A molecular taxonomy of colorectal cancer

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Wellcome Trust Sanger Institute
Disclosures

• Founder and consultancy, 14M Genomics
Progress in metastatic CRC

**Incidenc by Stage**

- **5-yr Survival %**
  - 93%
  - 77%
  - 48%
  - 11%
  - Unknown

**Median OS (months)**

- FOLFOX6 (Oxaliplatin/5FU) 2-weekly: 6 months
- FOLFIRI (Irinotecan/5FU) 2-weekly: 13 months
- FOLFOX/FOLFIRI: 13 months
- FOLFOX/Bev: 15 months
- FOLFIRI/Bev: 15 months
- FOLFOX/Bev/Cetux: 16 months
- FOLFIRI/Bev/Cetux: 17 months
- FOLFIRI/Bev/FOLFOX/Cetux: 21 months
- FOLFOX/Bev: 25 months
- FOLFIRI/Bev: 21 months
- FOLFOX/Cetux: 21 months
- FOLFIRI/Bev: 28.7 months

**Percent Surviving 5 Years**

- 64.7%

**Main chemotherapy regimens:**
- FOLFOX6 (Oxaliplatin/5FU) 2-weekly
- FOLFIRI (Irinotecan/5FU) 2-weekly

**Biologics:**
- Cetuximab (EGFR mAb)
- Bevacizumab (VEGFR mAb)
- Regorafenib (multi-kinase)
- Aflibercept (VEGF)
The adenoma-carcinoma sequence

**Normal epithelium**

**Early adenoma/dysplastic crypt**

**Intermediate adenoma**

**Late adenoma**

**Carcinoma**

**Metastasis**

**Activation:**
- CTNNB1
- KRAS
- BRAF
- PIK3CA

**Inactivation:**
- APC
- MMR genes (MSI)
- SMAD2/4
- TGFBR2
- TP53
- PTEN
- APC
- MMR genes (MSI)

**Other genetic alterations**

**MSI (right, CIMP)**

**MSS (chrom instability)**
Right versus Left Colon Cancer

Analysis of PETACC-3 samples (n=2849)

- **BRAF mut**
- **MSI**
- **KRAS**
- **PIK3CA**
- Mucinous differentiation

**High mutation Frequency**

**Poor Prognosis**

**Right**

- EREG expression
- 18q loss
- 20q Gain
- EGFR gain
- HER2 gain

**Sensitive to Cetuximab**

**Left**

**Good Prognosis**

Missiaglia, ASCO 2013
Adding ‘omics to classifying cancer

Nature Genetics 45, 1113–1120 (2013)
Mutation frequencies in colorectal cancer

A. Colorectal adenocarcinoma mutation rates (224 patients)

B. Hypermutated tumors vs. Non-hypermutated tumors
Pathways in colorectal cancer

**WNT signaling**
- **DKK1** (4% up, 33% down)
- **LRP5** (10% up, 19% down)
- **FZD10** (13% up)
- **APC** (81% down)
- **CTNNB1** (5% up, 7% down)
- **TCF7L2** (12% up, 30% down)
- **SOX9** (4% up, 7% down)
- **TCF7**

**TGFβ signaling**
- **TGFBR1** (17% up, 2% down)
- **TGFB2** (2% up, 43% down)
- **SMAD2** (2% up, 6% down)
- **SMAD3** (2% up, 17% down)
- **SMAD4** (15% up, 20% down)

**PI3K signaling**
- **IGF1R**
- **ERBB2** (6% up, 13% down)
- **ERBB3** (4% up, 20% down)
- **IRS2** (7% up, 3% down)
- **NRAS** (10% up, 10% down)
- **BRAF** (3% up, 7% down)

**RTK/RAS signaling**
- **KRAS** (48% up, 30% down)

**P53 signaling**
- **ATM** (7% up, 37% down)
- **TP53** (47% up, 59% down)

**Protein activation**
- **Transcriptional activation**
- **Complex**

**Transcriptional inhibition**
- **Protein inhibition**

EGFR therapy and KRAS in colorectal cancer

**KRAS wild-type**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Events</th>
<th>N</th>
<th>%</th>
<th>Median (weeks)</th>
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<tbody>
<tr>
<td>Panit + BSC</td>
<td>115</td>
<td>124</td>
<td>93</td>
<td>12.3</td>
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<tr>
<td>BSC alone</td>
<td>114</td>
<td>119</td>
<td>96</td>
<td>7.3</td>
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HR = 0.45  
(95% CI: 0.34 to 0.59)  
Stratified log-rank P < .0001

**KRAS mutant**

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<td>76</td>
<td>84</td>
<td>90</td>
<td>7.4</td>
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<tr>
<td>BSC alone</td>
<td>95</td>
<td>100</td>
<td>95</td>
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HR = 0.59  
(95% CI: 0.73 to 1.36)
RAS mutations in colon cancer

1060 CRC samples

- KRAS wild-type (620)
- KRAS mutant (440)
- NRAS wild-type (512)
- NRAS mutant (108)

FOLFOX vs FOLFOX/panitumumab

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B Overall Survival

<table>
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<th>Subgroup</th>
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<th>Hazard Ratio for Death from Any Cause (95% CI)</th>
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<td>Primary analysis</td>
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<td>Nonmutated KRAS exon 2</td>
<td>656</td>
<td>0.83 (0.67–1.02)</td>
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<td>1.24 (0.98–1.57)</td>
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RAS mutations in colon cancer

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BRAF mutant colon cancer

2. ASCO abstract 3534 (2010)

BRAF mutant colon cancer – BRAF/EGFR vs BRAF/MEK/EGFR
• 19 patients; Phase I/II
• BRAF/MEK/EGFR triplet = 4/6 patients achieved partial responses and 2 pts with stable disease
• BRAF/EGFR doublet = 7/8 achieved SD as the best overall response.
Immune-checkpoint ligands on tumour cells

Response of metastatic colorectal cancer to anti-PD-1 therapy

<table>
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<th>Colon cancer subtypes (n=87)</th>
<th>PD-1 expression (TILs) (%)</th>
<th>PD-L1 (tumour cells) (%)</th>
</tr>
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<tr>
<td>MSS colon cancers (n=60)</td>
<td>39%</td>
<td>13%</td>
</tr>
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<td>MSI-H colon cancers (n=27)</td>
<td>77%</td>
<td>38%</td>
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Molecular stratification of colon cancer

- **KRAS/NRAS/BRAF wild-type**
  - EGFR inhibitors

- **BRAF mutant**
  - BRAF/MEK/EGFR inhibitors

- **KRAS mutant**
  - EGFR/MEK inhibitors
  - IGF1R/MEK inhibitors

- **Microsatellite instability**
  - Anti-PD-1 or PD-L1 mAb

224 colorectal samples
# Genomically driven clinical trials

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<th>Genomic Profile</th>
<th>Strategy</th>
<th>Clinical Development</th>
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<td>KRAS wt anti-EGFR naive</td>
<td>Novel anti-EGFR mAbs</td>
<td>Phase II</td>
</tr>
<tr>
<td>MEK06945A + COX184 versus cetuximab</td>
<td>NCT0162482</td>
<td></td>
</tr>
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<td>Anti-EGFR mAbs + irreversible ERBB TKIs</td>
<td>Phase II</td>
<td></td>
</tr>
<tr>
<td>Cetuximab + afatinib versus cetuximab</td>
<td>NCT01995879</td>
<td></td>
</tr>
<tr>
<td>PI3K pathway inhibitors</td>
<td>Phase II</td>
<td></td>
</tr>
<tr>
<td>Cetuximab + irinotecan versus PF-05212384 + irinotecan</td>
<td>NCT0192574</td>
<td></td>
</tr>
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</table>

| KRAS wt progressing to anti-EGFR mAbs | Novel anti-EGFR mAbs with potent ADCC | Phase II |
| SYM034 | NCT0117478 |
| Anti-EGFR mAbs + MEK inhibitors | Phase II |
| Panitumumab + MEK62 | NCT01927341 |
| KRAS wt HER2 amplified progressing to anti-EGFR mAbs | Dual anti-HER2 therapy | Phase II |
| Trastuzumab + pertuzumab or lapatinib | Heracles trial |
| KRAS wt MET high progressing to anti-EGFR mAbs | Anti-EGFR mAbs + MET inhibitors | Phase II |
| Cetuximab + ARQ197 | NCT0192527 |
| Quadruple negative (KRAS, NRAS, BRAF, PIK3CA) progressing to anti-EGFR mAbs | Anti-EGFR mAbs + irreversible ERBB TKIs | Phase II |
| Cetuximab + neratinib | NCT01960233 |
| KRAS wt | Anti-EGFR mAbs + MEK inhibitors | Phase II |
| Panitumumab + MEK62 | NCT01927341 |
| Novel anti-EGFR/HER2 mAbs + MEK inhibitors | Phase II |
| MEK06945A + cobimetinib | NCT01960666 |
| Anti-EGFR mAbs + MEK inhibitors | Phase II |
| AMG 479 + MEK62 | NCT01602999 |

| KRAS G13D | Anti-EGFR mAbs | Phase II |
| Cetuximab | ICECREAM |

| KRAS wt FcγRIα genotype (CD32) | Anti-EGFR mAbs | Phase II |
| Cetuximab | NCT01450319 |

| BRAF mut (V600) anti-EGFR naive/refractory | BRAF TKIs + anti-EGFR mAbs | Phase II |
| PI3K pathway inhibitors | NCT0179380 |
| LOX18B + cetuximab | BYL719 |
| BRAF TKIs + anti-EGFR mAbs | Phase II |
| PI3K pathway inhibitors | NCT01750598 |
| COBRA-003 + panitumumab | trametinib |

| NRAS mut | MEK inhibitors | Phase II |
| PI3K pathway inhibitors | NCT01632332 |
| NRAS62 + BML20 | |

| PIK3CA mut | PI3K pathway inhibitors | Phase II |
| PFT-05212384 | irinotecan |
| NCT01747866 |

| MSI | Anti-POI mAb | Phase II |
| MK-2475 | NCT01876511 |

Abbreviations: ADCC, antibody-dependent cell mediated cytotoxicity; mAb, monoclonal antibody; MSI, microsatellite instability; mut, mutated; TKI, tyrosine kinase inhibitor; wt, wildtype.
...and more stratification

**Schlicker, 2012**
(n=1600)

- **Subtype 1.1**: Strongly mesenchymal, Ca-signalling
- **Subtype 1.2**: Mesenchymal, MSI, Immune system related
- **Subtype 1.3**: Mesenchymal, MSS, Transporters
- **Subtype 2.1**: Epithelial, Stress response and immune
- **Subtype 2.2**: Epithelial, MSS, Cell cycle and amino acid synthesis

**Vermeulen, 2013**
(n=1164)

- **CCS1**: KRAS/TP53 mutant, chromosomal instability, left-sided
- **CCS2**: MSI, CIMP, right-sided, BRAF mutant
- **CCS3**: Poorly differentiate, MSS, Poor prognosis

**Simon, 2013**
(n=543)

- **A-type**: MMR deficient, Good Prognosis, MSI, BRAF mutant
- **B-type**: MSS, High proliferative index, Poor prognosis, Chemotherapy benefit
- **C-type**: Mesenchymal, Poor prognosis, Chemotherapy resistant

**Delorenzi, 2013**
(n=1113)

- **A Surface crypt-like**: KRAS mutant, papillary or serrated
- **B Lower crypt-like**: Left-sided, Up-regulated Wnt
- **C CIMP-H-like**: Right-sided, MSI, BRAF mutant, High grade
- **D Mesenchymal**: Desmoplastic, Up-regulated EMT
- **E Mixed**: TP53 mutant, Left-sided

**Hanahan, 2013**
(n=1290)

- **Enterocyte**: Transit amplifying MSS, Poor vs Good prognosis subgroups
- **Stem-like**: MSS, Wnt signalling, Poor prognosis
- **Inflammatory**: MSI, Interferon-related genes
- **Goblet-like**: Good prognosis

**Marisa, 2013**
(n=1181)

- **C1**: CIN, Immune down, KRAS mutant, TP53 mutant, Up-regulated Wnt
- **C2**: dMMR, CIMP, BRAF mutant, Serrated, Up Proliferative
- **C3**: KRAS mutant
- **C4**: Stem cell-like, Up-regulated EMT
- **C5**: CIN, Up-regulated Wnt
- **C6**: CIN
A Consensus Molecular Classification

Background:
Recently, a number of independent groups reported novel molecular subtypes in colorectal cancer (CRC).
A formal comparison across these classifiers is needed to reconcile findings and accelerate clinical translation.

Methods:
6 groups (15+ institutions) that analyzed more than 30 patient cohorts with gene expression data, spanning multiple platforms and sample preparation methods,
Each of the 6 classifiers (with 3-6 subtypes) was applied to the collection of public and proprietary datasets,
Encompassing over 4,000 samples, mostly stage II-III CRC.

Results:
Subtype concordance analysis readily yielded a clear consensus on 4 CRC molecular subtypes (CMS1-4) in 84% of samples

Conclusions:
This is the first example of a large-scale, community based comparison of cancer subtypes,
Within the largest collection of CRC samples we identified recurrent signals of 4 biologically distinct subtype classes enriched for key clinical, pathway and molecular traits.
A Consensus Molecular Classification

Presented by: Rodrigo Dienstmann on behalf of the CRC Subtyping Consortium

J Clin Oncol 32:5s, 2014 (suppl; abstr 3511) Colorectal Cancer Subtyping Consortium
<table>
<thead>
<tr>
<th>CMS</th>
<th>%</th>
<th>Subtype</th>
<th>Outcome</th>
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<tr>
<td>CMS1</td>
<td>13%</td>
<td>Females, older age, right colon, MSI, hypermutation, <em>BRAF</em> mut, immune activation</td>
<td>Better RFS, intermediate OS, worse SaR</td>
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<td>CMS2</td>
<td>35%</td>
<td>Left colon, epithelial, MSS, high CIN, <em>TP53</em> mut, WNT/MYC pathway activation</td>
<td>Intermediate RFS, better OS, better SaR</td>
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<td>CMS3</td>
<td>11%</td>
<td>Epithelial, CIN/MSI, <em>KRAS</em> mut, <em>MYC</em> ampl, IGFBP2 overexpression</td>
<td>Intermediate RFS, OS and SaR</td>
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<td>CMS4</td>
<td>20%</td>
<td>Younger age, stage III/IV, mesenchymal, CIN/MSI, TGFβ/VEGF activation, <em>NOTCH3</em> overexpression</td>
<td>Worse RFS, worse OS Intermediate SaR</td>
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<td>Unclassified</td>
<td>21%</td>
<td>Mixed subtype with variable epithelial-mesenchymal activation?</td>
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Tomorrow’s stratification of colon cancer?

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  - EGFR inhibitors

- **BRAF mutant**
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- **KRAS mutant**
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- **Microsatellite instability**
  - Anti-PD-1 or PD-L1 mAb

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**CMS Table**

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<th>RFS/OS/SaR</th>
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Cancer diagnostics: now and then

The light microscope remains the central cancer diagnostic tool for 400 years

Zacharias and Hans Jansen (ca 1595)

Modern microscope (ca 2010)
Cancer diagnostics: now and then

Next-generation sequencing
- Genome
- Transcriptome
- Epigenome

Unified Classification?

Cellular origin
- Morphology
- Differentiation/grading
Conclusions

• Molecular subtypes in colorectal cancer that predict for drug response
• A subset of MSI tumours may respond to PD-1 / PD-L1 inhibitors
• The Sage consensus clusters provide additional stratification ? clinical significance
• Expect these clusters to be built into many future clinical trials
• Many clinical trials now appearing that stratify colorectal cancers for treatment