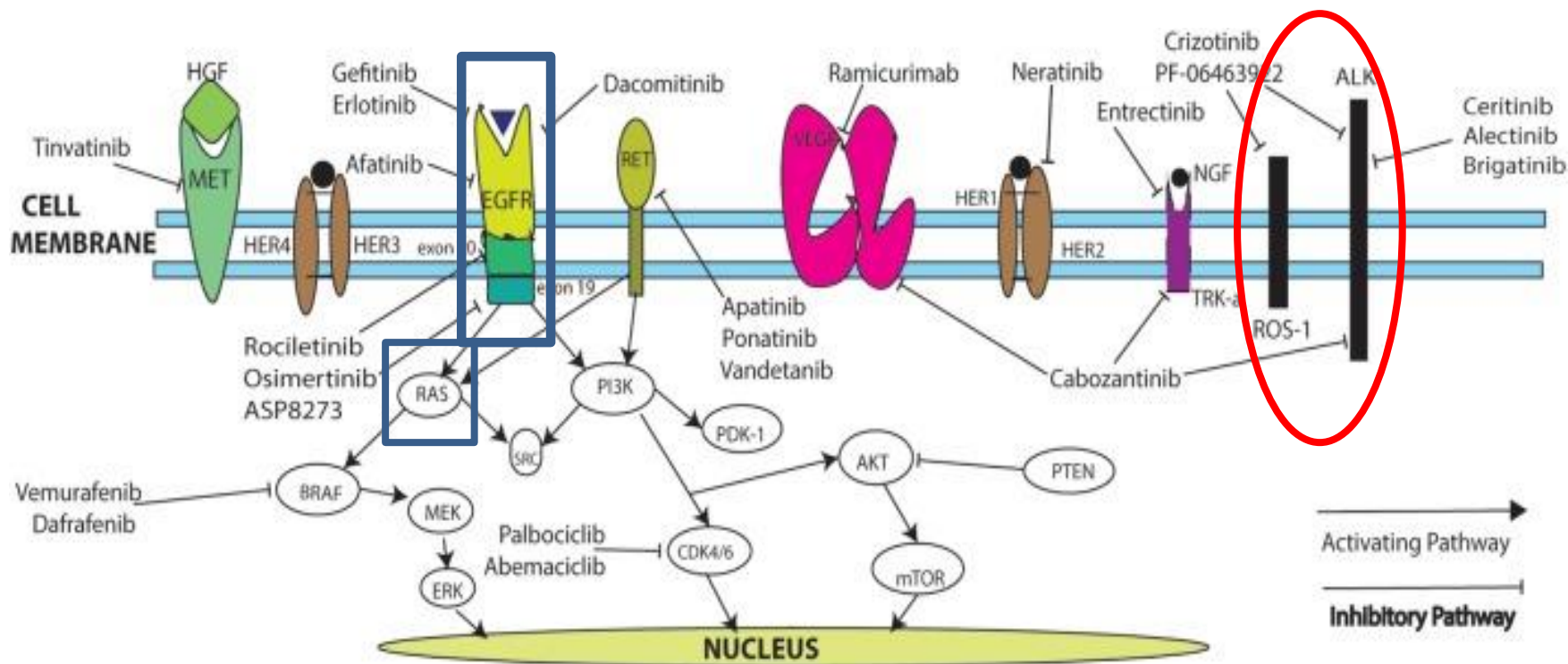
- Patients with EGFR mutations and treated with TKI show longer survival
- Resistance to TKI therapy
 - Primary:
 - KRAS exon 2 mutations (exclusive with az EGFR mutations)
 - Braf, ErbB2
 - Secondary:
 - EGFR e20 (T790M)
 - Met amplification



Main genetic abnormalities in lung cancer

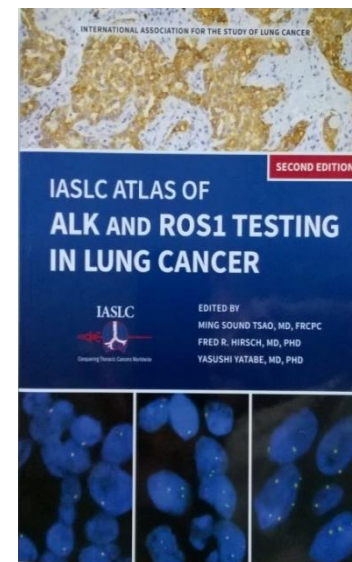
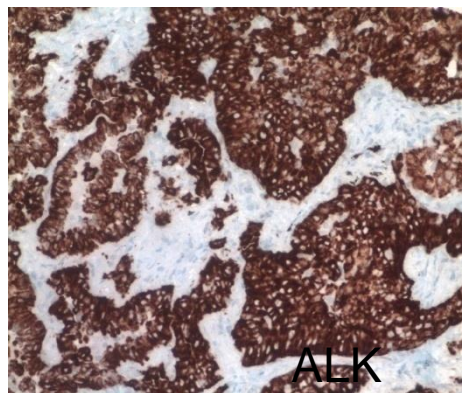
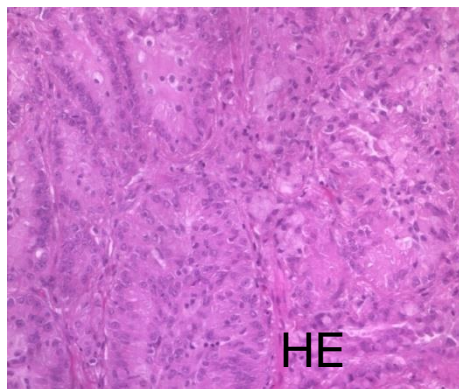
Gene abnormality	SCLC (%)	ACA (%)	SCC (%)
Mutation			
BRAF	0	< 5	0
EGFR Caucasian	< 1	10-20	< 1
Asian	< 5	35-45	< 5
ERBB2/HER2	0	< 5	0
KRAS Caucasian	< 1	15-35	< 5
Asian	< 1	5-10	< 5
PIK3CA	< 5	< 5	5-15
RB	> 90	5-15	5-15
TP53	> 90	30-40	50-80
Amplification			
EGFR	< 1	5-10	10
ERBB2/HER2	< 1	< 5	< 1
MET	< 1	< 5	< 5
MYC	20-30	5-10	5-10
FGFR1	< 1	< 5	15-25
Rearrangement			
ALK	0	5	< 1
RET	0	1-2	0
ROS1	0	1-2	0
NTRK1	0	< 1	0
NRG1	0	< 1	0

Targetable signaling pathways in nonsquamous NSCLC



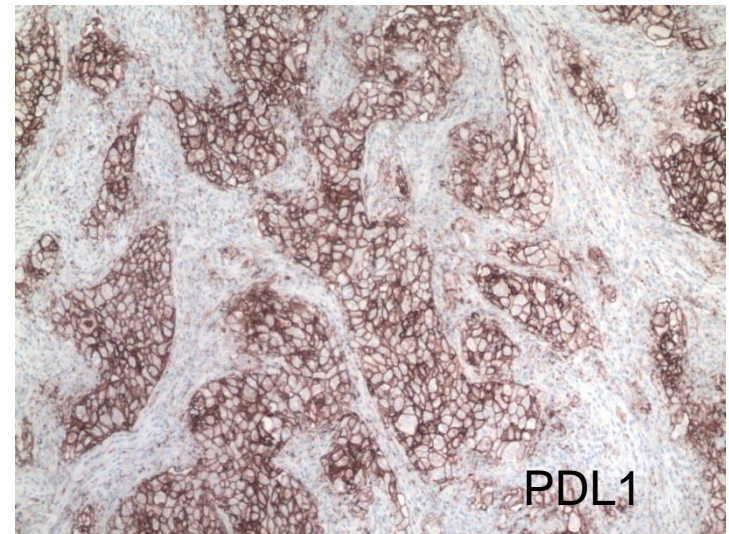
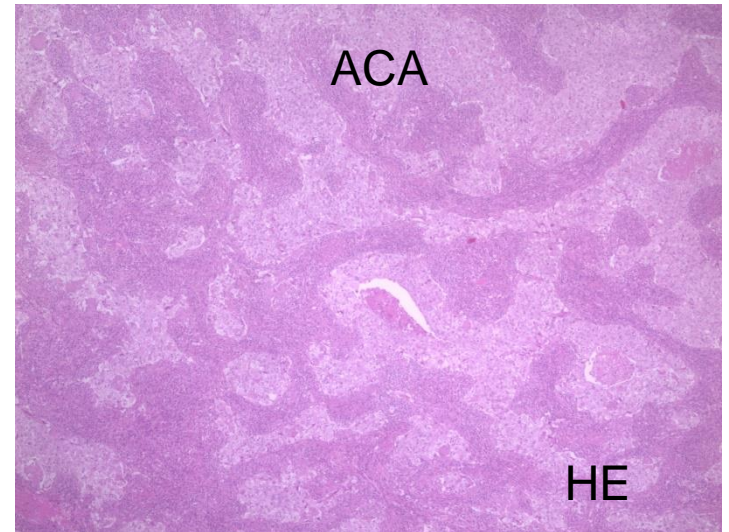
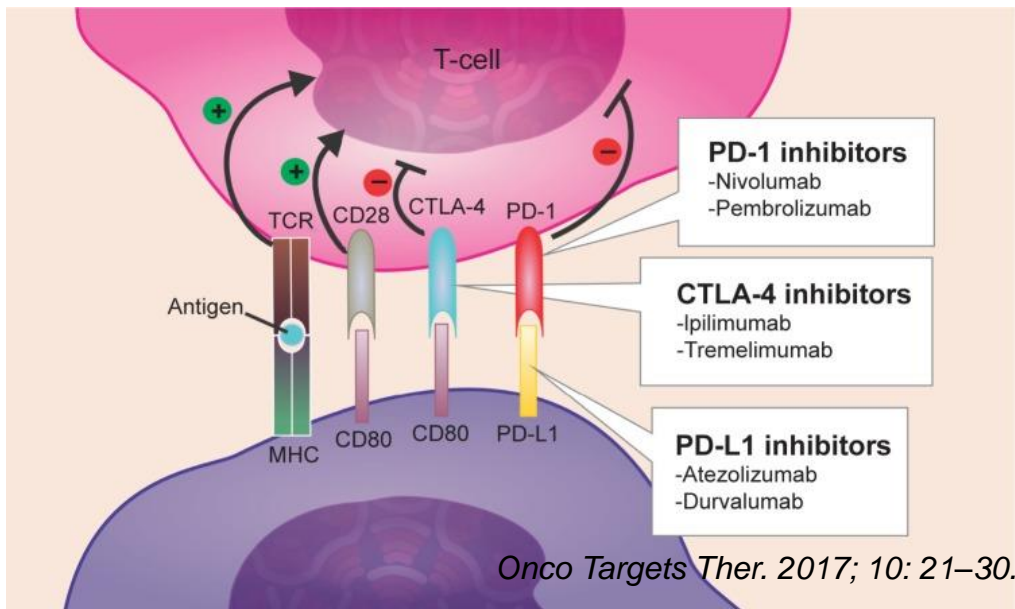
ALK és ROS1 gene rearrangements in lung adenocarcinoma

- EML4 (enichoderm microtubule-associated protein-like 4) 2p21 and ALK (anaplastic lymphoma kinase) 2p23 genes fusion
- 5% of lung adenocarcinomas
 - Younger age, non smoker
 - Signet ring cell, solid, solid, cribriform
- ALK inhibitor (crizotinib) therapy effective
- Detection
 - RT-PCR (fresh sample), sequencing
 - FISH (gold standard, at least 50-100 cells)
 - IHC (screening or diagnostic with validated antibodies)
- Reactive oxygen species 1 – ROS1 gén
- Tyrozine kinase receptor protein, similar to ALK
- 1-2% of lung adenocarcinomas (ROS1-CD74 fusion most often)
 - Non smoker females
 - No correlations with histological types
- ROS1 inhibitor (crizotinib) therapy effective
- Detetction:
 - RT-PCR (fresh sample), sequencing
 - FISH (gold standard, at least 50-100 cells)
 - IHC (screening or diagnostic with validated antibodies)

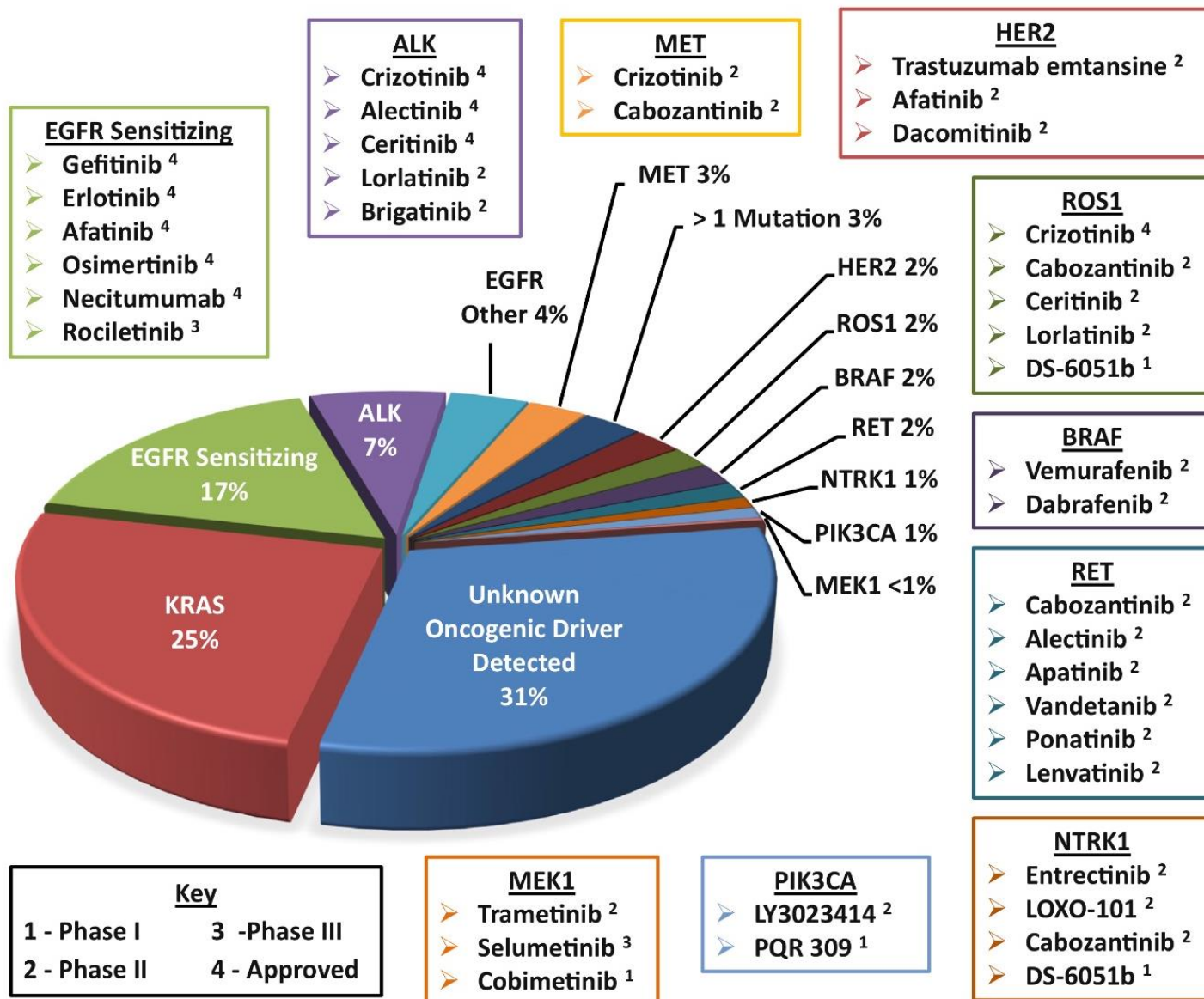


Immunotherapy

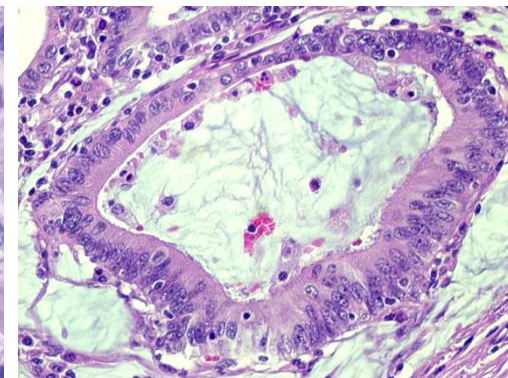
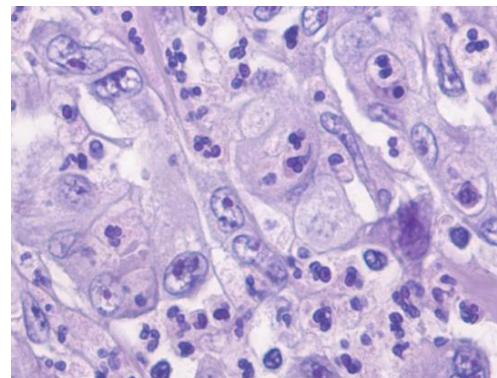
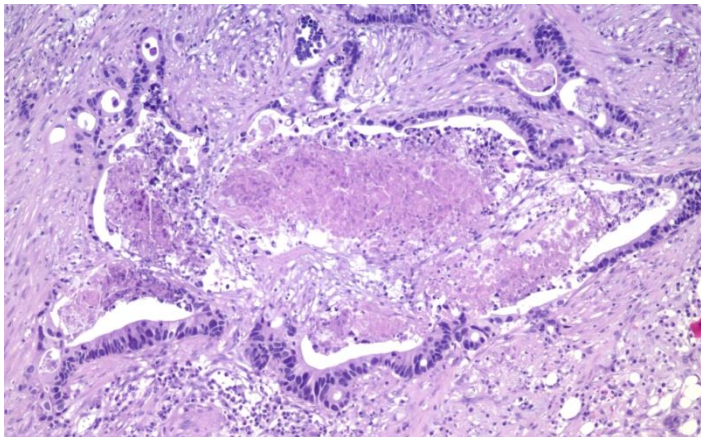
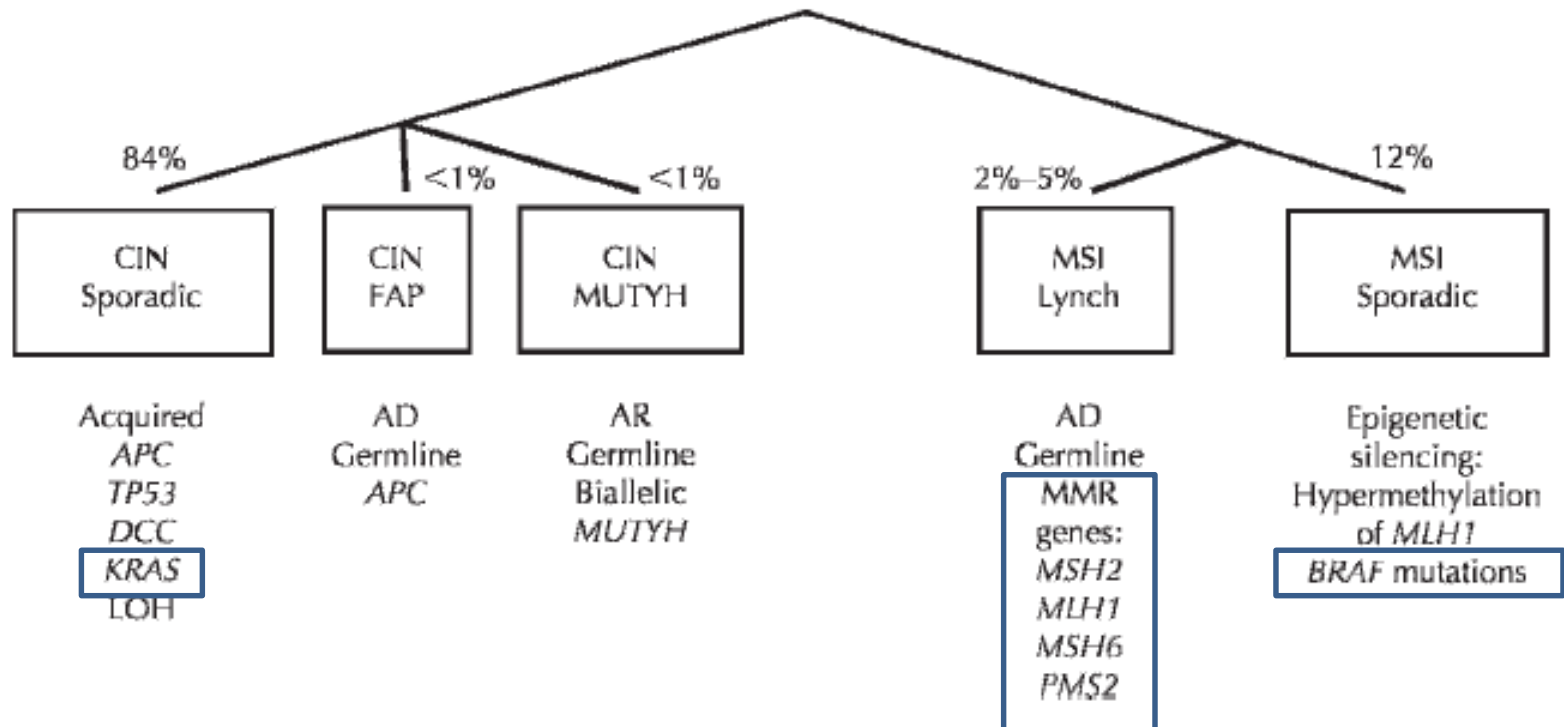
PD1-PDL1 inhibition in NSCLCs



Biomarker: PDL1 expression - IHC



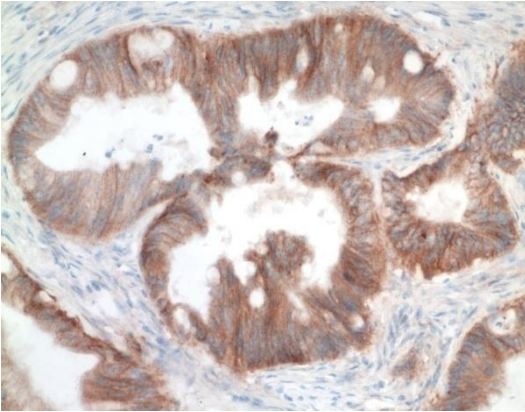
Colorectal cancer molecular classification



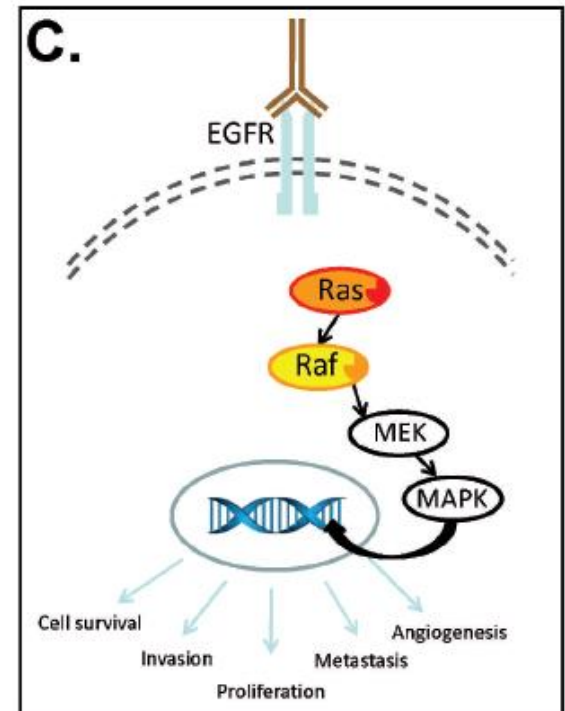
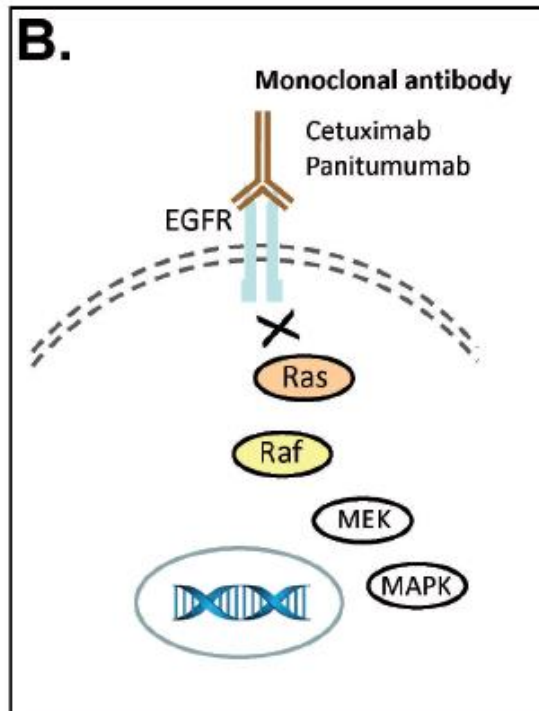
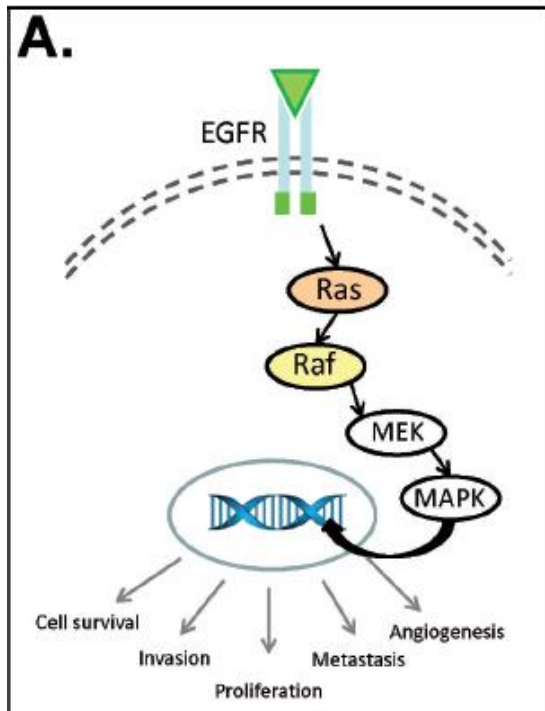
Colon adenocarcinoma

- **Before anti-EGFR therapy: testing KRAS and NRAS genes exon 2, codons 12, 13; exon 3, codons 59, 61; and exon 4, codons 117, 146. Mutation of the RAS gene indicates resistance against anti-EGFR therapy.**
 - 50% of colon adenocarcinomas carries RAS mutation
- **Testing for BRAF exon 15 mutation. The presence of mutation is a negative prognostic factor; tumours carrying this type of mutation exhibit rather unfavorable biological behavior. BRAF mutation may also indicate sporadic (non-hereditary) microsatellite instability.**
 - 10-15% of colon adenocarcinomas carries BRAF mutation
- **Microsatellite instability testing** may be done by testing **DNA repair enzyme proteins**, MLH1, MSH2, MSH6, PMS2 testing, or **microsatellite markers**. Recent studies reveal that tumors with microsatellite instability react favorably to immunotherapy.

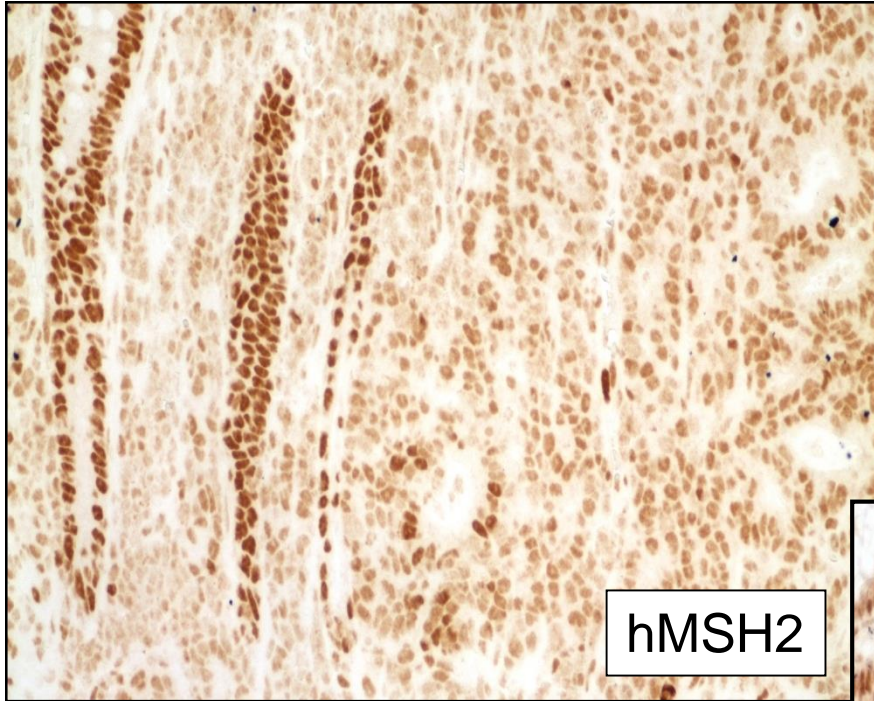
Molecular basis of anti-EGFR therapy in CRC



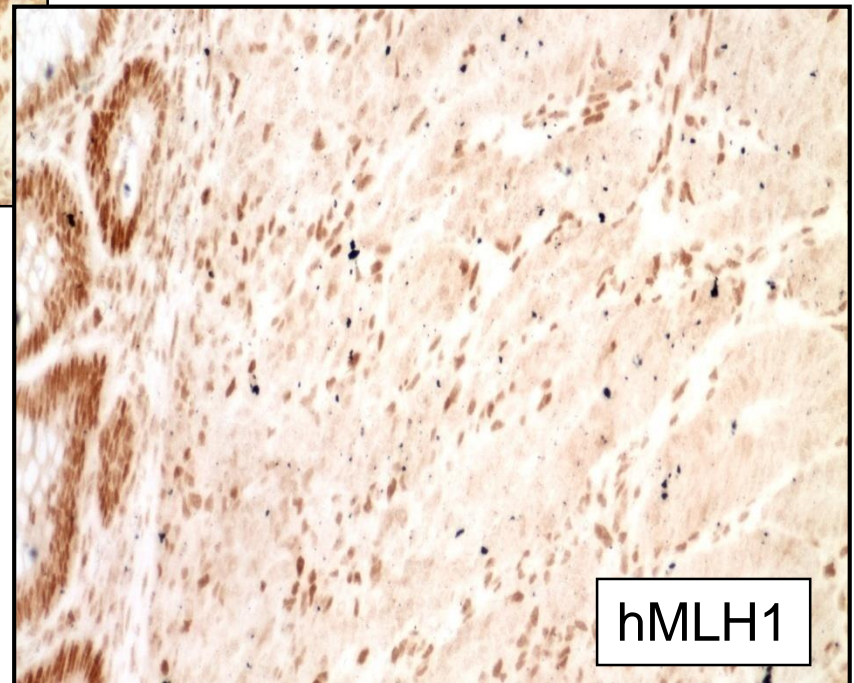
KRAS mutation: EGFR independent signal pathway activation



MSI detection with IHC

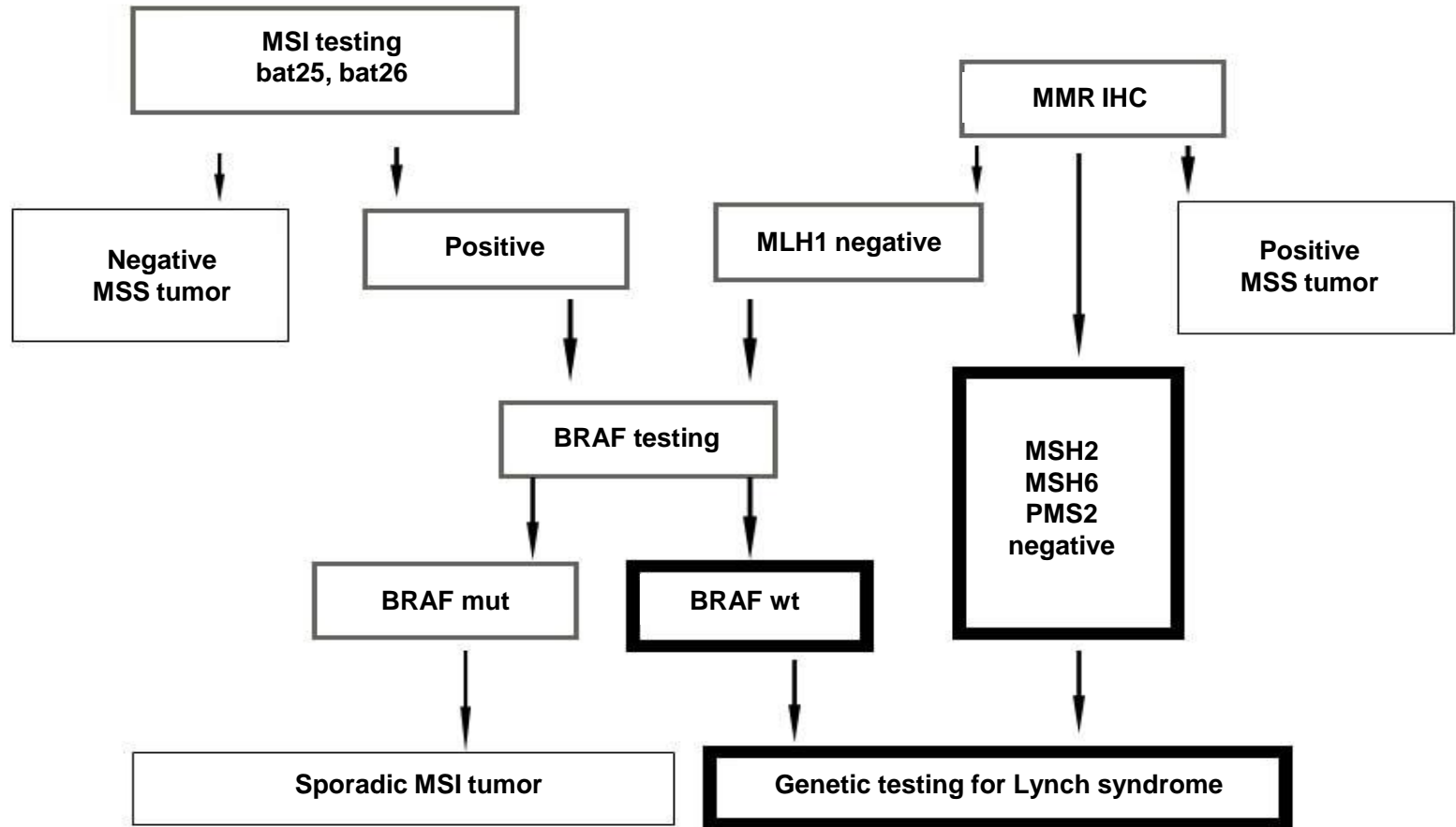


Sporadic CRC
MLH1 protein loss indicating MSI



Sensitivity 70-100%,
Specificity 98-100%.

MSI testing in colon adenocarcinomas

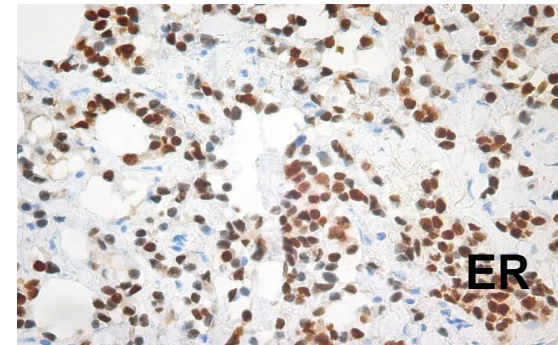
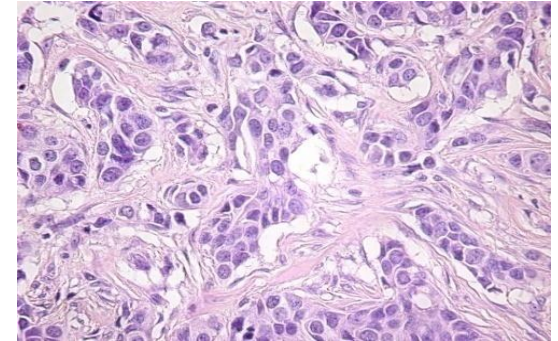
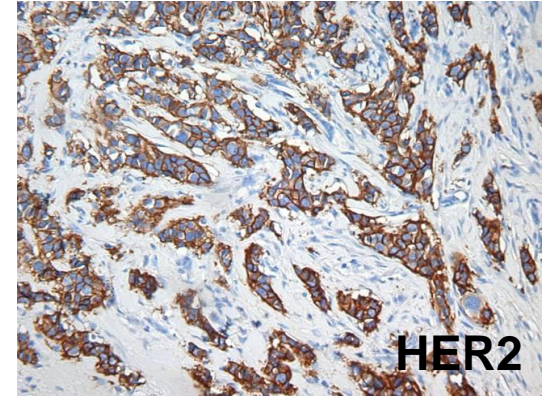


Breast cancer

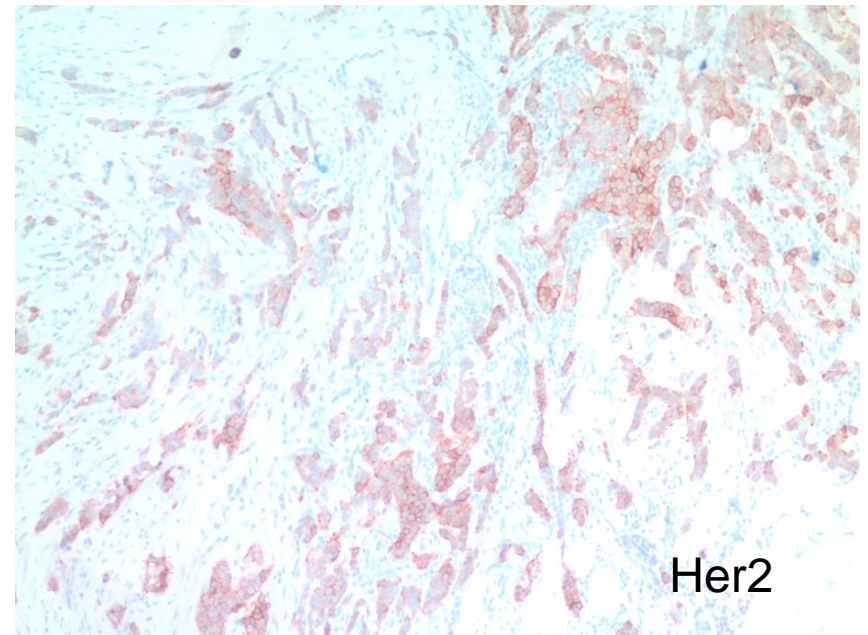
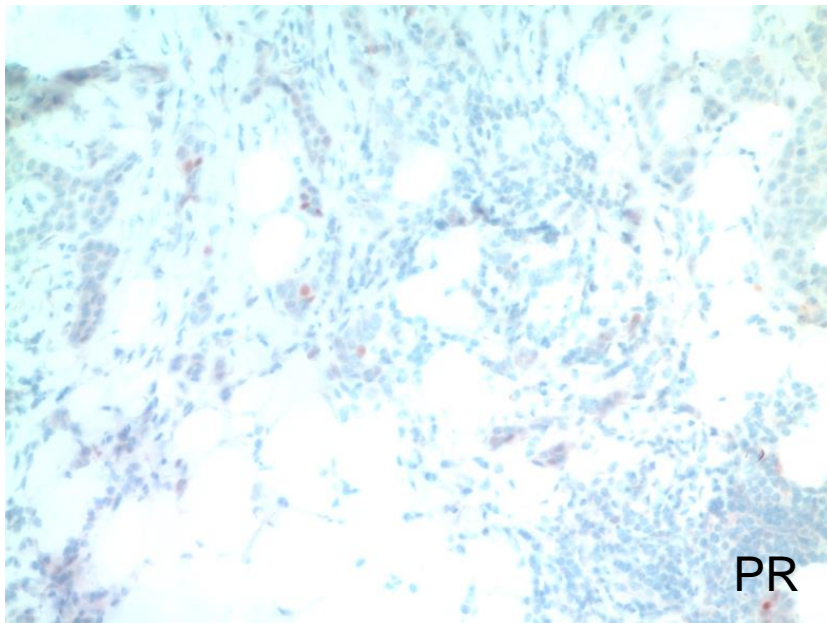
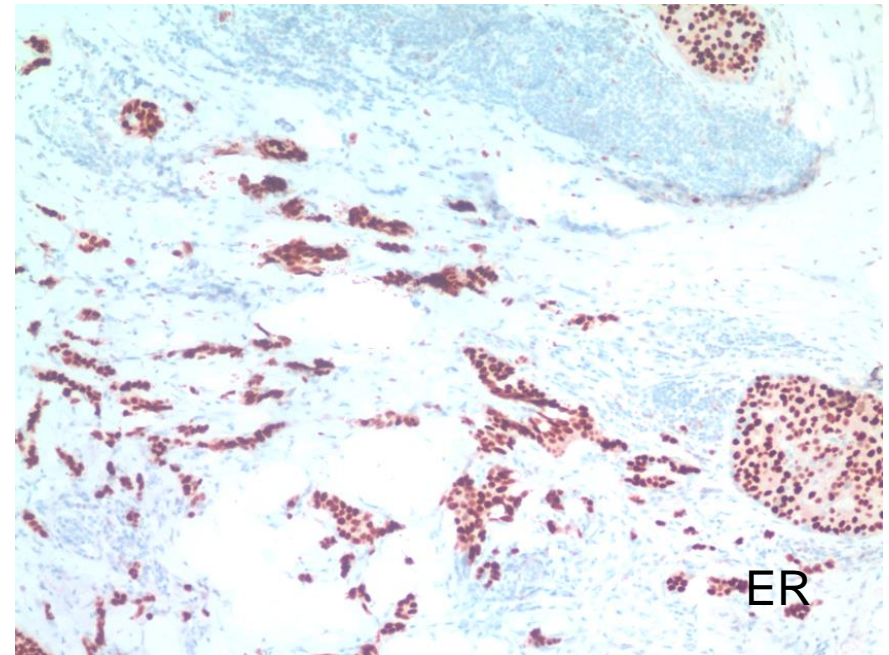
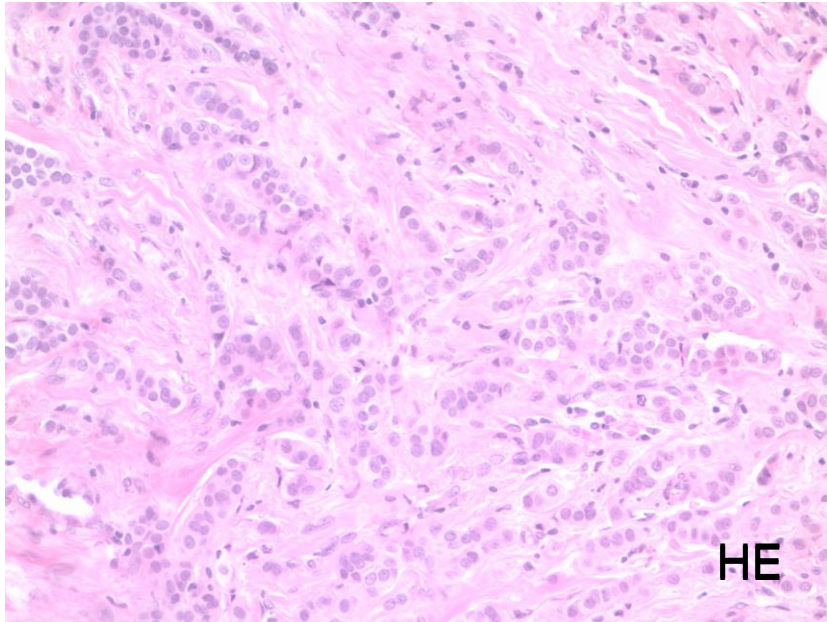
- **Her2 – amplification IHC, FISH**
 - Trastuzumab therapy
- **Other IHC**
 - ER
 - PR
 - Ki-67 : prognosis



Hormone therapy



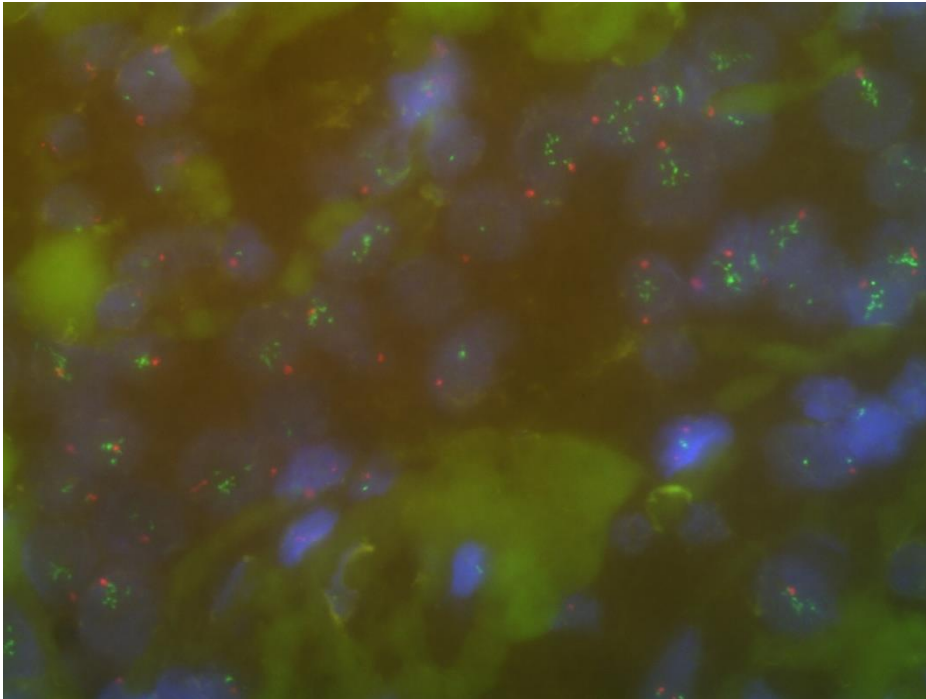
Well differentiated hormone positive breast carcinoma with equivocal Her2 expression



Her2- FISH amplified

Surgical pathology number: OOI, 7434/15

Histological diagnosis: Right breast, UIQ, invasive ductal carcinoma, Her2: 2+



Testing methods:

Her2 immunohistochemistry : clone 4B5 (Ventana, Pathway),
with external 3+ positive and 0, negative controls.

Fluorescent in situ hybridization: ZytoLight, Spec HER2/CEN 17
Dual Color Probe Kit with internal negative control.

Photo documentation number: breast FISH: 186-15

Results:

Her2 immunohistochemistry: 2+

FISH: 50 invasive tumor cells examined repeatedly: amplified

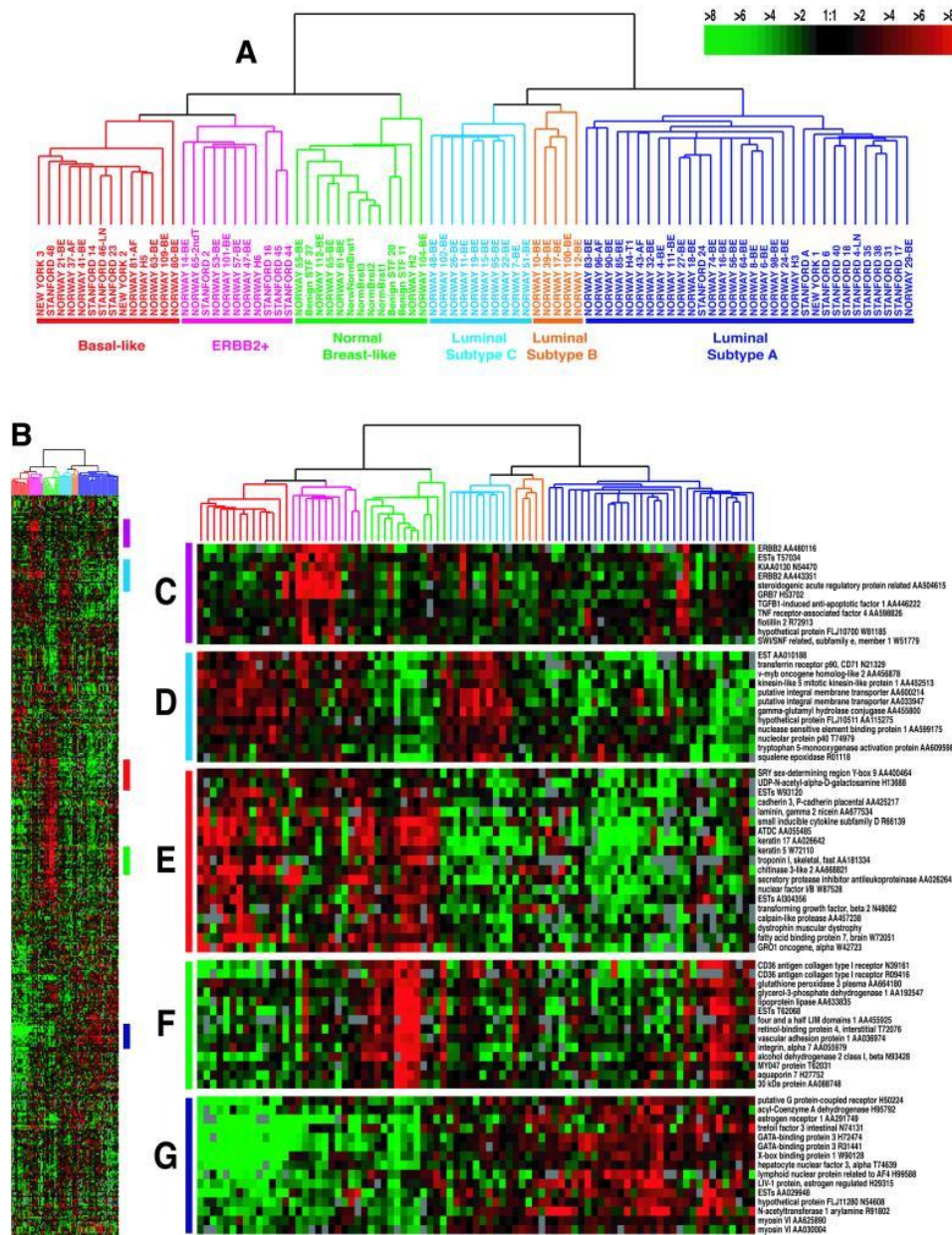
abs Her2 gene copy number: 10,5

abs Cen17 gene copy number: 0,9

Her2/Cen17 ratio: 11,6

**Diagnosis: By immunohistochemistry the tumor is Her2: 2+;
overexpression equivocal, with repeated FISH test
gene amplification is present.**

Breast cancer molecular classification



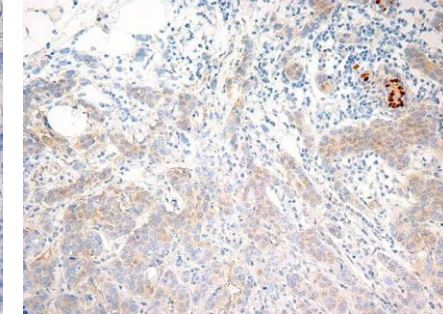
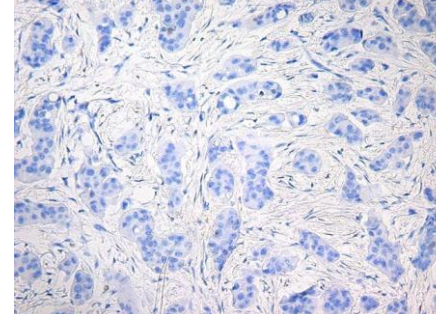
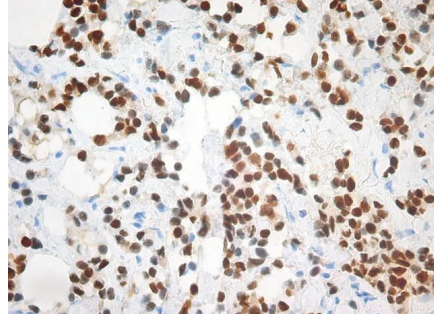
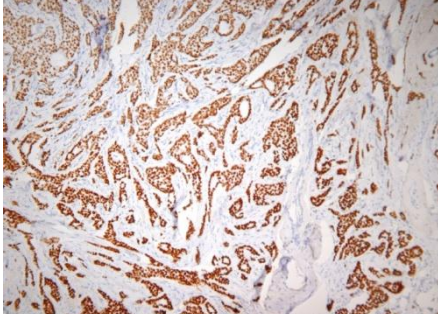
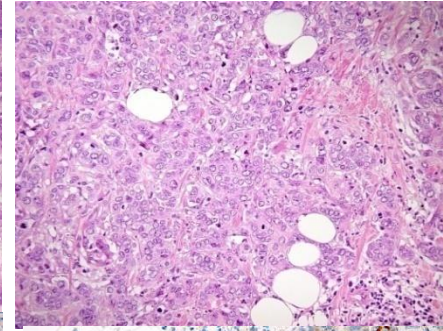
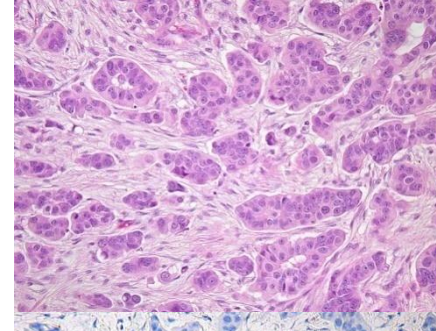
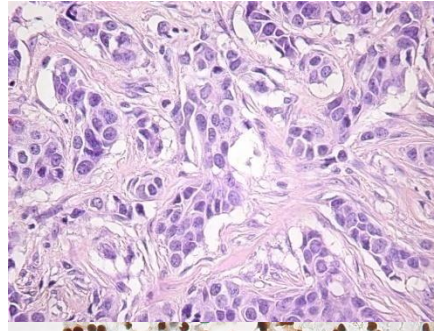
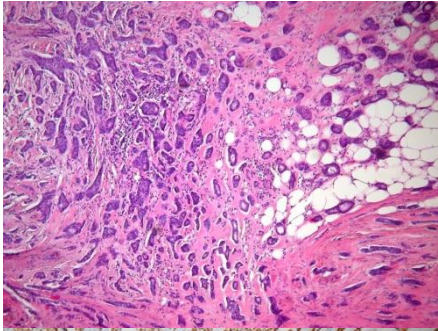
Molecular subtypes IHC

Luminal A

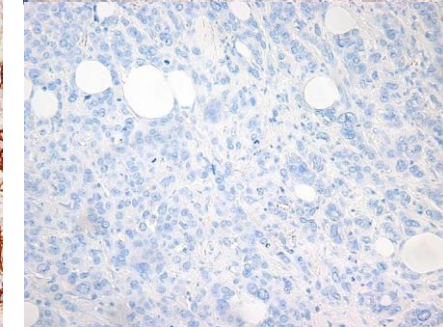
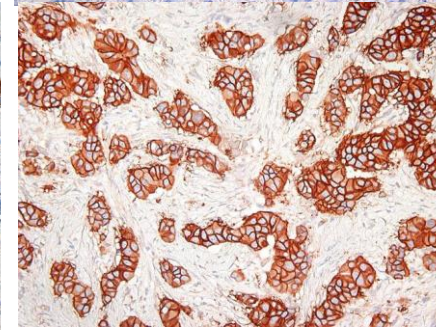
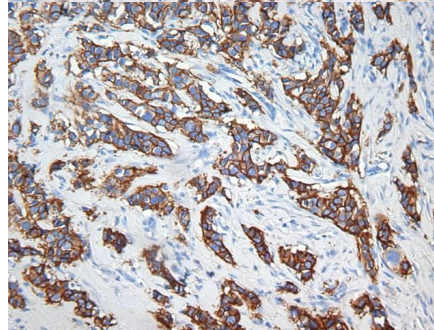
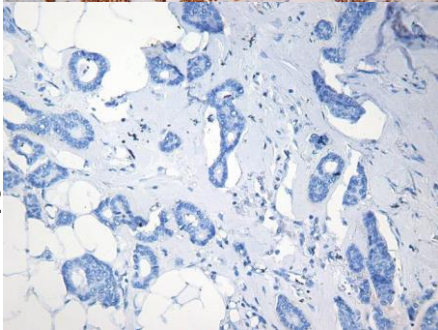
Luminal B

HER2

Basal



ER



HER2

Ki67<

CK5 +
EGFR +

Table 1 Major molecular subtypes of breast cancer determined by gene expression profiling.

	<i>Molecular subtype</i>		
	<i>Luminal</i>	<i>HER2</i>	<i>Basal</i>
Gene expression pattern	High expression of hormone receptors and associated genes (luminal A > luminal B)	High expression of HER2 and other genes in amplicon Low expression of ER and associated genes	High expression of basal epithelial genes, basal cytokeratins Low expression of ER and associated genes Low expression of HER2
Clinical features	~70% of invasive breast cancers ER/PR positive Luminal B tend to be higher histological grade than luminal A Some overexpress HER2 (luminal B)	~15% of invasive breast cancers ER/PR negative More likely to be high grade and node positive	~15% of invasive breast cancers Most ER/PR/HER2 negative ('triple negative') <i>BRCA1</i> dysfunction (germline, sporadic) Particularly common in African-American women
Treatment response and outcome	Respond to endocrine therapy (but response to tamoxifen and aromatase inhibitors may be different for luminal A and luminal B) Response to chemotherapy variable (greater in luminal B than in luminal A) Prognosis better for luminal A than luminal B	Respond to trastuzumab (Herceptin) Respond to anthracycline-based chemotherapy Generally poor prognosis	No response to endocrine therapy or trastuzumab (Herceptin) Appear to be sensitive to platinum-based chemotherapy and PARP inhibitors Generally poor prognosis (but not uniformly poor)

Oncotype DX® 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes

PROLIFERATION

Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN

ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

CD68

REFERENCE

Beta-actin
GAPDH
RPLPO
GUS
TFRC

INVASION

Stromelysin 3
Cathepsin L2

HER2

GRB7
HER2

$$\begin{aligned} \text{RS} = & + 0.47 \times \text{HER2 Group Score} \\ & - 0.34 \times \text{ER Group Score} \\ & + 1.04 \times \text{Proliferation Group Score} \\ & + 0.10 \times \text{Invasion Group Score} \\ & + 0.05 \times \text{CD68} \\ & - 0.08 \times \text{GSTM1} \\ & - 0.07 \times \text{BAG1} \end{aligned}$$

Risk Category	Recurrence score
Low risk	RS <18
Int risk	RS ≥18 and <31
High risk	RS ≥31

Summary

- The purpose of pathological diagnostics is **to provide oncologists with information** about the tumor they are treating.
- Pathological diagnosis is essential for determining **the prognosis of the tumor** and the **necessity of treatment**, the **selection** of appropriate **therapeutic modalities**, and the expected effectiveness of the therapy.
- **Tumour diagnosis is based on morphology.**
- For the majority of solid tumors, the **most important prognostic factor is the stage of the tumor.**
- Modern histological reports also contain other prognostic and predictive factors in a clearly structured form.
- **Molecular diagnostic methods have become increasingly important** in addition to conventional histopathological/surgical pathological diagnostics. This is true for both **establishing diagnosis** and **identifying gene/protein abnormalities that may serve as targets for targeted therapies**, which continue to gain prevalence.
- **Information collected by pathologists is used by many different specialists** (including oncologists, cancer registrars, and system planners) in order **to improve patient survival results.**