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

Reporting Template

Protocol Posting Date: June 2021
Select a single response unless otherwise indicated.

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

___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
___ NCI Panel
+BAT-25
___ Stable
___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
+BAT-26
___ Stable
___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
+D2S123
___ Stable
___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
+D5S346
___ Stable
___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
+D17S250
___ Stable
___ Unstable
___ Cannot be determined (explain): _____
___ Not performed
___ Other

Other Markers Tested (repeat as needed)
+Specify Marker: _____
___ Stable
___ Unstable
___ Cannot be determined (explain): _____

MLH1 Promoter Methylation (Note B)
+MLH1 Promoter Methylation Analysis
___ MLH1 promoter hypermethylation present
___ MLH1 promoter hypermethylation absent
___ Cannot be determined (explain): _____

KRAS (Note C)
+KRAS 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
___ 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
+Specify Type: _____
+Results
___ Low risk
___ Moderate risk
___ High risk
+Recurrence Score: _____

HER2 (Note F)
+Test(s) Performed (select all that apply)
___ HER2 (by immunohistochemistry)
HER2 by IHC
+Results
___ 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
+Results
___ 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 [HNPCC]), 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),[1] 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.[2] 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

1. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-268.
2. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998;58(22):5248-5257.
3. Bellizzi AM, Crowder CD, Marsh WL, Hampel H, Frankel WL. Mismatch repair status in a cohort of rectal adenocarcinomas before and after chemoradiation. Mod Pathol. 2010;23:137A.
4. Radu OM, Nikiforova MN, Farkas LM, Krasinskas AM. Challenging cases encountered in colorectal cancer screening for Lynch syndrome reveal novel findings: nucleolar MSH6 staining and impact of prior chemoradiation therapy. Hum Pathol. 2011;42(9):1247-1258.
5. Shia J, Zhang L, Shike M, et al. Secondary mutation in a coding mononucleotide tract in MSH6 causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency. Mod Pathol. 2013;26(1):131-138.

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

1. Domingo E, Niessen RC, Oliveira C, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. Oncogene. 2005;24(24):3995-3998.
2. Senter, L, Clendenning, M, Sotamaa, K, et al. The clinical phenotype of Lynch syndrome due to germline PMS2 mutations. Gastroenterology. 2008;135(2):419-428.
3. Bessa X, Balleste B, Andreu M, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. Clin Gastroenterol Hepatol. 2008;6(2):206-214.

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 cetuximab[1] 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.[4] 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

1. Lievre A, Bachet J-B, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66(8):3992-3995.
2. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(10):1626-1634.
3. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology Provisional Clinical Opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009;27(12):2091-2096.
4. Douillard JY, Oliner K, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369(11):1023–1034.

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

1. Nosho K, Kawasaki T, Ohnishi M, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia. 2008;10(6):534-541.
2. Abubaker J, Bavi P, Al-Harbi S, et al. Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. Oncogene. 2008;27(25):3539-3545.
3. Ogino S, Nosho K, Kirkner GJ, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol. 2009;27(9):1477-1484.
4. De Roock, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11(8):753-762.
5. De Roock, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol. 2011;12(6):594-603.
6. Prenen H, De Schutter J, Jacobs B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res. 2009;15(9):3184-3188.

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.[1,2,3,4] Loss of PTEN has been found to co-occur with KRAS, BRAF, and PIK3CA mutations.[1,4] 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.[3] There is currently no standardized method for PTEN expression analysis by immunohistochemistry.

References

1. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol. 2009;27(35):5924-5930.
2. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer. 2007;97(8):1139-1145.
3. Loupakis F, Pollina L, Stasi I, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol. 2009; 27(16):2622-2629.
4. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. PLoS One. 2009;4(10):e7287

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.[1,2] 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).[3] 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.[4,5,6,7] 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.[1] 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.[8] 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.[9,10]

References

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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.