Early (and not so early) colorectal cancer: The pathologist’s point of view

Daniela E. Aust, Institute for Pathology, University Hospital Dresden, Germany
Disclosure slide

- Member of advisory board for AMGEN, ROCHE
- Speaker honoraria from FALK Pharma, Lilly and ROCHE
- Third party funds from MERCK for immunohistochemistry in a clinical trial
What can (molecular) pathology offer for clinical decisions in colorectal cancer?

Better understanding of the disease

Prognostic markers

Predictive markers
Different pathways of colorectal carcinogenesis

- Adenoma-Carcinoma-Sequence (FAP)
- HNPCC, Lynch-Syndrom
- Serrated Pathway
- Alternate Pathway
Classical Adenoma-Carcinoma-Sequence (sporadic and FAP) (60-70%)
HNPCC, Lynch-Syndrom
(∼2-3%)

germline-mutation
MMR-Gene
(MSH2, MLH1)
gatekeeper

TGFβIIIR, IGF2R, Caspase 5, BAX, MSH3/6, others
Serrated Pathway of colorectal carcinogenesis (~15-20%)

- Normal colorectal epithelium
- Proliferation boost to ACF (serr.)
- Hyperplastic polyp (MVHP): senescence via p16, IGFBP7 etc.
- Progression to SSA w/o dysplasia
- Senescent lesion, no progression

BRAF: V600E

Alteration of Wnt-pathway: aberrant ß-Catenin via MCC-methylation

p16INK4a-Expr.↑ and IGFBP7-Sekr. ↑ (oncogene-induced senescence)

CIMP
p16INK4a, IGFBP7 methylation

Normal colorectal epithelium

p16

Hyperplastic polyp (MVHP): senescence via p16, IGFBP7 etc.

progression to SSA w/o dysplasia

senescent lesion, no progression

www.pathologie-universitaetsmedizin-dresden.de
Progression to carcinomas

Progression to SSA
/w dysplasia

Other CIMP-Targets
Wnt-pathway?
18q LOH?
p53-Mutation?

MLH1-loss in dysplastic epithelium;
MSI;
TGFβRII-Mut.

CIMP-H, BRAF mut.

Progression to MSS carcinoma
CIMP-H, BRAF mut.

Progression to MSI carcinoma
CIMP-H, BRAF mut.

www.pathologie-universitaetsmedizin-dresden.de
Alternate Pathway of sporadic colorectal carcinogenesis (~15-20%)
**Different pathways of sporadic colorectal carcinogenesis**

<table>
<thead>
<tr>
<th></th>
<th>Adenoma-Carcinoma-Sequence</th>
<th>Alternate (mixed type) pathway</th>
<th>Serrated pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precursor lesion</strong></td>
<td>Adenoma</td>
<td>Villous adenoma or traditional serrated adenoma</td>
<td>Sessile serrated adenoma</td>
</tr>
<tr>
<td><strong>Key mutation</strong></td>
<td>APC</td>
<td>KRAS</td>
<td>BRAF</td>
</tr>
<tr>
<td><strong>Secondary genetic alterations</strong></td>
<td>Mutations in KRAS, p53</td>
<td>CIMP low, mutations of APC, p53</td>
<td>CIMP high (silencing of hMLH1, MGMT and/or p16)</td>
</tr>
<tr>
<td><strong>MSI status</strong></td>
<td>MSS</td>
<td>MSS or MSI-L</td>
<td>MSI-H</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>60 %</td>
<td>15-20%</td>
<td>15-20%</td>
</tr>
<tr>
<td><strong>Localisation</strong></td>
<td>Left &gt; right</td>
<td>Left &gt; right</td>
<td>Right &gt; left</td>
</tr>
</tbody>
</table>
Different pathways of colorectal carcinogenesis

- Colorectal cancer is not *one* disease, it consists of different subentities, developed through different pathways of carcinogenesis.

- Certain mutations may be present as either drivers or passengers and thus may have different prognostic value in different pathways.
Prognostic markers in colorectal cancer

- pTNM
- Microsatellite instability
- BRAF
- Surgery
- Conflicting data: p53, loss of 18q, 17p, gain of 20q13, KRAS, etc.

<table>
<thead>
<tr>
<th>pT0</th>
<th>No primary tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTx</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>pT1</td>
<td>Infiltration into submucosa</td>
</tr>
<tr>
<td>pT2</td>
<td>Infiltration into M. propria</td>
</tr>
<tr>
<td>pT3</td>
<td>Infiltration into mesocolic/mesorectal fatty tissue</td>
</tr>
<tr>
<td>pT3a</td>
<td>≤ 5mm</td>
</tr>
<tr>
<td>pT3b</td>
<td>&gt; 5 mm, ≤ 15 mm</td>
</tr>
<tr>
<td>pT3c</td>
<td>&gt; 15mm</td>
</tr>
<tr>
<td>pT4</td>
<td>Penetration of serosa or infiltration of adjacent organs</td>
</tr>
<tr>
<td>pT4a</td>
<td>Penetration of serosa</td>
</tr>
<tr>
<td>pT4b</td>
<td>Infiltration of adjacent organs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pN0</th>
<th>No regional lymph node metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNx</td>
<td>Lymph node metastases cannot be assessed</td>
</tr>
<tr>
<td>pN1a</td>
<td>1 lymph node metastasis</td>
</tr>
<tr>
<td>pN1b</td>
<td>2-3 lymph node metastases</td>
</tr>
<tr>
<td>pN1c</td>
<td>Tumor nodule in subserosal mesocolic/mesorectal fatty tissue without lymph node metastases</td>
</tr>
<tr>
<td>pN2a</td>
<td>4-6 lymph node metastases</td>
</tr>
<tr>
<td>pN2b</td>
<td>≥ 7 lymph node metastases</td>
</tr>
</tbody>
</table>

12 lymph nodes should be assessed for pN0 staging!
If less than 12 lymph nodes without metastases are found, nodal status should be staged as pN0!
Number of lymph nodes with metastases and number of dissected lymph nodes should be stated in the pTNM classification!

<table>
<thead>
<tr>
<th>pM0</th>
<th>no distant metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>pM1</td>
<td>Distant metastases</td>
</tr>
<tr>
<td>pM1a</td>
<td>Distant metastases in one organ (liver, lung, ovary, etc.; not regional lymph nodes)</td>
</tr>
<tr>
<td>pM1b</td>
<td>Distant metastases in more than one organ or distant peritoneal metastases</td>
</tr>
</tbody>
</table>

Use of pMx is discouraged!

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rx</td>
<td>Residual tumor cannot be assessed</td>
</tr>
<tr>
<td>R0</td>
<td>No microscopic residual tumor</td>
</tr>
<tr>
<td>R1</td>
<td>Microscopic residual tumor at the margins</td>
</tr>
<tr>
<td>R2</td>
<td>Macroscopic residual tumor at the margins</td>
</tr>
</tbody>
</table>

**CRM (Circumferential margin) – concept for rectal cancer:**

- **CRM-**: R0, distance between tumor and circumferential margin > 1mm
- **CRM+**: R0, distance between tumor and circumferential margin ≤ 1mm

- Lymph vessel invasion (L0, L1)
- Blood vessel invasion (V0, V1, V2)
- Perineural invasion (Pn0, Pn1)

Example of a correct postoperative tumor classification (UICC 2010):

pT3a, pN1a (1/25 LN), L1, V0, Pn1, R0 (locally); G2
# UICC-Staging and stage adapted therapy

<table>
<thead>
<tr>
<th>UICC stage</th>
<th>T-stage</th>
<th>N-stage</th>
<th>M-stage</th>
<th>Therapeutic consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
<td>Endoscopic resection or surgery alone</td>
</tr>
<tr>
<td>I</td>
<td>T1, T2</td>
<td>N0</td>
<td>M0</td>
<td>Colon: Surgery alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rectum: Neoadjuvant treatment + surgery</td>
</tr>
<tr>
<td>II A</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
<td>Colon: Surgery alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rectum: Neoadjuvant treatment + surgery</td>
</tr>
<tr>
<td>II B</td>
<td>T4a</td>
<td>N0</td>
<td>M0</td>
<td>Colon: Surgery + adjuvant chemo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rectum: Neoadjuvant treatment + surgery</td>
</tr>
<tr>
<td>III A</td>
<td>T1, T2</td>
<td>N1</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td>III B</td>
<td>T3, T4a</td>
<td>N1</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td></td>
<td>T2, T3</td>
<td>N2a</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td></td>
<td>T1, T2</td>
<td>N2b</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td>III C</td>
<td>T4a</td>
<td>N2a</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td></td>
<td>T3, T4a</td>
<td>N2b</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td></td>
<td>T4b</td>
<td>N1-N2</td>
<td>M0</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td>IVA</td>
<td>any T</td>
<td>any N</td>
<td>M1a</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
<tr>
<td>IVB</td>
<td>Any T</td>
<td>any N</td>
<td>M1b</td>
<td>Palliative treatment, neoadjuvant treatment + surgery of metastases</td>
</tr>
</tbody>
</table>
Criteria for endoscopic resection vs. surgery in pT1

- Submucosal depth of invasion:
  - sm1-3 indicating the three thirds of the submucosa
  - Paris classification: cutoff > 1000µm
- Lymphangiosis carcinomatosa
- Grading
- Resection status

→ risk evaluation for lymphatic spread

*Update of the Paris-Classification of superficial neoplastic lesions* in the digestive tract, Lambert et al., Endoscopy 2005
Criteria for endoscopic resection vs. surgery in pT1

- Low risk for lymphatic spread:
  - Depth of submucosal infiltration ≤ 1000µm
  - No lymphangiosis carcinomatosa (L0)
  - Good differentiation (G1, G2)

  → **endoscopic resection is sufficient**

- High risk for lymphatic spread:
  - Depth of submucosal infiltration > 1000µm
  - Lymphangiosis carcinomatosa (L1)
  - Bad differentiation (G3, G4)

  → **oncologic surgery is the treatment of choice**
UICC-Staging and stage adapted therapy: locally resectable tumors

UICC-Stage

I

Low risk
sm1/2 ≤ 1000µ
G1/G2, L0, R0

Local excision suffices

High risk
Sm3 > 1000 µ
G3/G4, L1, R1

Oncological resection recommended

Endoscopical resection
UICC-Staging and stage adapted therapy: locally advanced tumors (rectum only)

UICC-Stage

II and III

T3

pT3

+/- LK-Met.

T4

pT4

Primary RCT

TME

Adjuvant therapy

Local recurrence rate!
The role of pathology in neoadjuvant treatment

1. Pretherapeutic biopsy
2. Quality control for TME
3. ypTNM-staging
4. Regression grading
5. Predictive and prognostic markers
UICC-Staging and stage adapted therapy: locally advanced tumors (rectum only)

UICC-Stage

II and III

Primary RCT TME Adjuvant therapy

Prognostic markers

favorable: pN0, R0 complete TME TRG 2-4

Unfavorable: pN+, R1 incomplete TME TRG 0-1

Local recurrence rate!
UICC-Staging and stage adapted therapy: metastasised tumors

UICC-Stadium

IV

+ hematogenous metastases

Resection of the primary (if symptomatic)

Resection of metastases (after neoadjuvant treatment)

Palliative chemotherapy
UICC-Staging and stage adapted therapy: metastasised tumors

- Perioperative chemotherapy of liver metastases
- Regression grading of the liver mets (Rubbia-Brandt, Blazer and others) correlates with outcome

Rubbia-Brandt et al., Ann Oncol 2007
Mismatch repair system
Microsatellite loci are used to diagnose mismatch repair

n = 13
CACACACACACACACACACACACACACACACACACACACAC

n = 9
CACACACACACACACACACACACACACACACACACACACAC

Normal
N  T
---
---

LOH
N  T
---
---

MSI phenotype
N  T
---
---

MSI
---
---

---
---
Microsatellite instability phenotype

**Molecular testing:** Genotyping 5 microsatellites allows the characterization of microsatellite tumor instability

- If at least 2 of the 5 microsatellites are unstable, the tumor phenotype is “MSI-high” or dMMR

**Immunohistochemical testing:** Tumor tissue can be checked for expression of DNA mismatch repair protein MLH1, MSH2, MSH6 or PMS1.

- Loss of expression indicates that the corresponding gene is not being appropriately expressed and suggests the existence of a mutation or epigenetic silencing

MSI: loss of MLH1 in tumor cells
**Bethesda criteria for hereditary non polyposis colorectal cancer (HNPCC)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CRC before the age of 50</td>
</tr>
<tr>
<td>2.</td>
<td>Syn- or metachronous CRC or other HNPCC-associated tumors, independent of patient age</td>
</tr>
<tr>
<td>3.</td>
<td>CRC before the age of 60 with typical MSI-H histology (tumor infiltrating lymphocytes (TILs), mucinous, medullary or signet ring differentiation)</td>
</tr>
<tr>
<td>4.</td>
<td>CRC-patient with first degree relative with diagnosis of CRC or another HNPCC-associated tumor before age 50</td>
</tr>
<tr>
<td>5.</td>
<td>CRC-patient with at least 2 first or second degree relatives with CRC or any other HNPCC-associated tumors (independent of age)</td>
</tr>
</tbody>
</table>
Algorithm for MSI-testing

CRC with ≥ 1 Bethesda-criterion

Immunohistochemistry MLH1, PMS2, MSH2, MSH6

Retained expression of MLH1, PMS2, MSH2, MSH6

Loss of MSH2-, MSH6- or PMS2-expression

Loss of MLH1-expression

BRAF-mutational analysis

MSS

MSI-H

WT

mutated

MSS-KRK

HNPCC-associated CRC possible

Sporadic MSI-H-CRC

fragment length analysis
MSI-H as a favorable prognostic marker in CRC

Molecular grading according to MSI (WHO 2010)

Morphological grading
- Gland-like
- Undifferentiated
  - G1
  - G2
  - G3
  - G4
  - Low grade
  - High grade

Molecular grading (MSI-status)
- Undifferentiated, signet-ring cell, mucinous carcinomas
  - MSI-H
  - MSS
  - Low grade
  - High grade
### MSI-H tumors have less metastases

<table>
<thead>
<tr>
<th></th>
<th>MSS n (%)</th>
<th>MSI-H n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UICC stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>146 (18,2)</td>
<td>13 (14,6)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>II</td>
<td>204 (25,4)</td>
<td>42 (47,2)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>237 (29,4)</td>
<td>27 (30,3)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>217 (27,9)</td>
<td>7 (7,9)</td>
<td></td>
</tr>
<tr>
<td><strong>lymphnode metastases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>423 (52,6)</td>
<td>33 (37,1)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>no</td>
<td>381 (47,4)</td>
<td>56 (62,9)</td>
<td></td>
</tr>
<tr>
<td><strong>distant Metastases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>217 (27,0)</td>
<td>7 (7,9)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>no</td>
<td>587 (73,0)</td>
<td>82 (92,1)</td>
<td></td>
</tr>
</tbody>
</table>


www.pathologie-universitaetsmedizin-dresden.de
CpG-Island-Methylator-Phenotype (CIMP)

- Definition CIMP+: Methylation of ≥ 3 loci
- CIMP-H: 4-5 loci
- CIMP-L: 1-3 loci
- No CIMP: 0 loci

Weisenberger, Nature Genetics 2006
Barault, Cancer Res 2008
MSI-H: prognostic value in association with CIMP-phenotype

Table 3. Crude and relative survival at 5 y in MSS and MSI groups according to methylation status

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>95% CI</th>
<th>Relative</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-CIMP</td>
<td>53.1</td>
<td>46.8–59.0</td>
<td>64.0</td>
<td>56.4–70.7</td>
</tr>
<tr>
<td>CIMP-Low</td>
<td>40.8</td>
<td>33.5–47.9</td>
<td>50.6</td>
<td>41.6–59.0</td>
</tr>
<tr>
<td>CIMP-High</td>
<td>27.9</td>
<td>14.5–43.0</td>
<td>37.7</td>
<td>18.9–56.6</td>
</tr>
<tr>
<td>MSI-H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-CIMP</td>
<td>54.3</td>
<td>19.1–79.8</td>
<td>61.2</td>
<td>18.5–86.7</td>
</tr>
<tr>
<td>CIMP-Low</td>
<td>52.9</td>
<td>23.8–75.4</td>
<td>74.3</td>
<td>18.6–94.9</td>
</tr>
<tr>
<td>CIMP-High</td>
<td>57.7</td>
<td>43.8–69.4</td>
<td>72.5</td>
<td>53.8–84.7</td>
</tr>
</tbody>
</table>

population-based study, UICC-stage I-IV, n=582

Barault, Cancer Res 2008
BRAF-Mutation

- Wild-type BRAF is required for response to Panitumumab or Cetuximab in metastatic CRC

→ predictive marker??

*Di Nicolantonio F et al., 2008
BRAF as a prognostic marker

Bokemeyer, EJC 2012
CRYSTAL- and OPUS-trials
n = 1535
UICC stage IV
No significant difference between treatment arms
Prognostic value of BRAF is dependent on MSI-Status

**CALGB-Study**

adjuvant therapy 5-FU vs. Irinotecan

UICC Stage III

n=506

Table 3. Combined BRAF mutation and MSI status and clinical outcome in stage III colon cancer

<table>
<thead>
<tr>
<th>BRAF mutation and MSI status</th>
<th>No.</th>
<th>RFS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
<td>Five-year survival probability</td>
</tr>
<tr>
<td>BRAF wild-type MSS</td>
<td>387</td>
<td>0.65</td>
<td>1 (referent)</td>
<td>0.63</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>BRAF wild-type MSI-high</td>
<td>43</td>
<td>0.74</td>
<td>0.57 (0.31–1.07)</td>
<td>0.74</td>
<td>0.51 (0.27–0.95)</td>
</tr>
<tr>
<td>BRAF-mutant MSS</td>
<td>41</td>
<td>0.48</td>
<td>1.38 (0.84–2.26)</td>
<td>0.45</td>
<td>1.38 (0.85–2.25)</td>
</tr>
<tr>
<td>BRAF-mutant MSI-high</td>
<td>34</td>
<td>0.74</td>
<td>0.63 (0.32–1.28)</td>
<td>0.67</td>
<td>0.81 (0.44–1.51)</td>
</tr>
</tbody>
</table>

Ogino, Clin Cancer Res 2012
Prognostic value of BRAF is dependent on MSI-Status

Disease free survival

整体 Wald 测试：p=0.1321 (df=3)

HR 0.37

HR 1.00

HR 0.70

HR 1.75

PETACC2
UICC stage III
adjuvant 5-FU
n = 385

<table>
<thead>
<tr>
<th>O</th>
<th>N</th>
<th>Number of patients at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>113</td>
<td>325</td>
<td>191</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>101</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

O 113
N 325

MSI/BRAF
MSS/BRAF WT
MSS/BRAF V600E
MSI-H/BRAF WT
MSI-H/BRAF V600E

www.pathologie-universitaetsmedizin-dresden.de
Prognostic value of BRAF is dependent on MSI-Status

BRAF-Mutation

UICC stage I  stage II/III  stage IV

BRAF V600E + MSI

12%  5%
MSI-H and BRAF : Prognostic Relevance for CRC with CIMP

**Good prognosis:**

- CIMP +
- MLH1-Methylation
- MSI-H
- ± BRAF-Mutation
- proximal colon
- old age
- elderly women
- mucinous or medullary cancers
- tumor infiltrating lymphocytes

**Bad prognosis:**

- CIMP-H +
- MSS
- + BRAF-Mutation
- 3,19fold higher risk for tumor-associated †
- proximal colon
- advanced pT
- mucinous carcinomas

Good prognosis:

- CIMP +
- MSS/MSI-L

Bad prognosis:

- CIMP-H +
- MSS
+ BRAF-Mutation
3,19fold higher risk for tumor-associated †
Summary prognostic markers

- pTNM is still the best validated prognostic marker in colorectal cancer and the basis for therapeutic decision making
- Regression grading for rectal cancer and liver metastases correlates with outcome
- MSI and BRAF are prognostic markers
- MSI-status must be tested for molecular grading in mucinous, undifferentiated and signet ring cell cancers (WHO 2010)
- MSI-status should be tested for its prognostic value and for detection of patients with Lynch-Syndrom
- Prognostic impact of BRAF depends on MSI-status