Histopathology and molecular pathology of cancer

János Szőke, MD
National Institute of Oncology
Center of Tumor Pathology

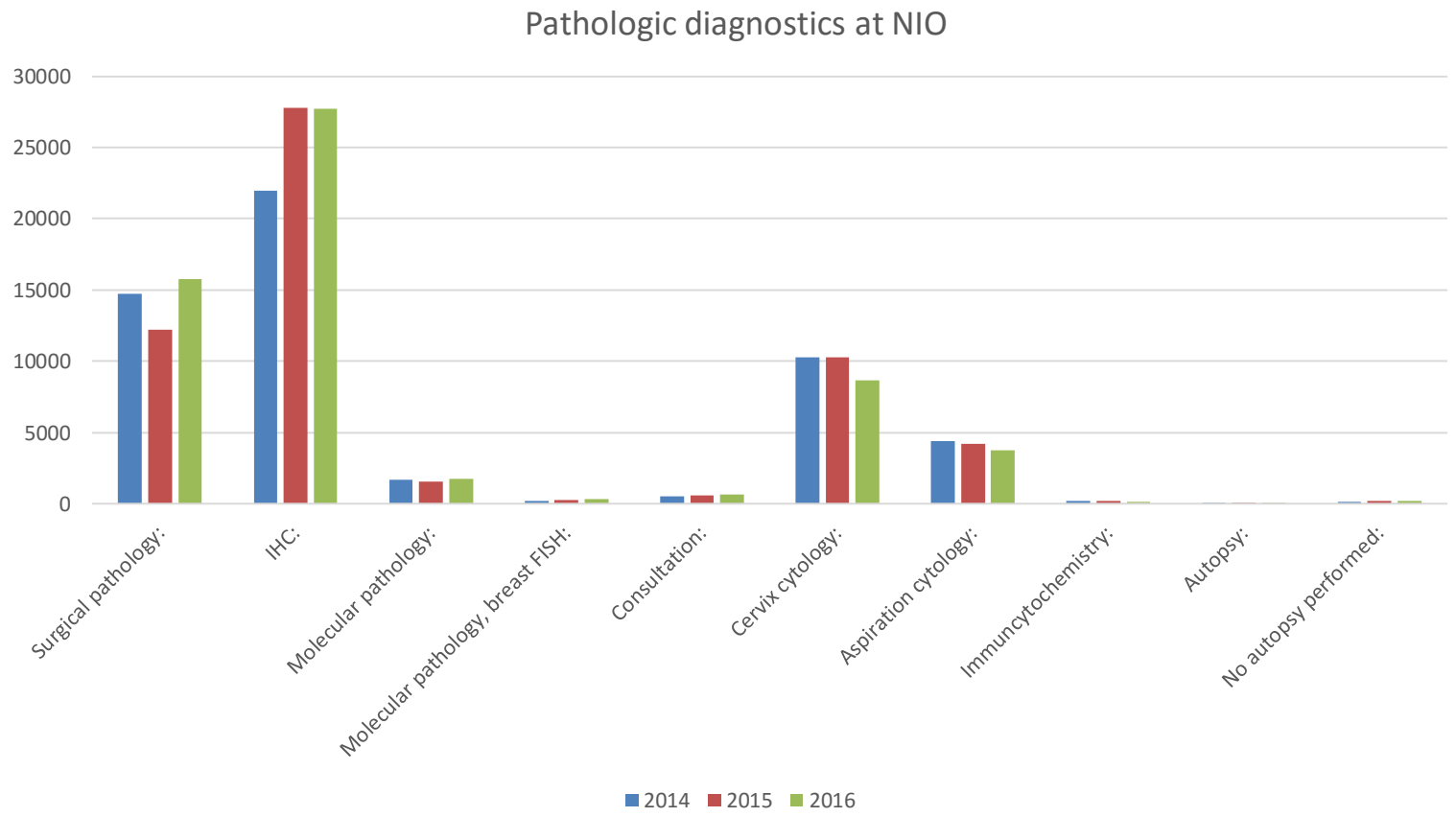
Budapest, 9/27/18.

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

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 Skean, http://www.albion.edu/biol/skean/haemcapg.htm)

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



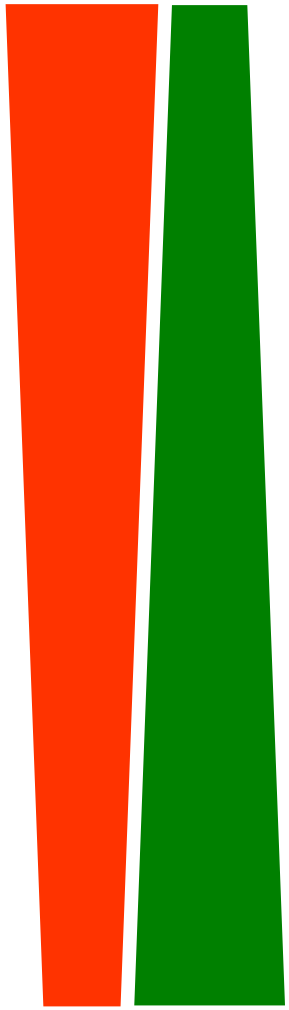
Patologic diagnostics:	2014	2015	2016
Surgical pathology:	14763	12210	15788
IHC:	21941	27759	27707
Molecular pathology:	1683	1554	1752
Molecular pathology, breast FISH:	222	287	322
Consultation:	565	578	689
Cervix cytology:	10278	10278	8661
Aspiration cytology:	4423	4202	3752
Immuncytochemistry:	246	220	175
Autopsies:	96	74	94
No autopsy performed:	150	201	195

Overview

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

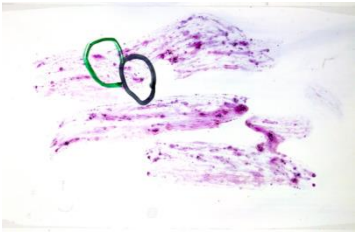
Diagnostic methods

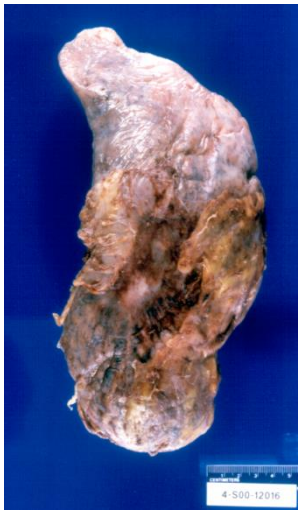
Dx



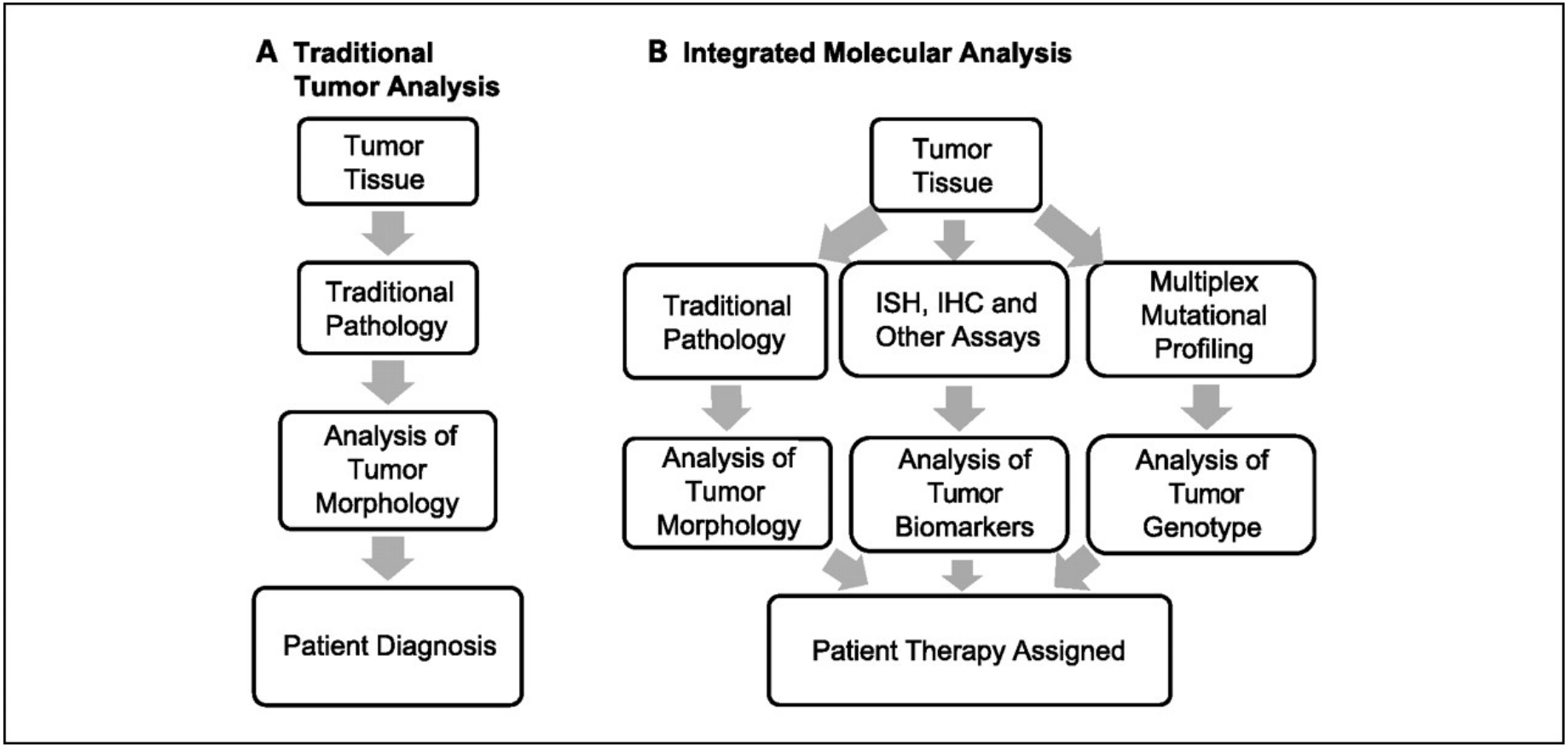
Staging

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





Role of pathology in oncology

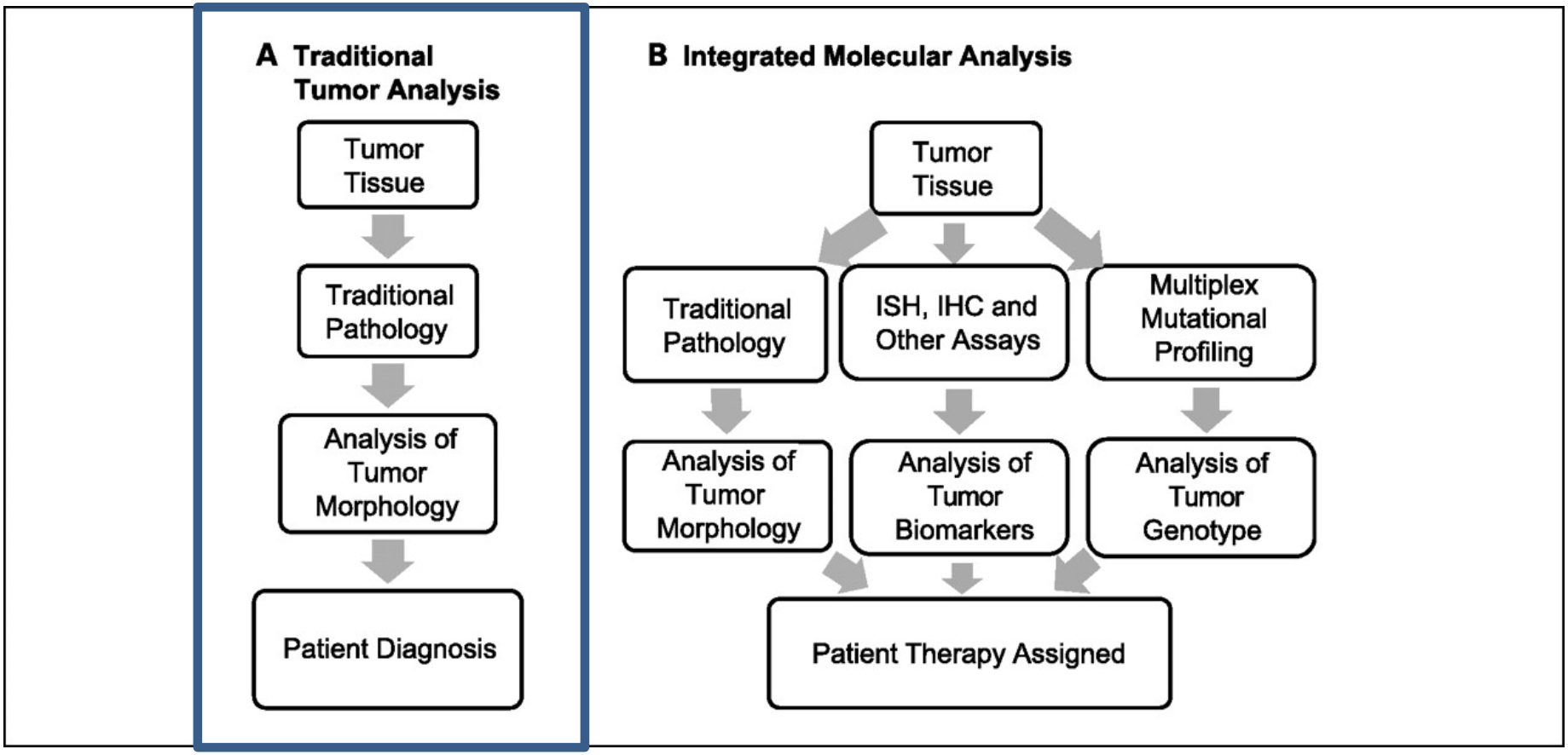


Pao W et al. Clin Cancer Res 2009;15:5317-5322

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

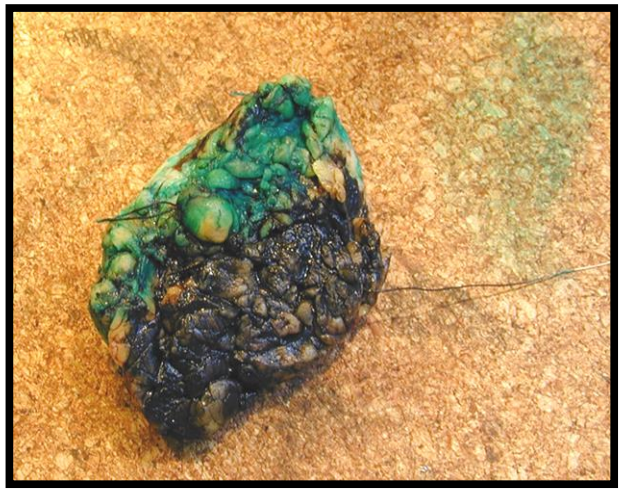
Role of pathology in oncology

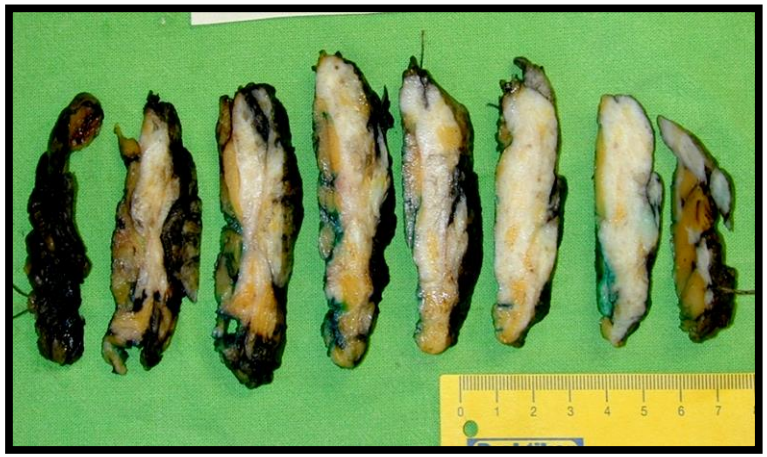


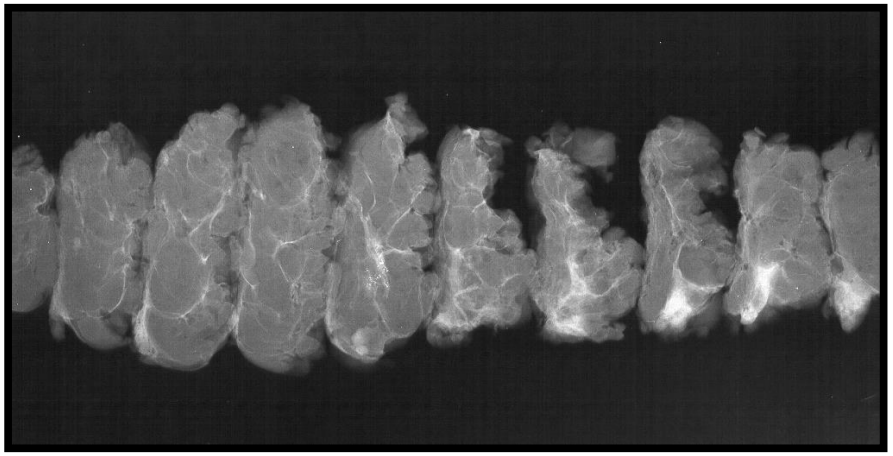
Pao W et al. Clin Cancer Res 2009;15:5317-5322

Clinical Cancer Research

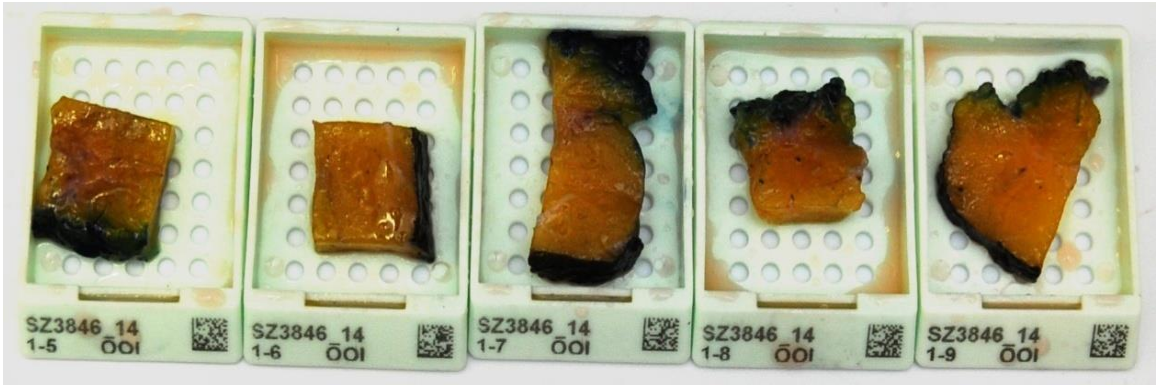
Macroscopic examination



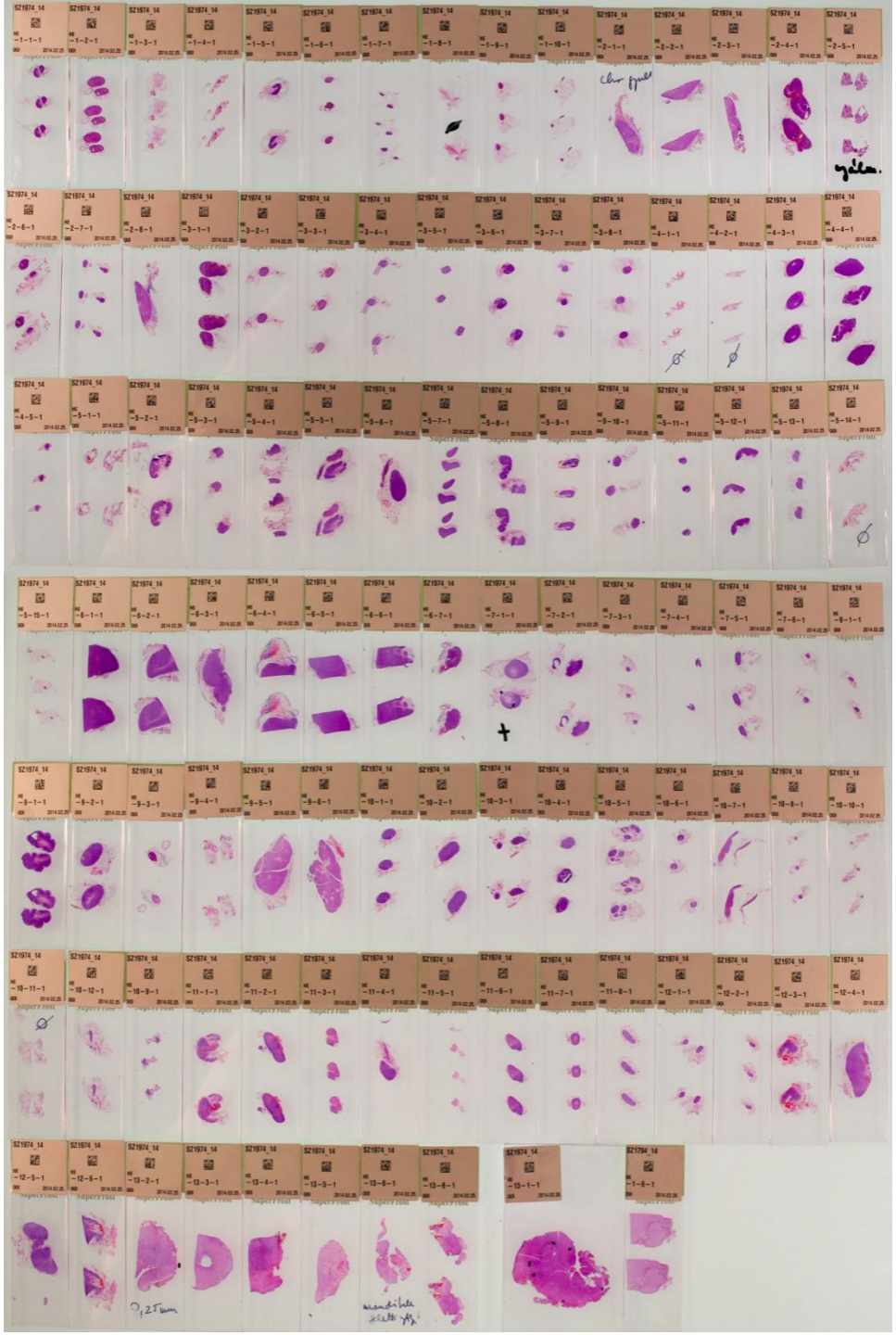




Macroscopic examination



Cutting sections of the blocks (FFPE)



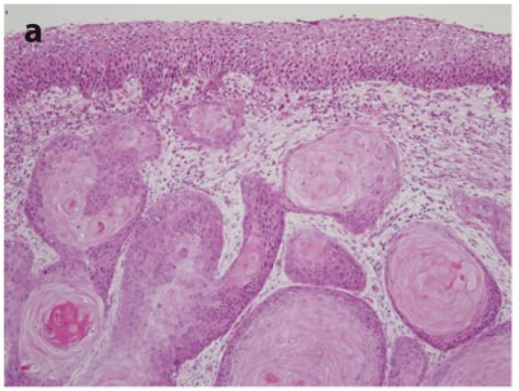
HE staining

„Traditional” histologic parameters

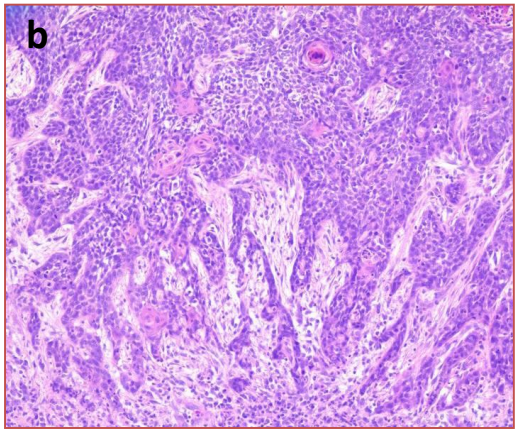
- **Diagnosis: What is the tissue/cell of origin, what is the phenotype?**
  - Dignity: Benign or malignant
  - Basic malignant tumor categories
    - Carcinoma
    - Sarcoma
    - Lymphoma
    - Melanoma
    - Germ cell
- **Tumor size**
- Histological grade
  - Differentiation – specialized function
    - 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

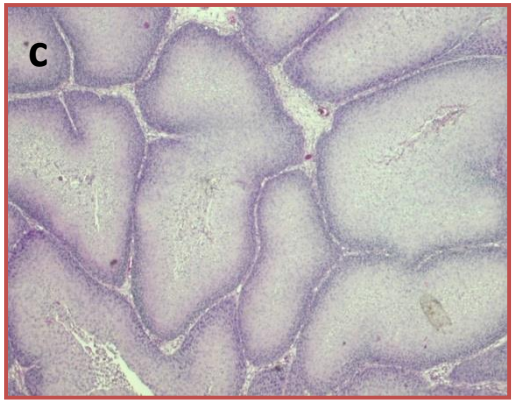
Conventional, NOS (a)



Basaloid (b)

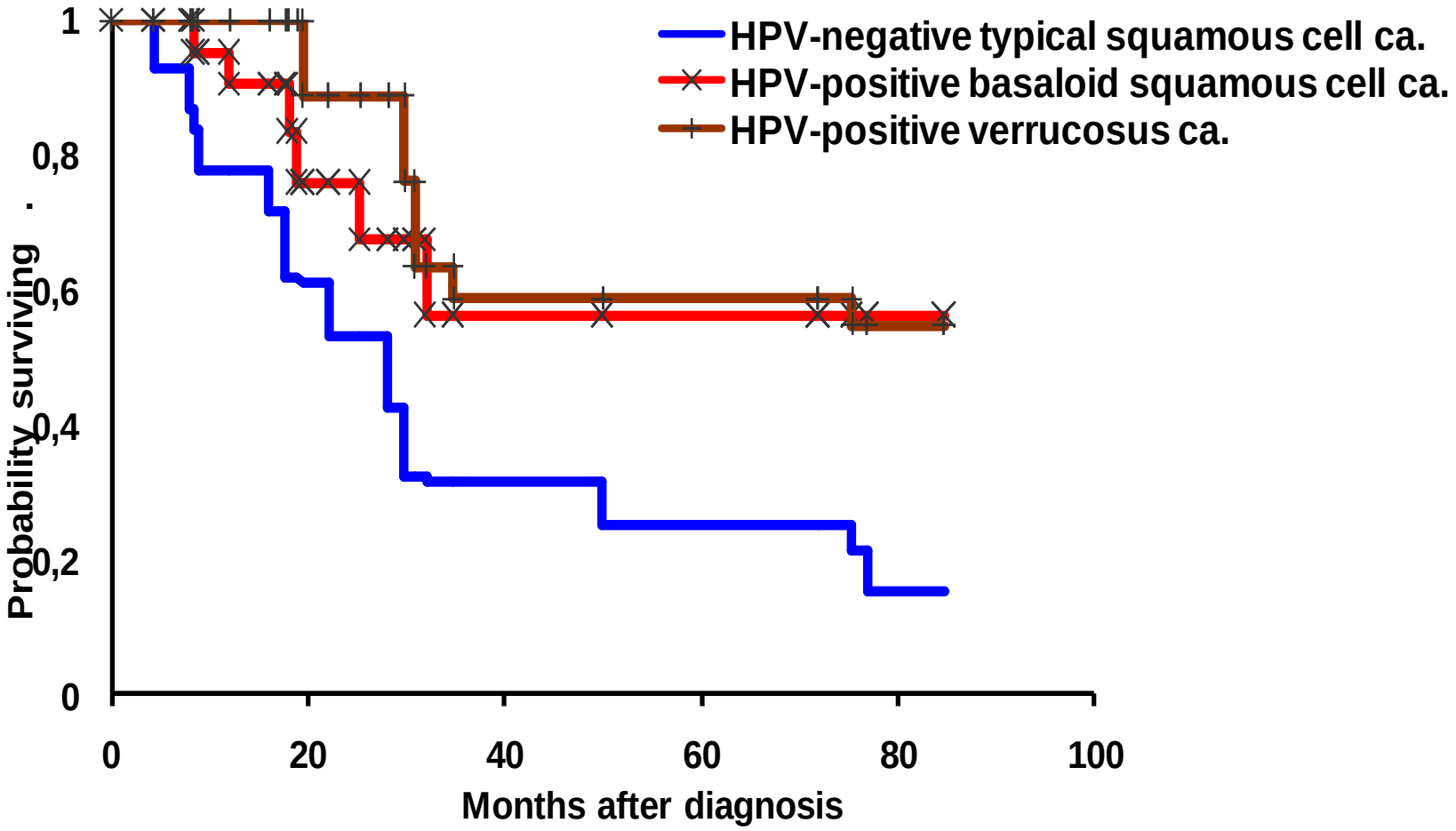


Verrucous (c)



Survival and Histology/HPV status

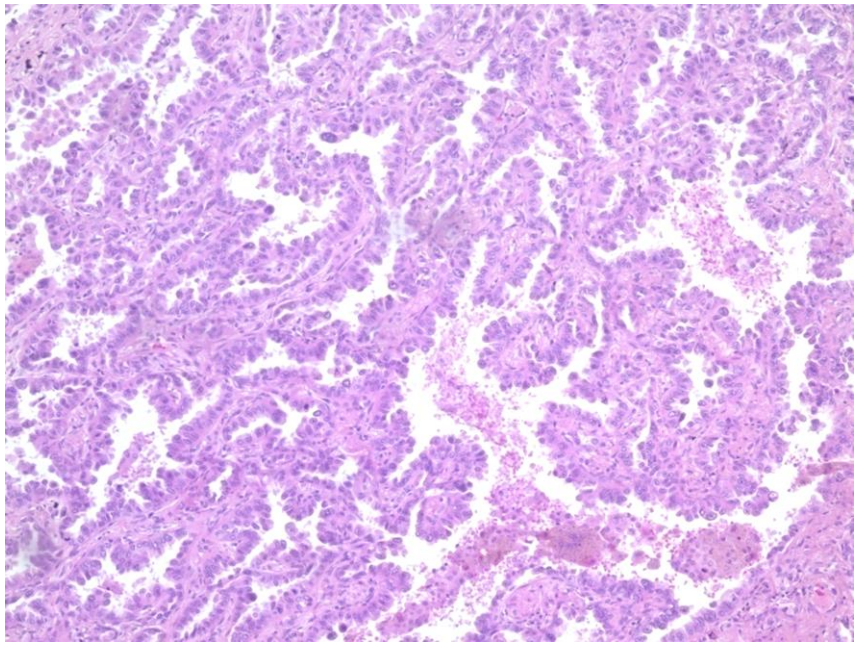
n=136



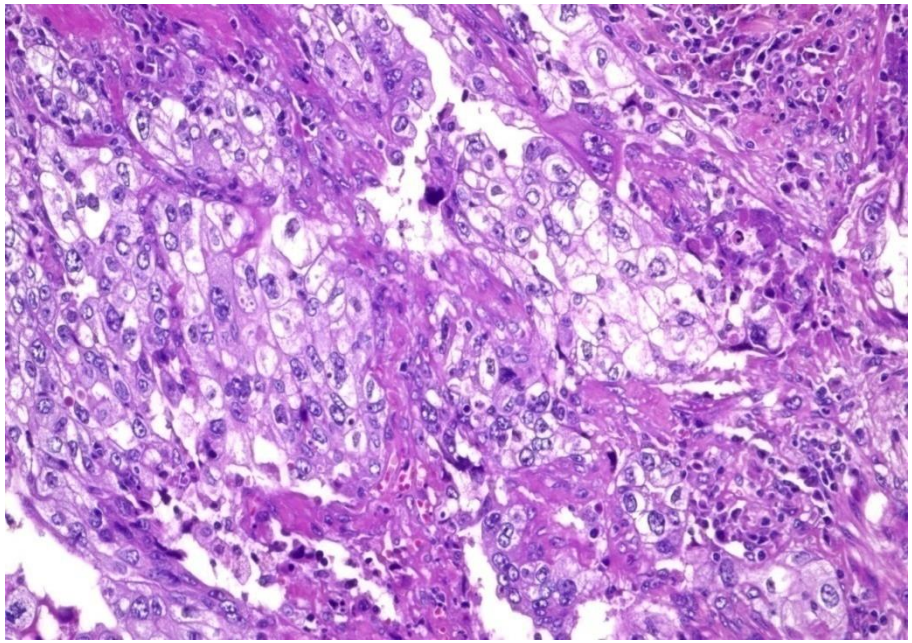
Szentirmay Z. et al, Cancer and Metastasis Reviews 24: 19-34, 2005

Differentiation – Histological grade

Lung adenocarcinoma

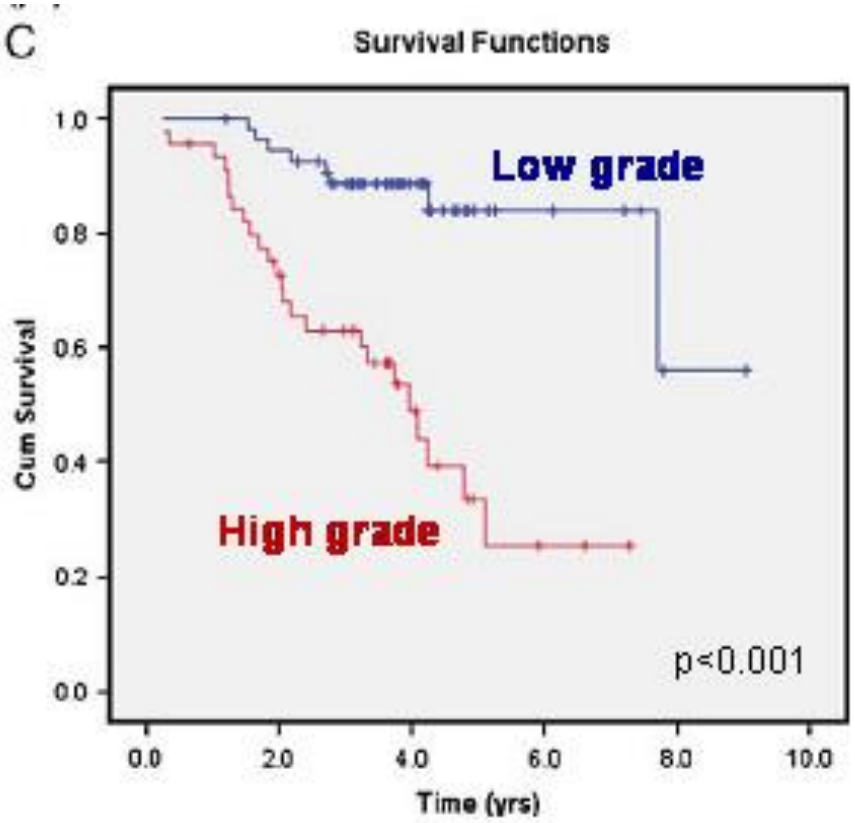


Low grade



High grade

100 resected primary lung adenocarcinoma
Survival and grade

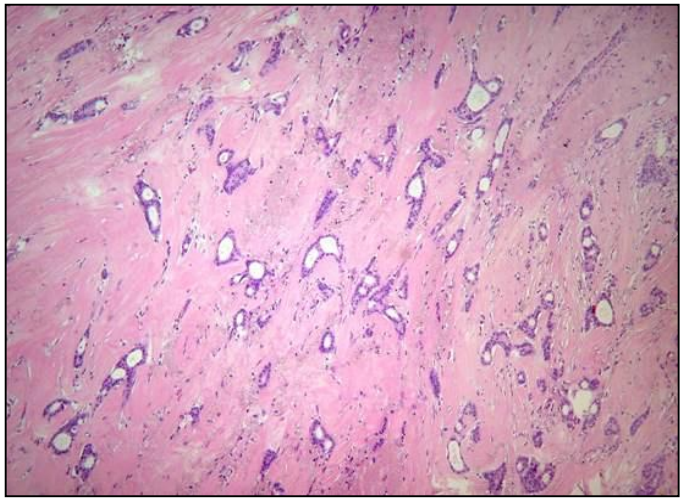


Motoi et al., Am. J. Surg. Pathol. 2008;32(6):810-27

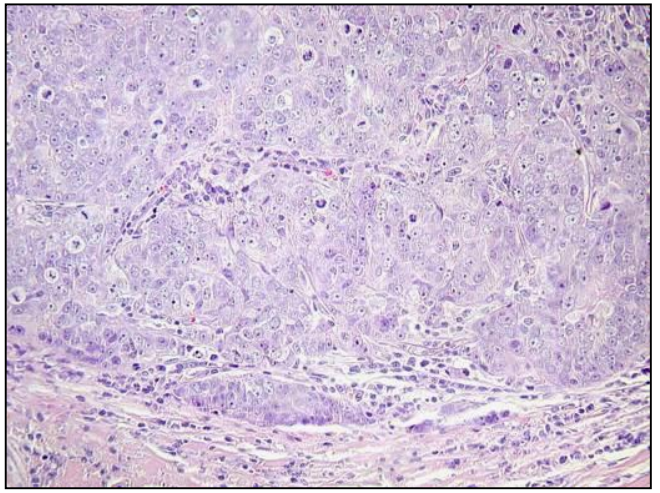
Histological grade
Breast cancer

Tumor grade 1-3:

Tubule formation + Nuclear grade + Mitotic count

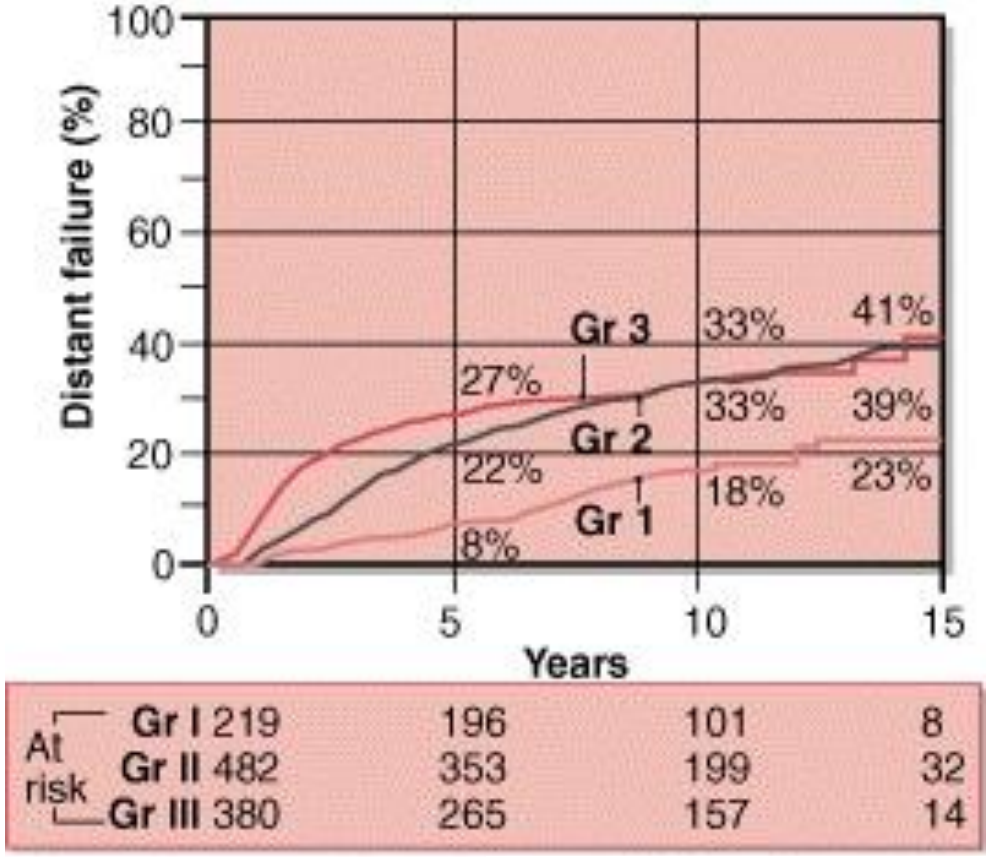


Grade I (well differentiated)



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.

Nottingham Prognostic Index

Size+Grade +LN status

Nottingham Prognostic Index: (0,2×0,9)+1+1= 2,18

Excellent prognostic group

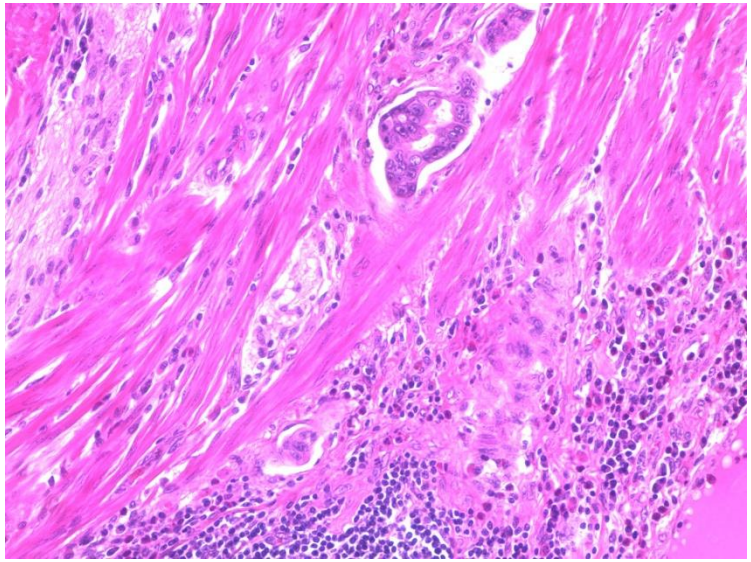
Prognostic value of NPI

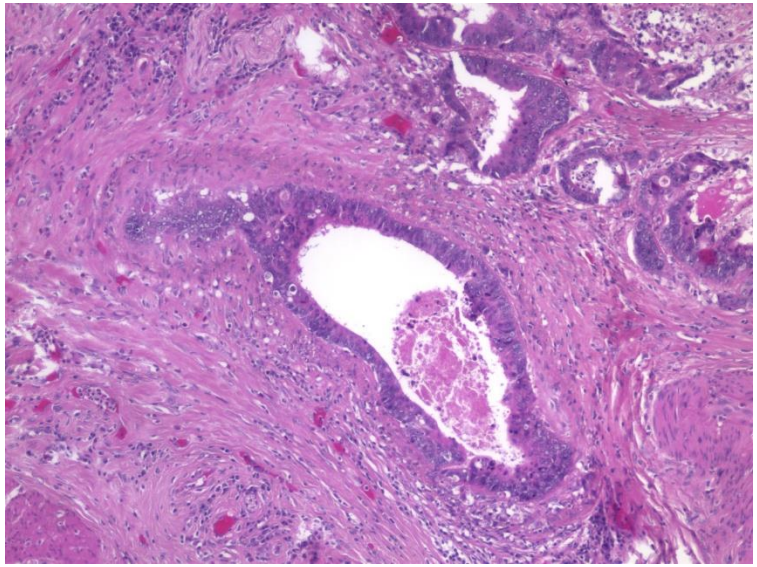
Group	NPI	10 yr survival
Excellent	2.02-2.4	96%
Good	2.41-3.4	93%
Moderate 1	3.41-4.4	81%
Moderate 2	4.41-5.4	74%
Poor	5.41-6.4	55%
Very poor	6.41-6.8	38%

Vascular invasion in CRC

- **Lymphovascular invasion**
  - Associated with local lymph node metastasis

- **Venous invasion**
  - 11%-89,5% prevalnec
  - Associated with tumor recurrence (mainly through hematogenous metastais) and decreased survival
  - **May be missed on HE**
    - Elasticus rostfestés (orcein, van Gieson)





Vascular invasion in CRC

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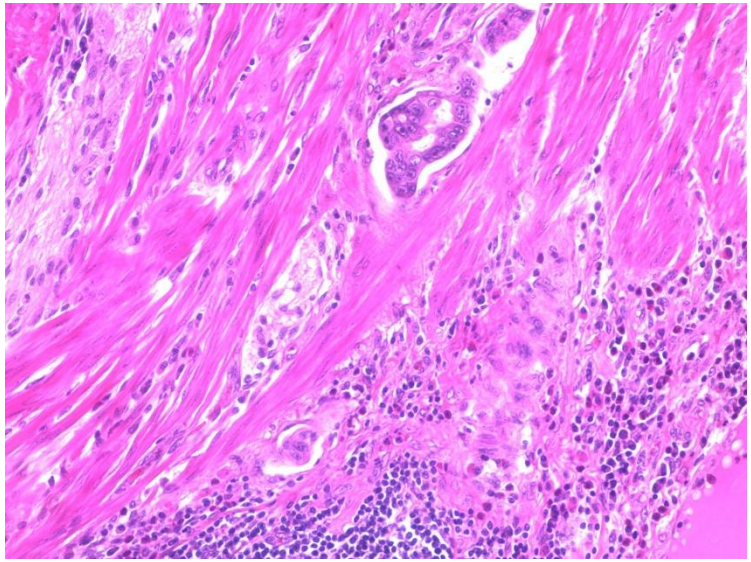
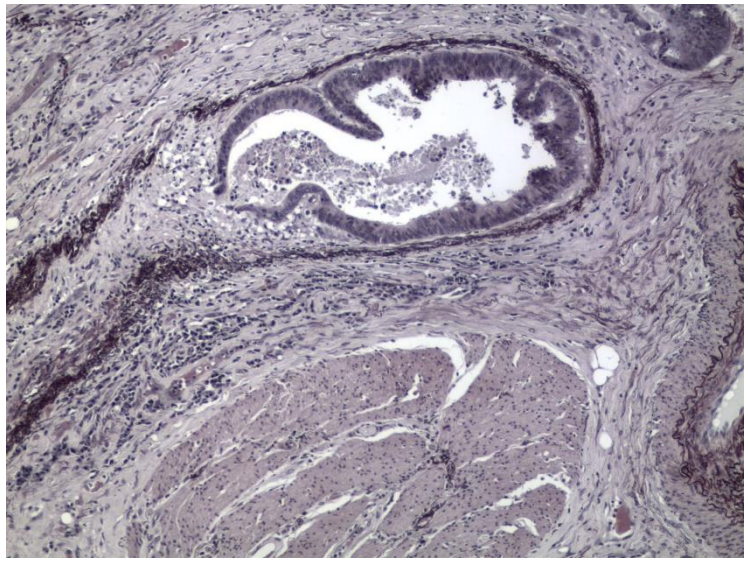
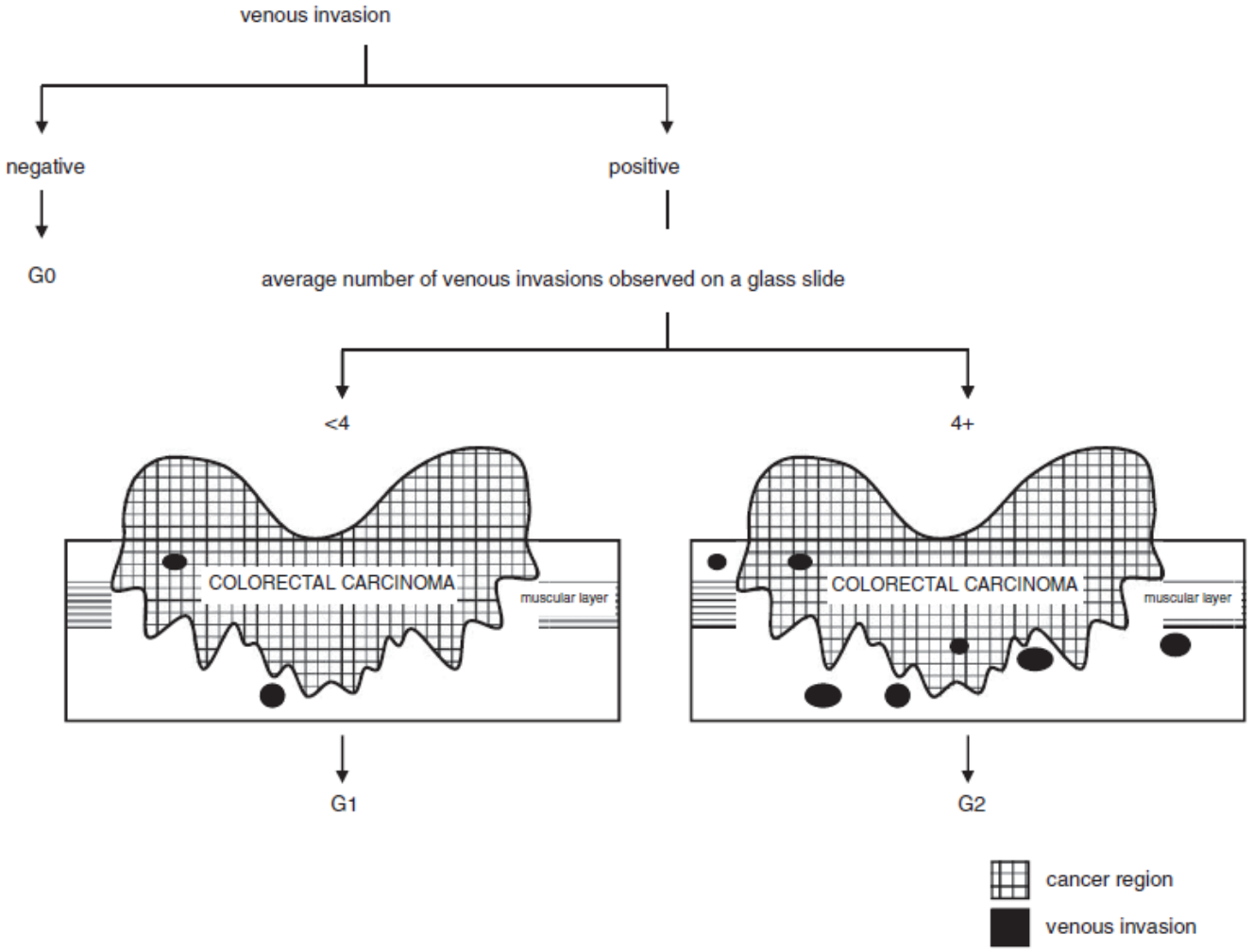


- **Venous invasion**
  - 11%-89,5% prevalence
  - Associated with tumor recurrence (mainly through hematogenous metastais) and decreased survival
  - May be missed on HE
    - **Elastic stain** (orcein, van Gieson)



Venous invasion as prognostic factor in CRC

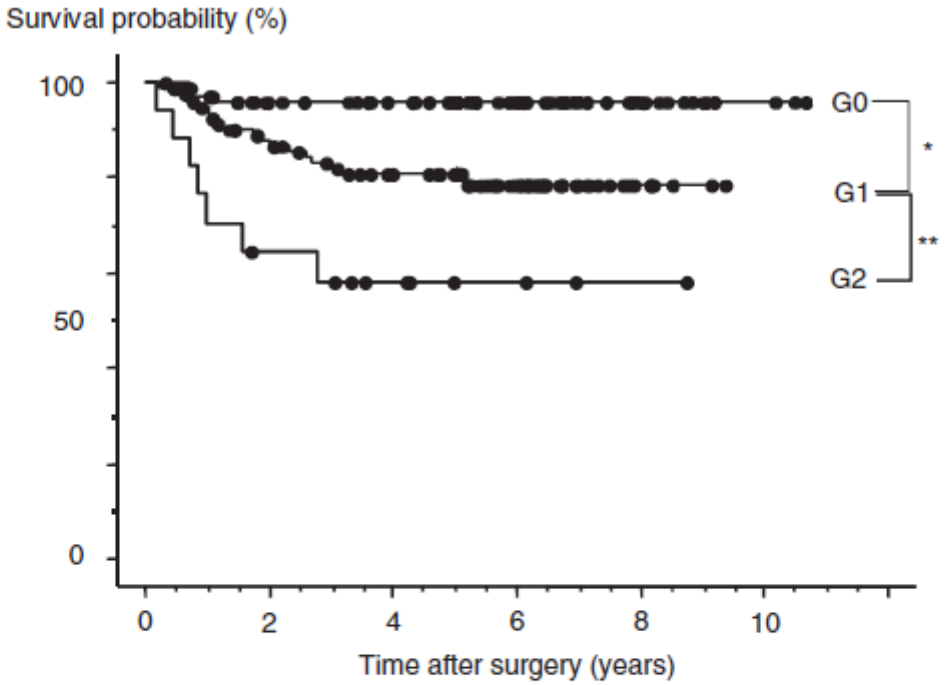
n=229 pT3, pT4 CRC



Sato et al AJSP 2010;34:454-462

Venous invasion as prognostic factor in CRC

n=191 pT3 CRC



Number of venous invasion		Number at risk				
0	(G0)	75	61	54	31	13
1-3	(G1)	99	74	58	30	6
4-	(G2)	17	10	6	3	1

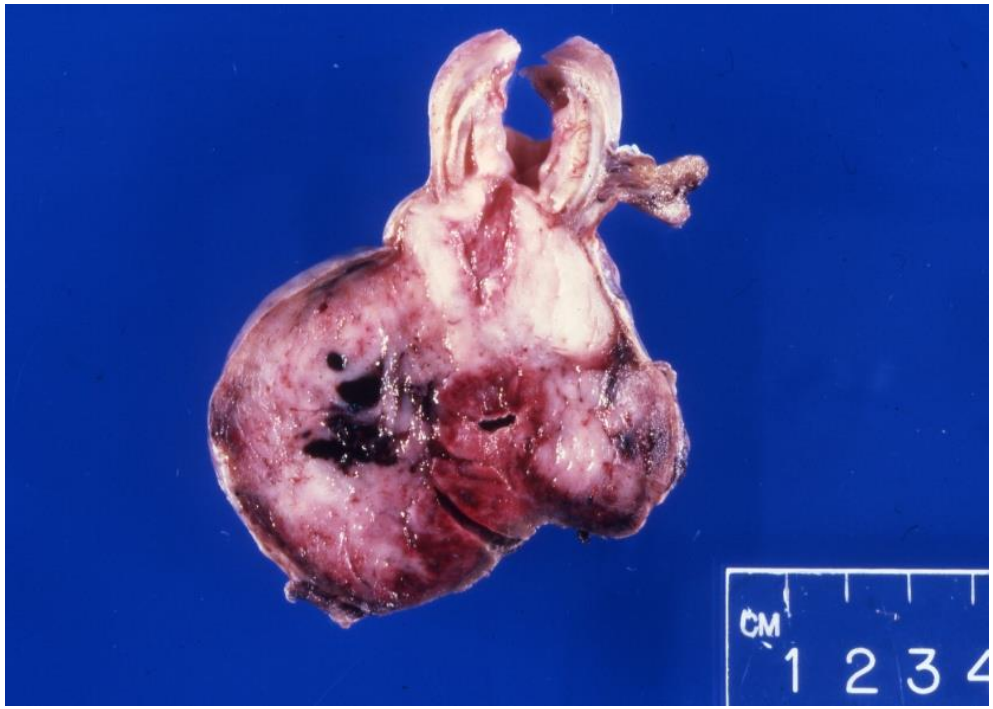
*p=0.004 **p=0.022

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

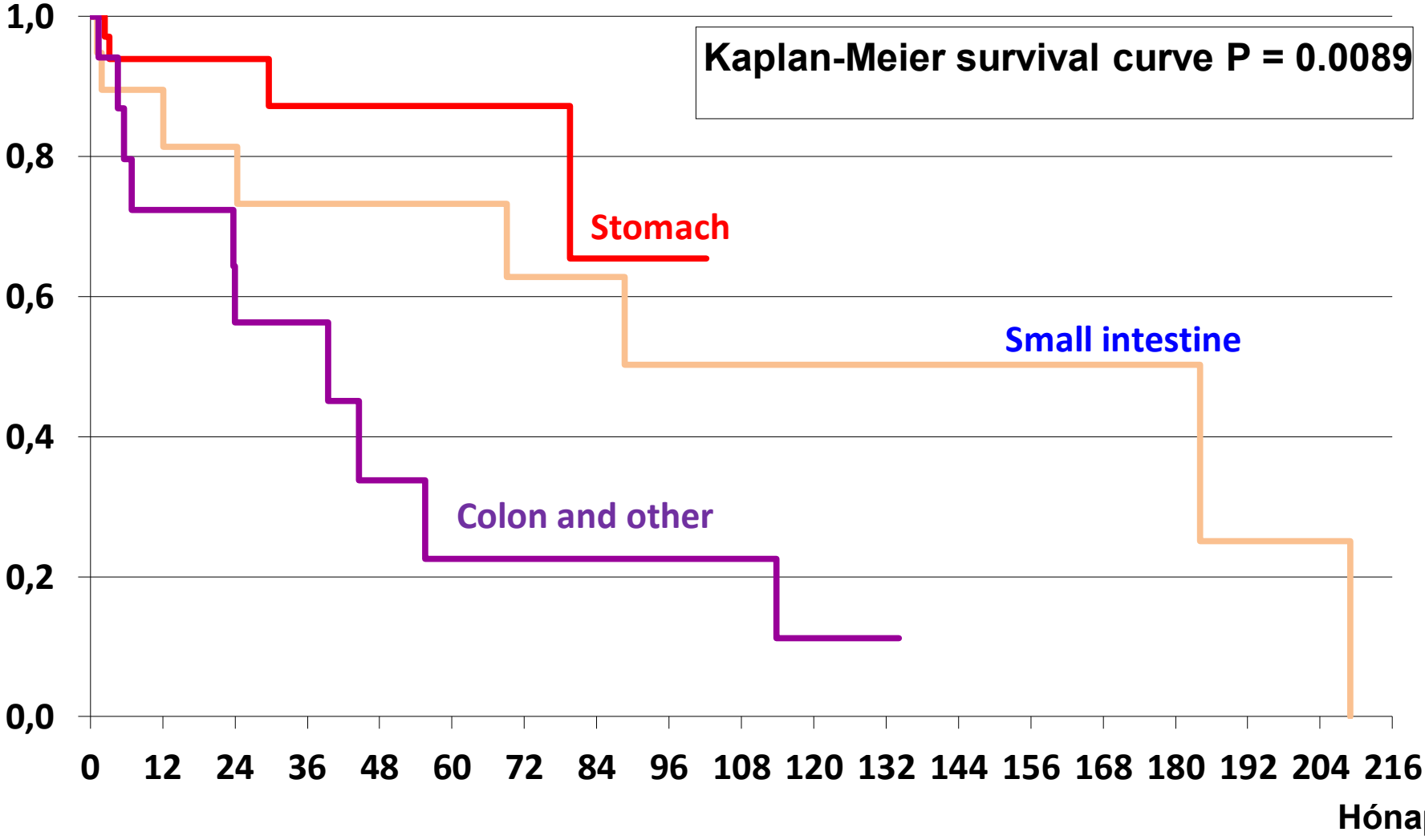
Sato et al AJSP 2010;34:454-462

Gastrointestinal stromal tumor (GIST)

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



GIST, localization and survial. No = 76



Dr. Szentirmay Zoltán

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%

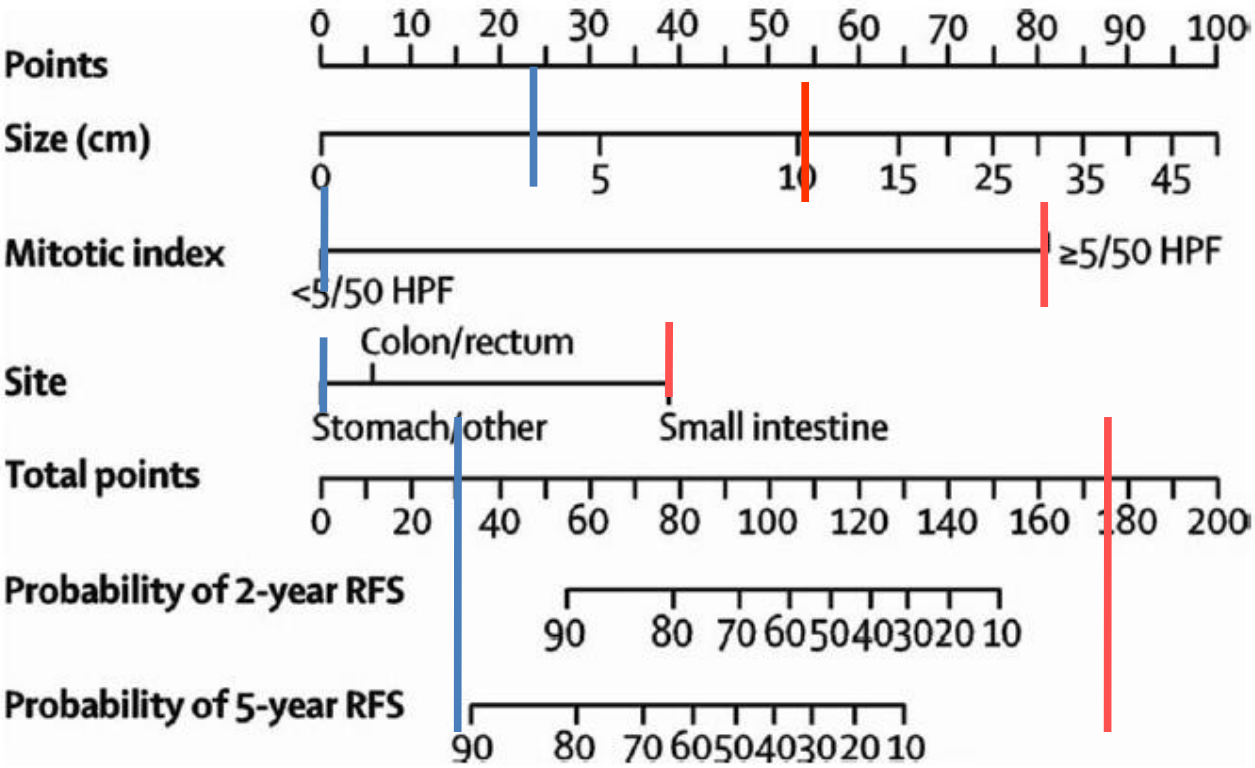


Figure 4.
Nomogram predicting 2 and 5-year recurrence-free survival in patients with resected primary GIST. Points are assigned based on tumor size, mitotic index, and site by drawing an upward vertical line to the "Points" bar. Based on the sum of the points generated, a downward vertical line is drawn 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.

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

5 year recurrence free survival: 90%

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

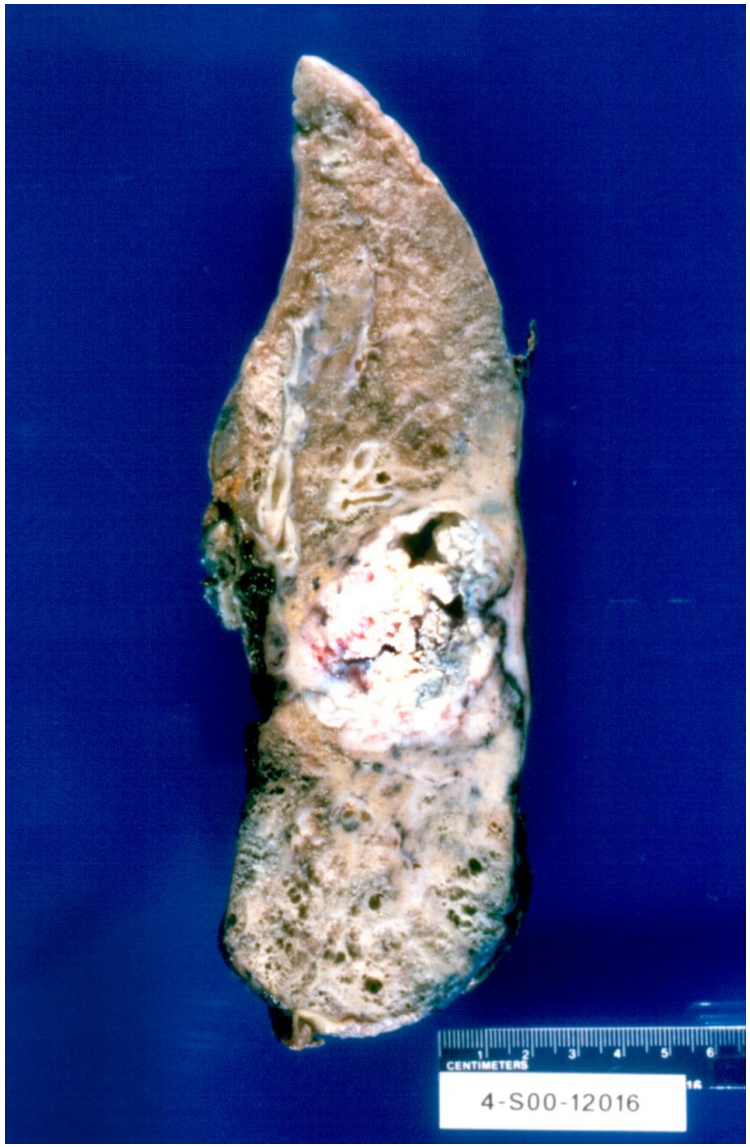
5 year recurrence free survival: <10%

„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
      - Breast: Nottingham Prognostic Index
      - Prostate: Gleason’s score
- Vascular invasion
- Perineural invasion
- Margins
- **Lymph node status**
- **pTNM: sum of the most important parameters**

TNM

- Components: T, N, M.
  - Tumor size
  - Lymph node status
  - Distant metastais
- Categories: T1a, …; N0, …; M1a, …
- Descriptors: Parameters that define categories



Baystate Pathology Associates
A Service of Baystate Health System

Baystate Health
Department of Pathology
759 Chestnut Street
Springfield, MA 01199
Tel: (413) 794-4500 Fax: (413) 794-5893
Pulmonary Pathology Service

Patient Name:
Med. Rec. #:
DOB: (Age: 78)
Gender: M
Physician(s):

Client: Baystate Medical Center
Location: PAHLD
Billing #:

Accession #:
Date Taken: 5/24/2000
Received: 5/24/2000
Signed Out: 5/29/2000

Final Diagnosis

Squamous cell carcinoma

PARTS 1 THROUGH 8:
LUNG, LEFT UPPER LOBE; LOBECTOMY:
- SQUAMOUS CELL CARCINOMA.
  A. TUMOR SIZE: 6.5 CM X 5.2 CM X 4.8 CM.
  B. HISTOLOGIC GRADE: G2.
  C. EXTENT OF PRIMARY TUMOR:
     - TUMOR INVADES VISCERAL PLEURA.
     - TUMOR IS 1 CM FROM BRONCHIAL MARGIN OF EXCISION.

6,5 cm

- 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:
     - LYMPHOPLASMACYTIC RESPONSE IS PRESENT, MILD.
     - STROMAL RESPONSE IS PRESENT, MODERATE.
     - TUMOR NECROSIS IS PRESENT, EXTENSIVE.
     - TUMOR INVADES 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.

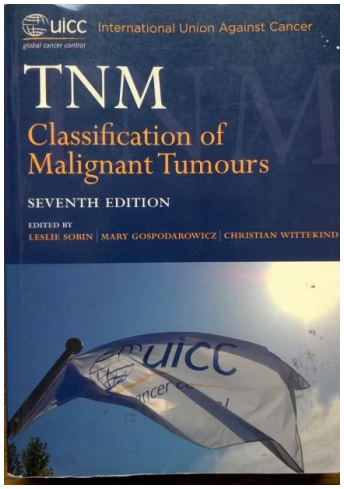
- pTN STAGE:
  A. PRIMARY TUMOR: pT2.
  B. REGIONAL LYMPH NODES: pN1.

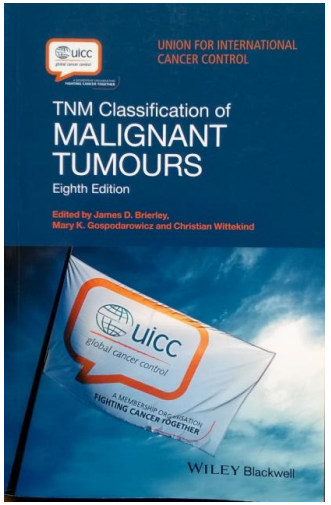
pT2N1

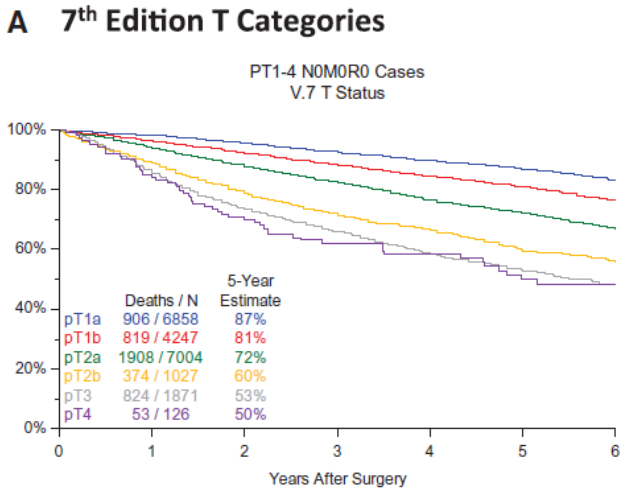
Electronically Signed Out

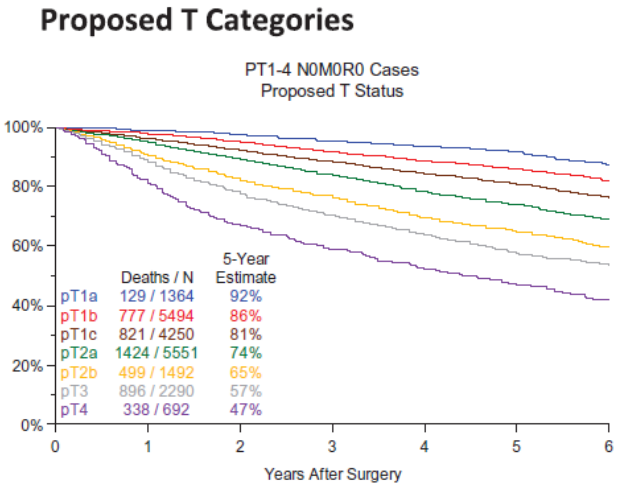
TNM v5,v6, v7 and v8
6,5 cm-es lung carcinoma

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







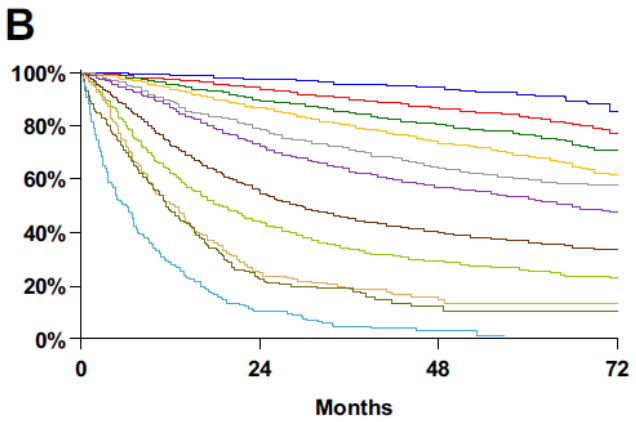


- UICC 8-ik ed. 2017 _ pT3

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



Goldstraw P et al. Thorac Oncol 2016; 11:39-51

Stage and therapy in NSCLC

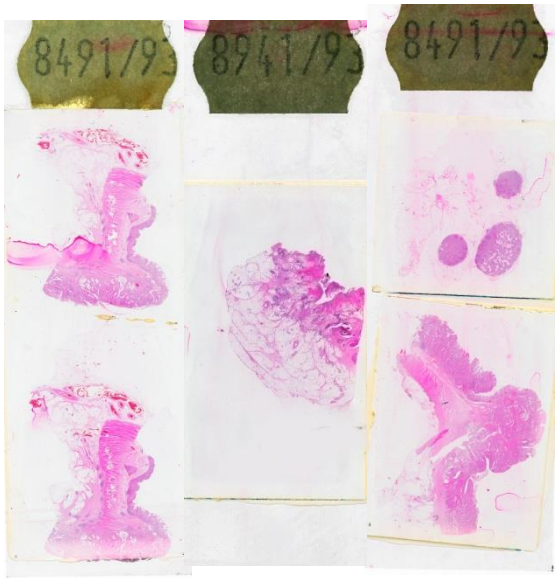
Stage	T	N	M	Surgery	Irrad.	Chemo.
I/A1,2,3	T1a,b,c	N0	M0	+	- (vagy: + !)	-
I/B	T2a	N0	M0	+	- (vagy: + !)	-
II/A	T2b	N0	M0	+	- (vagy: + !)	+
II/B	T1a-2b T3	N1 N0	M0 M0	+ +	- + / -	+ +
III/A	T1a-2b T3 T4	N2 N1 N0 – N1	M0 M0 M0	+ + / - + / -	+ + / - + / -	+ + +
III/B	T1a-2b T3-4	N3 N2	M0 M0	- + / -	+ +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

Dr. Bajcsay András

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



Érkezési idő: 11.25.
Anyag (localisatio): colon

WHO kód: 29ooo, 29o7o
Fagyasztásos szövettani vizsgálat, dg.:

13 cm hosszágú colon részlet. Középén 5 cm legnagyobb Ø-jü felhányt szélü kifekélyesedés van. Az egyik resctios vonaltól 3, a másiktól 3,5 cm-re kezödik az elváltozás- A metszlapon a bélfal kissé megvastagodott, úgy tünik tumorosan infiltralt. A környező zsirszövetben 4 db borsnyi és kisebb nyirokcsomó van.. A vastagbéllel összefüggő 15x2o cm nagyságú cspplesz részletben gócos elváltozás nincs.

Szövettani leírás: 1993.11.29./CSL

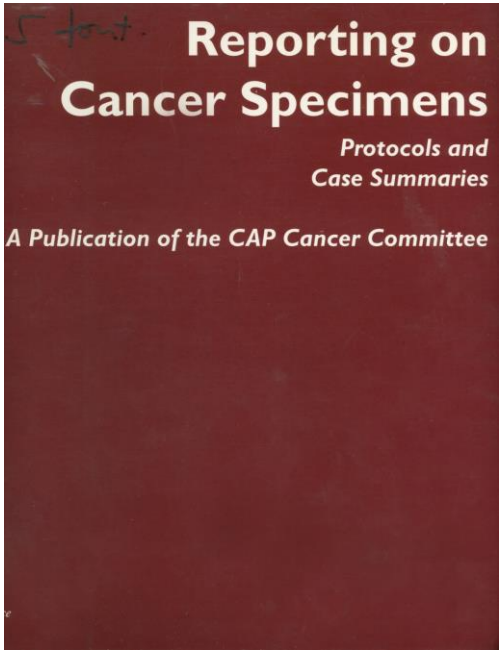
A preparátumban a vastagbél szöveti szerkezete csak a széli részeken ismerhető fel. A preparátum közepén a lumenbe domborodó és a bélfalat teljesen infiltráló daganat látható. A tumor változatos alakú és nagyságú mirigy-szerű lumeneket, hályenként cribriform mintázatot alkot. A daganatsejtek az atypia, 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 Dukes 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

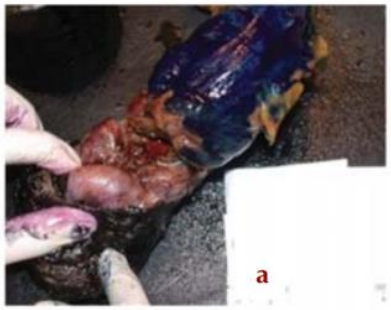
Lymph node status

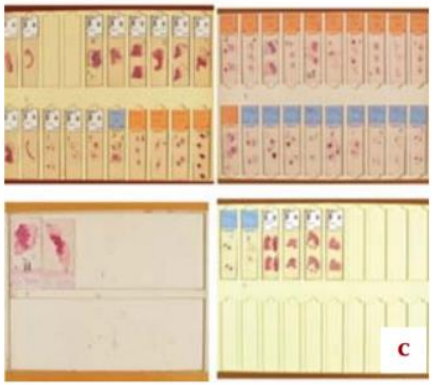
pTNM stage

Modified Astler-Coller stage

Other/notes

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

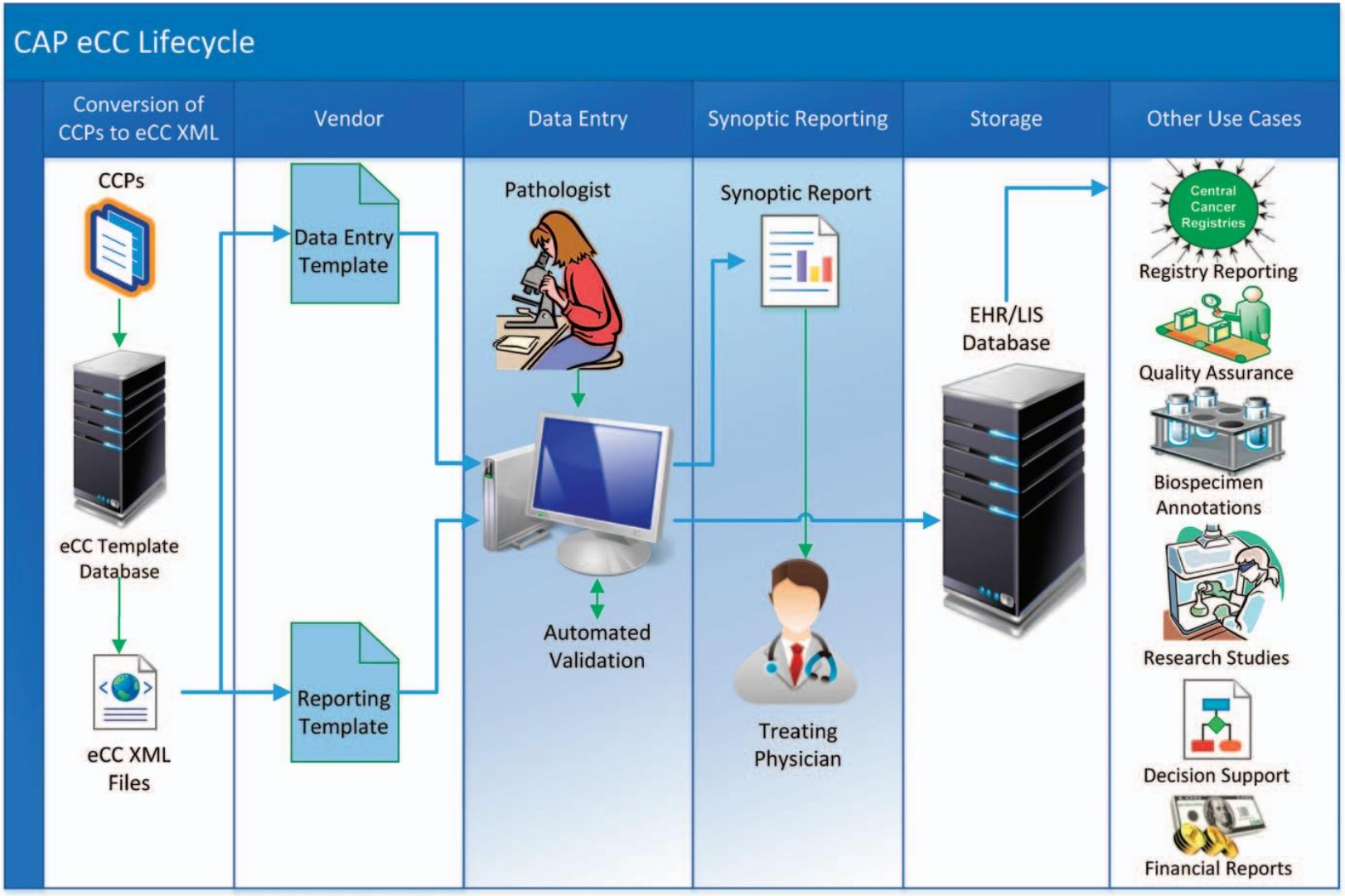




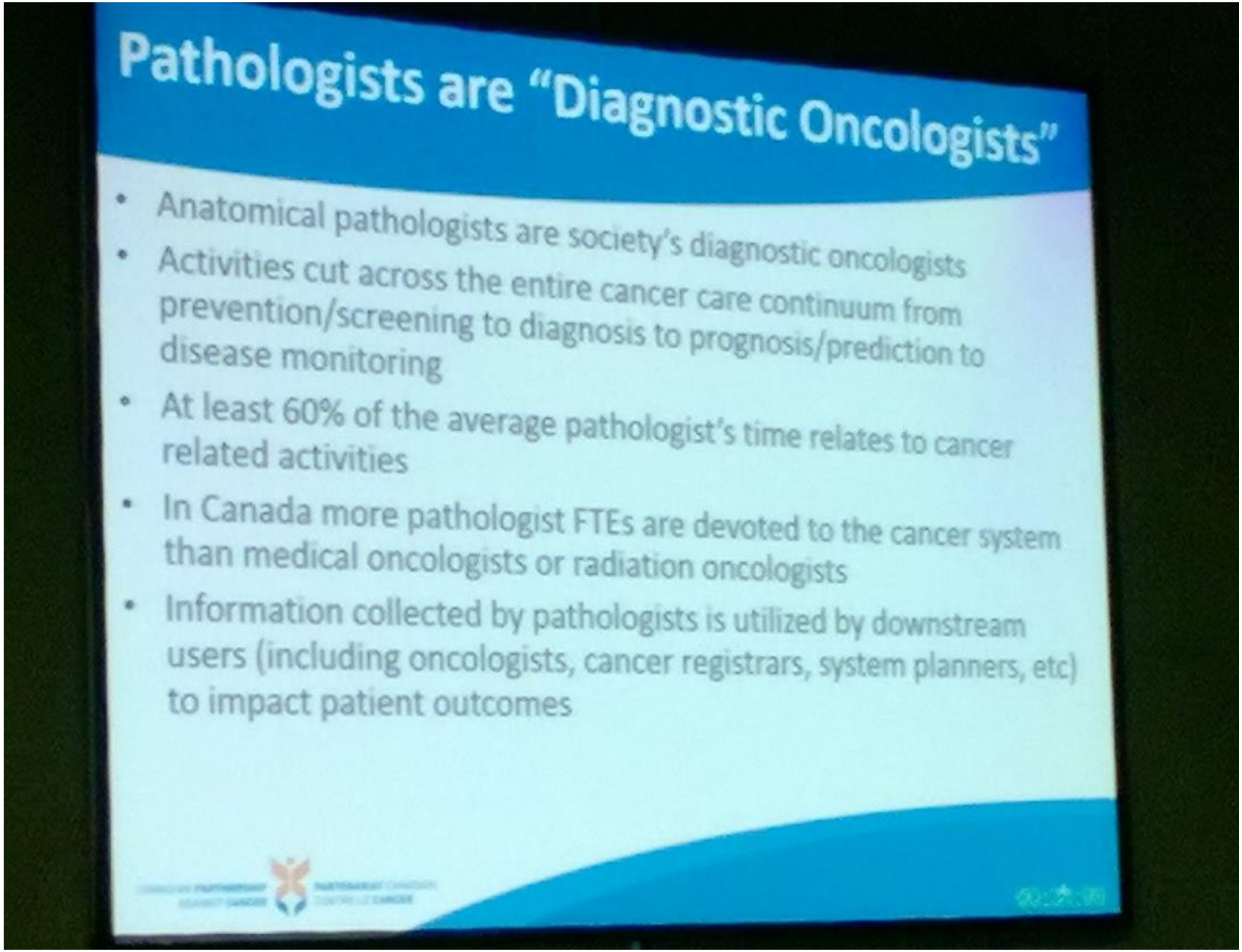
Rectal resection
Adenocarcinoma
Histological grade: poorly differentiated
Tumour localisation: upper third of the rectum
Macroscopic appearance: ulcerative infiltrative
Pre-existing polyps: not identified
Tumour size:
-largest diameter: 7 cm
-maximum tumour thickness: 2.5 cm
Local invasion: the tumour 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 metastasispresent
pTNM status: pT4a N2b M1b

d

Ecc-electronic cancer checklist



Arch Pathol Lab Med—Vol 139, May 2015



J. Srigley. Presentation at 27th ECP Congress, Belgrade, 2015

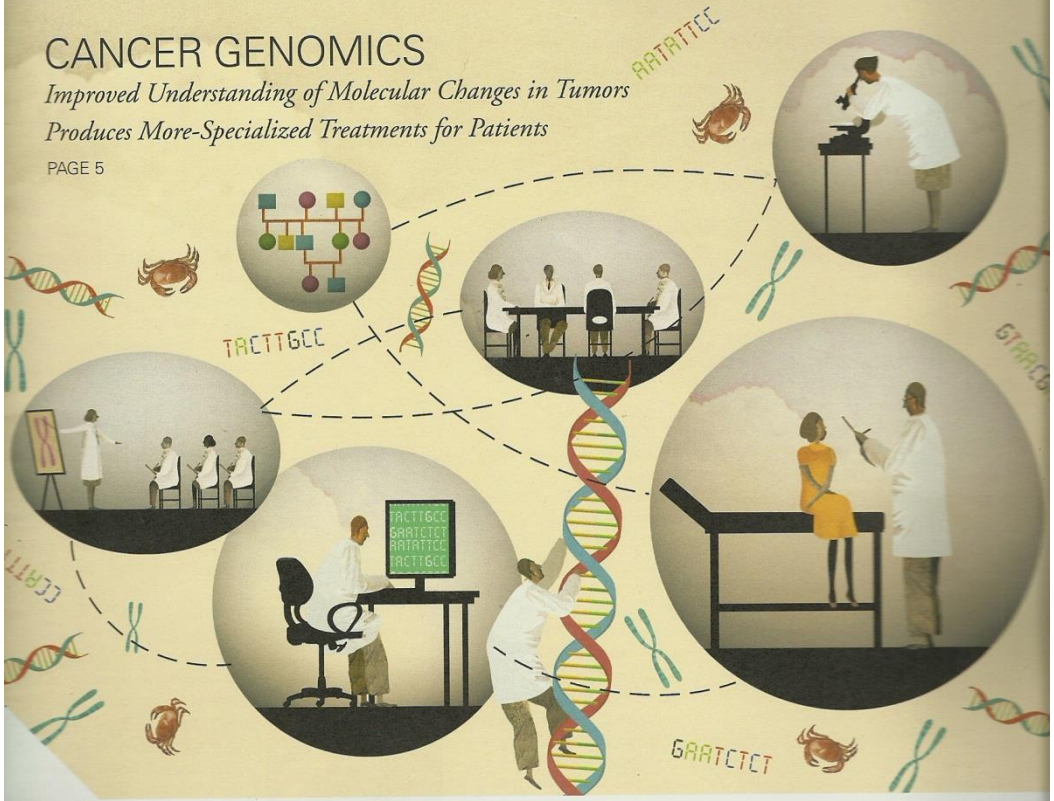
CenterNews

SEPTEMBER 2012
MEMORIAL SLOAN-KETTERING CANCER CENTER

CANCER GENOMICS

Improved Understanding of Molecular Changes in Tumors Produces More-Specialized Treatments for Patients

PAGE 5




2


2

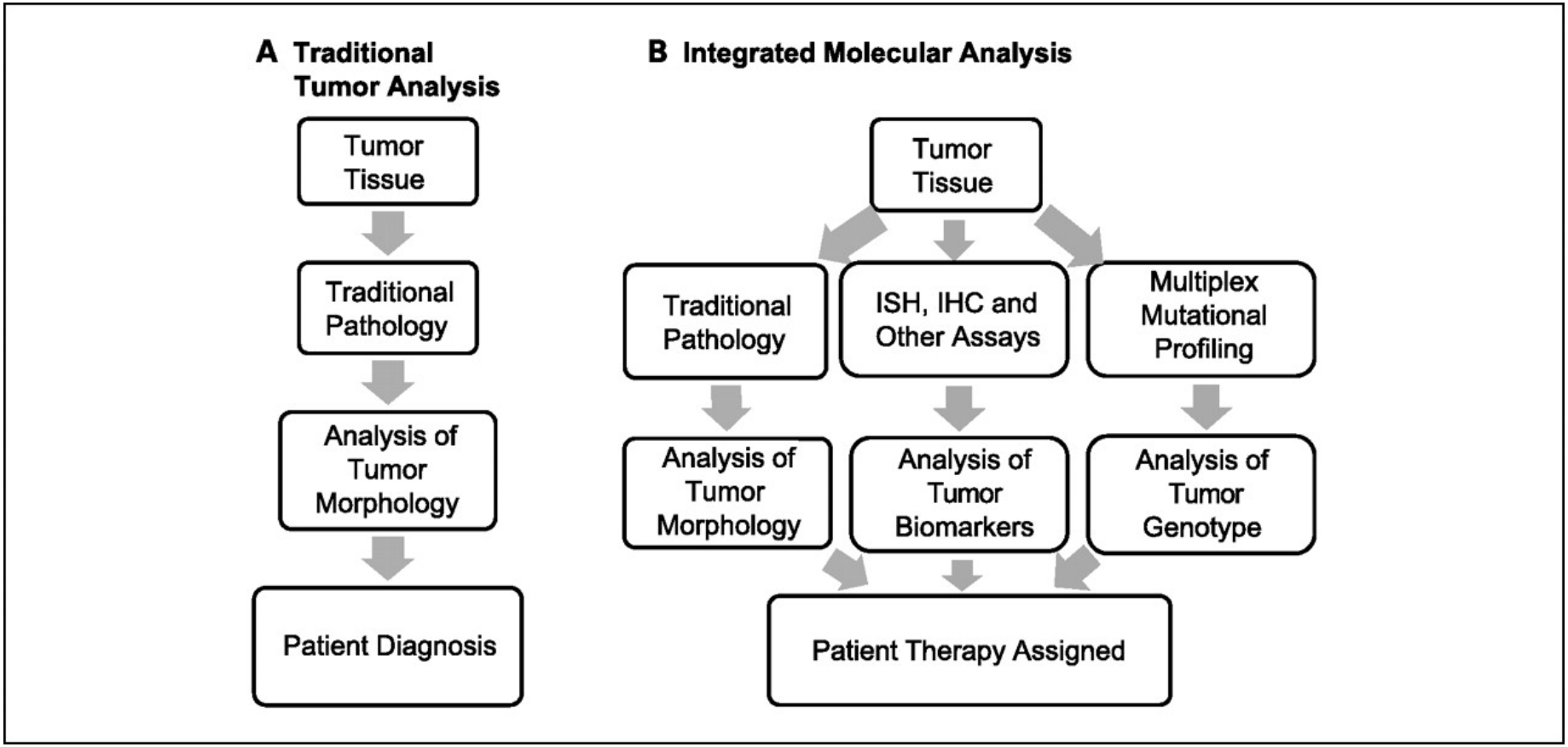

11


15


20

Memorial Sloan-Kettering Cancer Center

Role of pathology in oncology



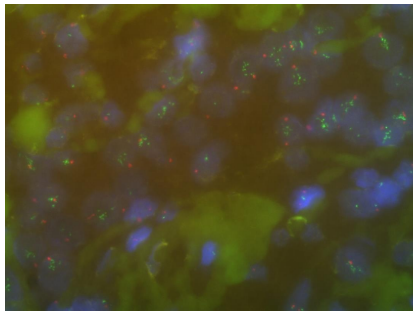
Pao W et al. Clin Cancer Res 2009;15:5317-5322

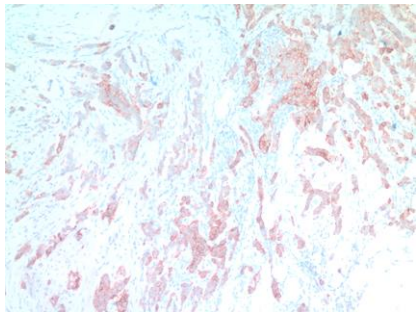
Practical use of molecular pathology

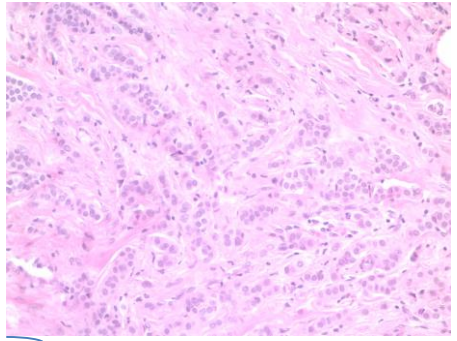
1. Ancillary study to support/make the diagnosis
2. Define genetic abnormalities associated with prognosis or predtictive for effectivness of therapy
   - Targeted therapy

Diagnostic methods in molecular pathology

- Immunohistochemistry
  - Detection of proteins
- In situ hybridisation
  - Longer DNA sequencies, 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

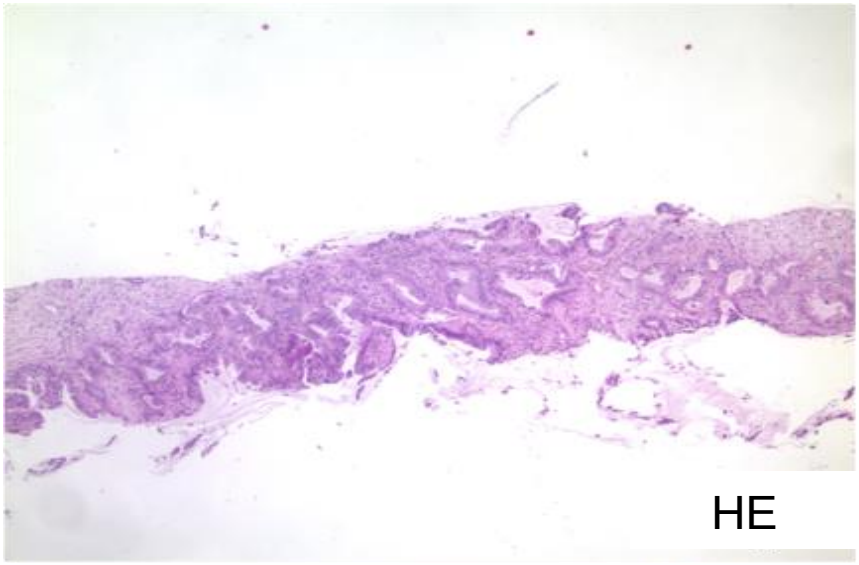
Immunohistochemistry

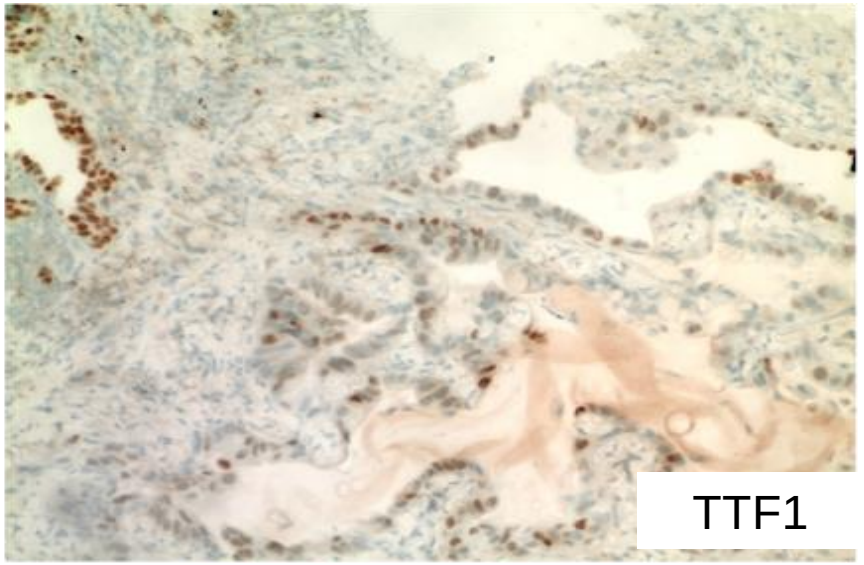
Molecular methods

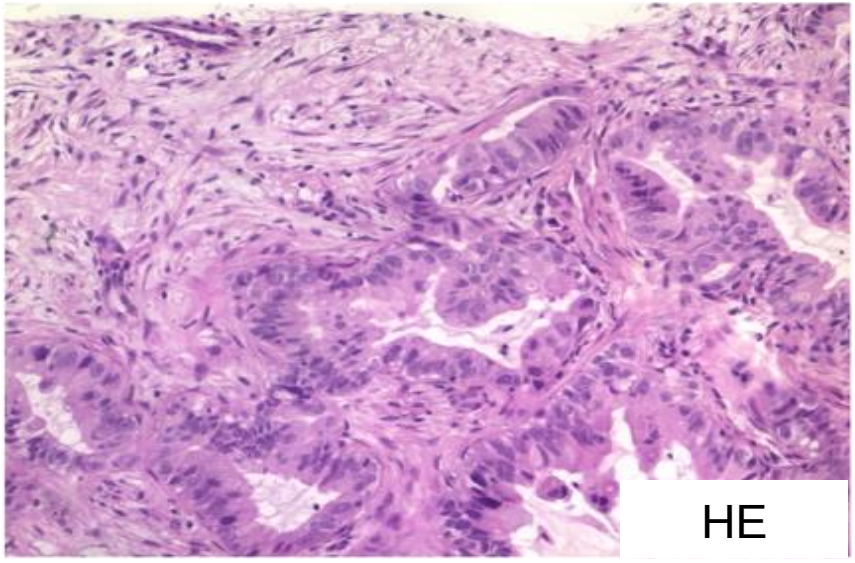
Practical use of molecular pathology

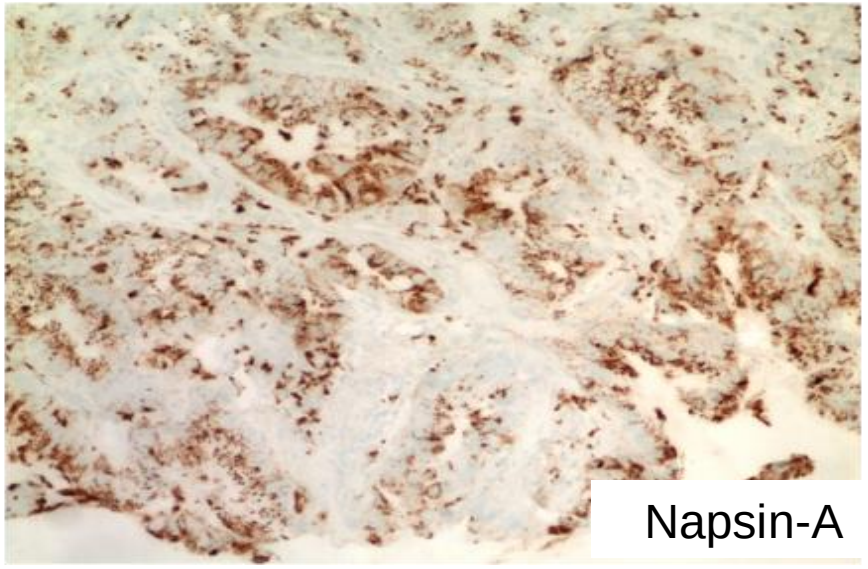
- **Ancillary study to support/make the diagnosis**
  - Detection of protein/geneteic abnormalities specific for a tumor type
    - Immunohistochemistry – tumor type specific protein expression
      - Breast cancer: ER, GATA3
      - Colon adenocarcinoma: CDX2
      - Lung adenocarcinoma: TTF1, Napsin-A
    - In situ hybridisation, RT-PCR, sequencing – tumor type specifc 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

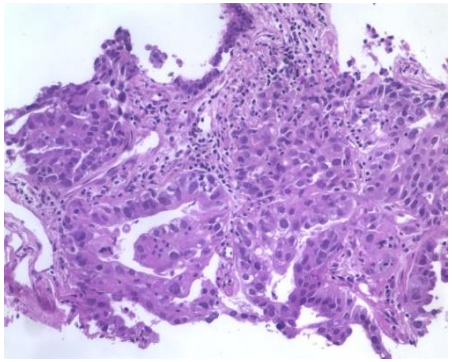
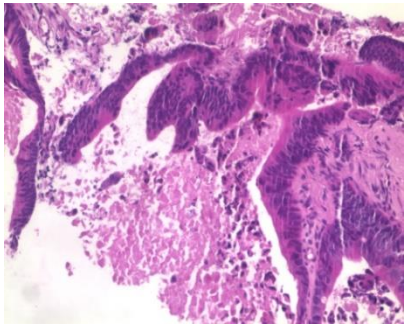
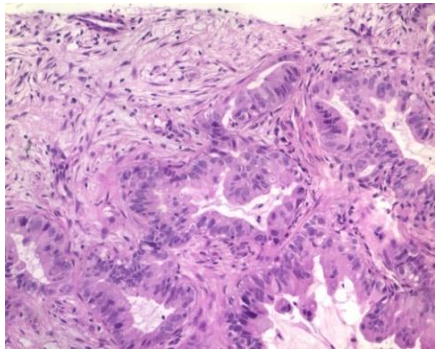
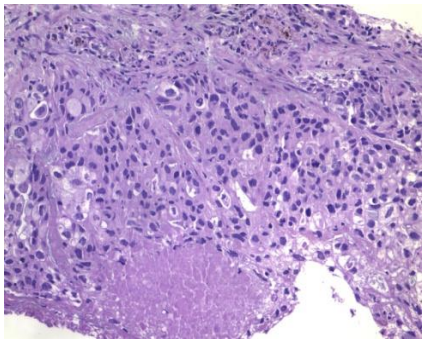
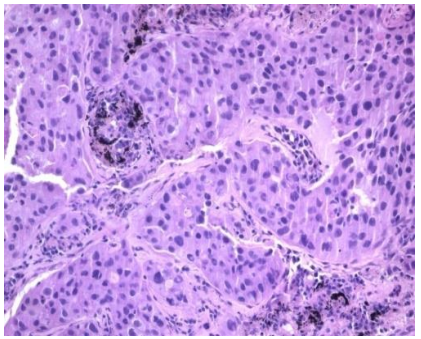
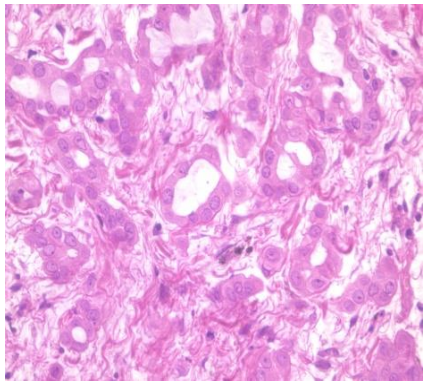
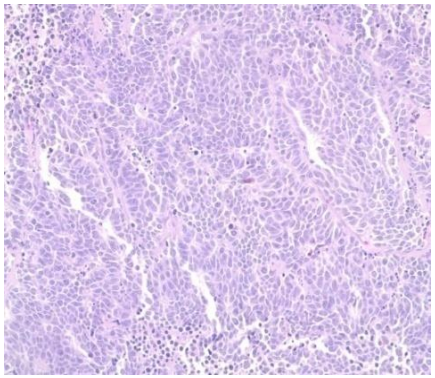
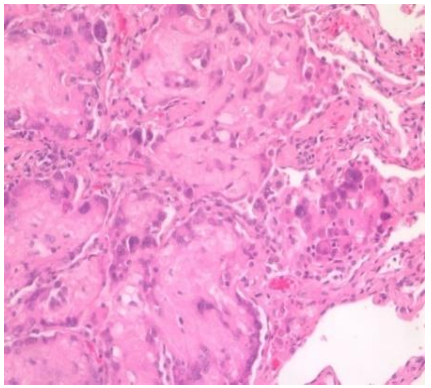




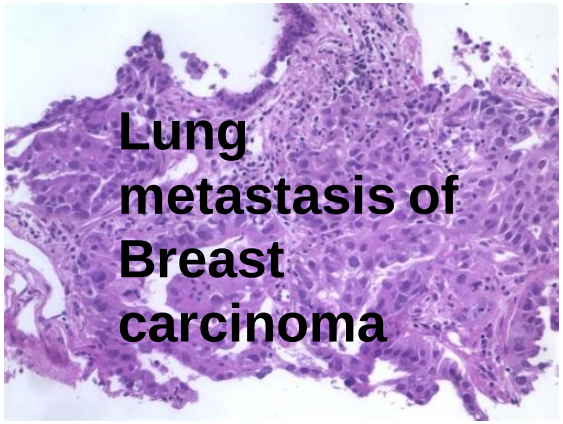


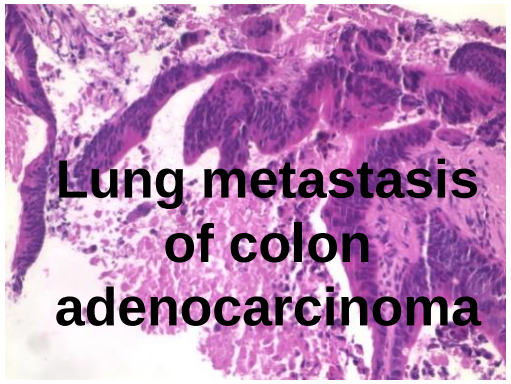


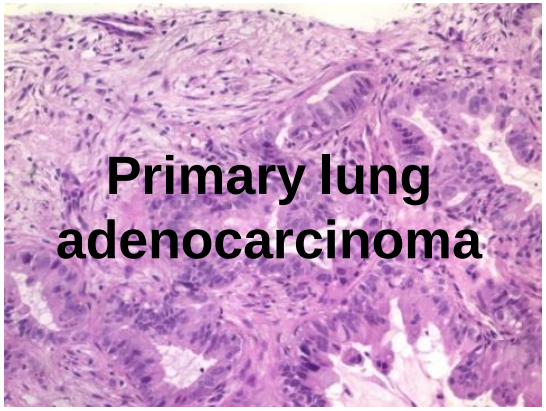
Lung tumors, HE

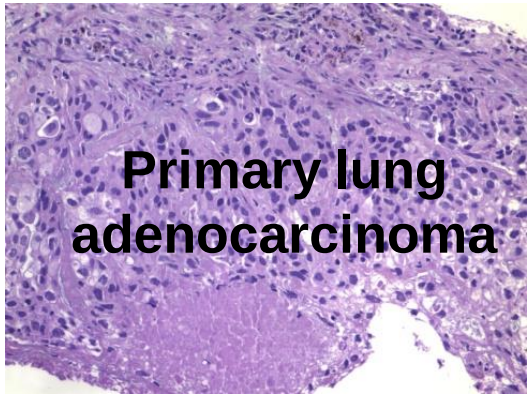









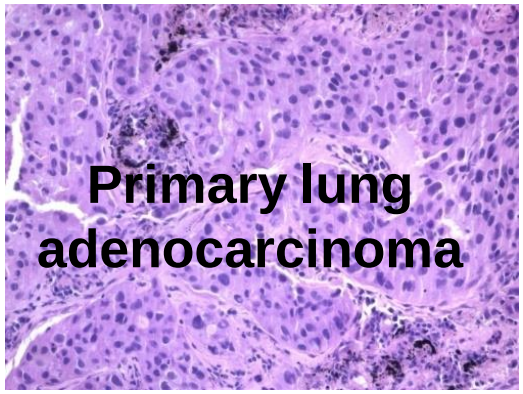

Breast
carcinoma

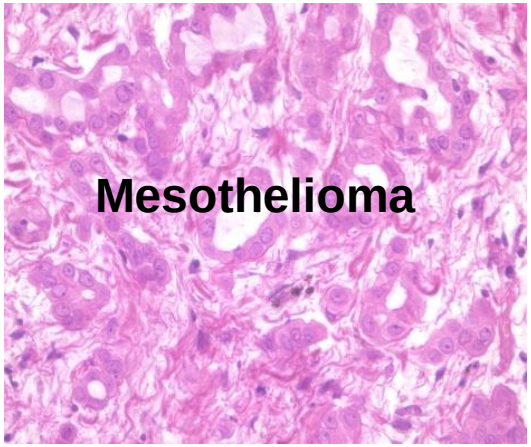

Lung
metastasis of
Breast
carcinoma

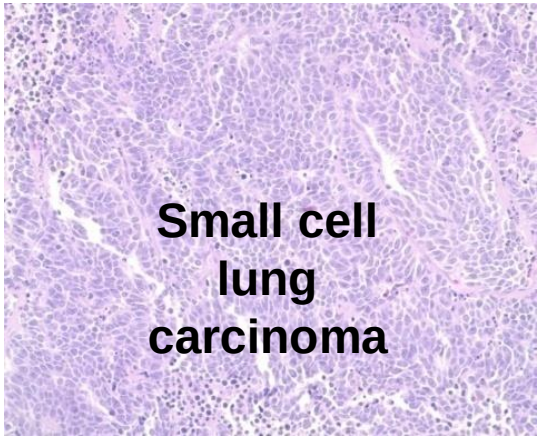

Lung metastasis
of colon
adenocarcinoma

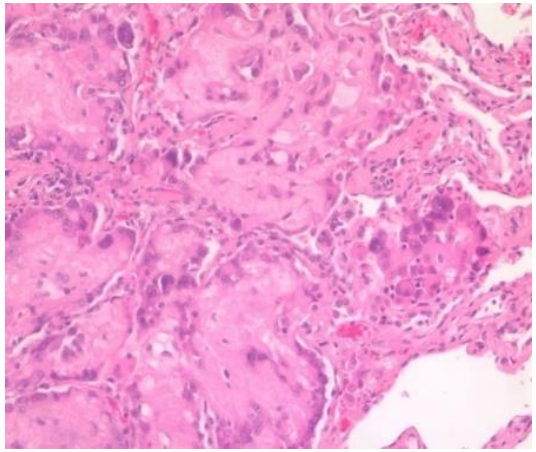

Primary lung
adenocarcinoma


Primary lung
adenocarcinoma

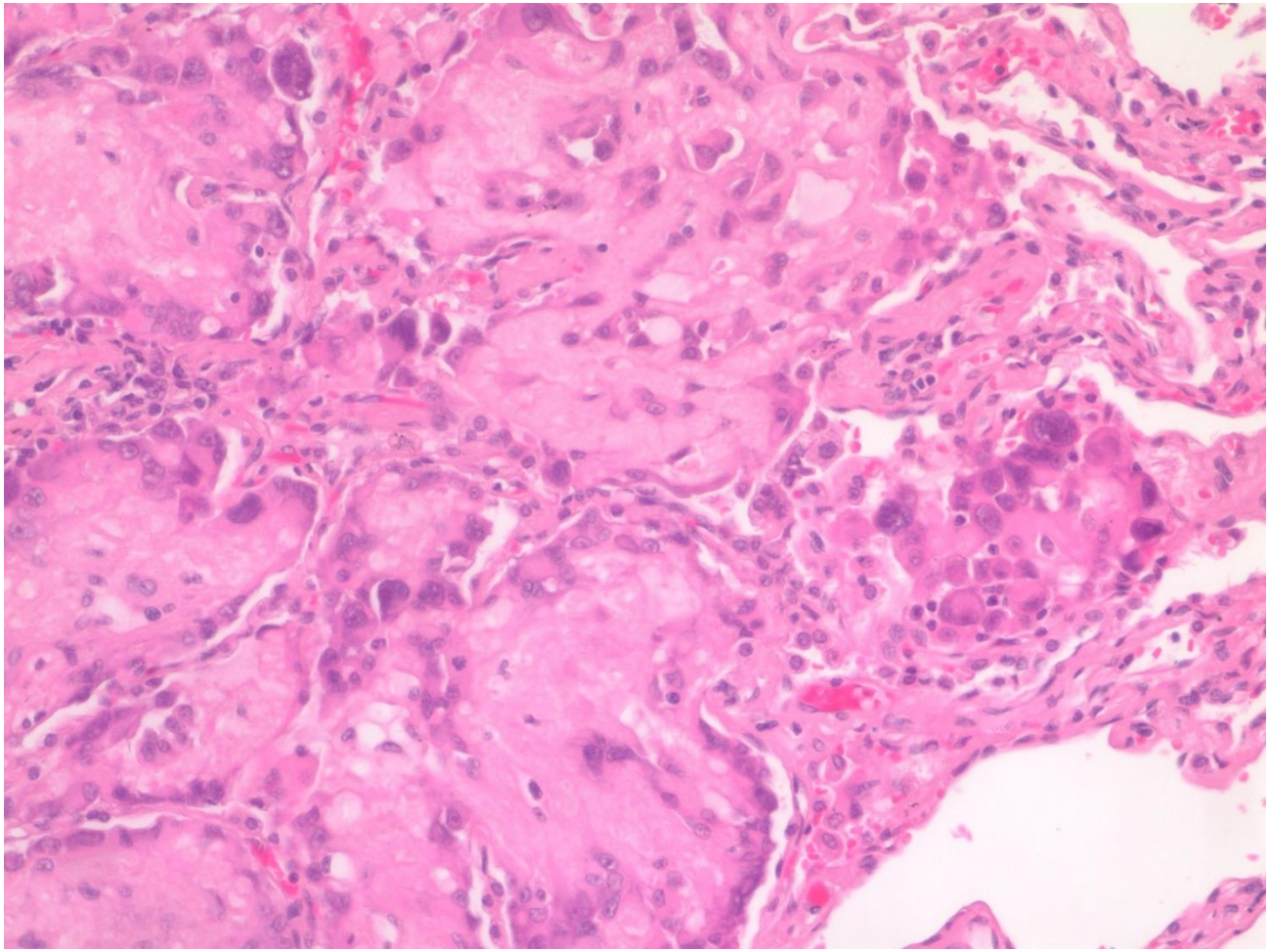

Primary lung
adenocarcinoma


Mesothelioma

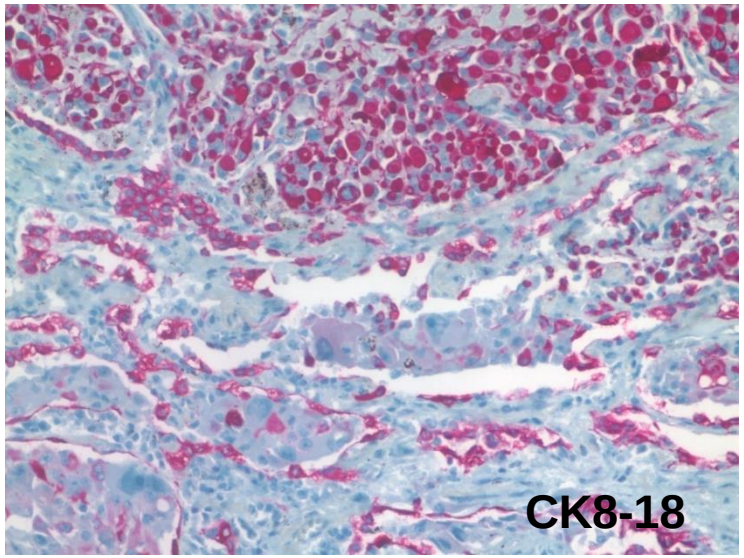

Small cell
lung
carcinoma

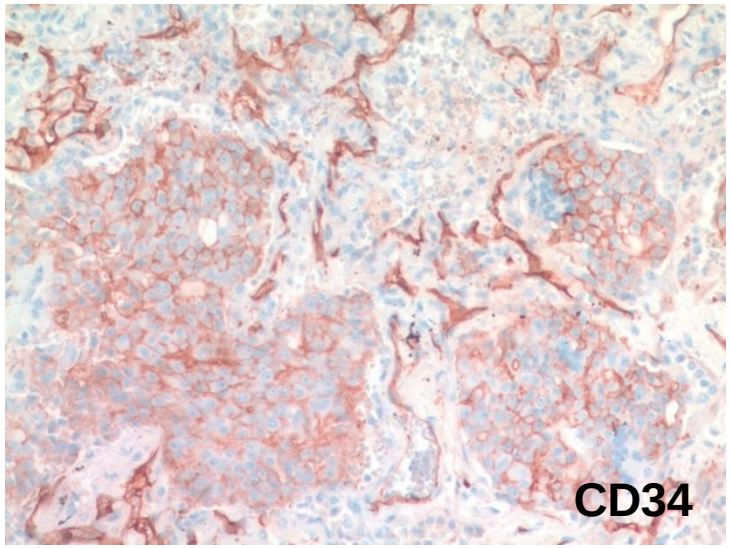


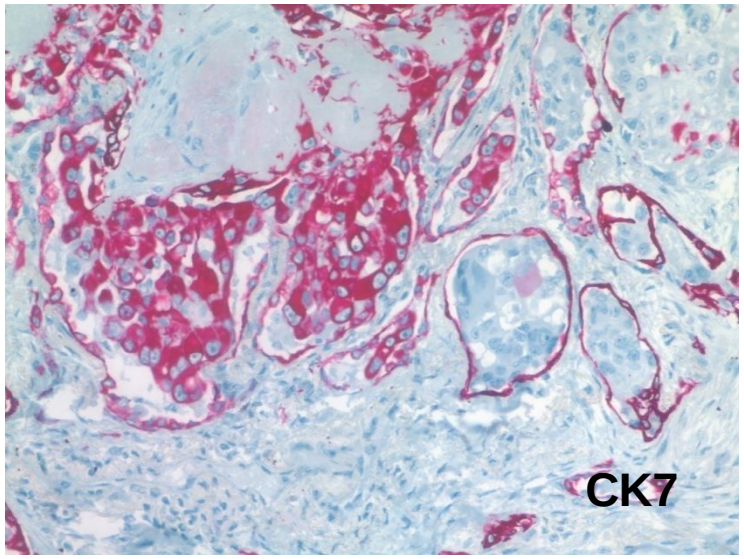
Immunhistochemistry in rare lung tumors

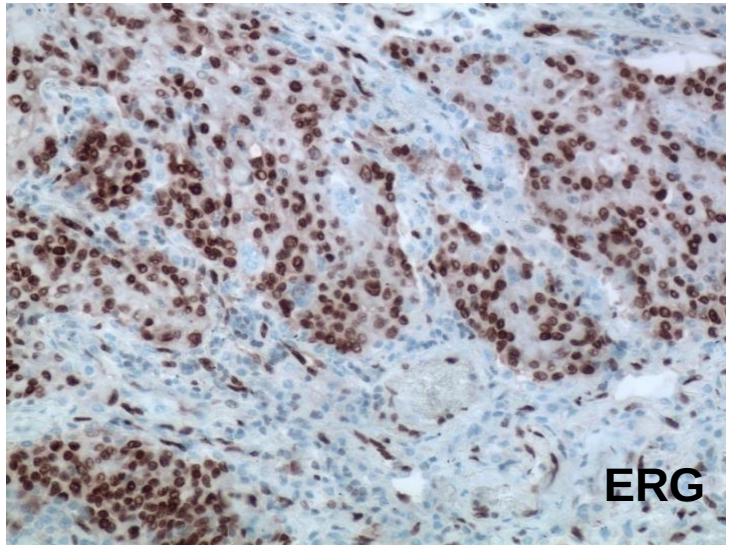


Immunohistochemistry









Epithelial markers

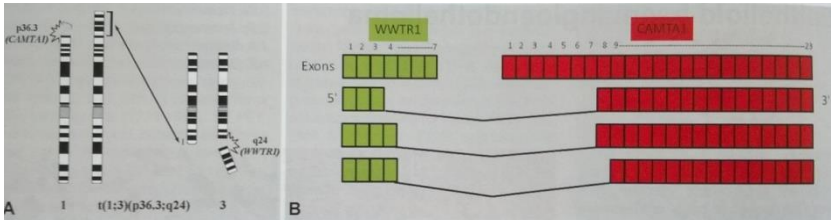
Vascular markers

Epithelioid hemangioendothelioma

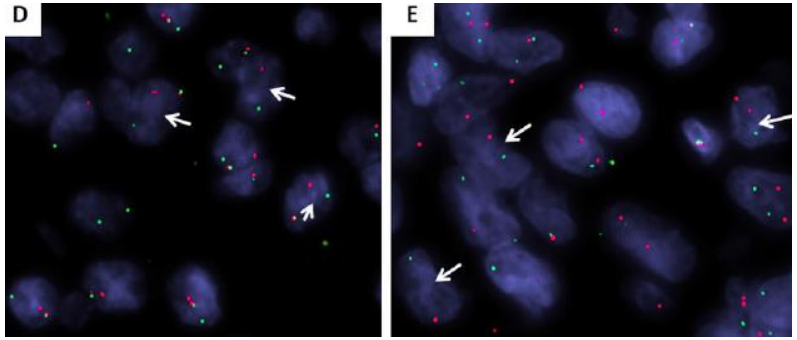
- Malignant vascular tumor, with realtively indolent behavior
- Soft tissue, bone, skin, lung and other parenchymal organs
- *Rare tumor*, wide age range, various anatomical locations, multifocality
  - *Differential diagnosis is wide*

Epithelioid hemangioendothelioma
Fusion genes

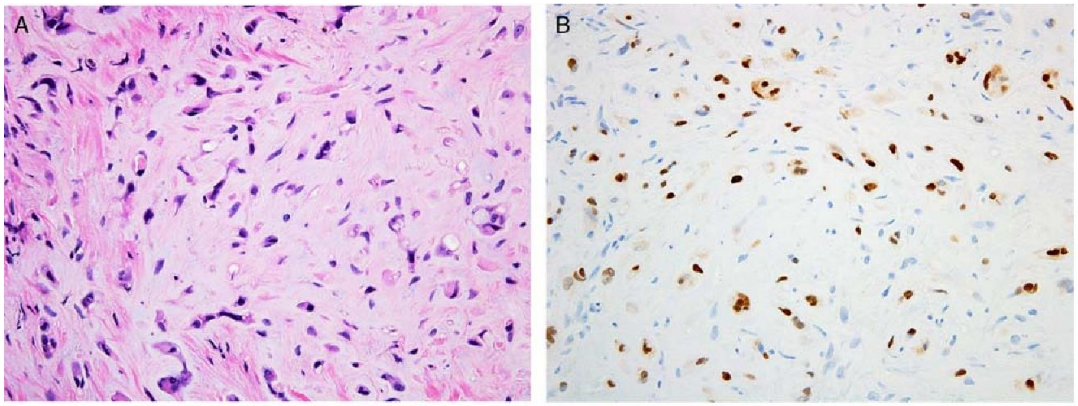
- 1. t(1;3) → ***WWTR1-CAMTA1*** (majority)
  - WWTR1 (TAZ): transcription co-activator
  - CAMTA1: calmodulin-binding transcription activator
- 2. t(11;X) → ***YAP1-TFE3*** (very rare)
  - YAP1: transcription co-activator
  - TFE3: transcription factor
- Detection:
  - RT-PCR
  - **FISH**
  - **Immunhistochemistry**



WHO 2015



AJSP.2015;39:132-139



www.pathologyoutlines.com

Practical use of molecular pathology

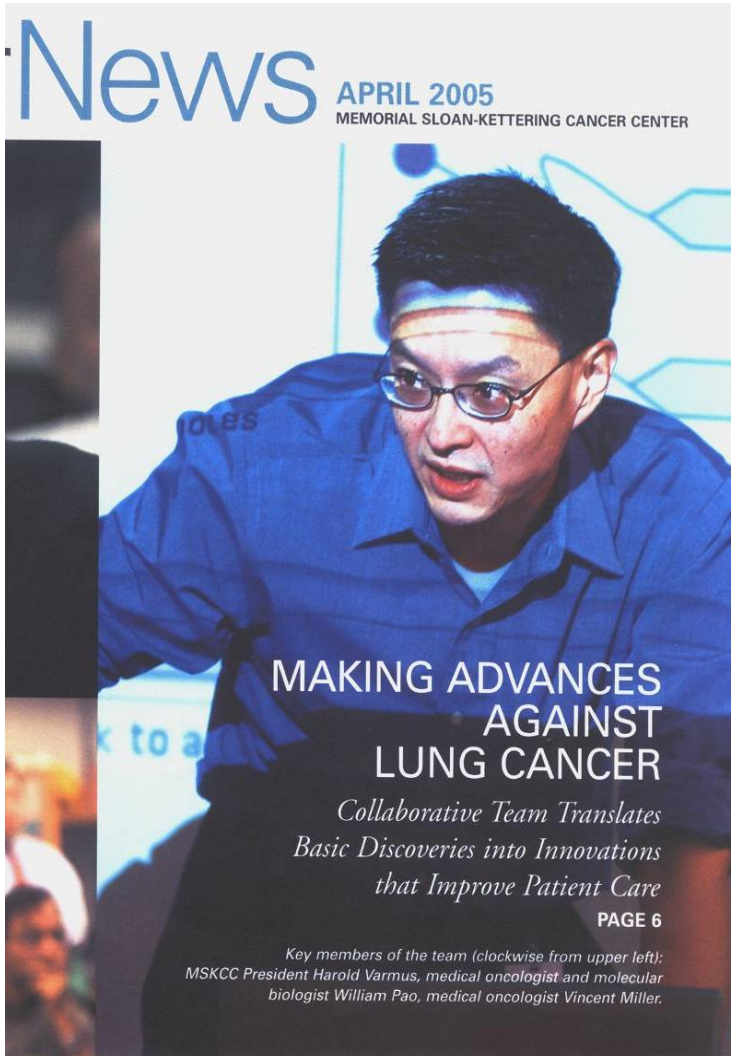
Define genetic abnormalities that are associated with prognosis or predictive for effectiveness of therapy

- Targeted therapy

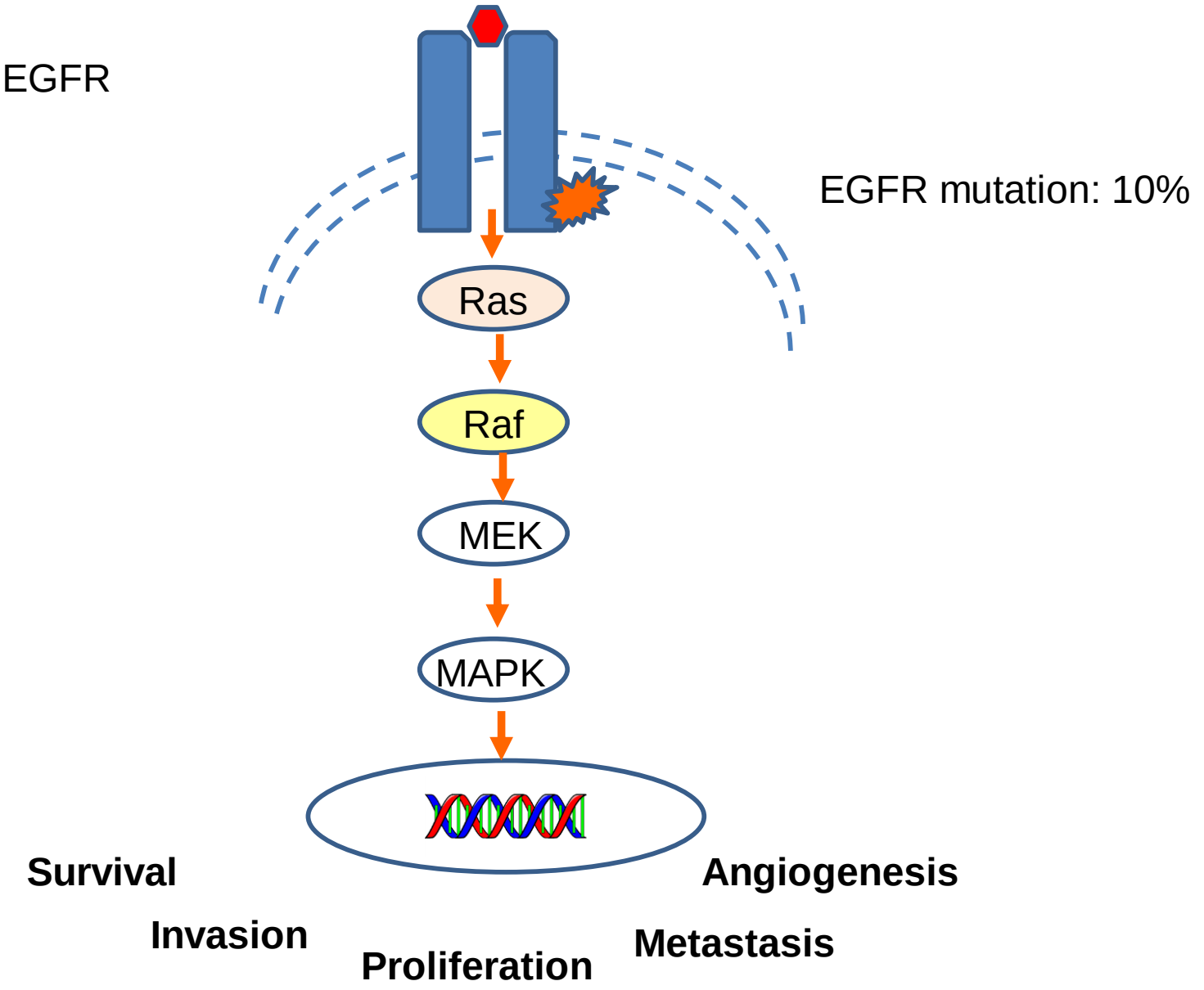
Tumour type	Gene	Abnormality	Drug/ indication
Lung adenocarcinoma	EGFR	mutation	EGFR TKI sensitivity
	RAS	mutation	EGFR TKI resistance
	ALK	translocation	Crizotinib sensitivity
	ROS1	translocation	Crizotinib sensitivity
Colon adenocarcinoma	KRAS	mutation	Anti-EGFR resistance
	NRAS	mutation	Anti-EGFR 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

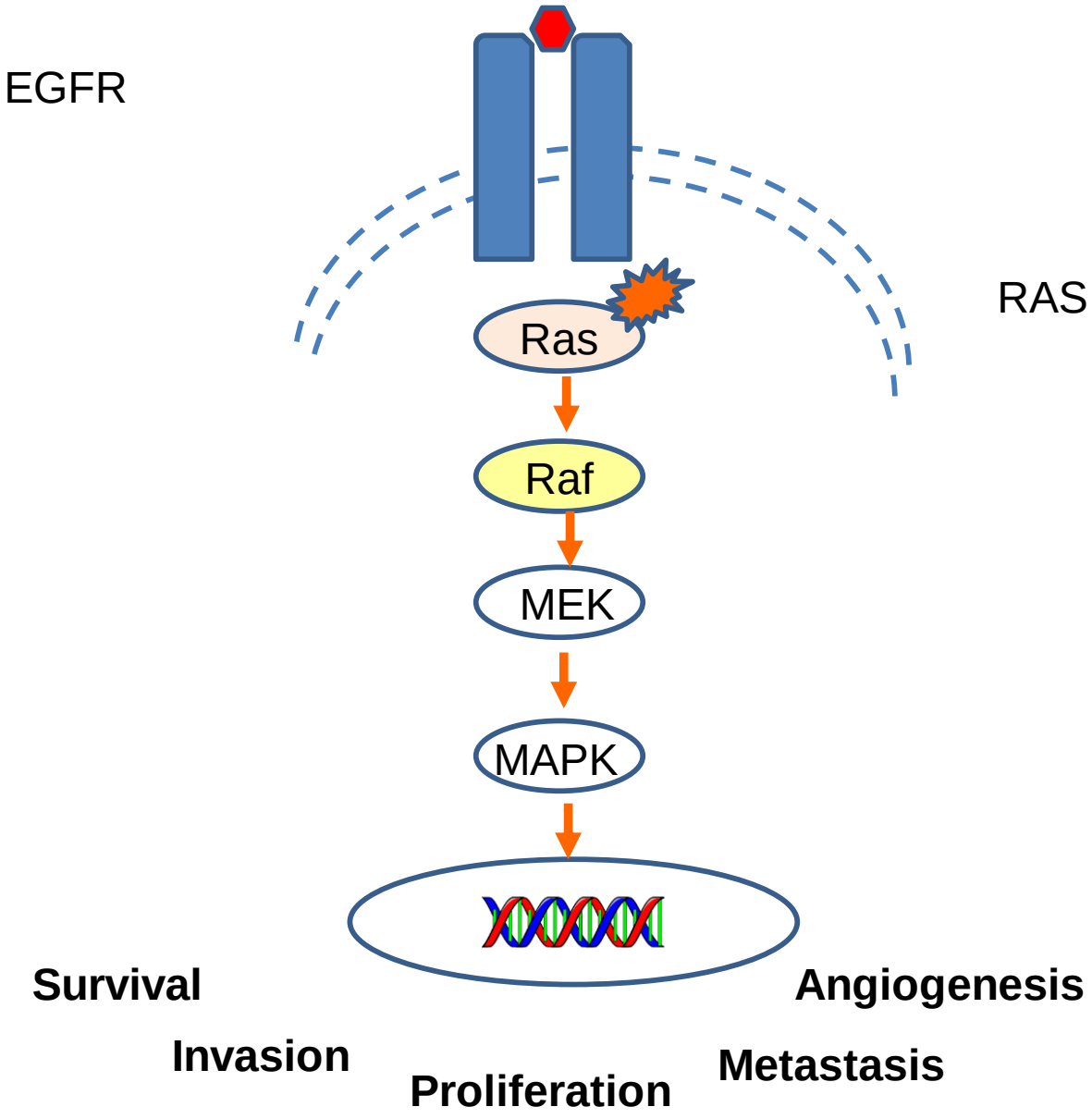
- Lynch, Paez, *Pao* - 2004
  - Somatic mutations of EGFR gene in exons 18-21-ben in lung NSCLC correlate with response to EGFR tirosine kinase inhibitor (TKI) therapy(erlotinib, gefitinb)
    - Female
    - Adenocarcinoma
    - Non-smokers



EGFR signaling pathway alteration in lung adenocarcinoma

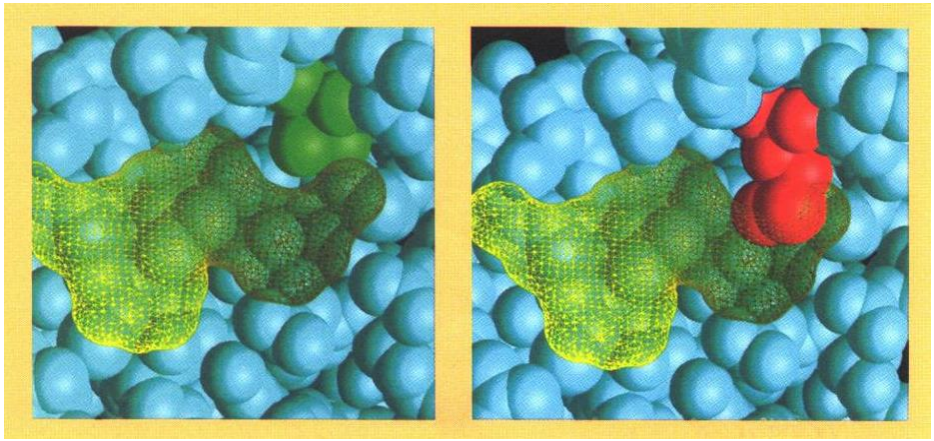


EGFR signaling pathway alteration in lung adenocarcinoma



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

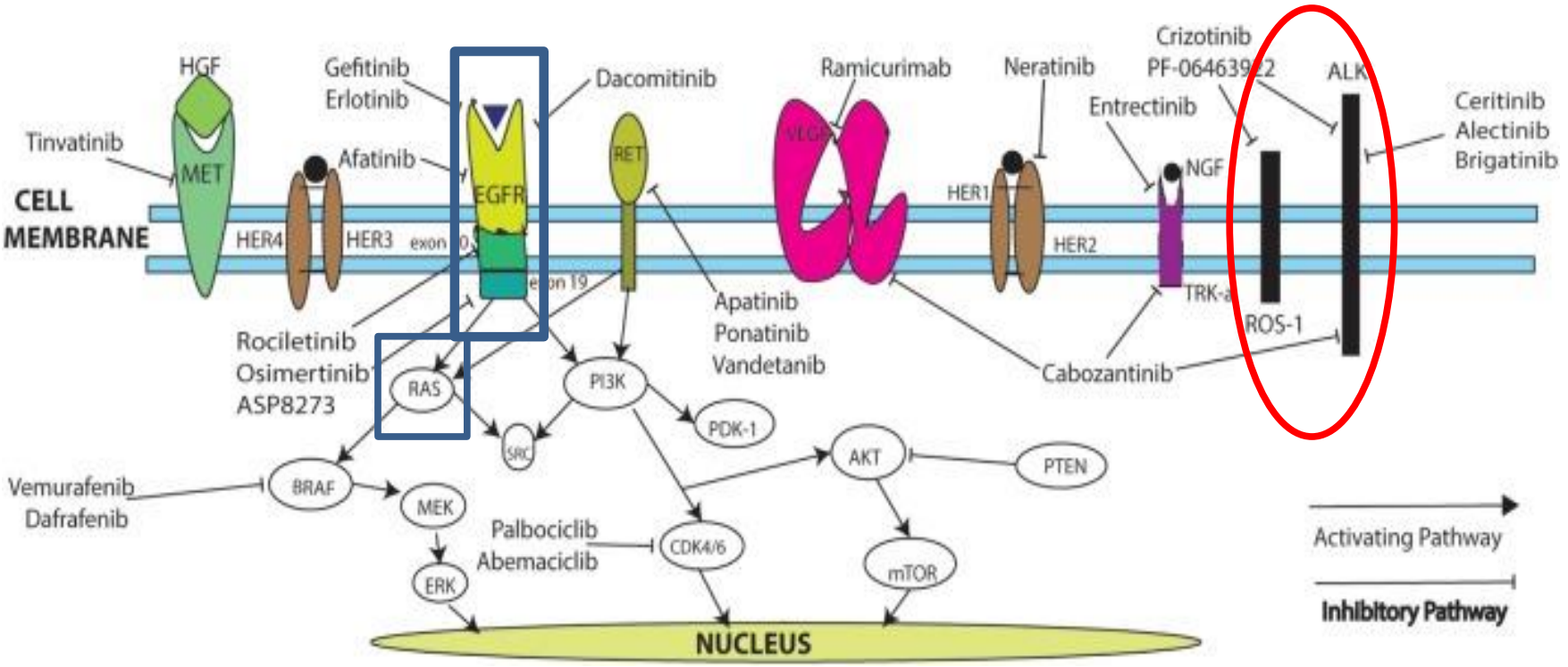


Main genetic abnormalities in lung cancer

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

WHO 2015

Targetable signaling pathways in nonsquamous NSCLC

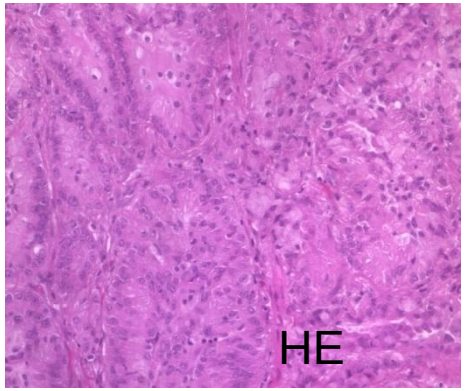


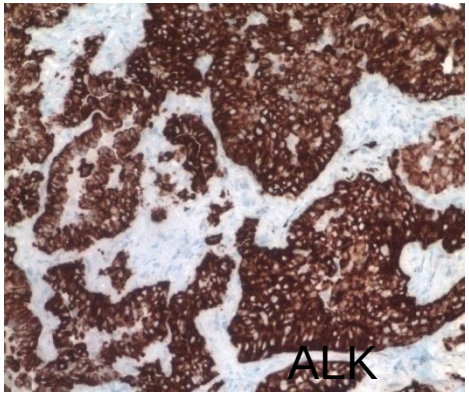
Bansal P, Front Oncol 2016

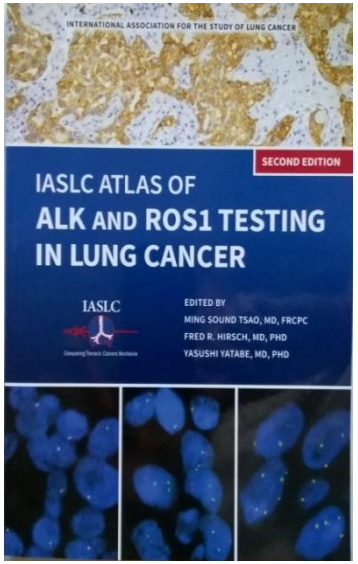
ALK és ROS1 gene rearrangements in lung adenocarcinoma

- 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) threapy effective
- Detection
  - RT-PCR (fresh sample), sequencing
  - FISH (gold standard, at least 50-100 cells)
  - IHC (screening or diagnostic with validated antibodies)

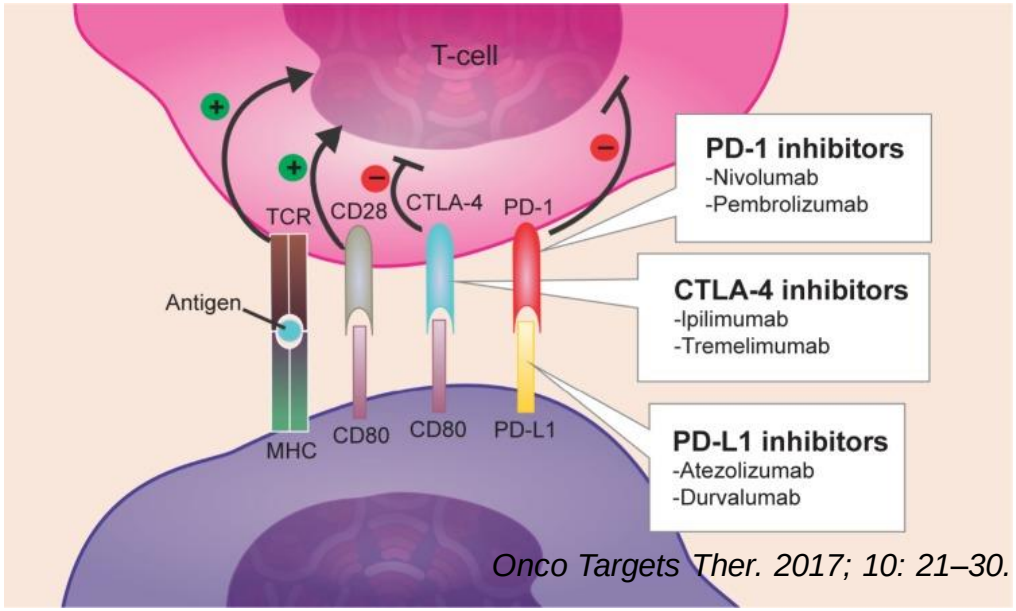
- Reactive oxygen species 1 – ROS1 gén
- Tyrozine kinase receptor protein, similar to ALK
- 1-2% of lung adenocarcinomas (ROS1-CD74 fusion most often)
  - Non smoker females
  - No correlations with histological types
- ROS1 gátló (crizotinib) terápiára reagál
- Detetction:
  - RT-PCR (fresh sample), sequencing
  - FISH (gold standard, at least 50-100 cells)
  - IHC (screening or diagnostic with validated antibodies)



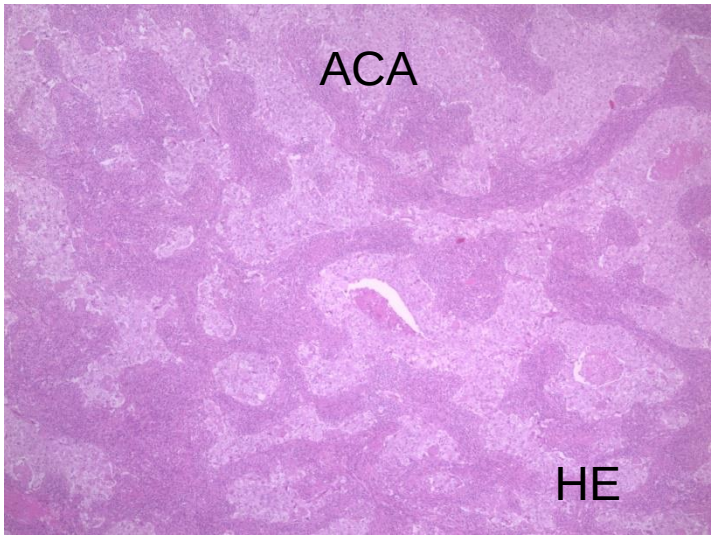


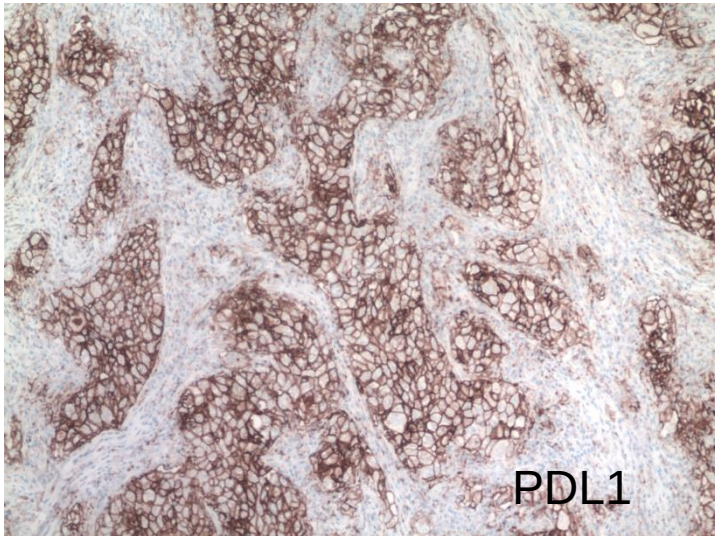


Immunotherapy
PD1-PDL1 inhibition in NSCLCs

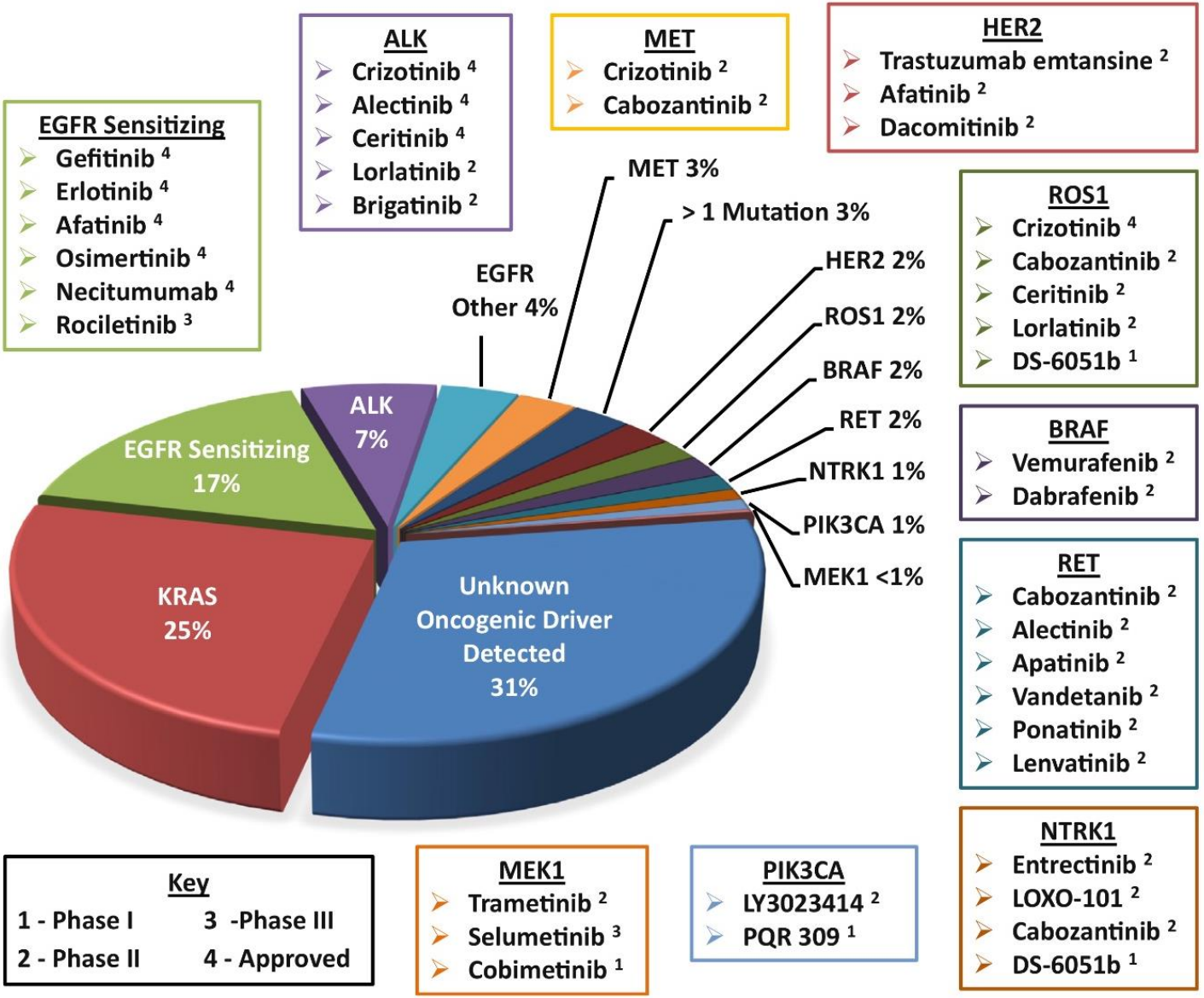


Onco Targets Ther. 2017; 10: 21–30.

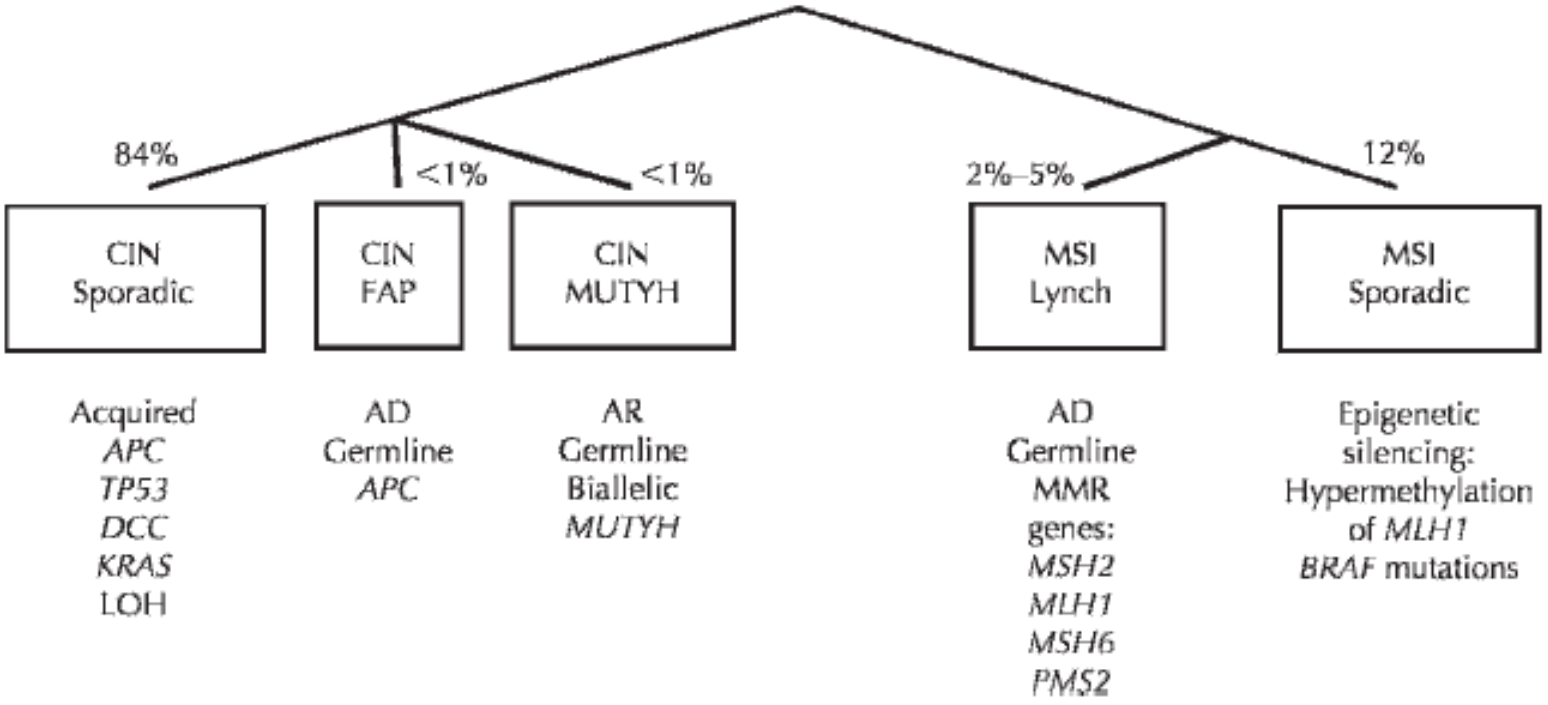


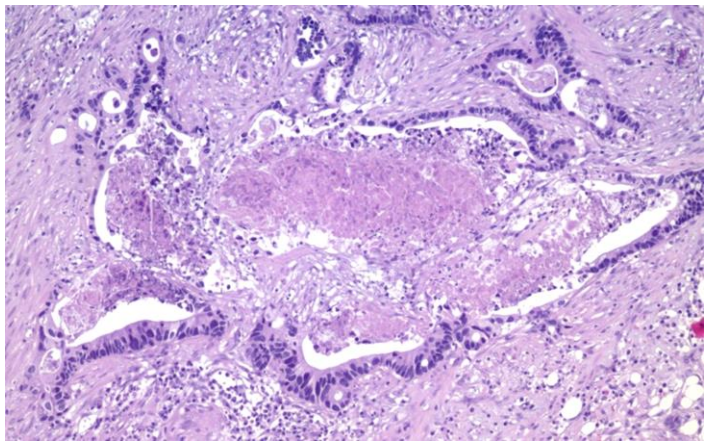


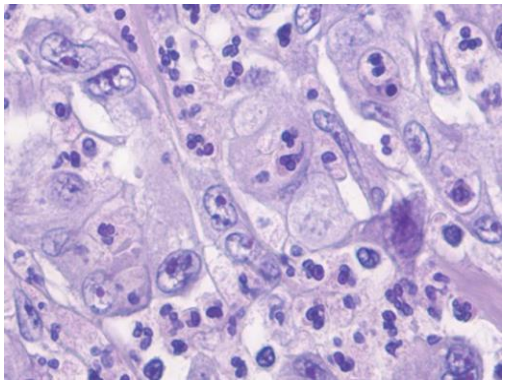
Biomarker: PDL1 expression - IHC

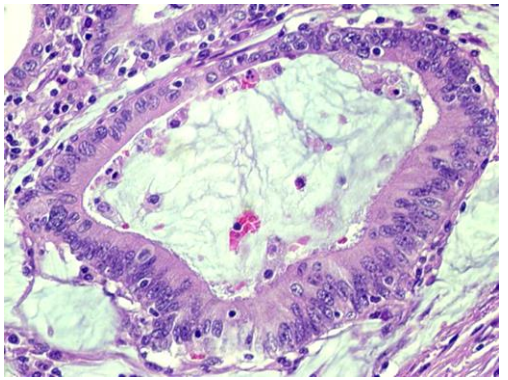

ALK
Crizotinib 4
Alectinib 4
Ceritinib 4
Lorlatinib 2
Brigatinib 2
MET
Crizotinib 2
Cabozantinib 2
HER2
Trastuzumab emtansine 2
Afatinib 2
Dacomitinib 2
EGFR Sensitizing
Gefitinib 4
Erlotinib 4
Afatinib 4
Osimertinib 4
Necitumumab 4
Rociletinib 3
MET 3%
> 1 Mutation 3%
EGFR Other 4%
HER2 2%
ROS1 2%
BRAF 2%
RET 2%
NTRK1 1%
PIK3CA 1%
MEK1 <1%
ALK 7%
EGFR Sensitizing 17%
KRAS 25%
Unknown Oncogenic Driver Detected 31%
ROS1
Crizotinib 4
Cabozantinib 2
Ceritinib 2
Lorlatinib 2
DS-6051b 1
BRAF
Vemurafenib 2
Dabrafenib 2
RET
Cabozantinib 2
Alectinib 2
Apatinib 2
Vandetanib 2
Ponatinib 2
Lenvatinib 2
NTRK1
Entrectinib 2
LOXO-101 2
Cabozantinib 2
DS-6051b 1
Key
1 - Phase I
2 - Phase II
3 -Phase III
4 - Approved
MEK1
Trametinib 2
Selumetinib 3
Cobimetinib 1
PIK3CA
LY3023414 2
PQR 309 1

Colorectal cancer molecular classification









Arch Pathol Lab Med 2011

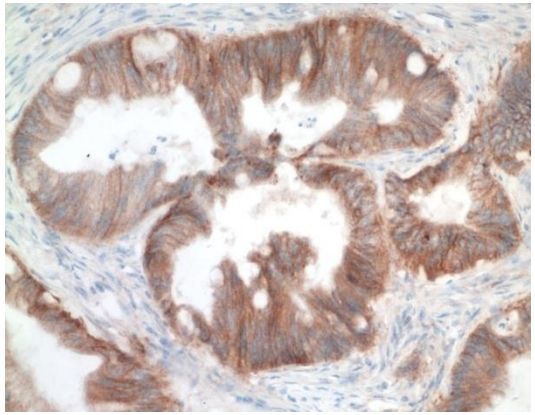
Colon adenocarcinoma

- **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

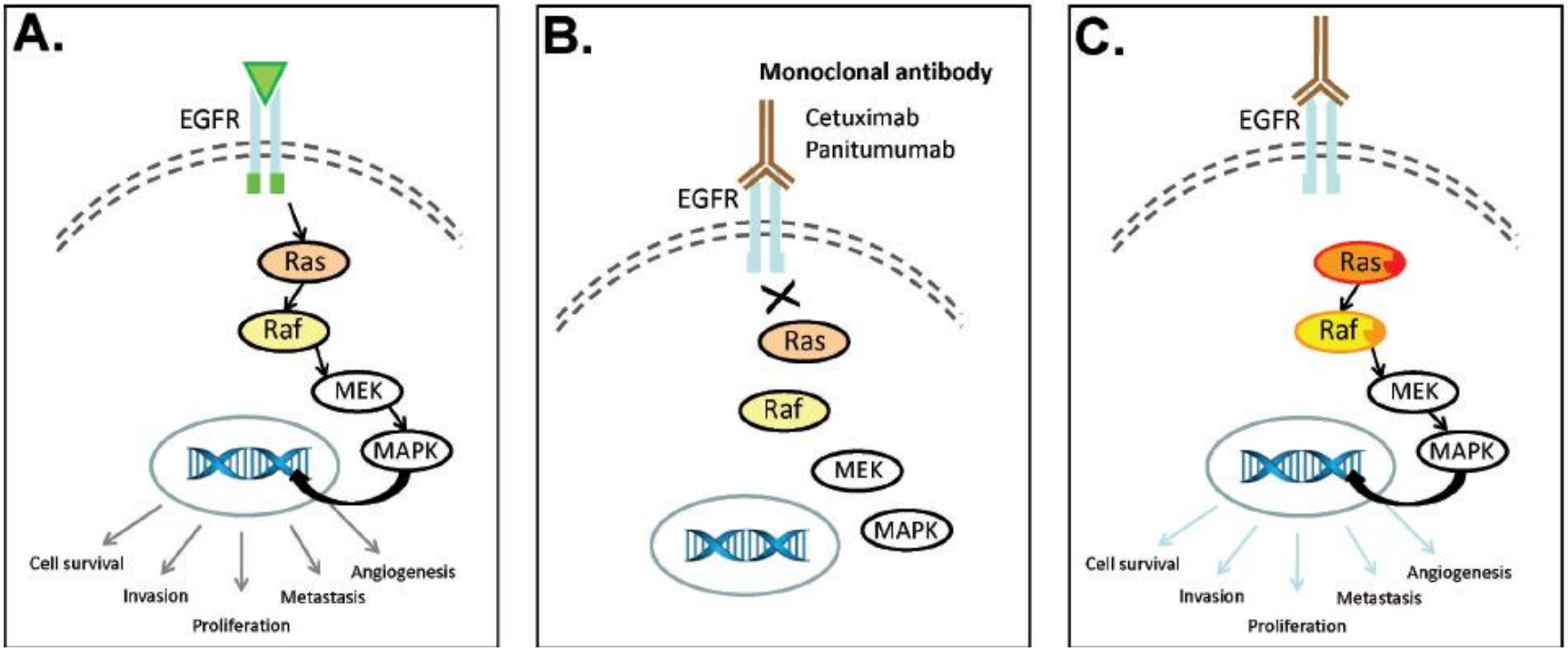
- **Testing for BRAF exon 15 mutation.** The **presence of mutation** is a **negative prognostic factor**; tumours carrying this type of mutation exhibit rather unfavorable biological behavior. BRAF mutation **may also indicate sporadic (non-hereditary) microsatellite instability**.
  - 10-15% of colon 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.

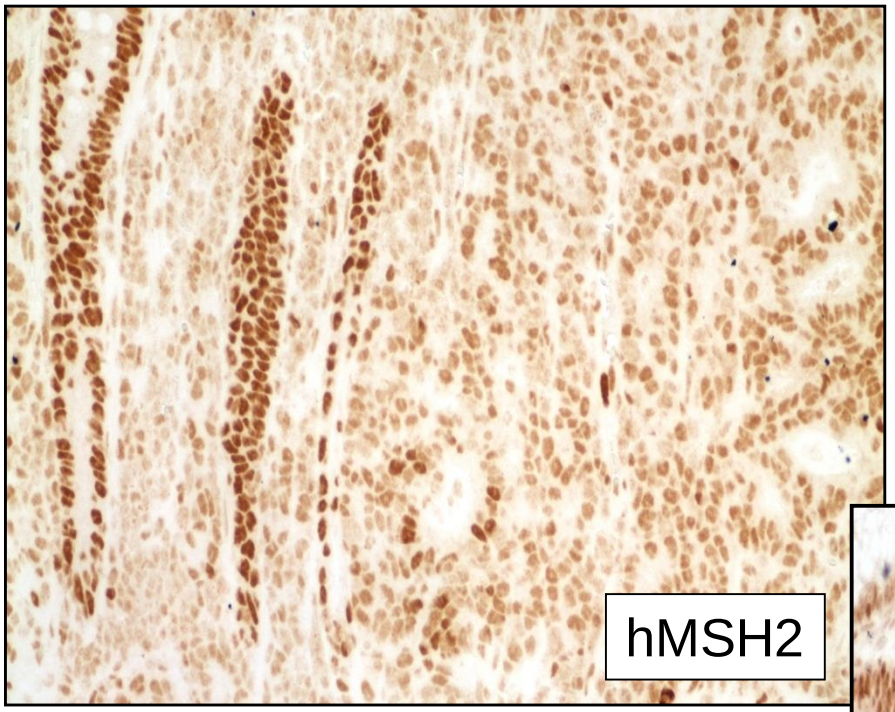
Molecular basis of anti-EGFR therapy in CRC



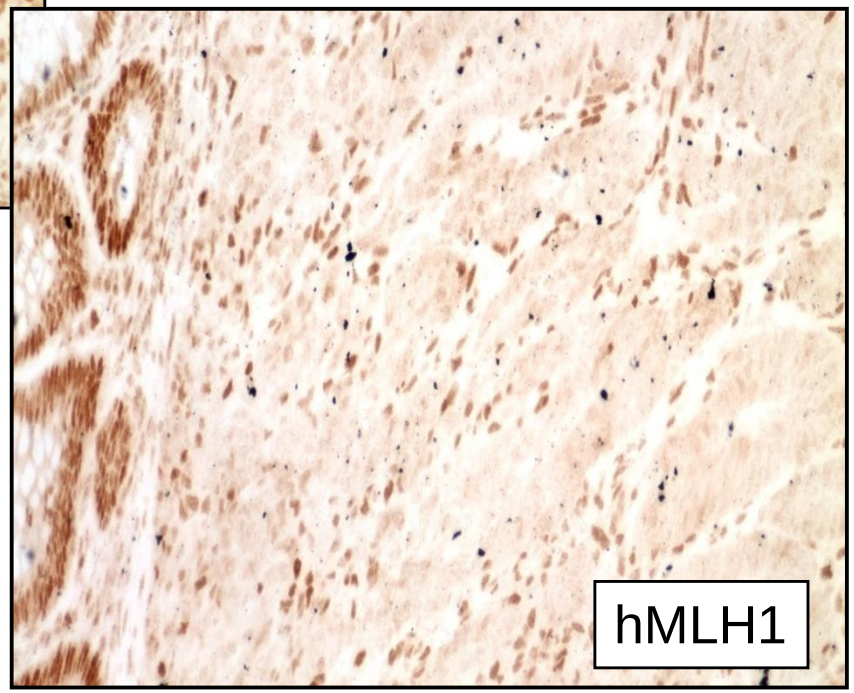
KRAS mutation: EGFR independent signal pathway activation



MSI detection with IHC

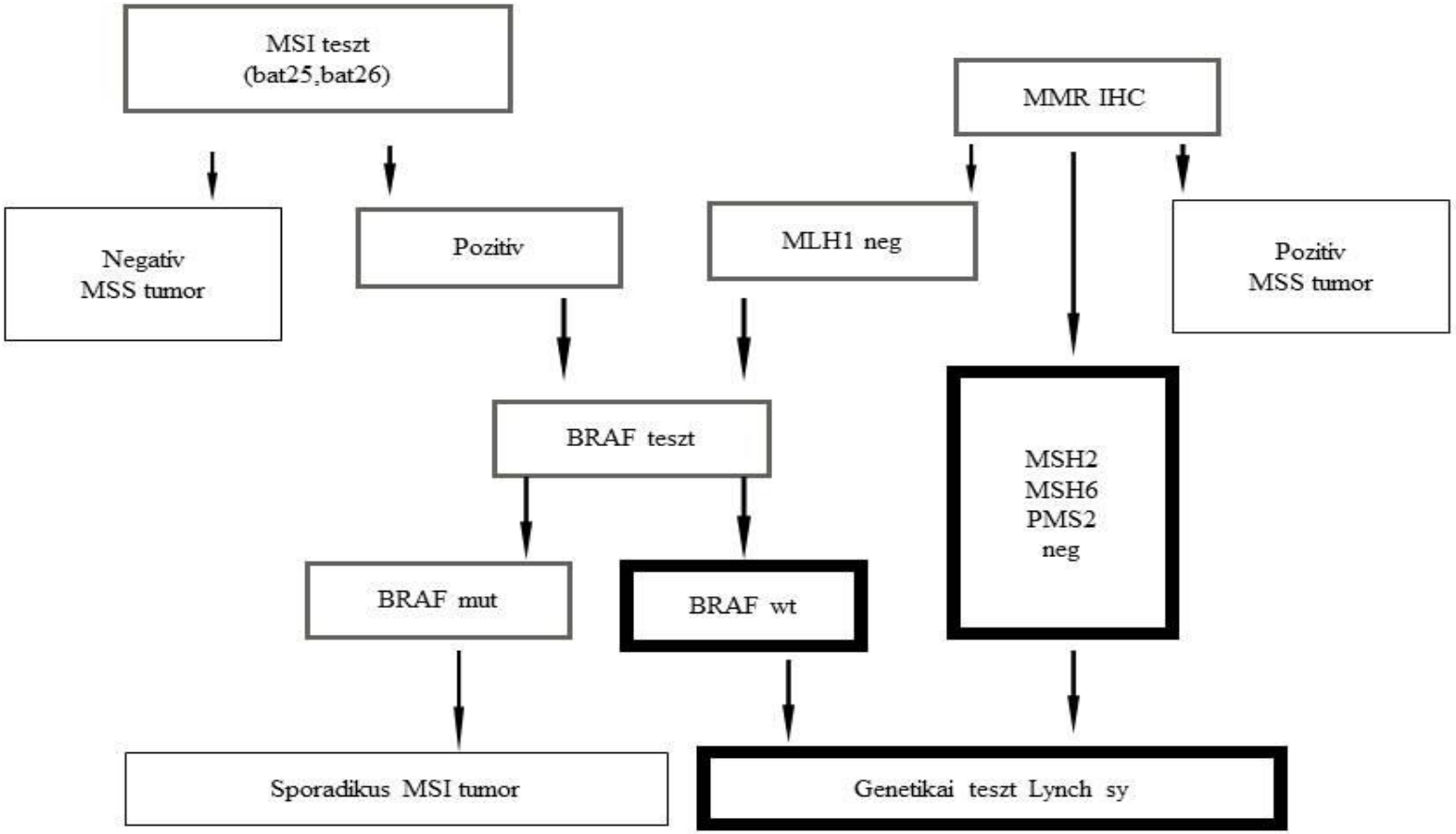


Sporadic CRC
MLH1 protein loss indicating MSI



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

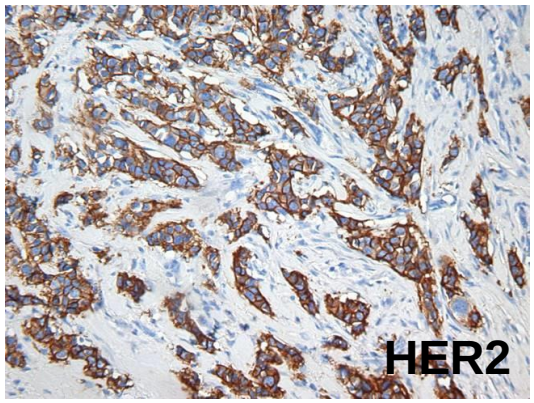
MSI testing in colon adenocarcinomas

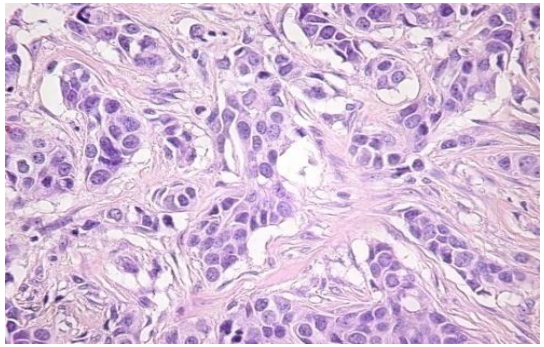


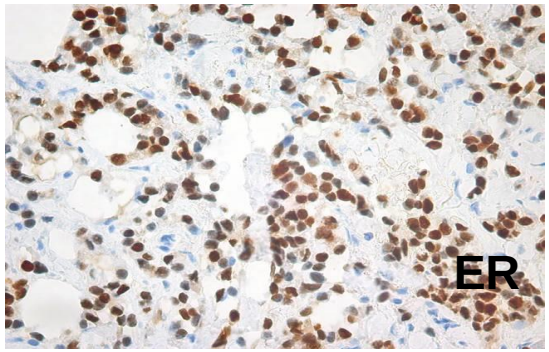
Breast cancer

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

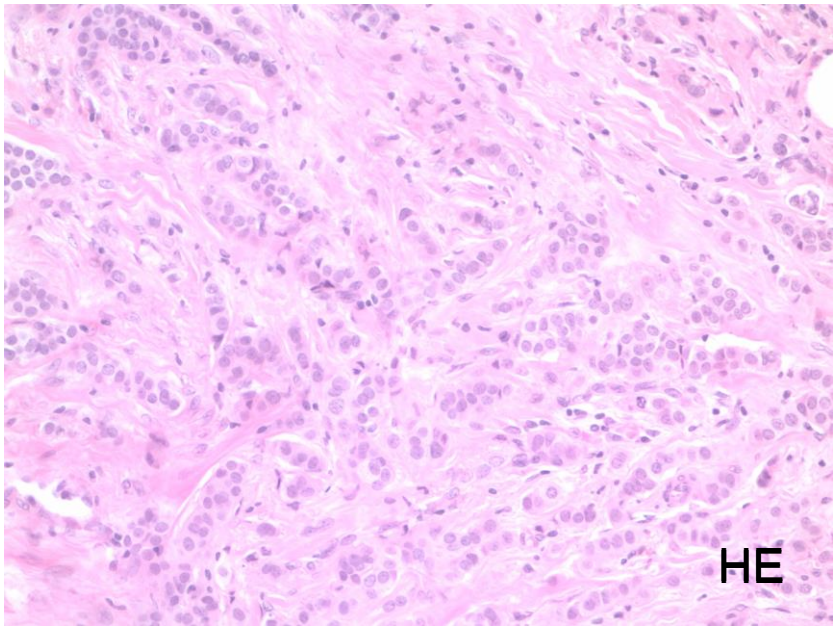
Hormone therapy

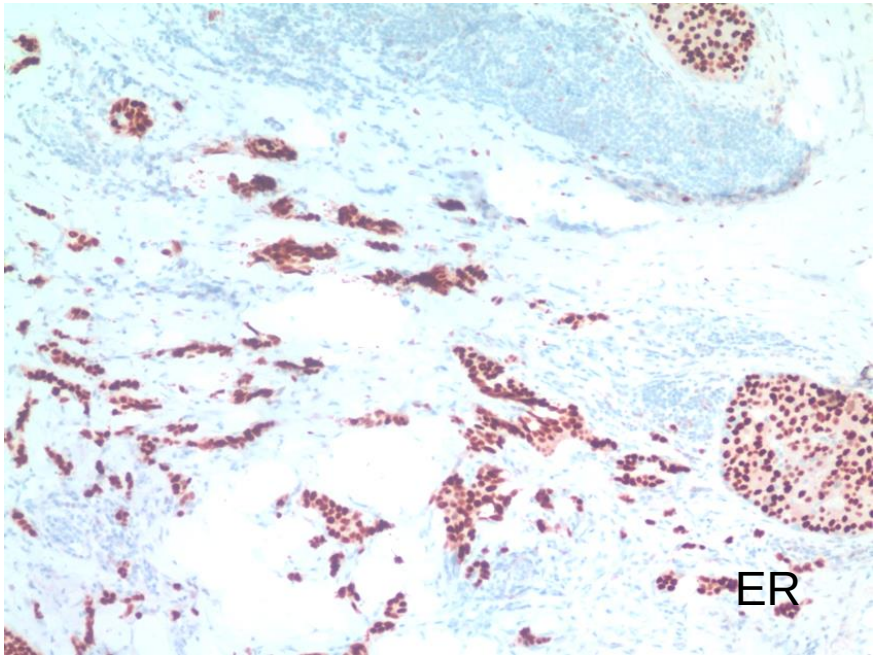


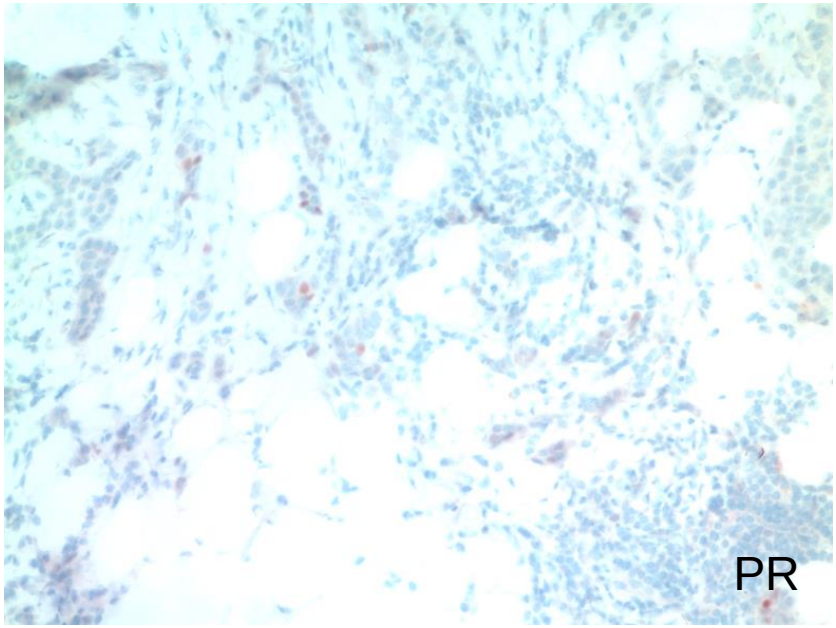


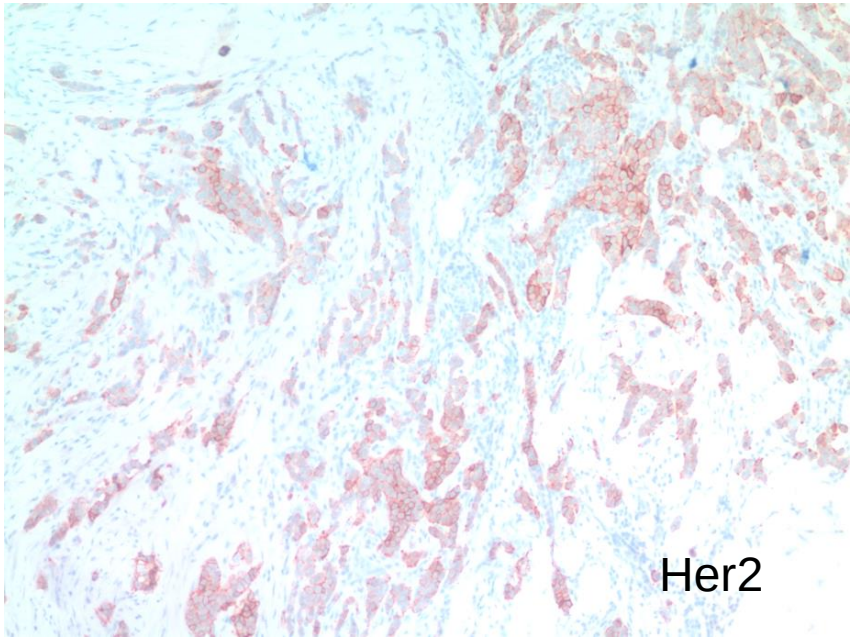


Well differentiated hormone positive breast carcinoma with equivocal Her2 expression

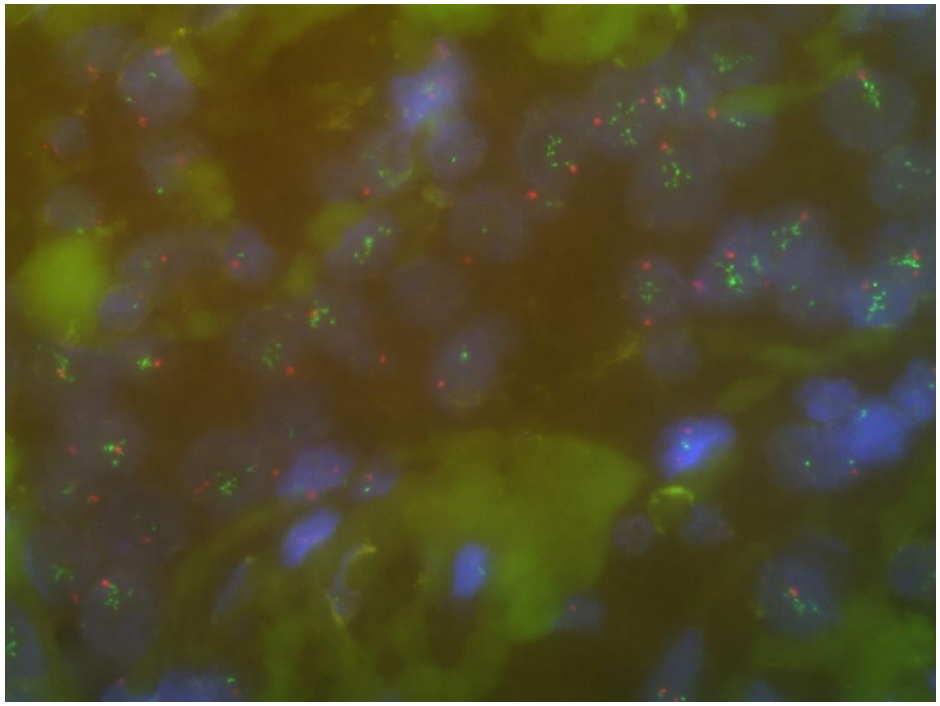








Her2- FISH
amplified



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 negatív control.

Photo documentation number: breast FISH: 186-15

Results:

Her2 immunohistochemistry: 2+

FISH: 50 invasive tumor cells examined repeatedly: amplified

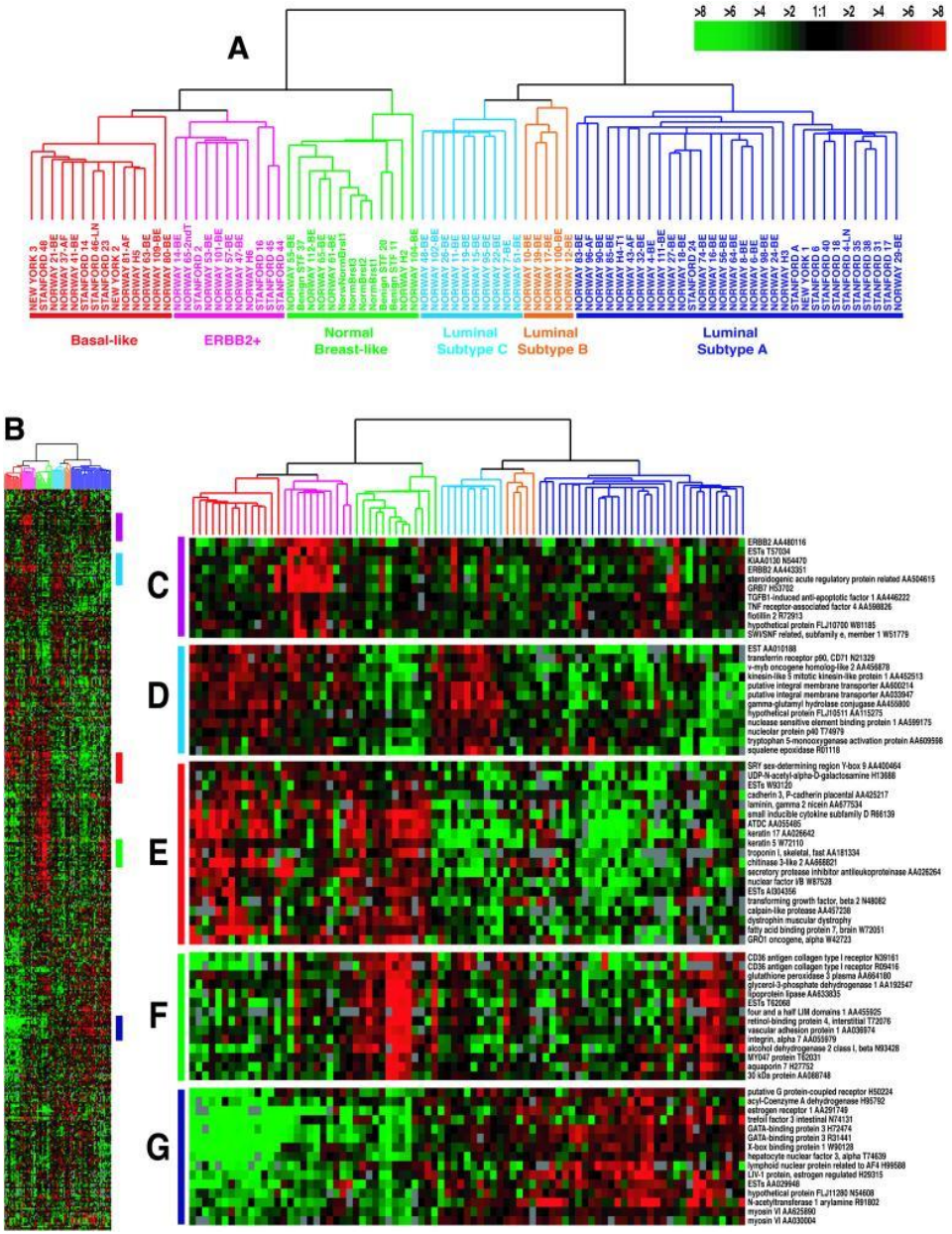
abs Her2 gene copy number: 10,5

abs Cen17 gene copy number: 0,9

Her2/Cen17 ratio: 11,6

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

Breast cancer molecular classfication



Sorlie, 2001

Molecular subtypes IHC

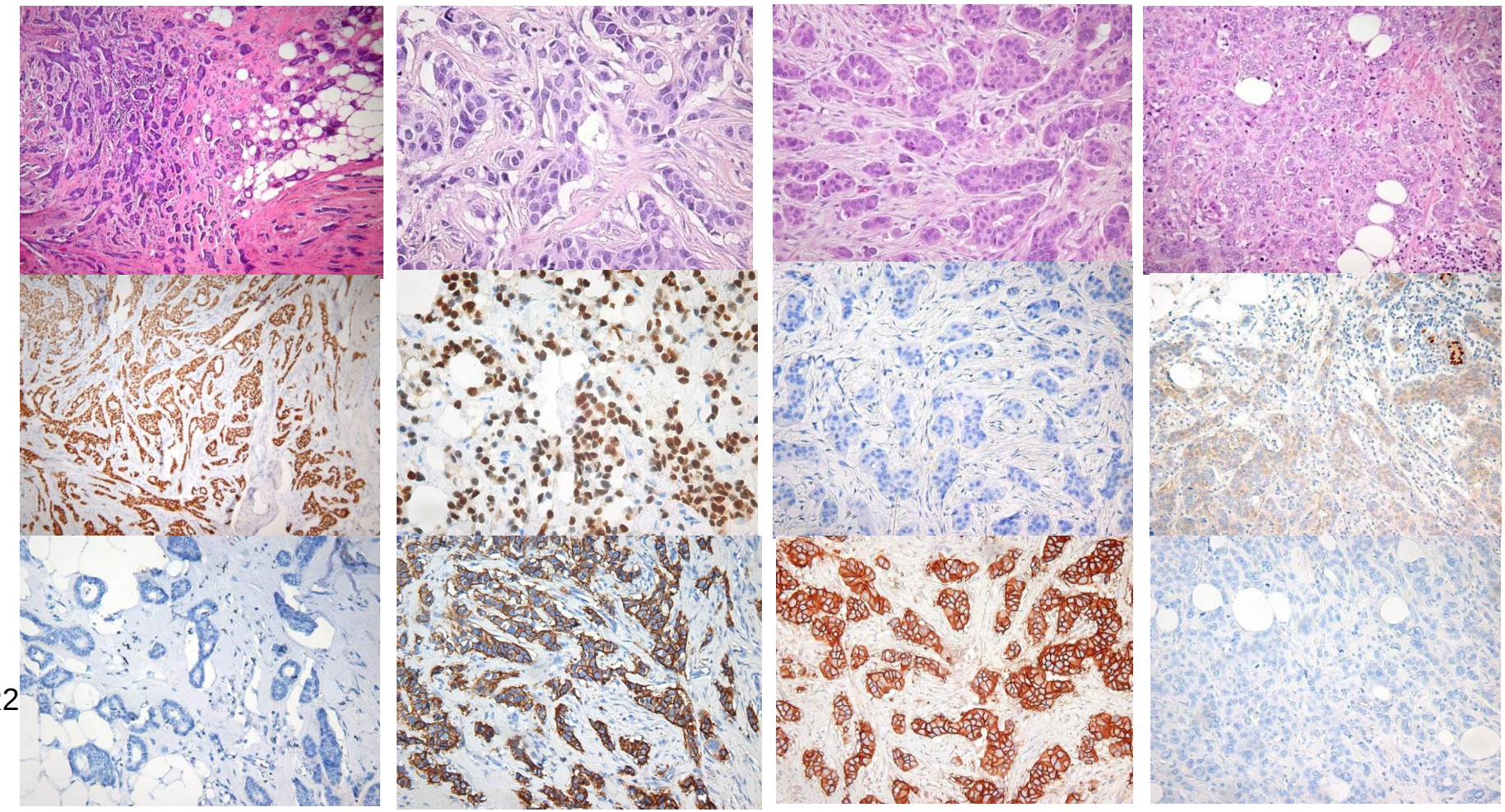


Table 1 Major molecular subtypes of breast cancer determined by gene expression profiling.

	Molecular subtype		
	Luminal	*HER2*	*Basal*
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') *BRCA1* 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

INVASION
Stromelysin 3
Cathepsin L2

HER2
GRB7
HER2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

CD68

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

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

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

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.**