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, BOEHRINGER
- Speaker honoraria from FALK Pharma, Pfizer, 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%)

TGFβIIIR, IGF2R, Caspase 5, BAX, MSH3/6, others

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

MSI

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Serrated Pathway of colorectal carcinogenesis (~15-20%)

Normal colorectal epithelium

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

Proliferation boost to ACF (serr.)

CIMP
p16INK4a, IGFBP7 methylation

BRAF: V600E

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

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

Progression to SSA w/o dysplasia

senescent lesion, no progression

STOP

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

Progression to SSA /w dysplasia

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

Progression to MSS carcinoma
CIMP-H, BRAF mut.

Progression to MSI carcinoma
CIMP-H, BRAF mut.
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>
Consensus molecular subtypes of CRC

Guinney et al., Nature Medicine 2015
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; 15 mm</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!
**pTNM (UICC 7th edition, 2010): distant metastases**

<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!

Rx  Residual tumor cannot be assessed
R0  No microscopic residual tumor
R1  Microscopic residual tumor at the margins
R2  Macroscopic residual tumor at the margins

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>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>I</td>
<td>T1, T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIA</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIB</td>
<td>T4a</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIA</td>
<td>T1, T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>IIIIB</td>
<td>T3, T4a</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2, T3</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1, T2</td>
<td>N2b</td>
<td>M0</td>
</tr>
<tr>
<td>IIIIC</td>
<td>T4a</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3, T4a</td>
<td>N2b</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T4b</td>
<td>N1-N2</td>
<td>M0</td>
</tr>
<tr>
<td>IVA</td>
<td>any T</td>
<td>any N</td>
<td>M1a</td>
</tr>
<tr>
<td>IVB</td>
<td>Any T</td>
<td>any N</td>
<td>M1b</td>
</tr>
</tbody>
</table>

## Therapeutic consequences

- **Endoscopic resection or surgery alone**

- Colon: Surgery alone  
  - Rectum: Neoadjuvant treatment + surgery

- Colon: Surgery + adjuvant chemo  
  - Rectum: Neoadjuvant treatment + surgery

- Palliative treatment, neoadjuvant treatment + surgery of metastases
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
UICC-staging and stage adapted therapy: locally resectable tumors

- Low risk: sm1/2 ≤ 1000µ G1/G2, L0, R0
  - Endoscopical resection suffices
- High risk: Sm3 > 1000 µ G3/G4, L1, R1
  - Oncological resection recommended
UICC-Staging and stage adapted therapy: locally advanced tumors (rectum only)

**UICC-Stage**

II and III

Local recurrence rate!

Primary RCT → TME → Adjuvant therapy
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

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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

**Figure 2.** Disease-free survival of 106 patients with complete clinical follow-up and with major (MjHR), partial (PHR), or no (NHR) histological tumor response in liver colorectal metastasis after neo-adjuvant chemotherapy.

Rubbia-Brandt et al., Ann Oncol 2007
Prognostic markers in colorectal cancer

- pTNM
- Microsatellite instability
- BRAF
Mismatch repair system
Microsatellite loci are used to diagnose mismatch repair

\[ \begin{align*}
\text{Normal} & : \quad N \quad T \\
\text{LOH} & : \quad N \quad T \\
\text{MSI phenotype} & : \quad N \quad T \\
\end{align*} \]
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)/Lynch Syndrome

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</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

Loss of MSH2-, MSH6- or PMS2-expression

Loss of MLH1-expression

Retained expression of MLH1, PMS2, MSH2, MSH6

fragment length analysis

MSS

MSI-H

BRAF-mutational analysis

WT

mutated

MSS-KRK

HNPCC-associated CRC possible

Sporadic MSI-H-CRC
MSI-H as a favorable prognostic marker in CRC


n = 893
UICC I-III

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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>


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CpG-Island-Methylator-Phenotype (CIMP)

- Definition CIMP+: Methylation of $\geq 3$ 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>
<thead>
<tr>
<th>Table 3. Crude and relative survival at 5 y in MSS and MSI groups according to methylation status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Crude</td>
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</tr>
<tr>
<td>MSS</td>
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<td></td>
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<td></td>
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<tr>
<td>MSI-H</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

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

Barault, Cancer Res 2008
Prognostic markers in colorectal cancer

- pTNM
- Microsatellite instability
- BRAF
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>
<thead>
<tr>
<th>BRAF mutation and MSI status</th>
<th>No.</th>
<th>RFS</th>
<th></th>
<th></th>
<th>DFS</th>
<th></th>
<th></th>
<th>OS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
<td></td>
<td>Five-year survival probability</td>
<td>Multivariate HR (95% CI)</td>
</tr>
<tr>
<td><strong>BRAF wild-type MSS</strong></td>
<td>387</td>
<td>0.65</td>
<td>1 (referent)</td>
<td></td>
<td>0.63</td>
<td>1 (referent)</td>
<td></td>
<td>0.75</td>
<td>1 (referent)</td>
</tr>
<tr>
<td><strong>BRAF wild-type MSI-high</strong></td>
<td>43</td>
<td>0.74</td>
<td>0.57 (0.31–1.07)</td>
<td></td>
<td>0.74</td>
<td>0.51 (0.27–0.95)</td>
<td></td>
<td>0.79</td>
<td>0.54 (0.27–1.08)</td>
</tr>
<tr>
<td><strong>BRAF-mutant MSS</strong></td>
<td>41</td>
<td>0.48</td>
<td>1.38 (0.84–2.26)</td>
<td></td>
<td>0.45</td>
<td>1.38 (0.85–2.25)</td>
<td></td>
<td>0.61</td>
<td>1.61 (0.96–2.69)</td>
</tr>
<tr>
<td><strong>BRAF-mutant MSI-high</strong></td>
<td>34</td>
<td>0.74</td>
<td>0.63 (0.32–1.28)</td>
<td></td>
<td>0.67</td>
<td>0.81 (0.44–1.51)</td>
<td></td>
<td>0.66</td>
<td>1.02 (0.54–1.93)</td>
</tr>
</tbody>
</table>

Ogino, Clin Cancer Res 2012

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Prognostic value of BRAF is dependent on MSI-Status

Disease free survival

Overall Wald test: $p=0.1321$ (df=3)

HR 0.37
HR 1.00
HR 0.70
HR 1.75

Number of patients at risk:

<table>
<thead>
<tr>
<th>MSI/BRAF</th>
<th>MSS/BRAF WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI/BRAF</td>
<td>MSS/BRAF V600E</td>
</tr>
<tr>
<td>MSS/BRAF V600E</td>
<td>MSS/H/BRAF WT</td>
</tr>
<tr>
<td>MSS/H/BRAF V600E</td>
<td></td>
</tr>
</tbody>
</table>
Prognostic value of BRAF is dependent on MSI-Status

BRAF-Mutation

<table>
<thead>
<tr>
<th>UICC stage I</th>
<th>stage II/III</th>
<th>stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E + MSI</td>
<td>12%</td>
<td>5%</td>
</tr>
<tr>
<td>12%</td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>
MSI-H and BRAF: Prognostic Relevance for CRC with CIMP

**Good prognosis:**

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

**Bad prognosis:**

- CIMP +
- MSS/MSI-L
- proximal colon
- old age
- mucinous carcinomas
- advanced pT

- 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