Personalized Medicine in Colorectal Cancer: Molecular Classifications and Biomarkers

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Overview

**Single marker molecular subtyping**
- KRAS/NRAS
- BRAF
- MSI-H
- HER2 amplification
- Fusions

**RNA-based molecular subtyping**
- Consensus molecular subtypes
- Intrinsic subtyping

**Immune subtyping**
- Immune quantification
- Tumor mutation burden
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MSI-H

**Prevalence:**

<table>
<thead>
<tr>
<th>Stage</th>
<th>MSI-H</th>
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<tbody>
<tr>
<td>II</td>
<td>22%</td>
</tr>
<tr>
<td>III</td>
<td>12%</td>
</tr>
<tr>
<td>IV</td>
<td>3.5%</td>
</tr>
</tbody>
</table>

**Enrichment:**

Right sided, bimodal age distribution

**Recommendation:**

Test all CRC patients of any stage

**Immunohistochemistry**

Complete loss of expression in one of the MMR proteins = MSI-high

**Polymerase Chain Reaction**

Panel of 5 or more microsatellites with allelic shift in 2 (>30%) or more markers = MSI-high

Tejpar et al BJC ‘09; Hall et al ASCO 2016 and GI ASCO 2016; Le Science 2017
Tumor Antigens:

1. Differentiation (melanocyte differentiation antigens…)
2. Overexpressed (HER-2…)
3. Viral (HPV proteins…)
4. Cancer/testis (MAGE, NY-ESO-1…)
5. Mutational (p53…)

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Durability of anti-PD1 +/- anti-CTLA-4 in dMMR

Nivolumab: Nivolumab + Ipiilimumab

12m PFS 77%

12m PFS 48%

N=86, MSI-high Cancers

Approximately 12m PFS 65%

Pembrolizumab (mandatory stop at 2 years)

18 pts (11 with CR and 7 with residual disease)

Median time off tx is 8 months

None have recurred

Mean EQ-5D VAS Score

Population Norm

EQ-5D QUALITY OF LIFE

No at Risk

NIVO 74 48 22 14 12 10 7 3

NIVO + IPI 84 65 35 17 13 8 1 0

Probability of Progression-free Survival

Progression-free Survival (%)
Locally Advanced/Recurrent dMMR CRC: Pathological Complete Response from anti-PD1

CASE 1
- Locally recurrent treated with irinotecan/cetuximab and then capox/panitumumab
- Then pembrolizumab x 4 cycles

CASE 2
- Locally advanced treated with FOLFOX with progression
- Then Nivolumab x 6 cycles
BRAF Mutations

Prevalence:
BRAF V600E : 4-6%
Atypical BRAF : 2%

Enrichment:
Right sided, older age

Recommendation:
Test all mCRC patients

Poor prognosis of BRAF V600E

Jones et al JCO ‘17; Phipps et al Gastroenterology ‘15; Lockhead et al JNCI ’13; Sinicrope ASCO ’14; Tran, et al, Cancer ‘11
BRAF V600E: Impact on Treatment Options

Vemurafenib, Irinotecan, Cetuximab

- Appendiceal Cancer
- Colorectal Cancer
- Prior cetuximab therapy

Binimetinib + Encorafenib + Cetuximab

Kopetz et al GI ASCO '18; Kopetz et al GI ASCO '19
BEACON Phase 3: Study Design

Results of Safety Lead-In led to the introduction of an additional primary endpoint of ORR and an interim OS analysis to allow for early assessment.

Patients with \(BRAF^{V600E}\) mCRC with disease progression after 1 or 2 prior regimens; ECOG PS of 0 or 1; and no prior treatment with any RAF inhibitor, MEK inhibitor, or EGFR inhibitor.

Safety Lead-in
- Encorafenib 300 mg PO daily
- Binimetinib 45 mg PO bid
- Cetuximab standard weekly dosing

Encorafenib 300 mg PO daily
Binimetinib 45 mg PO bid
Cetuximab standard weekly dosing

A separate Safety Lead-in cohort of \(n=7\) in Japan was enrolled subsequently. Results will be reported at a later time.

Phase 3

R 1:1:1

**Primary Endpoints:**
- OS
- ORR (Blinded Central Review)

**Triplet vs Control**
- Triplet therapy
  - ENCO + BINI + CETUX
  - \(n = 205\)

- Doublet therapy
  - ENCO + CETUX
  - \(n = 205\)

- Control arm
  - FOLFIRI + CETUX, or
  - irinotecan + CETUX
  - \(n = 205\)

Secondary Endpoints: Doublet vs Control OS & ORR, PFS, Safety

Randomization was stratified by ECOG PS (0 vs. 1), prior use of irinotecan (yes vs. no), and cetuximab source (US-licensed vs. EU-approved).
Primary Endpoint - Overall Survival: Triplet vs Control (all randomized patients)

Median OS in months (95% CI)

- **Triplet**: 9.0 (8.0–11.4)
- **Control**: 5.4 (4.8–6.6)

HR (95% CI): 0.52 (0.39–0.70)

2-sided \(P<0.0001\)
### Prevalence of Non-V600E BRAF mutations in CRC

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>MDA</th>
<th>FM</th>
<th>Totals</th>
<th>All BRAF mut %</th>
<th>% of all BRAF mut which are non-V600</th>
<th>% of total CRC which are non-V600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CRC Cases</td>
<td>1014</td>
<td>2276</td>
<td>6353</td>
<td>9643</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total BRAF Mutations</td>
<td>137</td>
<td>334</td>
<td>469</td>
<td>940</td>
<td>1147/9643 11.9%</td>
<td>207/940 22%</td>
<td>207/9643 2.1%</td>
</tr>
<tr>
<td>Non-V600 BRAF</td>
<td>27</td>
<td>54</td>
<td>126</td>
<td>207</td>
<td></td>
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</tr>
</tbody>
</table>

Jones et al., JCO ‘16
Atypical (Non-V600E) BRAF mutations

Prognosis is similar to BRAF wild-type

Recently identified as acquired alterations in post-EGFR inhibitor treated tumors


Johnson et al JCO PO ‘19
## Understanding Class II and Class III Non-V600E $BRAF^{\text{mut}}$

<table>
<thead>
<tr>
<th></th>
<th>Class I (BRAF V600E)</th>
<th>Class II BRAF</th>
<th>Class III BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td>BRAF monomer</td>
<td>BRAF dimers</td>
<td>BRAF/CRAF dimers</td>
</tr>
<tr>
<td><strong>RTK (EGFR) Dependency</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Kinase activity</strong></td>
<td>High</td>
<td>High/Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td><strong>EGFRi sensitivity</strong></td>
<td>No</td>
<td>Unlikely</td>
<td>Likely</td>
</tr>
<tr>
<td><strong>Potential Strategy</strong></td>
<td>BRAF, MEK, EGFR</td>
<td>RAF dimer inhibitors</td>
<td>RTK, MAPK combinations</td>
</tr>
</tbody>
</table>

**Diagram:**

1. **Class I (BRAF V600E):**
   - EGFR → KRAS → BRAF
   - BRAF → MEK → ERK

2. **Class II BRAF:**
   - EGFR → KRAS → BRAF
   - BRAF → MEK → ERK

3. **Class III BRAF:**
   - EGFR → KRAS → BRAF → CRAF
   - CAF → MEK → ERK

*Yao et al Nature '17*
HER2 Amplification

**Prevalence:** 2-4%

**Enrichment:**
RAS/BRAF wild-type patients

**Recommendation:**
Consider testing all mCRC patients

*Not yet universally recommended on biomarker guidelines

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**Immunohistochemistry** (Reflex ISH)

**NGS Panels**

High concordance between NGS testing and IHC/FISH results

*ctDNA testing can reliably detect and quantify amplifications*

(Raghav et al GI ASCO Poster #604)

Marx et al. Human Path ‘10; Siena et al GI ASCO ‘14
HER2 Amplifications: Potential predictive information

EGFR Inhibition

Trastuzumab + Pertuzumab

SWOG 1613

Arm 1
Trastuzumab + Pertuzumab

HER2 Amp
2nd, 3rd Line
RAS/RAF WT

Arm 2
Cetuximab + Irinotecan

Arm 3
Trastuzumab + Pertuzumab

After Progression

Raghav et al JCO PO, ‘18; Hurwitz GI ASCO ’17; Raghav, Fakih PI’s NCT03365882
KRAS/NRAS testing: Barriers in dissemination of best-practices
Codons 12, 13, 59, 61, 117, 146

Low rate of initial biomarker testing

Flat Iron Health: 13,437 patients with mCRC from 2013 to 2017, testing with 1st line therapy

- KRAS: 31% Tested, 69% Not tested
- NRAS: 10% Tested, 90% Not tested

Median time to obtain testing results: 26 days

Need for education/awareness

The best biomarker is one that is actually tested

Florea et al GI ASCO ‘18
Atypical KRAS and NRAS: What to do With the Rare Variant?

- Several notable atypical RAS mt with high activity included KRAS V14I, Q22K, D33E, N116S, and F156L (all >165% of WT activity).

- Conversely, within the typical mutations, KRAS G13C and K117R were not shown to increase activity above WT.
  - (However, these two mutations are very rare)

Direct targeting of KRAS: G12C inhibitors entering clinic

Inhibitors bind to the P2 pocket of KRAS adjacent to the mutant cysteine.

The inhibitor covalently modifies the cysteine residue.

Results in KRAS$^{G12C}$ locked in an inactive, GDP-bound conformation.

Distribution of KRAS$^{G12C}$

GDP, guanosine diphosphate

Unpublished data
AMG510 in CRC and other solid tumours

CRC, colorectal cancer; MTD, maximum tolerated dose; PD, progression of disease; PFS, progression-free survival; RR, response rate; SD, stable disease.

Fusions

**Prevalence:** <1% collectively

**Enrichment:**

MSI-H; low rates of APC, TP53, KRAS mutations

**Recommendation:**

*Consider* testing all refractory mCRC patients, especially MSI-H

*Not yet universally recommended on biomarker guidelines*
Larotrectinib FDA Approved for TRK Fusion, Including CRC


*Patient had TRK solvent front resistance mutation (NTRK3 G623R) at baseline due to prior therapy; Pathologic CR
Note: One patient not shown here. Patient experienced clinical progression and no post-baseline tumor measurements were recorded.
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- Tumor mutation burden
How do CRC differ by gene expression? Are there similarities in biology?

Each dot is one patient.

- **MSI/CIMP (30%):** BRAFm, hypermutated
- **CIN (30%):** Immune down, cell signaling, ECM and focal adhesion pathways up
- **Invasive (40%):**
  - CIN immune down (20%)
  - KRASm, CIMP+ (5%)
  - Poor prognosis
  - BRAFm, KRASm, CIMP+, CIN
- **CSC (10%):**
  - Malignant (7%)
  - EMT, CSC high
  - Poor prognosis
  - CIN

**TCGA**
- T:220
- 54 genes

**Swiss**
- T:445 V:774
- 30 genes

**PETACC3**
- T:113 V:720
- 54 genes

**AMC-AJCCII-90**
- T:50 V:1074
- 146 genes

**French**
- T:443 V:1058
- 57 genes

**Agenda**
- T:668 V:343
- 32/53/102 genes

**Melbourne**
- T:200 V:443
- 128 genes

**Good prognosis (40%):**
- Poor prognosis (60%): immune down, cell signaling, ECM and focal adhesion pathways up

Guinney et al, Nat Med ‘15
Philosophy: Consensus is required in order to move the field forward and transition to clinical application.
## Key Features of the CMS Subtypes

<table>
<thead>
<tr>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSI Immune</strong></td>
<td><strong>Canonical</strong></td>
<td><strong>Metabolic</strong></td>
<td><strong>Mesenchymal</strong></td>
</tr>
<tr>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
</tbody>
</table>

- **CMS1 (MSI Immune)**: MSI, CIMP high, Hypermethylation
- **CMS2 (Canonical)**: SCNA high
- **CMS3 (Metabolic)**: Mixed MSI status, SCNA low, CIMP low
- **CMS4 (Mesenchymal)**: SCN high

<table>
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<tr>
<td><strong>BRAF mutations</strong></td>
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<tr>
<td>Immune infiltration and activation</td>
<td></td>
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<tr>
<td>Worse survival after relapse</td>
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<td><strong>KRAS mutations</strong></td>
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<tr>
<td>WNT and MYC activation</td>
<td></td>
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<tr>
<td>Better survival after relapse</td>
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<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic deregulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stromal infiltration TGF beta activation Angiogenesis</td>
<td></td>
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<tr>
<td>Worse relapse-free and overall survival</td>
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</table>
CMS: Consistent Prognostic Information in mCRC

Despite being designed agnostic to outcomes, strong prognostic information.

Median overall survival: Differs from 15 months (CMS1) to 40 months (CMS2)

Progression-free survival: Differs from 5.7 months (CMS1) to 14.1 months (CMS2)
CMS Varies by Tumor Location

Further integrated analyses are needed to understand contributions of CMS and sidedness to prognosis, but appears to be independent information
CMS2/3 may Benefit from Addition of Bevacizumab

AGITG MAX Trial
Mesenchymal CMS4: Limited Benefit with Oxaliplatin?

C-07 study of FLOX vs FULV

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>No. of Patients</th>
<th>HR (95% CI)</th>
<th>Favors oxaliplatin plus fluorouracil-leucovorin</th>
<th>Favors fluorouracil plus leucovorin</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMS1</td>
<td>231</td>
<td>0.77 (0.46-1.29)</td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>CMS2</td>
<td>382</td>
<td>0.61 (0.43-0.87)</td>
<td></td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>CMS3</td>
<td>86</td>
<td>1.17 (0.54-2.53)</td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>CMS4</td>
<td>334</td>
<td>0.87 (0.64-1.19)</td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
</tbody>
</table>

Are there other subgroups or oxali-specific signatures that would perform better?

Song et al JAMA Onc ‘17
Differential Sensitivity to Oxaliplatin: Preclinical Data

Mouse co-clinical trial

Needs validation, but limited retrospective specimens available.

Linnekamp et al, Cell Death & Differentiation '18
CMS Strengths: Insights into Biologic / Immune Context

**CMS1: Immunogenic Tumors**
Infiltrating activated lymphocytes

**CMS2/3: Immune Desert**
No evidence of immune activation

**CMS4: Immune Excluded**
Immune system is engaged, but microenvironment prevents activity

Becht et al CCR ‘16
**CMS Strengths:** Insights into Biologic / Immune Context

**CMS1: Immunogenic Tumors**
- Infiltrating activated lymphocytes

**CMS2/3: Immune Desert**
- No evidence of immune activation

**CMS4: Immune Excluded**
- Immune system is engaged, but microenvironment prevents activity

CMS4 has a moderate cytotoxic T-cell infiltrate, but high myeloid, TGF-β signaling.
Molecular Subtypes in Premalignancy

Absence of CMS4 / Mesenchymal

Chang et al Annals Onc ’18, Dienstmann R. et al, Nat Rev Cancer ’17
Molecular Subtype Tools: PDXs and Cell Lines

Annotated models now available to support preclinical research

Linnekkamp et al, Cell Death & Differentiation ‘18
Example: Screens for subtype-specific vulnerabilities

- Topoisomerase inhibitors
- Heat-shock proteins

![Graph showing P value (log10) for CMS2 vs. CMS4 with circles highlighting EGFR and HER2 inhibitors](image)

Sveen et al CCR '18, Del Rio M Eur J Cancer '17, Sadanandam Nat Med '13
What is needed to move RNA classifiers into the clinic?

Clinical utility

- Validation of findings across multiple retrospective cohorts
- Integration into prospective studies

Clinical-grade, parsimonious assay

- To date, there is no broadly available CLIA assay
- MDACC and other academic labs have established FFPE-robust classifiers

Classifier robust to real-world sampling

- Works on small tissue and biopsies from metastatic sites
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Immunoscore and MSI/MSS subgroups

Stage II/III

High CD3+  Low CD3+

Stage IV, hepatectomy

Pagès et al Lancet May 2018; Wnag et al Cancer Immun ‘17
Tumor mutation burden as a molecular classification

QUASAR 2

CALGB/SWOG 80405: MSS

The data to date are only (modestly) prognostic, which limits the potential clinical utility. These will not be routinely utilized unless predictive applications can be identified.

Innocenti et al ASCO ‘17; Domingo et al Lancet G&H ‘18
Conclusions

- Molecular subtyping is a key mechanism to improve patient outcomes.
- Current molecular subtypes with clinical activity:
  - BRAF \( V600E \) mutation: Dual EGFR and BRAF inhibition (± MEK)
  - HER2 amplification: Trastuzumab with Lapatinib or Pertuzumab
  - NTRK fusions: Larotrectinib
  - MSI-H: Nivolumab/Ipilimumab, Pembrolizumab
- Future precision therapies may incorporate RNA-based classification
- We shouldn’t be discouraged by lack of immediate clinical applications
- Education and dissemination of existing best practices is critical!
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