Implementing Molecular Testing Guidelines for Colorectal Cancer

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PLEASE TURN OFF YOUR CELL PHONES
Colorectal Cancer (CRC): Scope of the problem

• Near 135K in 2016 in the US
• Overall 5 year survival 65%
• Half of CRC develop distant metastases a major unfavorable factor in survival
• New therapy regimens improved median survival from 6-7 to 24-30 months in patients with mCRC
Role of Molecular Pathology in Colorectal Cancer

• Testing CRC tissues
  • Predictive mutation biomarkers for targeted therapies
  • Prognostic molecular biomarkers for therapy decisions
  • Detection of HNPCC
• When and how to test? Need for guidelines
Molecular Biomarkers for the Evaluation of Colorectal Cancer

The CAP, ASCP, AMP, and ASCO convened an expert panel to systematically review published documents and develop an evidence-based guideline to:

- Establish evidence-based recommendations for the molecular testing of CRC tissues to guide targeted therapies and conventional chemotherapy regimens
- Summarize emerging molecular testing approaches for CRC and provide insight on needed studies
CRC Guideline Expert Panel

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Stanley Hamilton, MD, PhD – CAP
Carmen J. Allegra, MD – ASCO

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CRC Molecular Testing Guidelines
Approach

• Systematic literature search: 4,497 abstracts from Jan 1, 2008 through Aug 1, 2013 and literature refresh to Feb 12, 2015
• Full-text review of 866 and data extraction from 123 articles
• Over 70 systematic reviews and meta-analyses analyzed
• Addressed key questions and provided guideline recommendations
• Open comment period April 2015
Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

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2017
American Journal of Clinical Pathology
Journal of Molecular Diagnostics
Archives of Pathology and Laboratory Medicine
Journal of Clinical Oncology
Results:
Twenty-one guideline statements were established.

Conclusions:
- Evidence supports mutational testing for EGFR signaling pathway genes, since they provide clinically actionable information as negative predictors of benefit to anti-EGFR monoclonal antibody therapies for targeted therapy of CRC.
- Mutations in several of the biomarkers have clear prognostic value.
- Laboratory approaches to operationalize CRC molecular testing are presented.

2017, Sepulveda AR et al,
American Journal of Clinical Pathology
Journal of Molecular Diagnostics
Archives of Pathology and Laboratory Medicine
Journal of Clinical Oncology
<table>
<thead>
<tr>
<th>Guideline Statement</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patients with colorectal carcinoma being considered for anti-EGFR therapy must receive RAS mutational testing. Mutational analysis should include KRAS and NRAS codons 12 and 13 of exon 2, 59 and 61 of exon 3, and 117 and 146 of exon 4 (“expanded” or “extended” RAS).</td>
<td>Recommendation</td>
</tr>
<tr>
<td>2a. BRAF p.V600 (BRAF c.1799 p.V600) mutational analysis should be performed in colorectal cancer tissue in patients with colorectal carcinoma for prognostic stratification.</td>
<td>Recommendation</td>
</tr>
<tr>
<td>2b. BRAF p.V600 mutational analysis should be performed in deficient MMR tumors with loss of MLH1 to evaluate for Lynch syndrome risk. Presence of a BRAF mutation strongly favors a sporadic pathogenesis. The absence of a BRAF mutation does not exclude risk of Lynch syndrome.</td>
<td>Recommendation</td>
</tr>
<tr>
<td>3. Clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high risk for Lynch syndrome and/or prognostic stratification.</td>
<td>Recommendation</td>
</tr>
<tr>
<td>4. There is insufficient evidence to recommend BRAF c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-EGFR inhibitors.</td>
<td>No recommendation</td>
</tr>
<tr>
<td>5. There is insufficient evidence to recommend PIK3CA mutational analysis of colorectal carcinoma tissue for therapy selection outside of a clinical trial. Note: Retrospective studies have suggested improved survival with postoperative aspirin use in patients whose colorectal carcinoma harbors a PIK3CA mutation.</td>
<td>No recommendation</td>
</tr>
<tr>
<td>6. There is insufficient evidence to recommend PTEN analysis (expression by immunohistochemistry or deletion by fluorescence in situ hybridization) in colorectal carcinoma tissue for patients who are being considered for therapy selection outside of a clinical trial.</td>
<td>No recommendation</td>
</tr>
<tr>
<td>7. Metastatic or recurrent colorectal carcinoma tissues are the preferred specimens for treatment predictive biomarker testing and should be used if such specimens are available and adequate. In their absence, primary tumor tissue is an acceptable alternative and should be used.</td>
<td>Expert consensus opinion</td>
</tr>
</tbody>
</table>
CRC Guideline Statements (1)

• Recommendation 1
  • *RAS* mutational testing of colorectal carcinoma tissue must be performed for patients who are being considered for anti-EGFR therapy
  • Mutational analysis must include *KRAS* and *NRAS*
  • Codons 12, 13 of exon 2
  • Codons 59, 61 of exon 3
  • Codons 117 and 146 of exon 4
   ("expanded" or "extended" *RAS*)

• No Recommendation (4, 5, 6)
  • There is insufficient evidence to recommend *BRAF V600, PIK3CA*, mutational status and *PTEN IHC* as predictive molecular biomarkers for response to anti-EGFR inhibitors
All RAS Mutant CRC:

**KRAS** exon 2 c12 & c13 mutations and extended RAS mutations

Sorich MJ et al. 2014
# Prevalence of new RAS mutations across studies

<table>
<thead>
<tr>
<th>Study</th>
<th>New RAS Total</th>
<th>KRAS Exon 3</th>
<th>KRAS Exon 4</th>
<th>NRAS Exon 2</th>
<th>NRAS Exon 3</th>
<th>NRAS Exon 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPUS</td>
<td>26.3%</td>
<td>5.9%</td>
<td>117%</td>
<td>6.8%</td>
<td>5.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>PICCOLO</td>
<td>9.8%</td>
<td>NRb</td>
<td>13%</td>
<td>6.3%d</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>20020408</td>
<td>17.6%</td>
<td>4.8%b</td>
<td>9.3%</td>
<td>4.2%</td>
<td>3.0%</td>
<td>1.1%</td>
</tr>
<tr>
<td>20050181</td>
<td>20.5%</td>
<td>4.6%</td>
<td>7.9%</td>
<td>2.3%</td>
<td>5.8%</td>
<td>0.0%</td>
</tr>
<tr>
<td>PRIME</td>
<td>17.4%</td>
<td>3.7%b</td>
<td>5.6%</td>
<td>3.4%</td>
<td>4.1%b</td>
<td>0.0%</td>
</tr>
<tr>
<td>FIRE-3</td>
<td>16.0%</td>
<td>4.3%b</td>
<td>7.7%</td>
<td>3.8%</td>
<td>2.0%b</td>
<td>0.0%</td>
</tr>
<tr>
<td>PEAK</td>
<td>20.1%</td>
<td>4.1%</td>
<td>NE</td>
<td>5.4%</td>
<td>5.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>COIN</td>
<td>8.4%</td>
<td>2.1%b</td>
<td>5.6%</td>
<td>0.9%</td>
<td>3.0%</td>
<td>0.9%</td>
</tr>
<tr>
<td>CRYSTAL</td>
<td>14.7%</td>
<td>3.3%</td>
<td></td>
<td>3.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUMMARYf</td>
<td>19.9% (16.7%, 23.4%)</td>
<td>4.3% (3.3%, 5.5%)</td>
<td>6.7% (5.7%, 7.9%)</td>
<td>3.8% (3.0%, 4.8%)</td>
<td>4.8% (3.4%, 6.8%)</td>
<td>0.5% (0.2%, 1.2%)</td>
</tr>
</tbody>
</table>

\*a: proportion of the KRAS exon 2 wild-type group

Sorich MJ et al. 2014
CRC Guideline Statements (2)

- Recommendation 2a. *BRAF* c.1799 pV600 mutational analysis should be performed in CRC tissue in patients with CRC for prognostic stratification.
- Recommendation 2b. *BRAF* c.1799 pV600 mutational analysis should be performed in deficient DNA MMR tumors with loss of MLH1 to evaluate for Lynch syndrome risk.
  - Presence of *BRAF* mutation strongly favors a sporadic pathogenesis
  - Absence of *BRAF* mutation does not exclude risk of Lynch syndrome
- Recommendation 3. DNA mismatch repair status testing should be ordered in patients with CRC for:
  - Identification of patients at high risk of Lynch syndrome
  - And/or for prognostic stratification.
**BRAF and dMMR/MSI: Prognostic and Predictive Markers for Stage II/III CRC**

**A**

Prognostic: **BRAF** mut shorter survival time at recurrence

![Graph showing survival probability vs. SAR time (years)]

- No. at risk
  - **BRAFwt**: 510, 230, 93, 43, 8, 1
  - **BRAFmut**: 92, 13, 5, 3, 2

**B**

Prognostic: dMMR/MSI better OS survival (**BRAF wt**)

![Graph showing survival probability vs. OS time (years)]

- No. at risk
  - MMR-p, **BRAFwt**: 1,358, 1,271, 1,126, 830, 626, 100
  - MMR-d, **BRAFwt**: 130, 123, 114, 88, 67, 15
  - MMR-p, **BRAFmut**: 176, 145, 121, 93, 76, 11
  - MMR-d, **BRAFmut**: 71, 61, 57, 45, 35

CRC Guideline Statements (3): Specimens & Assays

Expert consensus opinion (ECO)

• 7. ECO: Metastatic or recurrent CRC tissues are the preferred specimens for treatment predictive biomarker testing... In their absence, primary tumor tissue is an acceptable alternative...

• 8. ECO: FFPE tissue is an acceptable specimen for ...mutational testing in CRC...other specimens (eg, cytology specimens) will require ... validation...

• 17. ECO: Pathologists must evaluate candidate specimens for biomarker testing to ensure specimen adequacy, taking into account tissue quality, quantity, and malignant tumor cell fraction. Specimen adequacy findings should be documented in the patient report.
9, 10, 11, 12. **Strong recommendations**: Labs must use validated...testing methods with sufficient performance characteristics for the intended clinical use.

...Biomarker testing ... must be validated in accordance with best lab practices.

Laboratories must provide clinically appropriate TATs and optimal utilization of tissue specimens by using appropriate techniques (e.g. multiplexed assays)... 

18. **ECO**: ...Use testing methods that detect mutations in specimens with at least 5% mutant allele frequency, taking into account the analytical sensitivity of the assay... and tumor enrichment (e.g. microdissection). ...Minimal neoplastic carcinoma cell content... at least 2x the assay LOD.
CRC Guideline Statements (4): Operational Aspects

• 13. ECO: Biomarker testing … should be initiated in a timely fashion based on the clinical scenario and in accordance with institutionally accepted practices. Note: Test ordering can occur on a case-by-case basis or by policies established by the medical staff.

• 14. ECO: Laboratories should establish policies to ensure efficient allocation and utilization of tissue for molecular testing, particularly in small specimens.

• 15. ECO: Members of the patient’s medical team, including pathologists, may initiate colorectal carcinoma molecular biomarker test orders in accordance with institutionally accepted practices.
CRC Guideline Statements (5): Operational TATs

Expert Consensus Opinion

• 16. ECO: …It is suggested that a benchmark of 90% of specimens should be sent out within 3 working days.

• 19. ECO: …It is suggested that a benchmark of 90% of reports be available within 10 working days from date of receipt in the molecular diagnostics laboratory.

• 20. ECO: … reports should include results & interpretation sections readily understandable by oncologists and pathologists. Appropriate … nomenclature.

• 21. ECO: Labs must incorporate CRC molecular biomarker testing methods into their…quality improvement program…must participate in formal proficiency testing programs, if available, or an alternative…
CRC emerging molecular biomarkers

- MSI/MMR status may have predictive value in patients with advanced CRC being considered for anti-PD-1/PD-L1 immune checkpoint inhibitor therapy
  - DNA MMR status tested by MSI DNA test
  - Pembrolizumab IV
  - 82% had HNPCC germline detected


| Table 2. Objective Responses According to RECIST Criteria. |
|---------------------------------|-----------------|-----------------|-----------------|
| Type of Response                | Mismatch Repair–Deficient Colorectal Cancer (N=10) | Mismatch Repair–Proficient Colorectal Cancer (N=18) | Mismatch Repair–Deficient Noncolorectal Cancer (N=7) |
| Complete response — no. (%)    | 0               | 0               | 1 (14)*         |
| Partial response — no. (%)     | 4 (40)          | 0               | 4 (57)†         |
| Stable disease at week 12 — no. (%) | 5 (50)       | 2 (11)          | 0               |
| Progressive disease — no. (%)  | 1 (10)          | 11 (61)         | 2 (29)          |
| Could not be evaluated — no. (%)‡ | 0              | 5 (28)          | 0               |
| Objective response rate (95% CI — %) | 40 (12–74)   | 0 (0–19)        | 71 (29–96)      |
| Disease control rate (95% CI — %§) | 90 (55–100)  | 11 (1–35)       | 71 (29–96)      |
| Median duration of response — wk | Not reached     | NA¶             | Not reached     |
| Median time to response (range) — wk | 28 (13–35) | NA¶             | 12 (10–13)     |
Considerations for implementation of CRC molecular testing guidelines

- **Setting:** Academic
- **Biomarker panel for CRC:**
  - IHC for DNA mismatch repair proteins (MLH1, MSH2, PMS2, MSH6).
  - MSI test should be ordered for all cases showing retained/preserved expression of all MMR proteins.
  - Molecular testing for KRAS and NRAS (codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 (“expanded” or “extended” RAS, BRAF, PIK3CA).
  - All colorectal biopsies with diagnosis of adenocarcinoma (including at least intramucosal).
  - If enough tissue with tumor is available order CRC panel “reflex” by pathologist/standard order per clinical team
  - Perform testing on colorectal resection specimen/or metastatic specimen if not done in a prior biopsy
  - Invasive colorectal cancer specimen (pN+, pM+ or pT3 or pT4 cases); Clinical stage 2 or greater. Test metastatic tissue if available.
- **Assay:** Next Generation Sequencing Panel
- **Report:** IHC for DNA MMR in diagnostic pathology report; NGS panel and MSI test: Molecular pathology report
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