Molecular classification of colon cancer: new insights

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Disclosures

Advisory role: Roche
                Boehringer-Ingelheim
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Speaker’s fee: Roche
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               Symphogen
               MSD
               IPSEN
               Sanofi
               Amgen

Research support: MERCK
Genomic markers

- **BRAF V600E**
- **BRAF non-V600**
- **MSI**
- **TMB high**
- **POLE mut**
- **HER2 ampl**
- **MET ampl**
- **Gene fusion**
- **RAS mut +/- PIK3CA/PTEN mut**
- **PIK3CA/PTEN mut**
- **Wild-type**
- **anti-EGFR therapies**
- **Kinase inh**
- **MEK inh?**
- **MEK inh + anti-EGFR?**
- **double anti-HER2**
- **anti-PD1/L1**
- **BRAF non-V600**
- **MEK inh + anti-EGFR +/- MEK inh**
- **TMB high**
- **POLE mut**
- **HER2 ampl**
- **Wild-type anti-EGFR therapies**

- **45%**
- **26%**
- **8%**
- **To be defined**
Transcriptomic classification

BULK-TUMOR subtypes (primary CRC)

- CMS1: 15%
- CMS2: 40%
- CMS3: 13%
- CMS4: 25%
- Mixed: 7%

BULK - TUMOR subtypes (primary CRC)
Consensus Molecular Subtype (CMS) groups

Molecular Enrichments

<table>
<thead>
<tr>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI Immune</td>
<td>Canonical</td>
<td>Metabolic</td>
<td>Mesenchymal</td>
</tr>
<tr>
<td>15%</td>
<td>40%</td>
<td>13%</td>
<td>25%</td>
</tr>
<tr>
<td>¾ MSI</td>
<td>CIN</td>
<td>¾ CIN</td>
<td>CIN</td>
</tr>
<tr>
<td>BRAF mut</td>
<td>Epithelial</td>
<td>Epithelial</td>
<td>Mesechymal</td>
</tr>
<tr>
<td>BRAF mut-like</td>
<td>WNT/MYC</td>
<td>RAS mut</td>
<td></td>
</tr>
<tr>
<td>Immune infiltration</td>
<td>BRAF mut-like</td>
<td>TGFβ, angiogenesis</td>
<td></td>
</tr>
<tr>
<td>Immune-activated</td>
<td>EGFR/ligands high</td>
<td>Metabolic</td>
<td>Stromal infiltration</td>
</tr>
<tr>
<td>Right-sided</td>
<td>Immune-desert</td>
<td>Immune-mixed</td>
<td>Immunosuppression</td>
</tr>
<tr>
<td>Left-sided</td>
<td>Right-sided</td>
<td>Right-sided</td>
<td>Both sides</td>
</tr>
</tbody>
</table>

Guinney et al, Nat Med 2015
Context matters

-Chang et al. Annals Oncol 2018
-Fontana et al. Annals Oncol 2018
Intra-tumor heterogeneity in early-stage CRC

CMS attribution is uncertain* for most samples (63%)
* i.e. RF classifier probability < 70%

...and most samples (57%) show intra-tumor heterogeneity*
* i.e. more than 1 CMS with a (WISP-derived) weight above 20%

Marisa et al, ESMO 2018
Intra-tumor heterogeneity in early-stage CRC

Adjusted multivariable analysis:
- CMS1 / CMS4  HR: 2.17 [1.36-3.5]
- CMS4 / CMS1  HR: 1.79 [1.14-2.8]

Marisa et al, ESMO 2018
Intra-tumor heterogeneity

Spatial stability

Tumor center

Invasive front

Primary-metastasis

Dunne et al, Nature Commun 2017

Piskol et al, Clin Cancer Res 2019
Microenvironment of CRC

Becht et al, Clin Cancer Res 2016
Microenvironment of CRC

Karpinski et al, Oncotarget 2017
Take-home messages

- CMS groups = **not driven by content** (*clustering of other classifiers*)
  highly reproducible in cohorts of **primary CRC**
  has **biology enrichments** (*not unique features from pathway and tumor microenvironment perspectives*)

- CMS classifiers = **technical** and **heterogeneity** issues
CRC Consensus Molecular Subtypes: prognostic value considerations
Prognosis of CMS groups in early-stage CRC

RFS in 1,785 stage II/III CRC

RFS in 1,151 stage III CRC (NSABP C-07 trial)

Guinney et al, Nat Med 2015

Song et al, JAMA Oncol 2016
Prognostic value of CMS groups in metastatic CRC

Survival after relapse (n=405)

Overall survival metastatic CRC (n=581)

Guinney et al, Nat Med 2015
Lenz et al, J Clin Oncol 2019
Prognostic value of CMS groups in multivariable models

Dienstmann et al, under review

Disease-free survival

ClinPath: pT, pN, age, sex, tumor location
Gen: MSI, KRAS, BRAF^{V600E}
CMS: CMSclassifier scores
MicroCells: MCPcounter scores (scaling 0-1)

No adjuvant chemotherapy
N=1,656

Imputation missing values
Variable selection with forward and backward stepwise regression (CMS scores and MCPcounter scores).

Adjuvant chemotherapy
N=980

Models stratified by sex, gene expression assay (Affymetrix fresh-frozen, Agilent fresh-frozen, Affymetrix-Almac FFPE) and adjuvant chemotherapy status.

Interaction terms explored

Final multivariable models (ANOVA, explained variation for survival models)
ClinPath + Gen;
ClinPath + Gen + CMS;
ClinPath + Gen + MicroCells;
ClinPath + Gen + CMS + MicroCells.
**Prognostic value of CMS groups in multivariable models**

### Disease free survival Cox models (all patients)\(^{\text{a}}\) (n=2,636, 769 events)

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>1.01 – 1.02</td>
</tr>
<tr>
<td>pT2/pT1 versus pT3</td>
<td>1.07</td>
<td>0.65 – 1.75</td>
</tr>
<tr>
<td>pT4 versus pT3</td>
<td>1.37</td>
<td>1.11 – 1.69</td>
</tr>
<tr>
<td>pN1 versus pN0</td>
<td>1.99</td>
<td>1.61 – 2.46</td>
</tr>
<tr>
<td>pN2 versus pN0</td>
<td>3.08</td>
<td>2.41 – 3.93</td>
</tr>
<tr>
<td>Rectum versus left</td>
<td>1.03</td>
<td>0.76 – 1.40</td>
</tr>
<tr>
<td>Right versus left</td>
<td>0.84</td>
<td>0.72 – 0.97</td>
</tr>
<tr>
<td>MSI versus MSS</td>
<td>0.76</td>
<td>0.61 – 0.93</td>
</tr>
<tr>
<td>KRAS mut versus wild-type</td>
<td>1.04</td>
<td>0.9 – 1.21</td>
</tr>
<tr>
<td>BRAF mut versus wild-type</td>
<td>0.9</td>
<td>0.72 – 1.13</td>
</tr>
<tr>
<td>CMS4 score</td>
<td>1.37</td>
<td>1.07 – 1.76</td>
</tr>
<tr>
<td>CAF infiltration score</td>
<td>1.6</td>
<td>0.93 – 2.74</td>
</tr>
<tr>
<td>CytoLym infiltration score</td>
<td>0.45</td>
<td>0.25 – 0.78</td>
</tr>
</tbody>
</table>

\(^{\text{a}}\)Dienstmann et al, under review
Prognostic value of CMS groups in multivariable models

Overall population (N=2,636)

Dienstmann et al, under review
Prognostic value of CMS groups in multivariable models

Dienstmann et al, under review
Prognostic value of Immunoscore in multivariable models

Pages et al, Lancet 2018
CRC Consensus Molecular Subtypes: predictive value considerations
Predictive value of CMS groups in early-stage disease

CRCA is a better classifier for oxaliplatin benefit in C-07

<table>
<thead>
<tr>
<th>Classifiers</th>
<th>Subtypes</th>
<th>N</th>
<th>HR</th>
<th>P-value</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>CRCA</td>
<td>Enterocyte</td>
<td>135</td>
<td>0.35</td>
<td>0.001</td>
<td>-</td>
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<tr>
<td></td>
<td>Goblet-like</td>
<td>103</td>
<td>1.02</td>
<td>0.95</td>
<td>-</td>
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<tr>
<td></td>
<td>Inflammatory</td>
<td>239</td>
<td>0.74</td>
<td>0.27</td>
<td>-</td>
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<tr>
<td></td>
<td>Stem-like</td>
<td>367</td>
<td>0.99</td>
<td>0.96</td>
<td>-</td>
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<tr>
<td></td>
<td>TA</td>
<td>307</td>
<td>0.82</td>
<td>0.32</td>
<td>-</td>
</tr>
<tr>
<td>CCS</td>
<td>CCS1</td>
<td>410</td>
<td>0.72</td>
<td>0.06</td>
<td>-</td>
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<tr>
<td></td>
<td>CCS2</td>
<td>245</td>
<td>0.73</td>
<td>0.23</td>
<td>-</td>
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<tr>
<td></td>
<td>CCS3</td>
<td>496</td>
<td>0.91</td>
<td>0.48</td>
<td>-</td>
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<tr>
<td>Consensus</td>
<td>CMS1</td>
<td>231</td>
<td>0.77</td>
<td>0.32</td>
<td>-</td>
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<tr>
<td></td>
<td>CMS2</td>
<td>382</td>
<td>0.61</td>
<td>0.006</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CMS3</td>
<td>86</td>
<td>1.17</td>
<td>0.68</td>
<td>-</td>
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<tr>
<td></td>
<td>CMS4</td>
<td>334</td>
<td>0.87</td>
<td>0.39</td>
<td>-</td>
</tr>
<tr>
<td>Stratify CCS1 to TA and enterocyte</td>
<td>CCS1-TA</td>
<td>251</td>
<td>0.79</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CCS1-ent</td>
<td>74</td>
<td>0.17</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>Stratify CMS2 to TA and enterocyte</td>
<td>CMS2-TA</td>
<td>261</td>
<td>0.77</td>
<td>0.24</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CMS2-ent</td>
<td>57</td>
<td>0.20</td>
<td>0.003</td>
<td>-</td>
</tr>
</tbody>
</table>

Oxaliplatin benefit seen in the CMS2 subtype
But significant benefit after further stratification
Restricted to the CMS2-enterocyte subtype

Song, Pogue-Geile et al. JAMA Oncol 2016

Pogue-Geile et al, ASCO 2019
Predictive value of CMS groups in early-stage disease

CMS Subtypes were not associated with oxaliplatin benefit in stage III pts in MOSAIC

Pogue-Geile et al, ASCO 2019
## Predictive value of CMS groups in metastatic disease

<table>
<thead>
<tr>
<th>Trial</th>
<th>Line</th>
<th>KRAS/BRAF Status</th>
<th>Study Type</th>
<th>Sample Size</th>
<th>Treatment Comparison</th>
<th>CMS Result in FOLFOX vs. FOLFOX-bevacizumab</th>
<th>CMS Result in FOLFOX-bevacizumab vs. FOLFOX-cetuximab</th>
<th>CMS Result in CAPOX-bevacizumab vs. CAPOX-bevacizumab-cetuximab</th>
<th>CMS Result in Bevacizumab vs. Irinotecan</th>
<th>Custom Prognostic Test</th>
<th>FFPE Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALGB80405</td>
<td>1st</td>
<td>KRAS wild-type</td>
<td>RCT (n=581)</td>
<td></td>
<td>FOLFOX-cetuximab vs. FOLFOX bevacizumab (75%)</td>
<td>CMS1 &gt; OS with FOLFOX-bevacizumab, CMS2 &gt; OS with FOLFOX-cetuximab</td>
<td>FOLFOX-cetuximab vs. FOLFOX bevacizumab</td>
<td>CAPOX-bevacizumab vs. CAPOX-bevacizumab-cetuximab (RAS/BRAF wt)</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Custom Nanostring FFPE</td>
<td>87%</td>
</tr>
<tr>
<td>FIRE-3</td>
<td>1st</td>
<td>RAS wild-type</td>
<td>RCT (n=438)</td>
<td></td>
<td>FOLFIRI-cetuximab vs. FOLFIRI bevacizumab</td>
<td>CMS4 &gt; OS with FOLFOX-bevacizumab</td>
<td>FOLFIRI-cetuximab vs. FOLFIRI bevacizumab</td>
<td>CAPOX-bevacizumab vs. CAPOX-bevacizumab-cetuximab (RAS/BRAF wt)</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Almac Xcell FFPE</td>
<td>100%</td>
</tr>
<tr>
<td>CAIRO2</td>
<td>1st</td>
<td>all-comers</td>
<td>RCT (n=311)</td>
<td></td>
<td>CAPOX-bevacizumab vs. CAPOX-bevacizumab-cetuximab</td>
<td>CMS2/CMS3 &gt; OS with cetuximab</td>
<td>CAPOX-bevacizumab vs. CAPOX-bevacizumab-cetuximab (RAS/BRAF wt)</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>IHC FFPE</td>
<td></td>
</tr>
<tr>
<td>MAX</td>
<td>1st</td>
<td>all-comers</td>
<td>RCT (n=237)</td>
<td></td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>CMS2/CMS3 &gt; PFS with bevacizumab</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Almac Xcell FFPE</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>1st</td>
<td>all-comers</td>
<td>Retrospective (n=193)</td>
<td></td>
<td>Oxaliplatin vs. Irinotecan</td>
<td>CMS4 &gt; PFS and OS with Irinotecan</td>
<td>Capecitabine +/- mitomycin +/- bevacizumab</td>
<td>Oxaliplatin vs. Irinotecan</td>
<td>Oxaliplatin vs. Irinotecan</td>
<td>Agilent FF</td>
<td></td>
</tr>
</tbody>
</table>

*Lenz et al, J Clin Oncol 2019; Stintzing et al, ASCO 2017; Trinh et al, Clinical Cancer Res 2017; Mooi et al, Annals Oncol 2018; Okita et al, Oncotarget 2018*
Predictive value of CMS groups in metastatic disease

CALGB 80405

Favors Bevacizumab

Favors Cetuximab

Favors Bevacizumab

Favors Cetuximab

Lenz et al, ASCO 2017
Predictive value of CMS groups in metastatic disease

CALGB 80405

Bevacizumab- treated

Cetuximab-treated

CMS1 interaction P value < 0.001

Lenz et al, J Clin Oncol 2019
Microenvironment targeting in metastatic CRC

Adaptive axis
Cytotoxic lymphocytes
Th1 orientation

Inflammatory axis
Myeloid cells
Angiogenesis

Adoptive T cell therapy
Anti-tumor and bi-specific Ab

CMS1
MSI Immune

CMS2
Canonical

CMS3
Metabolic

CMS4
Mesenchymal

Combination therapy:
anti-angiogenic
anti-checkpoint
anti-inflammatory

Becht et al, Advances Immunol 2016
Microenvironment of CRC

Primary-metastasis heterogeneity?

Karpinski et al, Oncotarget 2017
Take-home messages

• CMS prognostic value = largely explained by tumor microenvironment

• CMS predictive value = has driver pathway enrichments (maybe not the one that matters to a targeted/immunotherapy matched drug)
Future: combine CMS with pathway signatures

RPS: recombinant proficiency score – DNA damage repair

Pts with non-stem-like tumors AND low RPS scores DID receive significant oxaliplatin benefit in MOSAIC

P = 0.042
HR = 0.615

P = 0.209
HR = 1.375

Pogue-Geile et al, ASCO 2019
Future: Integrative CRC classification

- MSI
- CIN

Genomic:
- Copy number
- KRAS/NRAS mutations
- BRAF mutations

Driver genes:
- CMS1
  - Immune activation
  - JAK-STAT activation
  - Caspases
  - DNA damage repair
  - Glutaminolysis
- CMS2
  - Lipogenesis
  - Cell cycle
  - WNT/MYC targets
  - HER (ligands) expression
  - VEGF/VEGFR activation
  - Integrins activation
  - TGFβ activation
  - Mesenchymal transition
- CMS3
  - Complement activation
- CMS4
  - Immunosuppression

Transcriptomic:
- Methylation

Epigenomic:
- Cancer-associated fibroblasts

Stromal:
- Highly immunogenic
- Poorly immunogenic

Immune:
- Inflamed immunosuppressive

Clinical:
- Left (Tumor Location) to Right

Dienstmann et al, Nat Rev Cancer 2017
RAS targeting in CRC

COLOSSUS project – functional subtypes of MSS RAS mutant CRC for Precision Oncology

**Project Coordinator:** Annette Byrne  
**Scientific Leader:** Rodrigo Dienstmann

**Funding:** H2020 grant
CMS classifiers in the clinics

IHC FFPE

Classification of Colorectal Cancer in Molecular Subtypes by Immunohistochemistry

Sanne ten Hoorn, Anne Trinh, Joan de Jong, Lianne Koens, and Louis Vermeulen


NanoString FFPE

Analytical Validation of Multiplex Biomarker Assay to Stratify Colorectal Cancer into Molecular Subtypes

Chanthirka Ragulan1,2, Katherine Eason3, Elisa Fontana1,3, GiGi Nyamundanda1,2, Noelia Tarazona1, Yatish Patil1,2, Pawan Poudel1, Rita T. Lawlor1,2, Maguy Del Rio1, Si-Lin Koo2, Wah-Siew Tan1, Francesco Scafani3, Ruwaida Begum4, Larissa S. Teixeira Mendes4, Pierre Martineau5, Aldo Scarpa6,7, Andres Cervantes2, Iain Beehuiat Tan8,9,10,11, David Cunningham12 & Anguraj Sadanandam1,2

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