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

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Disclosure slide

- Member of advisory board for AMGEN, ROCHE
- 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%)

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

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

MSI

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

Normal colorectal epithelium

Proliferation boost to ACF (serr.)

BRAF: V600E

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

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

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

CIMP
p16INK4a, IGFBP7 methylation

Progression to SSA w/o dysplasia

STOP

Senescent lesion, no progression

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Progression to SSA /w dysplasia

Progression to MSI carcinoma
CIMP-H, BRAF mut.

Progression to carcinoma
CIMP-H, BRAF mut.

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

p53-Mutation?

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

Progression to MSS carcinoma

MLH1-loss

p16

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Institut für Pathologie
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

Figure 2: Identification of the
(a) Network of CRC subtypes across six classification systems: each node corresponds to a single subtype (colored according to group) and edge width corresponds to the Jaccard similarity coefficient. The four primary clusters, identified from the Markov cluster algorithm, are highlighted and correspond to the four CMS groups. (b) Per sample distribution of each of the six CRC subtyping systems (A–F), grouped by the four consensus subtyping clusters (n = 3,104), and the unlabeled non-consensus set of samples (n = 858). Colors within each row represent a different subtype. The n values shown in b correspond to the number of subtypes in the original independent classification published by each group. (c) Patient network: each node represents a single patient sample (n = 3,962). Network edges correspond to highly concordant (5 of 6) subtyping calls between samples. Nodes are colored according to their CMSs, with non-consensus samples in gray. (d) Final distribution of the CMS1–4 groups (solid colors), ‘mixed’ samples (gradient colors) and indeterminate samples (gray color) as per the classification framework.

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.
**pTNM (UICC 7th edition, 2010): primary tumor**

<table>
<thead>
<tr>
<th>pT</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT0</td>
<td>No primary tumor</td>
</tr>
<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>
## pTNM (UICC 7th edition, 2010): nodal status

<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!

<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>
</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>II A</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II B</td>
<td>T4a</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III A</td>
<td>T1, T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>III B</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>III C</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

*Update of the Paris-Classification of superficial neoplastic lesions in the digestive tract, Lambert et al., Endoscopy 2005

→ risk evaluation for lymphatic spread
Criteria for endoscopic resection vs. surgery in pT1

- Low risk for lymphatic spread:
  - Depth of submucosal infiltration $\leq 1000 \mu\text{m}$
  - No lymphangiosis carcinomatosa (L0)
  - Good differentiation (G1, G2)

  $\rightarrow$ endoscopic resection is sufficient

- High risk for lymphatic spread:
  - Depth of submucosal infiltration $> 1000 \mu\text{m}$
  - Lymphangiosis carcinomatosa (L1)
  - Bad differentiation (G3, G4)

  $\rightarrow$ oncologic surgery is the treatment of choice
UICC-Staging and stage adapted therapy: locally resectable tumors

UICC-Stage

Low risk
- sm1/2 ≤ 1000 µ
- G1/G2, L0, R0

High risk
- Sm3 > 1000 µ
- G3/G4, L1, R1

Local excision suffices

Oncological resection recommended

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

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

+/- LK-Met.

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 metastases (after neo-adjuvant treatment)

Resection of the primary (if symptomatic)

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

Rubbia-Brandt et al., Ann Oncol 2007
Mismatch repair system

**Mismatch Recognition**
- Single base mismatch
- Insertion-deletion loop
  - MutSα
  - ADP, ATP
  - MSH2, MSH6

**Sliding Clamp Translocation**
- MutLα
- PMS2, MLH1

**Excision of the Mismatch**
- EXO1
- POLYMERASE
- PCNA

**DNA Resynthesis by Polymerase**
Microsatellite loci are used to diagnose mismatch repair.

- **Normal**: 
  - N T
  - __ __
  - __ __

- **LOH**: 
  - N T
  - __ __
  - __

- **MSI phenotype**:
  - N T
  - __ __
  - __

where: 
- n = 13
- n = 9

The figure illustrates the comparison of normal (N T) and LOH (N T) states with MSI phenotype (N T), showing the characteristic changes in microsatellite repeats for microsatellite instability (MSI) identification.
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.
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

Loss of MSH2-, MSH6- or PMS2-expression

Loss of MLH1-expression

BRAF-mutational analysis

WT

mutated

fragment length analysis

Retained expression of MLH1, PMS2, MSH2, MSH6

HNPCC-associated CRC possible

MSS

MSI-H

MSS-KRK

Sporadic MSI-H-CRC

Retained expression of MLH1, PMS2, MSH2, MSH6

MSS

MSI-H

MSS-KRK

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

Molecular grading according to MSI (WHO 2010)

Morphological grading
- Gland-like
- Undifferentiated

G1 → low grade
G2 → high grade
G3 → low grade
G4 → high grade

Molecular grading (MSI-status)
- MSI-H
- MSS

- Undifferentiated, signet-ring cell, mucinous carcinomas
- Low grade
- High grade
<table>
<thead>
<tr>
<th></th>
<th>MSS</th>
<th>MSI-H</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>UICC stage</td>
<td>I 146 (18,2) 204 (25,4) 237 (29,4) 217 (27,9)</td>
<td>13 (14,6) 42 (47,2) 27 (30,3) 7 (7,9)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>II 423 (52,6) 381 (47,4)</td>
<td>33 (37,1) 56 (62,9)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>lymphnode metastases</td>
<td>yes 217 (27,0) 587 (73,0)</td>
<td>7 (7,9) 82 (92,1)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>Five-year survival probability</th>
<th>Multivariate HR (95% CI)</th>
<th>No.</th>
<th>Five-year survival probability</th>
<th>Multivariate HR (95% CI)</th>
<th>No.</th>
<th>Five-year survival probability</th>
<th>Multivariate HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF wild-type MSS</td>
<td>387</td>
<td>0.65</td>
<td>1 (referent)</td>
<td>387</td>
<td>0.63</td>
<td>1 (referent)</td>
<td>387</td>
<td>0.75</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>43</td>
<td>0.74</td>
<td>0.51 (0.27–0.95)</td>
<td>43</td>
<td>0.79</td>
<td>0.54 (0.27–1.08)</td>
</tr>
<tr>
<td>BRAF-mutant MSS</td>
<td>41</td>
<td>0.48</td>
<td>1.38 (0.84–2.26)</td>
<td>41</td>
<td>0.45</td>
<td>1.38 (0.85–2.25)</td>
<td>41</td>
<td>0.61</td>
<td>1.61 (0.96–2.69)</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>34</td>
<td>0.67</td>
<td>0.81 (0.44–1.51)</td>
<td>34</td>
<td>1.02</td>
<td>1.02 (0.54–1.93)</td>
</tr>
</tbody>
</table>

Ogino, Clin Cancer Res 2012

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

Disease free survival

PETACC2
UICC stage III
adjuvant 5-FU
n = 385

HR 0,37

HR 0,70

HR 1,75

HR 1,00

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

Number of patients at risk:

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>191</th>
<th>101</th>
<th>32</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>113</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>17</td>
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<td>7</td>
<td>27</td>
<td>20</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

MSI/BRAF
MSS/BRAF WT
MSS/BRAF V600E
MSI-H/BRAF WT
MSI-H/BRAF V600E
Prognostic value of BRAF is dependent on MSI-Status

BRAF-Mutation

UICC stage I  stage II/III  stage IV

BRAF V600E + MSI

12%

5%

BRAF V600E + MSS
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.