Histopathology and molecular pathology of cancer

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National Institute of Oncology
Center of Tumor Pathology

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
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 \((\text{predictive markers})\)
    - Surgery
    - Radiation therapy
    - Drugs
      - Traditional chemotherapy
      - Targeted therapy
  - \textit{Prognostic markers: TNM Stage}
Diagnostic methods

- **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

A Traditional Tumor Analysis

Tumor Tissue

Traditional Pathology

Analysis of Tumor Morphology

Patient Diagnosis

B Integrated Molecular Analysis

Tumor Tissue

Traditional Pathology

ISH, IHC and Other Assays

Analysis of Tumor Morphology

Analysis of Tumor Biomarkers

Analysis of Tumor Genotype

Multiplex Mutational Profiling

Patient Therapy Assigned

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

A Traditional Tumor Analysis
- Tumor Tissue
  - Traditional Pathology
  - Analysis of Tumor Morphology
  - Patient Diagnosis

B Integrated Molecular Analysis
- Tumor Tissue
  - Traditional Pathology
  - ISH, IHC and Other Assays
  - Multiplex Mutational Profiling
  - Analysis of Tumor Morphology
  - Analysis of Tumor Biomarkers
  - Analysis of Tumor Genotype
  - Patient Therapy Assigned

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

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
Kufe DW, Pollock RE, Weichselbaum RR, et al., editors.
Nottingham Prognostic Index: (0.2 \times 0.9) + 1 + 1 = 2.18

Excellent prognostic group
# Prognostic value of NPI

<table>
<thead>
<tr>
<th>Group</th>
<th>NPI</th>
<th>10 yr survival</th>
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<tr>
<td>Excellent</td>
<td>2.02-2.4</td>
<td>96%</td>
</tr>
<tr>
<td>Good</td>
<td>2.41-3.4</td>
<td>93%</td>
</tr>
<tr>
<td>Moderate 1</td>
<td>3.41-4.4</td>
<td>81%</td>
</tr>
<tr>
<td>Moderate 2</td>
<td>4.41-5.4</td>
<td>74%</td>
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<tr>
<td>Poor</td>
<td>5.41-6.4</td>
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</tr>
<tr>
<td>Very poor</td>
<td>6.41-6.8</td>
<td>38%</td>
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</table>
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

Sato et al AJSP 2010;34:454-462
Venous invasion as prognostic factor in CRC

n=191 pT3 CRC

**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 survival. No = 76

Kaplan-Meier survival curve P = 0.0089

- Stomach
- Small intestine
- Colon and other

Dr. Szentirmay Zoltán
Gastrointestinal Stromal Tumor (GIST)

Grading / Staging / Report

Grading

Risk for Metastasis/Progressive Disease

<table>
<thead>
<tr>
<th>Size (cm)</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Jejunum &amp; Ileum</th>
<th>Rectum</th>
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<tbody>
<tr>
<td>≤5 mits/50 hpf</td>
<td>0 none</td>
<td>0 none</td>
<td>0 none</td>
<td>0 none</td>
</tr>
<tr>
<td>&gt;2 cm</td>
<td>very low</td>
<td>low</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>&gt;5 cm ≤10 cm</td>
<td>low</td>
<td>high</td>
<td>moderate</td>
<td>high</td>
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<td>&gt;10 cm</td>
<td>moderate</td>
<td>high</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>&gt;5 mits/50 hpf</td>
<td>few cases</td>
<td>no cases</td>
<td>few cases</td>
<td>high</td>
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<tr>
<td>&gt;2 cm</td>
<td>moderate</td>
<td>high</td>
<td>high</td>
<td>high</td>
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<td>&gt;5 cm ≤10 cm</td>
<td>high</td>
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<td>high</td>
</tr>
<tr>
<td>&gt;10 cm</td>
<td>high</td>
<td>high</td>
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<td>high</td>
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</table>

Large Intestine tumors are rare, risk appears similar to Jejunum & Ileum
Esophageal tumors are too rare to develop criteria
With wide field microscope view (5mm x 5mm), count 25 fields with same cutoff of 5 as above
Based on Miettinen and Lasota 2006

Progressive Disease or Death Risk Groups

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<th>Group</th>
<th>Approximate Progression Incidence</th>
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<tr>
<td>Moderate</td>
<td>10-30%</td>
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<tr>
<td>High</td>
<td>&gt;50%</td>
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</table>
4 cm GIST in stomach, MI<5/50 HPF

5 year recurrence free survival: 90%

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

5 year recurrence free survival: <10%

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.
“Traditional” histologic parameters

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- Vascular invasion
- 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
Final Diagnosis

Squamous cell carcinoma

- Tumor Size: 6.5 cm x 5.2 cm x 4.8 cm.
- Histologic Grade: 2.
- Margin of Resection: Negative for tumor.
- Vascular Margin of Excision: Negative for tumor.
- Nodal Status: N1.
- Slightly Positive for Tumor.
- Lymph Nodes (Hilar):
  - Number Examined: 11.
  - Number Positive: 4.
- Comment: Two of the nodes are positive as a result of direct extension of tumor into hiliar nodes.
  - Number Examined: 7.
  - Number Positive: 5.
- Additional Tumor Features:
  - Lymphatic Vessel Invasion in Lung: Not identified.
  - Blood Vessel Invasion is Lung: Identified in a large hilar artery branch (Block 4-7).
  - 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:
  - Associates Obstructive Pneumonia: Extending to Hilar Region, Acute and Organizing.
- 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.

pT2N1
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 8th ed. 2017 _ pT3
Final Diagnosis

Squamous cell carcinoma

- Tumor size: 6.5 cm x 5.2 cm x 4.8 cm
- Histologic grade: I
- Tumor invades visceral pleura
- Tumor is 5 cm from bronchial margin of excision
- Margins of excision:
  - Bronchial margin of resection: negative for tumor
  - Vascular margin of excision: negative for tumor
- Lymph nodes ( hilus):
  - Number examined: 11
  - Number positive: 3
  - Comment: Two of the nodes are positive as a result of direct extension of tumor into fat filled nodes
- (Pan 1, 2, 3, 9): Lymph nodes (mediastinal L4, L5, L6, L9, L10, L11):
  - Number examined: 7
  - Number positive: 6
- Additional tumor features:
  - Lymphatic vessel invasion in lung: NOT IDENTIFIED
  - Blood vessel invasion in lung: IDENTIFIED IN A LARGE PULMONARY ARTERY BRANCH (BLOCK 4-1)
  - Comment:
    - Lymphoplasmacytic response is present, mild
    - Stromal response is present, moderate
    - Tumor necrosis is present, extensive
    - Tumor invades a segmental bronchus
- Non neoplastic lung tissue:
  - Associate obstructive pneumonitis: extending to hilar region, acute and organizing
- 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:
  - Primary tumor: pT2
  - Regional lymph nodes: pN1

pT3N1
### Stage groups and survival, lung cancer

**TNM v8**

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<thead>
<tr>
<th>Stage</th>
<th>N0</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>M1a any N</th>
<th>M1b any N</th>
<th>M1c any N</th>
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<td>IA1</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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<td>T1b</td>
<td>IA2</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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<tr>
<td>T1c</td>
<td>IA3</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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<tr>
<td>T2a</td>
<td>IB</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
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<td>IVB</td>
</tr>
<tr>
<td>T2b</td>
<td>IIA</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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<tr>
<td>T3</td>
<td>IIB</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IIIC</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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<tr>
<td>T4</td>
<td>IIIA</td>
<td>IIIA</td>
<td>IIIB</td>
<td>IIIC</td>
<td>IVA</td>
<td>IVA</td>
<td>IVB</td>
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#### Survival Analysis

![Survival Curve](image)

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<th>Proposed</th>
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<th>24 Month</th>
<th>60 Month</th>
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<td>IA1</td>
<td>68 / 781</td>
<td>NR</td>
<td>97%</td>
<td>92%</td>
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<tr>
<td>IA2</td>
<td>505 / 3106</td>
<td>NR</td>
<td>94%</td>
<td>83%</td>
</tr>
<tr>
<td>IA3</td>
<td>546 / 2417</td>
<td>NR</td>
<td>90%</td>
<td>77%</td>
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<tr>
<td>IB</td>
<td>560 / 1928</td>
<td>NR</td>
<td>87%</td>
<td>68%</td>
</tr>
<tr>
<td>IIA</td>
<td>215 / 585</td>
<td>NR</td>
<td>79%</td>
<td>60%</td>
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<td>605 / 1453</td>
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<td>72%</td>
<td>53%</td>
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<tr>
<td>IIIA</td>
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<td>1551 / 2140</td>
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<td>IIIC</td>
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<td>13%</td>
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<td>IVA</td>
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<td>23%</td>
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<td>IVB</td>
<td>328 / 398</td>
<td>6.0</td>
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## Stage and therapy in NSCLC

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<td>T1a,b,c</td>
<td>N0</td>
<td>M0</td>
<td>+</td>
<td>- (vagy: + !)</td>
<td>-</td>
</tr>
<tr>
<td>I/B</td>
<td>T2a</td>
<td>N0</td>
<td>M0</td>
<td>+</td>
<td>- (vagy: + !)</td>
<td>-</td>
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<td>- (vagy: + !)</td>
<td>+</td>
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<tr>
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<td>M0</td>
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<td>+</td>
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<tr>
<td></td>
<td></td>
<td>N0</td>
<td>M0</td>
<td>+</td>
<td>+ / -</td>
<td>+</td>
</tr>
<tr>
<td>III/A</td>
<td>T1a-2b T3 T4</td>
<td>N2</td>
<td>M0</td>
<td>+</td>
<td>+ / -</td>
<td>+</td>
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<td>N1</td>
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<td>+ / -</td>
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<td>M0</td>
<td>+</td>
<td>+ / -</td>
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<td>T1a-2b T3-4</td>
<td>N3</td>
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<td>N1- 3</td>
<td>M1a,b</td>
<td>-</td>
<td>+ / -</td>
<td>+</td>
</tr>
<tr>
<td>IV/B</td>
<td>T1-4</td>
<td>N1-3</td>
<td>M1c</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
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

1 cm hosszágú colon részlet. Középen 5 cm legnagyobb 0-jú felhányt méli kifekélyéssedés van. Az egyik resctios vonaltól 3, a másként 35 cm-re kezdődik az elváltozás. A metszalpon a belfal kissé meg-vastagodott, úgytűnik tumorosan infiltrált. A környező záriszövö accept bán, kisboronyos és kisebb nyirokcsomók van. A vastagbéllel összeefüggő 2 cm nagyságú capillasz részletben gócos elváltozás nincs.

Szövettani leírás: 1993.11.29./CSL


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 metastasis present
pTNM status: pT4a N2b M1b
Ecc-electronic cancer checklist

### CAP eCC Lifecycle

<table>
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<th>Conversion of CCPs to eCC XML</th>
<th>Vendor</th>
<th>Data Entry</th>
<th>Synoptic Reporting</th>
<th>Storage</th>
<th>Other Use Cases</th>
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<td>Reporting Template</td>
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<td>Automated Validation</td>
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<tr>
<td>EHR/LIS Database</td>
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</tbody>
</table>

**Other Use Cases**
- Central Cancer Registries
- Registry Reporting
- Quality Assurance
- Biospecimen Annotations
- Research Studies
- Decision Support
- Financial Reports

Arch Pathol Lab Med—Vol 139, May 2015
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.
CANCER GENOMICS
Improved Understanding of Molecular Changes in Tumors
Produces More-Specialized Treatments for Patients

PAGE 5

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

2 2 11 15 20
Role of pathology in oncology

A Traditional Tumor Analysis
- Tumor Tissue
- Traditional Pathology
- Analysis of Tumor Morphology
- Patient Diagnosis

B Integrated Molecular Analysis
- Tumor Tissue
  - Traditional Pathology
  - ISH, IHC and Other Assays
  - Multiplex Mutational Profiling
    - Analysis of Tumor Genotype
    - Analysis of Tumor Biomarkers
      - Analysis of Tumor Morphology
      - Patient Therapy Assigned

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

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

Morphology

PCR
ISH
Sequencing

RNA

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

HE

TTF1

HE

Napsin-A
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
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) $\rightarrow$ **WWTR1-CAMTA1** (majority)
   - WWTR1 (TAZ): transcription co-activator
   - CAMTA1: calmodulin-binding transcription activator
2. t(11;X) $\rightarrow$ **YAP1-TFE3** (very rare)
   - YAP1: transcription co-activator
   - TFE3: transcription factor

Detection:
- RT-PCR
- FISH
- Immunohistochemistry

[Images of cellular structures and gene fusions related to pathology]
Practical use of molecular pathology

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

- Targeted therapy
<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Gene</th>
<th>Abnormality</th>
<th>Drug/indication</th>
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<tbody>
<tr>
<td>Lung adenocarcinoma</td>
<td>EGFR</td>
<td>mutation</td>
<td>EGFR TKI sensitivity</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>mutation</td>
<td>EGFR TKI resistance</td>
</tr>
<tr>
<td></td>
<td>ALK</td>
<td>translocation</td>
<td>Crizotinib sensitivity</td>
</tr>
<tr>
<td></td>
<td>ROS1</td>
<td>translocation</td>
<td>Crizotinib sensitivity</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>KRAS</td>
<td>mutation</td>
<td>Anti-EGFR resistance</td>
</tr>
<tr>
<td></td>
<td>NRAS</td>
<td>mutation</td>
<td>Anti-EGFR resistance</td>
</tr>
<tr>
<td></td>
<td>BRAF</td>
<td>mutation</td>
<td>Negative prognostic factor</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF</td>
<td>mutation</td>
<td>Vemurafenib sensitivity</td>
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<tr>
<td>Breast carcinoma</td>
<td>ERBB2 (HER2)</td>
<td>amplification</td>
<td>Trastuzumab, Lapatinib sensitivity</td>
</tr>
</tbody>
</table>
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, gefitinib)
    - Female
    - Adenocarcinoma
    - Non-smokers
EGFR signaling pathway alteration in lung adenocarcinoma

EGFR

Ras

Raf

MEK

MAPK

Survival

Invasion

Proliferation

Metastasis

Angiogenesis

EGFR mutation: 10%
EGFR signaling pathway alteration in lung adenocarcinoma

EGFR

Ras

Raf

MEK

MAPK

Survival

Invasion

Proliferation

Metastasis

Angiogenesis

RAS mutation: 30%
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

<table>
<thead>
<tr>
<th>Gene abnormality</th>
<th>SCLC (%)</th>
<th>ACA (%)</th>
<th>SCC (%)</th>
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</thead>
<tbody>
<tr>
<td><strong>Mutation</strong></td>
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<tr>
<td>BRAF</td>
<td>0</td>
<td>&lt; 5</td>
<td>0</td>
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<tr>
<td><strong>EGFR</strong></td>
<td>&lt; 1</td>
<td>10-20</td>
<td>&lt; 1</td>
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<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>&lt; 5</td>
<td>35-45</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>ERBB2/HER2</td>
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<td>&lt; 5</td>
<td>0</td>
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<tr>
<td><strong>KRAS</strong></td>
<td>&lt; 1</td>
<td>15-35</td>
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<tr>
<td>Caucasian</td>
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<tr>
<td>Asian</td>
<td>&lt; 1</td>
<td>5-10</td>
<td>&lt; 5</td>
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<tr>
<td>PIK3CA</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>5-15</td>
</tr>
<tr>
<td>RB</td>
<td>&gt; 90</td>
<td>5-15</td>
<td>5-15</td>
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<tr>
<td>TP53</td>
<td>&gt; 90</td>
<td>30-40</td>
<td>50-80</td>
</tr>
<tr>
<td><strong>Amplification</strong></td>
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<td></td>
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<tr>
<td>EGFR</td>
<td>&lt; 1</td>
<td>5-10</td>
<td>10</td>
</tr>
<tr>
<td>ERBB2/HER2</td>
<td>&lt; 1</td>
<td>&lt; 5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>MET</td>
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<tr>
<td>MYC</td>
<td>20-30</td>
<td>5-10</td>
<td>5-10</td>
</tr>
<tr>
<td>FGFR1</td>
<td>&lt; 1</td>
<td>&lt; 5</td>
<td>15-25</td>
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<td><strong>Rearrangement</strong></td>
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<tr>
<td><strong>ALK</strong></td>
<td>0</td>
<td>5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>RET</td>
<td>0</td>
<td>1-2</td>
<td>0</td>
</tr>
<tr>
<td><strong>ROS1</strong></td>
<td>0</td>
<td>1-2</td>
<td>0</td>
</tr>
<tr>
<td>NTRK1</td>
<td>0</td>
<td>&lt; 1</td>
<td>0</td>
</tr>
<tr>
<td>NRG1</td>
<td>0</td>
<td>&lt; 1</td>
<td>0</td>
</tr>
</tbody>
</table>
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) 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
- Tyrosine 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
- Detection:
  - 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

- **84%**
  - CIN Sporadic
  - Acquired
    - APC
    - TP53
    - DCC
    - KRAS
    - LOH

- **<1%**
  - CIN FAP
  - AD Germline
    - APC

- **<1%**
  - CIN MUTYH
  - AR Germline
    - Biallelic
    - MUTYH

- **2%-5%**
  - MSI Lynch
    - AD Germline
      - MMR genes:
        - MSH2
        - MLH1
        - MSH6
        - PMS2

- **12%**
  - MSI Sporadic
    - Epigenetic silencing:
      - Hypermethylation of MLH1
        - BRAF mutations

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%,
Specificity 98-100%.
MSI testing in colon adenocarcinomas

- MSI testing: bat25, bat26
  - Negative MSS tumor
  - Positive
    - MLH1 negative
      - MMR IHC
        - Positive MSS tumor
  - BRAF testing
    - BRAF mut
      - Sporadic MSI tumor
    - BRAF wt
      - Genetic testing for Lynch syndrome
  - Genetic testing for Lynch syndrome

- MSH2, MSH6, PMS2 negative
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

Sorlie, 2001
<table>
<thead>
<tr>
<th>Molecular subtypes IHC</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2</th>
<th>Basal</th>
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</thead>
<tbody>
<tr>
<td>ER</td>
<td><img src="ER.png" alt="Image" /></td>
<td><img src="Luminal_B.png" alt="Image" /></td>
<td><img src="HER2.png" alt="Image" /></td>
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<tr>
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<td><img src="HER2.png" alt="Image" /></td>
<td><img src="Luminal_B.png" alt="Image" /></td>
<td><img src="HER2.png" alt="Image" /></td>
<td><img src="Basal.png" alt="Image" /></td>
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<tr>
<td>Ki67&lt;</td>
<td><img src="Ki67.png" alt="Image" /></td>
<td><img src="Ki67.png" alt="Image" /></td>
<td><img src="Ki67.png" alt="Image" /></td>
<td><img src="Ki67.png" alt="Image" /></td>
</tr>
<tr>
<td>CK5 + EGFR +</td>
<td><img src="CK5.png" alt="Image" /></td>
<td><img src="EGFR.png" alt="Image" /></td>
<td><img src="CK5.png" alt="Image" /></td>
<td><img src="EGFR.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Table 1: Major molecular subtypes of breast cancer determined by gene expression profiling.

<table>
<thead>
<tr>
<th>Molecular subtype</th>
<th>Luminal</th>
<th>HER2</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression pattern</td>
<td>High expression of hormone receptors and associated genes (luminal A &gt; luminal B)</td>
<td>High expression of HER2 and other genes in amplicon Low expression of ER and associated genes</td>
<td>High expression of basal epithelial genes, basal cytokeratins Low expression of ER and associated genes Low expression of HER2</td>
</tr>
<tr>
<td>Clinical features</td>
<td>~70% of invasive breast cancers ER/PR positive Luminal B tend to be higher histological grade than luminal A Some overexpress HER2 (luminal B)</td>
<td>~15% of invasive breast cancers ER/PR negative More likely to be high grade and node positive</td>
<td>~15% of invasive breast cancers Most ER/PR/HER2 negative (‘triple negative’) BRCA1 dysfunction (germline, sporadic) Particularly common in African-American women</td>
</tr>
<tr>
<td>Treatment response and outcome</td>
<td>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</td>
<td>Respond to trastuzumab (Herceptin) Respond to anthracycline-based chemotherapy Generally poor prognosis</td>
<td>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)</td>
</tr>
</tbody>
</table>
**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

**Invasion**
- Stromelysin 3
- Cathepsin L2

**Her2**
- GRB7
- HER2

**Reference**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

**Risk Category**
- Low risk: RS <18
- Int risk: RS ≥18 and <31
- High risk: RS ≥31

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

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.**