Diagnosis and management issues in colorectal cancer

• What can molecular pathology offer for optimal decision making?

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Disclosure slide

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

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

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

MSI

www.esmo2012.org
Serrated Pathway of colorectal carcinogenesis

~15-20%

Normal colorectal epithelium

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

BRAF: V600E

CIMP
p16INK4a, IGFBP7 methylation

Proliferation boost to ACF (serr.)

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

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

progression to SSA w/o dysplasia

senescent lesion, no progression

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

Progression to SSA /w dysplasia

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

Progression to SSA /w dysplasia

Progression to MSI carcinoma CIMP-H, BRAF mut.

Progression to MSS carcinoma CIMP-H, BRAF mut.
Alternate Pathway of 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
- Conflicting data: p53, loss of 18q, 17p, gain of 20q13, KRAS, etc.
Microsatellite instability (MSI): definition

- 2/5 panel-markers instable or > 30% of tested markers instable \( \Rightarrow \) MSI-H
- 1/5 panel-markers instable or < 30% of tested markers instable \( \Rightarrow \) MSI-L
- All markers stable \( \Rightarrow \) MSS

Boland & Goel, Gastroenterology 2010

Table 2. Microsatellite Markers Used in Diagnosis of Microsatellite Instability in Colorectal Cancer

- MS, microsatellite; NCI, National Cancer Institute.
# MSI-H frequency in CRC

<table>
<thead>
<tr>
<th>Author and journal</th>
<th>year</th>
<th>n</th>
<th>method</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watanabe et al., NEJM</td>
<td>2001</td>
<td>229</td>
<td>MSI/IHC</td>
<td>20%</td>
</tr>
<tr>
<td>Samowitz et al., Cancer Epid Prev</td>
<td>2001</td>
<td>1986</td>
<td>MSI</td>
<td>12%</td>
</tr>
<tr>
<td>Barratt et al., Lancet</td>
<td>2001</td>
<td>368</td>
<td>MSI/IHC</td>
<td>24%</td>
</tr>
<tr>
<td>Ribic et al., NEJM</td>
<td>2003</td>
<td>570</td>
<td>MSI/IHC</td>
<td>17%</td>
</tr>
<tr>
<td>Westra et al., J Clin Oncol</td>
<td>2005</td>
<td>273</td>
<td>MSI</td>
<td>16%</td>
</tr>
<tr>
<td>Sinicrope et al., Gastroenterology</td>
<td>2006</td>
<td>528</td>
<td>IHC</td>
<td>18%</td>
</tr>
<tr>
<td>Malesci et al., Clin Cancer Res</td>
<td>2007</td>
<td>893</td>
<td>MSI</td>
<td>10%</td>
</tr>
<tr>
<td>Deschoolmeester et al., EJC</td>
<td>2008</td>
<td>241</td>
<td>MSI</td>
<td>12%</td>
</tr>
<tr>
<td>Nehls et al., IJCD</td>
<td>2009</td>
<td>344</td>
<td>MSI</td>
<td>15%</td>
</tr>
<tr>
<td>Kim et al., Cancer ChemoPrev</td>
<td>2010</td>
<td>134</td>
<td>MSI</td>
<td>9%</td>
</tr>
<tr>
<td>Qui tal., CancGenProt</td>
<td>2011</td>
<td>803</td>
<td>MSI</td>
<td>10%</td>
</tr>
<tr>
<td>Lin et al., IJCD</td>
<td>2011</td>
<td>709</td>
<td>MSI</td>
<td>9%</td>
</tr>
<tr>
<td>Yoon et al., JournalGastroHepatol</td>
<td>2011</td>
<td>2028</td>
<td>IHC</td>
<td>10%</td>
</tr>
</tbody>
</table>

8914 10%
MSI-H as a favorable prognostic marker in CRC

<table>
<thead>
<tr>
<th>Source</th>
<th>Stage / Treatment</th>
<th>Endpoint</th>
<th>MMR-D vs MMR-P HR (95% CI); p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribic et al¹</td>
<td>II/III Surgery alone</td>
<td>Overall survival</td>
<td>0.31 (0.14-0.72); p=0.004</td>
</tr>
<tr>
<td>Sargent et al²</td>
<td>II/III Surgery alone</td>
<td>Disease-free survival</td>
<td>0.46 (0.22-0.95); p=0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall survival</td>
<td>0.51 (0.24-1.10); p=0.06</td>
</tr>
<tr>
<td>Gray et al³ (QUASAR)</td>
<td>II Surgery alone</td>
<td>Recurrence-free interval</td>
<td>0.31 (0.15-0.63); p&lt;0.001</td>
</tr>
<tr>
<td>Roth et al⁴ (PETACC-3)</td>
<td>II 5FU ± irinotecan</td>
<td>Relapse-free survival</td>
<td>0.30 p=0.004</td>
</tr>
</tbody>
</table>
MSI-H as a favorable prognostic marker in CRC


n = 893
UICC I-III
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>

Molecular grading according to MSI (WHO 2010)

Morphological grading

- Gland-like
- Undifferentiated

Molecular grading (MSI-Status)

- Undifferentiated, signet-ring cell, mucinous carcinomas

G1
- Low grade
- Low grade

G2
- High grade

G3
- High grade

G4

- MSI-H
- MSS

- High grade

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MSI-H: prognostic value in association with CIMP-phenotype

<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>

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

Barault, Cancer Res 2008
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
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>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Five-year survival</td>
<td>Multivariate HR (95% CI)</td>
<td>Five-year survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>probability</td>
<td></td>
<td>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>
</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>
</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>
</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>
</tr>
</tbody>
</table>

Ogino, Clin Cancer Res 2012
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

PETACC2
UICC stage III
adjuvant 5-FU
n = 385
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

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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

<table>
<thead>
<tr>
<th>CIMP + MSS/MSI-L</th>
<th>CIMP-H + MSS</th>
<th>+ BRAF-Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>proximal colon</td>
<td></td>
<td>3,19fold higher</td>
</tr>
<tr>
<td>old age</td>
<td></td>
<td>risk for tumor-</td>
</tr>
<tr>
<td>mucinuous</td>
<td></td>
<td>associated †</td>
</tr>
<tr>
<td>carcinomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>advanced pT</td>
<td>ca. 50%</td>
<td>ca. 50%</td>
</tr>
</tbody>
</table>

**Bad prognosis:**
Summary prognostic markers

- MSI and BRAF are prognostic markers (for the serrated pathway)
- MSI-H is a strong prognostic indicator in stage II and may lead to a better risk stratification
- 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
- For the adenoma-carcinoma-sequence and the alternate pathway, there is abundant but conflicting data on various markers (p53, 18q, 17p-, EGFR, KRAS, etc.)
Predictive markers

• MSI for 5-FU, irinotecan ?
• TS, TP, DPD for 5-FU-therapy
• ERCC1 for oxaliplatin
• KRAS for anti-EGFR-therapy
•
# MSI-H: Predictive value for 5-FU

## Table 3. Chemotherapy in Colorectal Cancer with Microsatellite Instability

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Study design</th>
<th>Adjuvant chemotherapy regimen</th>
<th>No. of patients (MSI/MSS)</th>
<th>Benefit of chemotherapy in patients with MSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elsaleh⁹³</td>
<td>2000</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>63/669</td>
<td>Yes</td>
</tr>
<tr>
<td>Ribic⁸⁹</td>
<td>2003</td>
<td>Randomized controlled study</td>
<td>5-FU</td>
<td>95/475</td>
<td>No</td>
</tr>
<tr>
<td>Carethers⁹⁴</td>
<td>2004</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>36/168</td>
<td>No</td>
</tr>
<tr>
<td>de Vos tot Nederveen Cappell⁹⁴</td>
<td>2004</td>
<td>Lynch syndrome patients</td>
<td>5-FU</td>
<td>28/0</td>
<td>No</td>
</tr>
<tr>
<td>Storojeva⁹⁶</td>
<td>2005</td>
<td>Randomized controlled study</td>
<td>5-FU/mitomycin</td>
<td>21/139</td>
<td>No</td>
</tr>
<tr>
<td>Benatti⁹²</td>
<td>2005</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>256/1007</td>
<td>No</td>
</tr>
<tr>
<td>Popat⁹¹</td>
<td>2005</td>
<td>Pooled data from multiple studies</td>
<td>5-FU</td>
<td>1277/6365</td>
<td>No</td>
</tr>
<tr>
<td>Lanza⁹⁷</td>
<td>2006</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>75/288</td>
<td>No</td>
</tr>
<tr>
<td>Jover⁹⁸</td>
<td>2006</td>
<td>Consecutive patients</td>
<td>5-FU</td>
<td>66/688</td>
<td>No</td>
</tr>
<tr>
<td>Kim⁹⁶</td>
<td>2007</td>
<td>Prospective study</td>
<td>5-FU/leucovorin</td>
<td>98/444</td>
<td>No</td>
</tr>
<tr>
<td>Des Guetz⁹⁹</td>
<td>2009</td>
<td>Meta-analysis</td>
<td>5-FU/irinotecan/leucovorin</td>
<td>454/2871</td>
<td>No</td>
</tr>
<tr>
<td>Bertagnoli⁹⁰</td>
<td>2009</td>
<td>Randomized controlled study</td>
<td>5-FU/irinotecan/leucovorin</td>
<td>106/677</td>
<td>No</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; MSS, microsatellite stable.

Boland & Goel, Gastroenterology 2010
MSI-H: negative predictive value for 5-FU therapy?

Sargent, JCO 2008
N = 1027
UICC stage II and III

Fig 1. (A) Disease-free survival (DFS) in untreated patients by DNA mismatch repair (MMR) status. (B) DFS in treated patients by MMR. dMMR, defective DNA mismatch repair; pMMR, proficient DNA mismatch repair.
Predictive value of MSI-H dependent on background?

Sporadic MSI

"Hereditary" MSI
(BRAF-WT, <55y, MSH-2)

>> no benefit from 5-FU

Benefit from 5-FU?
Sinicrope, J Natl Cancer Inst 2011
n = 778 UICC stage 2
Summary MSI-H

• The predictive value of MSI-H is questionable and may depend on background (hereditary vs. sporadic)
RAS-/RAF-pathway
KRAS-mutation as a negative predictor for anti-EGFR-treatment

- KRAS, BRAF, NRAS, PIK3CA wild type, no PTEN loss
- Non-Response 85%
- KRAS mutation 32%
- Molecular aberration not yet identified 23%
- PIK3CA mutation / PTEN loss 12%
- NRAS mutation 3%
- BRAF mutation 5%
- BRAF + PIK3CA mutation 2%
Association of **KRAS** p.G13D Mutation With Outcome in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer Treated With Cetuximab

**Figure 1.** Overall Survival: Predictive Analysis by KRAS Status for Patients Receiving Any Cetuximab-Based Therapy vs No Cetuximab

The no cetuximab group for all patients from the pooled data set is the best supportive care group from the CO.17 trial.

De Roock W et al., JAMA 2010
Summary KRAS and EGFR

• KRAS-Mutation is a negative predictor of response to anti-EGFR-therapy, but
• Other members of the pathway may also contribute to non-response: PI3K, PTEN, NRAS, EGFR, etc.
• Different KRAS-mutations may have varying predictive impact
• Amphiregulin and epiregulin may prove to be the first positive predictive markers for anti-EGFR-treatment
Molecular signatures in CRC – do we need them?

YES

• To select stage II patients who are at risk of recurrence (~15%)
• To select stage III patients who are at low risk of recurrence (~50%)
• To select stage II and III patients who will benefit from adjuvant chemotherapy
UICC Stage II and Stage III prognosis

• Inside each tumour stage the risk of recurrence is depending of various risk factors
• For UICC stage II: obstruction/perforation, emergent admission, T4 stage, high-grade, less than 12 LN are indicative of poor prognosis
• For stage III the number of positive lymph-nodes are associated with the risk of recurrence
• The only validated prognostic biomarker is the MSI status in stage II patients

O’Connor J Clin Oncol 2011;29:3381-88
Weisser J Clin Oncol 2011; 29:4796-802
Roth J Cin Oncol 2009; 28:466-74
The different signatures for UICC stage II

- 114 genes → MD Anderson
- 12 genes → Recurrence score™ Genomic Health
- 18 genes → Coloprint Agendia
- 634 genes → Colorectal DSA Almac
- 13 genes → ColoGuideEX
114 genes signatures Fresh frozen tissues (MD Anderson)

Method: unsupervised signature
National Center for Biotechnology Information Gene Expression Omnibus database

One set of training Moffit cancer Center (n=177)
Two cohorts of validation
Vanderbilt and Max Planck Institute (VMP) cohort (117)
Melbourne hospital cohort (96)

Oh et al. Gut 2011 on line
114 genes signatures (MD Anderson)

Method: unsupervised signature
National Center for Biotechnology Information Gene Expression Omnibus database

One set of training Moffit cancer Center (n=177)

Two cohorts of validation
Vanderbilt and Max Planck Institute (VMP) cohort (117)
Melbourne hospital cohort (96)

Validation cohorts
Stage II & III
Impact of chemotherapy stage III

Oh et al. Gut 2011 on line

www.esmo2012.org
12 genes signature FFPE tissues: Recurrence score™

Method: Supervised signature
RT-PCR from FFPE

Development: 1,851 patients with stage II and stage III colon cancer in four independent studies: (NSABP C-01/C-02 (n = 270), Cleveland Clinic (n = 765), NSABP C-04 (n = 308), NSABP C-06 (n = 508)

Unsupervised hierarchical clustering of the 48 genes significantly related to recurrence-free interval → 7 genes and 5 control genes

O’connell J Clin Oncol 2010;28:3937-44
12 genes signature FFPE tissues: 
Recurrence score™

Three cohorts of validation 
Stage II Colon Cancer QUASAR (n = 1436) 
Stage II Colon Cancer CALGB 9581 (n = 690) 
Stage II/III Colon Cancer 5FU vs 5FU+Oxaliplatin NSABP C-07 (n = 892)

• Validated in stage II patients included in QUASAR and CALBG 9581
• Significant association between the recurrence score™ and the risk of recurrence HR per interquartile range, 1.38; Cl_{95%} [1.1- to 1.7]; p=0.004
• Remains significant in multivariate analysis

Gray J Clin Oncol 2011;29:4611-19; Venook ASCO 2011 abst 3818
12 genes signature FFPE tissues: Recurrence score™

Three cohorts of validation
Stage II Colon Cancer QUASAR (n = 1436)
Stage II Colon Cancer CALGB 9581 (n = 690)
Stage II/III Colon Cancer 5FU vs 5FU+Oxaliplatin NSABP C-07 (n = 892)

Gray J Clin Oncol 2011;29:4611-19; Venook ASCO 2011 Abstract 3518
Contribution of Recurrence Score® Result Beyond Clinical and Pathologic Covariates

Pre-specified Multivariate Analysis (n=892)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>HR</th>
<th>HR 95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage (by nodal status)</td>
<td>Stage III A/B vs II</td>
<td>0.97</td>
<td>(0.55,1.71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Stage III C vs II</td>
<td>2.07</td>
<td>(1.16,3.68)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>5FU+Ox vs 5FU</td>
<td>0.82</td>
<td>(0.64,1.06)</td>
<td>0.12</td>
</tr>
<tr>
<td>MMR</td>
<td>MMR-D vs MMR-P</td>
<td>0.27</td>
<td>(0.12,0.62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-stage</td>
<td>T4 st II &amp; T3-T4 st III vs T3 st II &amp; T1-T2 st III</td>
<td>3.04</td>
<td>(1.84,5.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nodes examined</td>
<td>&lt;12 vs ≥12</td>
<td>1.51</td>
<td>(1.17,1.95)</td>
<td>0.002</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>High vs Low</td>
<td>1.36</td>
<td>(1.02,1.82)</td>
<td>0.041</td>
</tr>
<tr>
<td>RS</td>
<td>per 25 units</td>
<td>1.57</td>
<td>(1.19,2.08)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

- The Recurrence Score value is significantly associated with risk of recurrence after controlling for effects of T and N stage, MMR status, number of nodes examined, grade and treatment.
18 genes signature Fresh Frozen Tissues: Coloprint

Method: Unsupervised selection
RT-PCR from Fresh frozen tissues

Development: Training Set (stage I-IV) (n=188)
Netherlands Cancer Institute, Leiden Medical Center, Slotervaart
Clinical Validation Study 1 (stage I-III) Institute Catala d’Oncologia Barcelona
In-silico Validation Study (stage I-III) public datasets (n=322)

Whole Genome Array 44K Agilent \(\rightarrow\) defined three groups of tumors

Salazar J Clin Oncol 2011;29:17-24

Selection of 18 genes

www.esmo2012.org
18 genes signatures fresh frozen tissues

Method: Unsupervised selection
RT-PCR from Fresh frozen tissues

Development: Training Set (stage I-IV) (n=188)
Netherlands Cancer Institute, Leiden Medical Center, Slotervaart
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In-silico Validation Study (stage I-III) public datasets (n=322)

Salazar J Clin Oncol 2011;29:17-24
Colorectal DSA Almac

634 transcript signature from FFPE

Development: Training Set stage II (n=215)
Validation stage II (n=144)

Method: Supervised selection
Microarray Colorectal Cancer DSA from fresh frozen tissues

Kennedy J Clin Oncol 2011;35:4620
ColoGuideEX
13 genes signature fresh frozen tissues

Method: Supervised selection
Affymetrix array from fresh frozen tissues

207 training set (stage I-IV)
53 and 108 validation sets (stage II)

Validation series I, 53 stage II CRC
Low risk (n=45)
High risk (n=7)
p=0.02; HR=3.6

Validation series II, 108 stage II CRC, external dataset
Low risk (n=91)
High risk (n=17)
p=0.001; HR=6.5

Agesen Gut 2012
Summary Signatures in CRC II

- There are multiple prognostic signatures
  - for stage II and sometimes for stage III
- All signatures seem to be validated
  - The level of “validation” is different
- The overlap between these different signatures is weak
- None of these signatures is able to predict the benefit of adjuvant chemotherapy
- They all make the hypothesis that colon cancer is an homogenous cancer → which is clearly not the case
Different groups of colorectal cancer

<table>
<thead>
<tr>
<th>CIT CCMST</th>
<th>C2 (n=83)</th>
<th>C4 (n=46)</th>
<th>C3 (n=56)</th>
<th>C6 (n=45)</th>
<th>C5 (n=118)</th>
<th>C1 (n=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI</td>
<td>2.1e-42</td>
<td>68%</td>
<td>12%</td>
<td>7%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>CIMP+</td>
<td>1.1e-23</td>
<td>59%</td>
<td>34%</td>
<td>18%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>CIN+</td>
<td>1.7e-15</td>
<td>44%</td>
<td>73%</td>
<td>65%</td>
<td>63%</td>
<td>95%</td>
</tr>
<tr>
<td>BRAF mut</td>
<td>4.1e-19</td>
<td>40%</td>
<td>22%</td>
<td>6%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>KRAS mut</td>
<td>1.1e-12</td>
<td>28%</td>
<td>50%</td>
<td>87%</td>
<td>28%</td>
<td>27%</td>
</tr>
<tr>
<td>TP53 mut</td>
<td>7.3e-03</td>
<td>41%</td>
<td>45%</td>
<td>35%</td>
<td>59%</td>
<td>71%</td>
</tr>
<tr>
<td>Proximal Location</td>
<td>5e-17</td>
<td>72%</td>
<td>57%</td>
<td>59%</td>
<td>16%</td>
<td>21%</td>
</tr>
</tbody>
</table>
Oncotype signature versus the different groups of CRC

Stage II and III
775 patients

---

C1

C2

C3

C4

C5

C6
Summary Conclusion I

- CRC is a complex disease with several subentities derived through different pathways
- MSI-H is a prognostic indicator in stage II
- The prognostic impact of BRAF is dependent on MSI-status
- KRAS is still the only validated predictive marker for anti-EGFR treatment
- The role of different KRAS-mutations needs to be verified in large prospective trials
- Signatures need to be developed for the different subentities, rather than „one size fits all“
The division of CRC in various subentities generates the necessity of multicenter trials, since subgroups will be small.

FFPE-material should be collected in these trials and investigated for potential prognostic and predictive markers.

The gold standard of risk-stratification is still correct pTNM-staging and thorough histopathological workup.

The addition of molecular data will – hopefully – allow the development of a more personalized treatment of CRC.