Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Colon and Rectum

Version: 1.3.0.0
Protocol Posting Date: June 2021

This biomarker template is not required for accreditation purposes but may be used to facilitate compliance with CAP Accreditation Program Requirements

Authors
Lawrence J. Burgart, MD*; William V. Chopp, MD*; Dhanpat Jain, MD*; Andrew M. Bellizzi, MD*; Patrick L. Fitzgibbons, MD, FCAP*.

With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

* Denotes primary author.

Accreditation Requirements
Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team. This template is not required for accreditation purposes.

Summary of Changes

v 1.3.0.0

- General Reformatting
- Addition of HER2 Reporting
CASE SUMMARY: (Colon and Rectum Biomarker Reporting Template)
Includes elements from the 2016 HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. Completion of the template is the responsibility of the laboratory performing the biomarker testing and / or providing the interpretation. When both testing and interpretation are performed elsewhere (e.g., a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.
Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report.
Gene names should follow recommendations of The Human Genome Organization (HUGO) Nomenclature Committee (www.genenames.org).
All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/rec).

RESULTS
Mismatch Repair (Note A)
+Immunohistochemistry (IHC) Testing for Mismatch Repair (MMR) Proteins (select all that apply)
___ MLH1
+MLH1 Result
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): ___________________
___ MSH2
+MSH2 Result
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): ___________________
___ MSH6
+MSH6 Result
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): ___________________
___ PMS2
+PMS2 Result
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): ___________________
___ Background nonneoplastic tissue / internal control with intact nuclear expression

+IHC Interpretation#
___ No loss of nuclear expression of MMR proteins: low probability of MSI-H
___ Loss of nuclear expression of MLH1 and PMS2: testing for methylation of the MLH1 promoter and / or mutation of BRAF is indicated (the presence of a BRAF V600E mutation and / or MLH1 methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both MLH1 methylation and of BRAF V600E mutation suggests the possibility of Lynch
syndrome and sequencing and / or large deletion / duplication testing of germline MLH1 may be indicated)

___ Loss of nuclear expression of MSH2 and MSH6: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline MSH2 may be indicated and, if negative, sequencing and / or large deletion / duplication testing of germline MSH6 may be indicated)

___ Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline MSH6 may be indicated)

___ Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline PMS2 may be indicated)

# There are exceptions to the above IHC interpretations. These results should not be considered in isolation, and clinical correlation with genetic counseling is recommended to assess the need for germline testing.

+Microsatellite Instability (MSI)

___ MSI-Stable (MSS)

___ MSI-Low (MSI-L)

___ 1-29% of the National Cancer Institute (NCI) or mononucleotide markers exhibit instability

___ 1 of the NCI or mononucleotide markers exhibits instability

___ Other (specify): _________________

___ MSI-High (MSI-H)

___ Greater than equal to 30% of the NCI or mononucleotide markers exhibit instability

___ 2 or more of the NCI or mononucleotide markers exhibit instability

___ Other (specify): _________________

___ MSI-Cannot be determined: _________________

+Loci Testing (select all that apply)

___ Mononucleotide Panel

+BAT-25

___ Stable

___ Unstable

___ Cannot be determined (explain): _________________

___ Not performed

+BAT-26

___ Stable

___ Unstable

___ Cannot be determined (explain): _________________

___ Not performed

+NR-21

___ Stable

___ Unstable

___ Cannot be determined (explain): _________________

___ Not performed

+NR-24

___ Stable

___ Unstable

___ Cannot be determined (explain): _________________

___ Not performed

+Mono-27

___ Stable
<table>
<thead>
<tr>
<th></th>
<th>Stable</th>
<th>Unstable</th>
<th>Cannot be determined (explain):</th>
<th>Not performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI Panel</td>
<td>+BAT-25</td>
<td>+BAT-26</td>
<td>+D2S123</td>
<td>+D5S346</td>
</tr>
<tr>
<td></td>
<td>+D17S250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>+Specify Marker:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other Markers Tested (repeat as needed)

MLH1 Promoter Methylation (Note B)

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
<th>Cannot be determined (explain):</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1 Promoter Methylation Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KRAS (Note C)

<table>
<thead>
<tr>
<th></th>
<th>Detected</th>
<th>Identified</th>
<th>Cannot be determined (explain):</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS Mutational Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codon 12</td>
<td>Gly12Asp</td>
<td>Gly12Val</td>
<td></td>
</tr>
</tbody>
</table>
Gly12Cys (GGT>TGT)
Gly12Ser (GGT>AGT)
Gly12Ala (GGT>GCT)
Gly12Arg (GGT>CGT)
Codon 12 mutation, not otherwise specified
Other codon 12 mutation (specify): 

Codon 13
Gly13Asp (GGC>GAC)
Gly13Arg (GGC>CGC)
Gly13Cys (GGC>TGC)
Gly13Ala (GGC>GCC)
Gly13Val (GGC>GTC)
Codon 13 mutation, not otherwise specified
Other codon 13 mutation (specify): 

Codon 61
Gln61Leu (CAA>CTA)
Gln61His (CAA>CAC)
Codon 61 mutation, not otherwise specified
Other codon 61 mutation (specify): 

Codon 146
Ala146Thr (G436A) (GCA>ACA)
Codon 146 mutation, not otherwise specified
Other codon 146 mutation (specify): 

Other Codon (specify): 
Cannot be determined (explain): 

Codons Assessed (select all that apply)
12
13
61
146
Other (specify): 

NRAS (Note C)
+NRAS Mutational Analysis
No mutation detected
Mutation(s) identified
Codon 12
Gly12Asp (GGT>GAT)
Gly12Val (GGT>GTT)
Gly12Cys (GGT>TGT)
Gly12Ser (GGT>AGT)
Gly12Ala (GGT>GCT)
Gly12Arg (GGT>CGT)
Codon 12 mutation, not otherwise specified
Other codon 12 mutation (specify): 

Codon 13
Gly13Asp (GGC>GAC)
Gly13Arg (GGC>CGC)
Gly13Cys (GGC>TGC)
___ Gly13Ala (GGC>GCC)
___ Gly13Val (GGC>GTC)
___ Codon 13 mutation, not otherwise specified
___ Other codon 13 mutation (specify): _________________

+Codon 61
___ Gln61Lys (CAA>AAA)
___ Gln61Arg (CAA>CGA)
___ Codon 61 mutation, not otherwise specified
___ Other codon 61 mutation (specify): _________________

+Codon 146
___ Ala146Thr (G436A) (GCA>ACA)
___ Codon 146 mutation, not otherwise specified
___ Other codon 146 mutation (specify): _________________

+Other Codon (specify): _________________
___ Cannot be determined (explain): _________________

+Codons Assessed (select all that apply)
___ 12
___ 13
___ 61
___ 146
___ Other (specify): _________________

BRAF (Note B)
+BRAF Cytoplasmic Expression (by immunohistochemistry) (Note B)
___ Positive
___ Negative
___ Cannot be determined (explain): _________________

+BRAF Mutational Analysis
___ No mutations detected
___ BRAF V600E (c.1799 T>A) mutation
___ Other BRAF mutation identified (specify): _________________
___ Cannot be determined (explain): _________________

+BRAF Mutations Assessed (select all that apply)
___ V600E
___ Other BRAF V600 mutation (specify): _________________
___ Other (specify): _________________

PIK3CA (Note D)
+PIK3CA Mutational Analysis
___ No mutations detected
___ Exon 9 mutation present (specify): _________________
___ Exon 20 mutation present (specify): _________________
___ Cannot be determined (explain): _________________

PTEN (Note E)
+PTEN Expression (by immunohistochemistry)
___ Positive cytoplasmic and / or nuclear expression
___ Negative for cytoplasmic and nuclear expression
___ Cannot be determined (explain): ________________

**PTEN Mutational Analysis**
___ No mutations detected
___ Exon 1-9 mutation present (specify): ______________
___ Cannot be determined (explain): ________________

**Multiparameter Gene Expression / Protein Expression Assay**
___ Low risk
___ Moderate risk
___ High risk
___ Cannot be determined (explain): ________________

**HER2 (Note F)**
___ HER2 (by immunohistochemistry)

**HER2 by IHC**
___ Negative (Score 0)
___ Negative (Score 1+)
___ Equivocal (Score 2+)
___ Positive (Score 3+)
___ Cannot be determined (explain): ________________

**Scoring System**
___ ASCO / CAP HER2 Breast Cancer 2018
___ CAP / ASCP / ASCO HER2 Gastroesophageal Adenocarcinoma 2016 (aka Ventana)
___ HERACLES
___ Other (specify): ______________

**Staining Intensity**
___ 0 (none)
___ 1+ (faint or barely perceptible)
___ 2+ (weak to moderate)
___ 3+ (strong)
___ Other (specify): ______________

**Percentage of Tumor Cells with Specific Membrane Staining (i.e., complete, basolateral, or lateral membrane)**
___ Less than 10%
___ 10-49%
___ Greater than or equal to 50%
___ Specify percentage: ______________%

___ HER2 (ERBB2) (by in situ hybridization)

**HER2 (ERBB2) by ISH**
___ Negative (not amplified)
___ Positive (amplified)
___ Cannot be determined (explain): ________________

**Scoring System**
___ ASCO / CAP HER2 Breast Cancer 2018
___CAP/ASCP/ASCO HER2 Gastroesophageal Adenocarcinoma 2016 (aka Ventana)
___HERACLES
___Other (specify): _________________

+Number of Invasive Cancer Cells Counted: _________________ cells

+Method (select all that apply)
___Using dual-probe assay
+HER2 (ERBB2):CEP17 Ratio: _________________

+Average Number of HER2 (ERBB2) Signals per Cancer Cell: _________________ signals/cell

+Average Number of CEP17 Signals per Cancer Cell: _________________ signals/cell

+Range of Number of HER2 (ERBB2) Signals per Cancer Cell: _________________

___Using single-probe assay
+Average Number of HER2 (ERBB2) Signals per Cancer Cell: _________________ signals/cell

+Range of Number of HER2 (ERBB2) Signals per Cancer Cell: _________________

___HER2 (ERBB2) (by genomic test for amplification or mutation)
HER2 (ERBB2) by Genomic Test
+Results
___Negative
___Positive (specify): _________________
___Cannot be determined (explain): _________________

METHODS

Specimen Processing (Note G)
+Dissection Method(s)# (select all that apply)
___Laser capture microdissection (specify test name): _________________
___Manual under microscopic observation (specify test name): _________________
___Manual without microscopic observation (specify test name): _________________
___Cored from block (specify test name): _________________
___Whole tissue section - no tumor enrichment procedure employed (specify test name): _________________

# If more than 1 dissection method used, please specify which test was associated with each selected dissection method.

Cellularity
+Percent Tumor Cells Present in Specimen: _________________%

Sequencing
+Whole Genome or Exome Sequencing
___Whole genome sequencing (specify): _________________
___Whole exome sequencing (specify): _________________
Microsatellite Instability (MSI)

+Number of MSI Markers Tested (specify number): ______

MLH1 Promoter Methylation

+MLH1 Promoter Methylation Testing Method
___ Methylation-specific real time polymerase chain reaction (PCR)
___ Other (specify): ______

KRAS Mutational Analysis

+KRAS Testing Method(s)# (select all that apply)
___ Direct Sanger sequencing (specify applicable codons): ______
___ Pyrosequencing (specify applicable codons): ______
___ High resolution melting analysis (specify applicable codons): ______
___ PCR, allele specific hybridization (specify applicable codons): ______
___ Real-time PCR (specify applicable codons): ______
___ Other (specify test and applicable codons): ______

# Please specify if different testing methods are used for different codons.

NRAS Mutational Analysis

+NRAS Testing Method(s)# (select all that apply)
___ Direct Sanger sequencing (specify applicable codons): ______
___ Pyrosequencing (specify applicable codons): ______
___ High resolution melting analysis (specify applicable codons): ______
___ PCR, allele specific hybridization (specify applicable codons): ______
___ Real-time PCR (specify applicable codons): ______
___ Other (specify test and applicable codons): ______

# Please specify if different testing methods are used for different codons.

BRAF Mutational Analysis

+BRAF Testing Method(s) (select all that apply)
___ Direct Sanger sequencing
___ PCR, allele-specific hybridization
___ Pyrosequencing
___ Real-time PCR
___ Immunohistochemistry for V600E gene product: ______
___ Other (specify): ______

PIK3CA Mutational Analysis

+PIK3CA Testing Method
___ Direct Sanger sequencing
___ Other (specify): ______

PTEN Expression and Mutational Analysis

+PTEN Testing Method (select all that apply)
___ Immunohistochemistry (specify antibody): ______
___ In situ hybridization (specify probe): ______
___ Direct Sanger sequencing
___ Duplication / deletion testing (MLPA)
___ Other (specify): ______
HER2 Expression and Genetic Analysis

+HER2 protein expression by immunohistochemistry (select all that apply)
___ Food and Drug Administration (FDA) cleared (specify test / vendor): __________________
___ Laboratory-developed test

+Primary Antibody
___ A0485
___ CB11
___ DG44
___ EP3
___ SP3
___ HercepTest
___ Oracle
___ PATHWAY
___ Other (specify): __________________

+HER2 (ERBB2) by in situ hybridization (select all that apply)
___ Food and Drug Administration (FDA) cleared (specify test / vendor): __________________
___ Laboratory-developed test

+Method (select all that apply)
___ Dual-probe assay
___ Single-probe assay

+HER2 (ERBB2) by genomic test for amplification or mutation (select all that apply)
___ Food and Drug Administration (FDA) cleared (specify test / vendor): __________________
___ Laboratory-developed test

COMMENTS

Comment(s): __________________
Explanatory Notes

A. Mismatch Repair Testing: Microsatellite instability and Immunohistochemistry

Detection of defective mismatch repair in colorectal carcinomas is important for detection of Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome [HNPPC]), which accounts for approximately 2% to 3% of all colorectal carcinomas and has clinical implications for treatment of the affected patient and family members. Microsatellite instability (MSI) testing can be used to cost-effectively screen colorectal cancer patients for possible Lynch syndrome. Patients with a microsatellite instability-high (MSI-H) phenotype that indicates mismatch repair deficiency in their cancer may have a germline mutation in one of several DNA mismatch repair (MMR) genes (eg, MLH1, MSH2, MSH6, or PMS2) or an altered EPCAM (TACSTD1) gene. After appropriate genetic counseling, patients may want to consider testing to identify the causative heritable abnormality. An MSI-H phenotype is more frequently observed in sporadic colorectal cancer (about 15% of cases) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter. The specificity of MSI testing can be increased by using it primarily on at-risk populations, such as colorectal cancer patients younger than 50 years, or patients with a strong family history of Lynch-associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma), but with sacrifice of sensitivity, since a sizeable minority of cases lacks these clinical characteristics.

MSI testing of tumor DNA is generally performed with at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers. In 1998, a National Institutes of Health consensus panel proposed that laboratories use a 5-marker panel consisting of 3 dinucleotide and 2 mononucleotide repeats for MSI testing. Recent data suggests that dinucleotide repeats may have lower sensitivity and specificity for identifying tumors with an MSI-H phenotype. As a consequence, there has been a move towards including more mononucleotides and fewer dinucleotides in MSI testing panels. Many laboratories now use a commercially available kit for MSI testing that utilizes 5 mononucleotide markers.

MSI testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS expression). If DNA MMR IHC has not been performed, this testing should be recommended for any case that shows an MSI-H phenotype, because this information will help identify the gene that is most likely to have a germline mutation (eg, a patient whose tumor shows loss of MSH2 and MSH6 expression, but retention of MLH1 and PMS2 expression, is likely to have an MSH2 germline mutation). If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC or abnormal IHC with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up. However, MSI-H may not occur in colorectal cancers of patients with germline MSH6 mutation. Intact expression of all 4 proteins indicates that MMR enzymes tested are intact but does not entirely exclude Lynch syndrome, as approximately 5% of families may have a missense mutation (especially in MLH1) that can lead to a nonfunctional protein with retained antigenicity. Defects in lesser-known MMR enzymes may also lead to a similar result, but this situation is rare.

Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy. An interpretation of expression loss in tumor cells should be made only if a positive reaction is seen in internal control cells, such as the nuclei of stromal, inflammatory, or nonneoplastic epithelial cells. Loss of expression of MLH1 may be due to Lynch syndrome or methylation of the MLH1 promoter region (as occurs in sporadic MSI colorectal carcinoma). Genetic testing is ultimately required for this distinction, although a specific BRAF gene mutation (V600E) is present in many sporadic cases, but not familial cancers. Loss of MSH2 expression strongly suggests Lynch syndrome. PMS2 loss is often associated with loss of MLH1 and is only independently meaningful
if MLH1 is intact. MSH6 is similarly related to MSH2. One should also keep in mind that nucleolar staining or complete loss of MSH6 staining has been described in colorectal cancer cases with prior radiation or chemotherapy,3,4 and a significant reduction of MSH6 staining has been described in a small percentage of colorectal carcinomas with somatic mutations of the coding region microsatellites of the MSH6 gene in MLH1/PMS2-deficient carcinomas.5

References

B. MLH1 Promoter Hypermethylation Analysis and BRAF Mutational Analysis
Defective mismatch repair in sporadic colorectal cancer is most often due to inactivation of the MLH1 gene promoter by hypermethylation (epigenetic silencing). The V600E mutation of the BRAF gene may be present in up to 70% of tumors with hypermethylation of the MLH1 promoter. In colorectal cancer, this mutation has been associated with a limited clinical response to epidermal growth factor receptor (EGFR) targeted therapies (cetuximab or panitumumab). Analysis for somatic mutations in the V600E hot spot in BRAF may also be indicated for tumors that show MSI-H, as this mutation has been found in sporadic MSI-H tumors, but not in Lynch-associated cancers with MLH1 or MSH2 mutations.1 BRAF V600E mutations have been described in probands with monoallelic PMS2 mutations.2 Direct testing of MLH1 promoter hypermethylation and/or the use of BRAF V600E mutational analysis prior to germline genetic testing in patients with MSI-H tumors and loss of MLH1 by IHC may be a cost-effective means of identifying patients with sporadic tumors for whom further testing is not indicated.3

References

C. RAS Mutational Analysis
The presence of a KRAS mutation has been shown to be associated with lack of clinical response to therapies targeted at EGFR, such as cetuximab1 and panitumumab.2 While clinical guidelines for KRAS mutational analysis are evolving, current provisional recommendations from the American Society of Clinical Oncology are that all patients with stage IV colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations.3 Anti-EGFR antibody
therapy is not recommended for patients whose tumors show mutations in KRAS codon 12, 13, or 61, but data on codon 146 are currently insufficient. A recent study has shown that NRAS mutation, like KRAS mutation, has influence on response to anti-EGFR therapy. Although more studies are needed, these findings may lead to broad KRAS and NRAS panels to include codons 12, 13, 61, and 146 of both genes.

References

D. PIK3CA Mutational Analysis
PIK3CA mutations activate the PI3K-PTEN-AKT pathway that is downstream from both the EGFR and the RAS-RAF-MAPK pathways. PIK3CA mutation and subsequent activation of the AKT pathway has been shown to play an important role in colorectal carcinogenesis and have been associated with KRAS mutation\(^1\) and microsatellite instability.\(^2\) PIK3CA mutation has further been associated with poor survival in resectable stage I to III colon cancer, with the adverse effect of PIK3CA mutation potentially limited to patients with KRAS wild-type tumors.\(^3\) PIK3CA mutations have been associated with resistance to anti-EGFR therapy in several studies,\(^4\)\(^5\) but not in others.\(^6\) The reasons for the discrepancy are not clear. Mutations of exons 1, 9, and 20 of the PIK3CA gene represent >95% of known mutations.

A European consortium recently suggested that only PIK3CA exon 20 mutations are associated with a lack of cetuximab activity in KRAS wild-type tumors and with a shorter median progression-free survival and overall survival.\(^5\) By contrast, exon 9 PIK3CA mutations are associated with KRAS mutations and do not have an independent effect on cetuximab efficacy.\(^5\) More studies are needed to establish the prognostic and predictive roles of PIK3CA exon-9 and exon-20 mutations.

References
E. PTEN Mutational Analysis
The role of PTEN loss in colorectal cancer prognosis and therapy is unclear. It has been suggested that loss of PTEN expression, as determined by immunohistochemistry, is associated with lack of benefit from cetuximab in metastatic colorectal cancer. Loss of PTEN has been found to co-occur with KRAS, BRAF, and PIK3CA mutations. The recorded frequency of loss of PTEN expression varies from 19% to 36%, with some studies reporting an effect on response rate and survival, whereas others found an effect only on progression-free or overall survival. Moreover, data on the loss of PTEN expression are not concordant in primary and metastatic tissues. There is currently no standardized method for PTEN expression analysis by immunohistochemistry.

References

F. HER2 Testing
Although HER2 is most familiar as an oncogenic driver and therapeutic target in breast and gastroesophageal adenocarcinoma, it is receiving increased attention in other cancer types, including colorectal cancer. HER2 overexpression is identified in approximately 2% of colorectal cancers, and overexpression is generally mutually exclusive of RAS/RAF mutation such that HER2-positivity is seen in up to 5% of KRAS wild-type tumors. Although anti-HER2 therapy is still only FDA-approved in breast and gastroesophageal adenocarcinoma, the current (2021) NCCN colon cancer guideline recommends testing all metastatic colon cancers for HER2 (with the exception of those that are known to be RAS/RAF mutant). Phase II clinical trials of dual anti-HER2 therapy—trastuzumab combined with lapatinib (a small molecule inhibitor of HER2 and EGFR) or pertuzumab (an anti-HER2 monoclonal antibody that inhibits HER2/HER3 dimerization) or conjugated to deruxtecan (a topoisomerase inhibitor)—have shown positive signal, with objective response rates in the 15-45% range. Based on these emerging efficacy data, the NCCN has included dual anti-HER2 therapy as a potential first-line therapy in HER2-positive patients not appropriate for intensive therapy and as a potential second-line therapy or beyond in patients well enough to receive standard intensive first-line therapy.

Anti-HER2 colon cancer trials have used differing criteria to define HER2-positivity, which has led to confusion among pathologists. The HERACLES trial had the unprecedented requirement of 3+ immunohistochemistry positivity in ≥50% of tumor cells or in situ hybridization amplification in ≥50% of tumor cells in patients who were initially found to be HER2 2+ by immunohistochemistry. Other clinical trials, including MyPathway and DESTINY, which enrolled patients based on more permissive criteria adapted from the 2016 CAP/ASCP/ASCO HER2 Testing in Gastroesophageal Adenocarcinoma Guideline, have shown anti-HER2 therapy to be similarly effective. The MyPathway trial also enrolled patients based on increased HER2 copy number or activating mutations as determined by molecular methods, and a recent study has shown good correlation between next generation sequencing and immunohistochemistry results.
References

G. Dissection Method
*Please denote the manner in which the tissue was dissected and specify the biomarker test only if different dissection methods are used for different tests.*

1. Laser capture microdissection (LCM): Use of a laser-equipped microscope to isolate and retrieve specific cells of interest from a histopathologic region of interest.
2. Manual under microscopic observation: hematoxylin and eosin (H&E) slide is examined under a light microscope and marked by a pathologist for subsequent tumor dissection and retrieval.
3. Manual without microscopic observation: H&E slide is examined without a microscope and marked by a pathologist for subsequent tumor dissection and retrieval.
4. Cored from block: Area of interest is cored from a paraffin-embedded tissue block.
5. Whole tissue section: No tumor enrichment procedure employed for tissue retrieval.