GIPS at **ASCP**: Pathologic **Considerations for HER2** Testing in **Colon Cancer**

Andrew M Bellizzi, MD University of Iowa Hospitals and Clinics

ASCP

OCTOBER 27–29

ASCP



• None



Funder Statement

Funded by an independent educational grant from Merck Sharp and Dohme Corp.



This 63-year-old man presented with 6months of change in bowel habits and 25-pound weight loss. Colonoscopy demonstrated a circumferential <u>rectal</u> <u>mass</u>, with biopsy showing adenocarcinoma Imaging highlighted metastatic disease in the lungs, liver, and L2 vertebral body. The tumor was <u>RAS/RAF wild-type and showed</u> <u>proficient DNA mismatch repair</u> <u>status</u>. *HER2* amplification was identified on <u>circulating tumor DNA</u> <u>testing</u>, which medical oncology asked us to confirm in the tissue. HER2 immunostain demonstrates areas of

strong, basolateral-predominant staining and absent staining.

Overall, 50% of the tumor was HER2overexpressing. The patient was initially treated with FOLFOX, to which bevacizumab was added for the third cycle. The tumor initially responded and the patient was transitioned to capecitabine/bevacizumab maintenance therapy. The patient was switched to FOLFIRI + panitumumab on disease progression. The tumor progressed on this therapy, and the patient, now 16-months from initial diagnosis, was recently begun on trastuzumab and lapatinib.



Key Reference

Q&A column

in 2020 Issues, February 2020, In Every Issue

💟 🗗 💊 🍸 📴 💟

Create PDF

ASCP2@21

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Submit a Question "Q&A" is devoted this month to a question about HER2 testing in colorectal cancer. **NEED RAPID TESTS?** Browse our selection of COVID-19 tests, urinalysis tests, and others designed for CLIA-waived facilities. Learn More!»

I am a community pathologist and would like to know if the CAP has recommendations on diagnostic criteria for evaluating HER2 in colorectal carcinoma. There appears to be more than one set of criteria in various references (i.e. HERACLES, Ventana), and when clinicians request the test, I am not sure how best to evaluate these specimens.

February 2020-The CAP has no official position on HER2 testing in colorectal

https://www.captodayonline.com/qa-column-0220/

Outline

- Scientific rationale
- Status of FDA approvals
- NCCN Guidelines colon
- Frequency of positivity/clinicopathologic correlates
- HERACLES and MyPathway trials
- DESTINY-CRC01 Trial
- Scoring Criteria
- NGS as a surrogate for overexpression/amplification
- NCCN Guidelines all other tumor types
- What tissue to test
- My approach
- Low HER2
- Reflex biomarker testing at the University of Iowa Hospitals and Clinics SCP 2 2 21

HER2 Signaling Cascade



DOI:<u>10.1200/JCO.2009.25.8624</u>



Anti-HER2 Therapeutics

- Trastuzumab (T): monoclonal antibody to HER2 extracellular subdomain IV
- T + lapatinib (HER2 and EGFR small molecule inhibitor) HERACLES
- T + pertuzumab (monoclonal ab to HER2 extracellular subdomain II, which inhibits HER2/HER3 dimerization) – MyPathway
- T-deruxtecan (antibody drug conjugate; topoisomerase I inhibitor) DESTINY
- T-emtansine (antibody drug conjugate; microtubule inhibitor; aka DM1) HERACLES B (T-emtansine + pertuzumab)



HER2 Activation Pan-Cancer



https://www.cbioportal.org/

ASCP2

21

Precision Oncology Clinical Trial Designs



	<u>Webpage</u>	Description \$	<u>Date</u> 🚽	
	<u>FDA grants accelerated approval to pembrolizumab for HER2-</u> positive gastric cancer	Food and Drug Administration granted accelerated approval to pembrolizumab (Keytruda, Merck & Co.) in combination with trastuzumab, fluoropyrimidine- and platinum-containing chemotherapy for the first-line treatment of patients with locally advanced unresectable or metastatic HER2 positive gastric or gastroesophageal junction (GEJ) adenocarcinoma.	5/5/2021	
FDA L A	<u>FDA approves fam-trastuzumab deruxtecan-nxki for HER2-positive</u> g <u>astric adenocarcinomas</u>	Food and Drug Administration approved fam-trastuzumab deruxtecan- nxki (Enhertu, Daiichi Sankyo) for adult patients with locally advanced or metastatic HER2-positive gastric or gastroesophageal (GEJ) adenocarcinoma who have received a prior trastuzumab-based regimen.	1/15/2021	/ Hemato
	<u>FDA approves margetuximab for metastatic HER2-positive breast</u> <u>cancer</u>	Food and Drug Administration approved margetuximab-cmkb (MARGENZA, MacroGenics) in combination with chemotherapy, for the treatment of adult patients with metastatic HER2-positive breast cancer who have received two or more prior anti-HER2 regimens, at least one of which was for metastatic disease.	12/16/2020	\ }
	<u>FDA approves combination of pertuzumab, trastuzumab, and hyaluronidase-zzxf for HER2-positive breast cancer</u>	Food and Drug Administration approved a new fixed-dose combination of pertuzumab, trastuzumab, and hyaluronidase–zzxf (PHESGO, Genentech, Inc.) for subcutaneous injection	6/29/2020	
	<u>FDA approves tucatinib for patients with HER2-positive metastatic</u> <u>breast cancer</u>	Food and Drug Administration approved tucatinib (TUKYSA, Seattle Genetics, Inc.) in combination with trastuzumab and capecitabine, for adult patients with advanced unresectable or metastatic HER2- positive breast cancer, including patients with brain metastases, who have received one or more prior anti-HER2-based regimens in the metastatic setting.	4/17/2020	
https://www. hematologic-r	FDA approves neratinib for metastatic HER2-positive breast cancer	Food and Drug Administration approved neratinib (NERLYNX, Puma Biotechnology, Inc.) in combination with capecitabine for adult patients with advanced or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2 based regimens in the metastatic setting.	2/25/2020	er- 2 sep 21



NCCN Guidelines

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)



Version 3.2021 — September 10, 2021

NCCN.org

NCCN Guidelines for Patients® available at www.nccn.org/patients

Continue



NCCN Guidelines Version 3.2021 Colon Cancer

NCCN Guidelines Index Table of Contents Discussion

*AI B. Benson, III, MD/Chair † Robert H. Lurie Comprehensive Cancer Center of Northwestern University

*Alan P. Venook, MD/Vice-Chair † ‡ UCSF Helen Diller Family Comprehensive Cancer Center

Mahmoud M. Al-Hawary, MD ¢ University of Michigan Rogel Cancer Center

Nilofer Azad, MD † The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

*Yi-Jen Chen, MD, PhD § City of Hope National Medical Center

Kristen K. Ciombor, MD † Vanderbilt-Ingram Cancer Center

Stacey Cohen, MD † Fred Hutchinson Cancer Research Center/ Seattle Cancer Care Alliance

Harry S. Cooper, MD ≠ Fox Chase Cancer Center

Dustin Deming, MD † University of Wisconsin Carbone Cancer Center

Linda Farkas, MD ¶ UT Southwestern Simmons Comprehensive Cancer Center

Ignacio Garrido-Laguna, MD, PhD † Huntsman Cancer Institute at the University of Utah

Jean L. Grem, MD † Fred & Pamela Buffett Cancer Center

Andrew Gunn, MD φ O'Neal Comprehensive Cancer Center at UAB

J. Randolph Hecht, MD † UCLA Jonsson Comprehensive Cancer Center

NCCN Guidelines Panel Disclosures

Sarah Hoffe, MD § Moffitt Cancer Center

Joleen Hubbard, MD ‡ Mayo Clinic Cancer Center

Steven Hunt, MD ¶ Siteman Cancer Center at Barnes-Jewish Hospital and Washington University School of Medicine

Kimberly L. Johung, MD, PhD § Yale Cancer Center/Smilow Cancer Hospital

Natalie Kirilcuk, MD ¶ Stanford Cancer Institute

Smitha Krishnamurthi, MD † Þ Case Comprehensive Cancer Center/ University Hospitals Seidman Cancer Center and Cleveland Clinic Taussig Cancer Institute

Wells A. Messersmith, MD † University of Colorado Cancer Center

*Jeffrey Meyerhardt, MD, MPH † Dana-Farber Brigham and Women's Cancer Center

*Eric D. Miller, MD, PhD § The Ohio State University Comprehensive Cancer Center - James Cancer Hospital and Solove Research Institute

Mary F. Mulcahy, MD ‡ † Robert H. Lurie Comprehensive Cancer Center of Northwestern University

Steven Nurkin, MD, MS ¶ Roswell Park Comprehensive Cancer Center

Michael J. Overman, MD † ‡ The University of Texas MD Anderson Cancer Center



Aparna Parikh, MD † Massachusetts General Hospital Cancer Center

Hitendra Patel, MD † UC San Diego Moores Cancer Center

Katrina Pedersen, MD, MS † Siteman Cancer Center at Barnes-Jewish Hospital and Washington University School of Medicine

Elizabeth Raskin, MD ¶ UC Davis Comprehensive Cancer Center

*Leonard Saltz, MD † ‡ Þ Memorial Sloan Kettering Cancer Center

Charles Schneider, MD † Abramson Cancer Center at the University of Pennsylvania

David Shibata, MD ¶ The University of Tennessee Health Science Center

John M. Skibber, MD ¶ The University of Texas MD Anderson Cancer Center

*Constantinos Τ. Sofocleous, MD, PhD φ Memorial Sloan Kettering Cancer Center

Elena M. Stoffel, MD, MPH ¤ University of Michigan Rogel Cancer Center

Eden Stotsky-Himelfarb, BSN, RN † ¶ ¥ The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

*Christopher G. Willett, MD § Duke Cancer Institute

NCCN Kristina Gregory, RN, MSN Lisa Gurski, PhD

ф Diagnostic/Interventional	≠ Pathology
radiology	¥ Patient advocacy
Gastroenterology	§ Radiotherapy/Radiation
# Hematology/Hematology	oncology
oncology	¶ Surgery/Surgical oncology
Internal medicine	* Discussion Section Writing
† Medical oncology	Committee

Version 3.2021, 09/10/21 @ 2021 National Comprehensive Canoer Network® (NCCN®). All rights reserved. NCCN Guidelines® and this illustration may not be reproduced in any form without the express written permission of NCCN.

NCCN Guidelines

NCCN NCCN NCCN Network[®]

NCCN Guidelines Version 3.2021 Colon Cancer

CLINICAL WORKUP FINDINGS PRESENTATION See Treatment Resectableh Colonoscopy and Adjuvant Chest/abdominal/pelvic CT^b Synchronous Therapy (COL-5) CBC, chemistry profile liver only and/or CEA lung only Determination of tumor gene status metastases for RAS and BRAF mutations and Unresectable See Treatment HER2 amplifications (individually or (potentially and Adjuvant as part of next-generation sequencing convertible^h or Therapy (COL-6) [NGS panel])^{v,w} NCCN unconvertible) Determination of tumor MMR or MSI Suspected or status^e (if not previously done) proven metastatic Synchronous See Primary synchronous Biopsy, if clinically indicated abdominal/peritoneal **Guidelines:** Freatment (COL-7) Consider PET/CT scan (skull base adenocarcinoma metastases (any T, any N, M1) to mid-thigh) if potentially surgically curable M1 disease in selected cases^b Consider MRI of liver for liver Who to Test metastases that are potentially resectableb If potentially resectable, then Synchronous See Systemic multidisciplinary team evaluation. unresectable metastases Therapy (COL-D) including a surgeon experienced in of other sites^x the resection of hepatobiliary or lung

^bSee Principles of Imaging (COL-A).

See Principles of Pathologic Review (COL-B 4 of 8) - MSI or MMR Testing.

metastases

^hSee Principles of Surgery (COL-C 2 of 3).

^v See Principles of Pathologic Review (COL-B 4 of 8) - KRAS, NRAS, and BRAF Mutation Testing.

^w If known RAS/RAF mutation, HER2 testing is not indicated. NGS panels have the ability to pick up rare and actionable mutations and fusions. *Consider colon resection only if imminent risk of obstruction, significant bleeding, perforation, or other significant tumor-related symptoms.

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

NCCN Guidelines: How to Test



NCCN Guidelines Index Table of Contents Discussion

PRINCIPLES OF PATHOLOGIC REVIEW

HER2 Testing

- Diagnostic testing is via immunohistochemistry, fluorescence in situ hybridization (FISH), or NGS.
- Positive by immunohistochemistry is defined as: 3+ staining in more than 50% of tumor cells. 3+ staining is defined as an intense membrane staining that can be circumferential, basolateral, or lateral. Those that have a HER2 score of 2+ should be reflexed to FISH testing.⁶²⁻⁶⁴ HER2 amplification by FISH is considered positive when the HER2:CEP17 ratio is ≥2 in more than 50% of the cells.⁶²⁻⁶⁴ NGS is another methodology for testing for HER2 amplification.⁶⁵
- Anti-HER2 therapy is only indicated in HER2-amplified tumors that are also RAS and BRAF wild type.



NCCN Guidelines: Treatment Algorithm for mCRC



ASCP2@21

Frequency of Positivity/Clinicopathologic Correlates

- HER2 overexpression in CRC uncommon, though slightly more common in stage IV (2.2%; 29/1342) than stage II-III (1.3%; 25/1914) disease
- HER2+ is more common in KRAS-wild-type (5%) than KRAS-mutant tumors (0-1%) and is probably also largely mutually exclusive of NRAS and BRAF
- Because of this HER2+ is more common in the left colon
- HER2+ may confer resistance to anti-EGFR therapy
- Acquired HER2+ has been reported in the setting of acquired cetuximab resistance

PMIDs: 26690310, 26449765, 30952821

Phase II Clinical Trials of Anti-HER2 Therapy in mCRC

- HERACLES (HER2 Amplification for Colorectal Cancer Enhanced Stratification) (2016)
 - Phase II trial enrolling 27 *KRAS*-wild-type, HER2+ patients for treatment with trastuzumab/lapatinib
 - ORR 30% (n=8); 1 complete response, 7 partial responses, 12 stable disease
 - HERACLES Diagnostic Criteria
 - Central testing
- MyPathway (2019)
 - Phase II multiple basket trial for targeted HER2, BRAF, EGFR, or hedgehog signaling tx
 - 57 HER2+ patients enrolled regardless of KRAS status for treatment with trastuzumab/pertuzumab
 - ORR 32% (n=18); 1 complete response, 17 partial responses, 7 stable disease
 - Broad inclusion criteria
 - HER2 IHC 3+ in >10% of cells
 - HER2:CEP17 ≥2.0 or HER2 count >6 per cell in 20 intact tumor cells showing highest count
 - Increased HER2 gene copy number by molecular methods
 - *HER2* activating mutations (exon 20 insertions, deletions around aa 755-759, G309A, G309E, S310F, D769H, D769Y, V777L, P780-Y781insGSP, V842L, R896C, activating mutations reported in COSMIC)

 ΔSCF

Local testing

DOI:https://doi.org/10.1016/S1470-2045(16)00150-9; DOI: 10.1016/S1470-2045(18)30904-5

Phase II Clinical Trials of Anti-HER2 Therapy in mCRC

• DESTINY-CRC01 (2021)

- Phase II trial enrolling 78 RAS/RAF-wild-type, HER2-expressing patients for treatment with trastuzumab deruxtecan
 - Cohort A (n=53): HER2 IHC 3+ or IHC2+/ISH+
 - Cohort B (n=7): HER2 IHC 2+/ISH-
 - Cohort C (n=18): HER2 IHC 1+
- Cohort A ORR 45.3% (n=26); 1 complete response, 23 partial responses, 20 stable disease
- Cohorts B and C had no objective responses
- CAP/ASCP/ASCO 2016 GEA Criteria
- Central testing

DOI:https://doi.org/10.1016/S1470-2045(21)00086-3



Ventana/ToGA/Ruschoff/Hoffman vs HERACLES IHC Criteria

HER2 IHC Result	CAP/ASCP/ASCO GEA Interpretation (for resections)	Consequence	HERACLES Diagnostic Criteria Interpretation	Consequence
No reactivity or membranous reactivity in <10%	Negative (0)	No further testing required; not eligible for therapy	Negative	No further testing required; not eligible for therapy
Faint/barely perceptible reactivity in ≥10%	Negative (1+)	No further testing required; not eligible for therapy	Negative	No further testing required; not eligible for therapy
Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% but <50%	Equivocal (2+)	Perform ISH testing	Negative	No further testing required; not eligible for therapy
Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥50%	Equivocal (2+)	Perform ISH testing	Equivocal	Mandatory IHC retesting to confirm staining in ≥50% of cells; ISH testing required; eligible for therapy if ISH positive
Strong complete, basolateral, or lateral membrane staining in 10-50%	Positive (3+)	Eligible for therapy; no further testing required	Conditionally positive	Mandatory IHC retesting to confirm staining in ≥10% of cells; ISH testing required; eligible for therapy if ISH positive
Strong complete, basolateral, or lateral membrane staining in >50%	Positive (3+)	Eligible for therapy; no further testing required	Positive	Eligible for therapy; no further testing required

CAP/ASCP/ASCO vs HERACLES ISH Criteria

CAP/ASCP/ASCO	HERACLES
• HER2:CEP17 ratio ≥2.0 in >10% of	HER2:CEP17 ratio ≥2.0 in ≥50% of cells
cells	
• HER2 count >6 per cell in >10% of	
cells	
(if HER2:CEP17 ratio is <2.0 and HER2	
count is 4-6, count another 20 cells)	



50% Threshold for IHC-Positivity was Not a Slam Dunk

Assessment of a HER2 scoring system for colorectal cancer: results from a validation study

Emanuele Valtorta^{1,19}, Cosimo Martino^{2,19}, Andrea Sartore-Bianchi¹, Prédérique Penaullt-Llorca³, Giuseppe Viale⁴, Mauro Risio², Massimo Rugge⁵, Walter Grigion⁶, Katia Bencardino⁴, Sara Lonardi², Viltorina Zagonel⁷, Francesco Leone², Johannes Noe⁸, Fortunato Ciardiello⁹, Carmine Pinto⁶, Roberto Labianca¹⁰, Stefania Mosconi¹⁰, Claudio Graiff¹¹, Giuseppe Aprile¹², Barbara Frau¹³, Carlo Garufi¹⁴, Fotios Loupakis¹⁵, Patrizia Racca¹⁶, Giuseppe Tonini¹⁷, Calogero Lauricella¹, Silvio Veronese¹, Mauro Truini³, Salvatore Siena^{1,18,20}, Silvia Marsoni^{2,20} and Marcello Gambacorta^{1,20}

¹Niguardu Gancar Conter, Onpedule Niguarda Ga' Grandra, Milano, Ibaly; ²Istituto di Gandiolo, FPO-IRCCS, Gandiolo, Italy; ²Contro Jean Parini, University of Aavengue, Clarmont's Formod, France, "Diluto Europeo Oncologia, Milano, Italy; ³Anotomin Patologica, Attorneh Oepedulo-Università di Padova, Pudova, Italy; ⁴Podeclinico: Sant'Oraola-Malpighi, Bologna, Italy; ⁴Oacologia Modica I, Istituto Oncologia; Vanoto – IRCCS, Padova, Ibay; ⁴F. Hoffmann-La Rocha, Basel, Switzentende, ⁴Seconda Università di Napoli, Napoli, Italy; ⁴⁹Oapadale Papa Giovanni XXII, Bergamo, Italy; ⁴¹Oapadale di Bolzano Vin Boehler, Bolzano, Italy; ⁴²Oapadale Santo Maria della Minoricordia, Piezzale Santa Maria della Misaricordia, Udine, Italy; ⁴²Oapadale Santo Maria della Minoricordia, Mansertuta, Italy; ⁴⁴Ationada Oopodalioro-Universitaria Tapoliclinico Universitario di Cagliari, Monsertuta, Italy; ⁴⁴Ationda Oopodalioro-Universitaria Gittà della Salute e della Scienza, Tarino, Italy; ¹¹Policlinico Universiturio Campus Biomedico, Bonne, Baly ⁴⁴Atienda Oupodaliero-Universitaria Pienna Via Roma, Pisa, Rohy; ⁴⁴Anienda Oupodalioro-Universitaria Gittà della Salute e della Scienza, Tarino, Italy; ¹¹Policlinico Universitario Campus Biomedico, Roma, Baly

We sought to develop criteria for ERB82-positivity (HER2) in colorectal cancer to ensure accurate identification of ERB82-amplified metastatic colorectal cancer patients auitable for enrolment in a phase II trial of ERB82targeted therapy (HERACLES trial). A two-step approach was used. In step 1, a consensus panel of pathologiats adapted existing protocols for use in colorectal cancer to text ERBB2 expression and amplification. Collegial revision of an archival test cohort of colorectal cancer samples led to specific recommendations for adapting current breast and gastric cancer criteria for acoring ERBB2 in colorectal cancer. In step 2, from September 2012 to January 2015, colorectal-apecific ERBB2 testing protocols and ERBB2 scoring criteria were used to centrally acreen for ERBB2-positive KRAS wild-type colorectal cancer patients to be enrolled in the HERACLES trial (clinical validation cohort). In both archival test (N=256) and clinical validation (N=830) cohorts, a clinically sizeable 5% fraction of K/RAS wild-type colorectal cancer patients was found to be E/RBS2-positive according to the colorectal cancer-specific ERB82 scoring criteria. ERB82-positive tumora showed ERB82 immunostaining consisting of intense membranous ERB82 protein expression, corresponding to homogenous ER882 amplification, in >50% of cells. None of the immunchistochemistry 0 or 1+ cases was amplified. Concordance between SISH and FISH was 100%. In conclusion, we propose specific criteria for defining ER882-positivity in colorectal cancer (HERACLES Diagnostic Criteria). In a phase il trial of trastuzumab and lapatinib in a cetuximabresistant population, HERACLES Diagnostic Criteria shaped the selection of patients and defined ERBB2 as a predictive marker for response to ERBB2-targeted therapy in metastatic colorectal cancer Modern Pathology (2015) 28, 1481-1491; doi:10.1038/modpathol.2015.98; published online 9 October 2015

Carrespondences Dr 5 Marseni, MD, fatimits di Candielo, Fondazione del Piennante per l'Oncología-BCCS, Strada Previnciale 142, Kus 3.85, Candielo 19896, July. Fendi cittu camenolificto. It

¹⁰These authors contributed separity as first eathors.

²⁰These authors contributed separity as senior authors. Received 2 April 2015; revised 13 July 2015; accepted 13 July 2015; published online 9 October 2015.





- Compared Ventana Pathway (4B5) FDAapproved kit to Dako A0485 polyclonal antibody to predict ISH positivity at the 50% ISH threshold
- Ventana assay most accurate at 50% IHC threshold, while Dako assay most accurate at 10% IHC threshold



NGS as a Surrogate for Overexpression/Amplification

AJCP / Onepost Attents

Detection of *ERBB2* Amplification by Next-Generation Sequencing Predicts HER2 Expression in Colorectal Carcinoma

Odise Cenaj, MD, Azna H. Ligon, PhD, Jason L. Hornick, MD, PhD, and Lynette M. Sholl, MD

From the Department of Pathology, Brigham and Women's Hospital, Boston, MA.

Key Wards: ERBID2; HER2; Amplification; Colonectal carcinoms; Colon; Next-generation sequencing; NGS; Immunohistochemistry; Transaumsb; Lapatinib

Jon J Clin Pathol J Jy 2018, NO 811 108 DOI: 10.1080/J.C.YALUDDI

ABSTRACT

Objectives: ERBB2 (human epidermal growth factor receptor 2 {HER2}) amplification/orcrexpression in colorectal carcinomat (CRCs) may predict response to HER2 inhibitors: We correlated ERBR2 amplification by next-generation supporting (NGS) with HER2 orcrexpression by immunohistochemistry.

Methods: NGS was performed on specimens containing 20% or more turnor. HER2 immunohistischemisiny (closer SP3) was normd nonispantitatively by H-score. ERB82 phorescence is site hybrikhzation (FISH) was performed to examine copy alterations in one HER2-beirrogeneous turnos.

Results: ERBR2 complification was detected in 2% of 1,300 CRCs analyzed by NGS. HER2 immensiohistochemistry was examined to 15 cause with ERBR2 complification (six or more copies), 10 with low gain (three to five copies), and 77 copy neutral. ERBR2 complification and HER2 immensiohistochemistry showed perfect concendance at an H-score of 105 or more. FISH confirmed homogeneous ERBR2 complification in a tumor showing HER2 protein expression hotercopyneity ERBR2 complification anticorrelated with RAS/RAF maturitors (P = .0001). No ERBR2-couplified cause showed mismatch repair deficience.

Conclusions: NGS-detected ERBB2 amplification highly correlates with HER2 overexpression in CRC, but immunohistochomistry is required to capture protein-level heterogeneity. Colorectal carcinoma (CRC) is a leading cause of cancer-related deaths worldwide. Adjuvant chermotherapy regimens, as well as targeted therapies via humanized monoclonal antibodies (cetaximah and panitumumah for epidermal growth factor receptor (EGFR), bexacianand for vascalar endothetial growth factor receptor), have shown clinical benefit in treating advanced-stage CRCs, with significant improvement in overall and progression-free arvival.¹⁶

Another major therapeutic target of interest in human cancer is the human epidemul growth factor receptor 2 (HER2), which is encoded by the Erb-b2 receptor tyrosine kinase 2 (ERBR2) gene located on chromosomal region 17q12.³ HER2 is a transmembrane receptor tyrosine kinase in the HER family, which includes EGFR.⁸¹⁰ *ERBR2* gene amplification and HER2 protein overexpression lead to homodimerization and heterodimerization with other members of the HER family.¹¹ triggering activation of P13K/AKT and mitogen-activated probein kinase (MAPK) pathwaya¹² and resulting in tumor proliferation, differentiation, survival, apoptosis, angiogenesis, and invasion.^{40,10}

ERBR2 amplification with HER2 overexpression is an established therapeutic target in breast and gastric cancer, where anti-HER2 monoclonal antibody therapies (trasturamah, perturaumab) have led to significant improvement in outcomes.^{11,13} ERBR2 amplification and/or HER2 overexpression predict response to trastuamah; therefore, detection by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization and

- HER2 amplification in 2% of 1300 CRCs analyzed by NGS
- Defined amplification as ≥6 copies
- OncoPanel on Illumina HiSeq2500
- CN= (2*((AGCR-1)/P +2)
- Correlated IHC and NGS in 15 amplified, 10 "low gain" (3-5 copies), and 77 copy neutral cases
- HER2 IHC: SP3 on Dako Autostainer; H-score reported



ASCP2@21

6 Anseican Saciety for Clinical Pathology 2018. All rights received. For permissions, please e-mail: journals, permissionellisup.com Am J Cliv Pathol 2018;152:97-108 97 201:10:1000;event

Correlation of NGS/IHC (H-Score & HERACLES "Calls")

HER2 Staining HER2			ERBB2 Copy	ERAR2 Calculated		Matarion Status by NGS (8 = Wild-Typs) MMR			_				
Na.		Diagnostic Criteria	Number Status by NG25	Copy Number by NGS*	KR45	NRAS BRAF	ERAN	PERICA		BIC Z			
1	3+	300 285		Amplified Amplified	123	0	0 0	8	0		tact B		
2	3+	290		Amplified	152	0	0 0	ō	0	in	tact S		
4	2+	190	En	Amplified	55	0	0 0	0	Ð	les	tati Da		
57	2+	150	HER2	Immunohis	tochemis	stry and ERE	BB2 Amp	lification	on by NO	GS			
8	3+ 3+	100					ERBB2	Copy N	umber by	NGS			
11	3+	110	7										
13	2+	340	Charac	teristic		Amplified	Low Gain	I N	eutral	Total No	onamplified	Sensitivity, % (95% CI)	Specificity, % (95% Cl
54	3+	105											
15	3+	300	HER2	IHC result by	HERACI	ES diagnosti	c criteria						
17	1+	10	Posit	tive		7	0	0		0		47 (0.21-0.73)	100 (0.96-1.00)
18	2+	30 25	Equi	vocal		7	0	0		0			
20	1+	5				1	10	7	7	87			
21	1+	10		ative			10						
23	1+	5	HER2	IHC result by	HERACI	ES diagnosti	c criteria (includi	ng equivo	ocal)			
24	1+	5	Posit	tive or equivo	cal	14	0	0		0		93 (0.68-1.00)	100 (0.96-1.00)
26	1+	2.5		ative		1	10	7	7	87			
27	1+	1 25				and a set in t		· · · ·					
28	14	4.0	HERZ	IHC result by	cutpoint	proposed in	this study	(H-SCC	re 2105)				
			Posit	tive	10 C 12	15	0	0		0		100 (0.78-1.00)	100 (0.96-1.00)
29	2+	75	Neg	ative	(D	10	7	7	87			
35	-	0	-										
31	-	0	-	Low gain-arm level	3	c.38GoA (p.G13D)		0	0		tect		
32	-	0	-	Low gain-arm level	NED	c-836G>A (p.A146T) 0	0 0	0	0		tact tact		
34	-	0	-	Low gain-arm level	3	c.34G>A (p.G125)		0	0		tact		
35	-	0	-	Low gain Low gain—focal	NID	0 c.355>C to 612Al	0 0	0	0		tact		

Eq. equivocal, HERACLES, HER2Amplification for Coloractal Concer Tohonced Statification, HER2, based epideronal growth factor surgetor 2, HEC, immunohistochemistry, MMR, minuratik repair, NCE not done, NCR, not generation suggeneration suggeneration suggeneration suggeneration.

'Cases labeled NOP twee segament on the evelocit version of the NGB panel and do not have now converge data available.

HER2 in NCCN Guidelines - Non-Breast/GEA/CRC

- Head and Neck: Salivary duct carcinoma highly expressing and responsive
- Hepatobiliary: Overexpressed in 15% of extrahepatic cholangiocarcinomas and gallbladder carcinomas and demonstrated response in retrospective series
- Non-Small Cell Lung Cancer: Recommends T-DM1 in HER2-mutant tumors (2%)
- Pancreas: Recommends NGS in locally advanced/metastatic tumors to identify actionable fusions (ALK, NRG1, NTRK, ROS1) and mutations (BRAF, BRCA1/2, HER2, KRAS, PALB2) and testing for dMMR/MSI-H
- Small Bowel: Cites more frequent HER2+, dMMR/MSI-H, PD-L1+, & TMB vs CRC
- <u>Uterine Neoplasms:</u> HER2 IHC with reflex to FISH for equivocals is recommended for advanced serous endometrial cancer (30%+); carboplatin/paclitaxel/trastuzumab is preferred therapy



HER2 Testing in Salivary Duct Carcinoma and Uterine Serous Carcinoma

					-	
Ado-trastuzumab emtansine in patients with HER2 amplified salivary gland cancers (SGCs): Results from a phase II basket trial. © Check for update Bob T. U. Bonglai Shen, Michael Offin, Darren J. Buonocore, Mackenzie L. Myers, Alshwarya Venkatedy, Show More Abstract bot T. U. Bonglai Shen, Michael Offin, Darren J. Buonocore, Mackenzie L. Myers, Alshwarya Venkatedy, Show More Abstract 6001 Background: SGCs are rare tumors with no approved therapy for metastatic disease. HER2 amplification occurs in B% among all SGC histologies, and 25-33% of the aggressive salivary duct carcinoma (SDC) histologic subtype. We hypothesized that ado-trastuzumab emtansine, a HER2 targeted antibody drug conjugate, may be clinically active in these patients. Methods: A cohort of patients with HER2 amplified SGCs were enrolled into a multi-histology basket trial of ado-trastuzumab emtansine, treated at 3.6mg/kg IV every 3 weeks. The primary endpoint was overall response rate (ORR) by RECIST v1.1 or PERCIST. A Eirone head these in events and bland bland. Jores treated at 3.6mg/kg IV every 3 weeks.	 HER2 targeted therapy for HER2+ tumors^a Trastuzumab^{b,6} Ado-trastuzumab emtansine (TDM-1)⁷ Trastuzumab/pertuzumab^{b,8} Docetaxel/trastuzumab^{b,9} 	<section-header><section-header>DOLUME 28 - NUMBER 20 - JU JOURNAL OF CLINICAL DURNAL OF CLINICAL Sector Autor State And Sector Carbo Carb</section-header></section-header>	Time for Standardized Pathology Pr Martar 1 - Ontext.—Endometrial serous carcinoma is an aggressive subtype one andometrial cancer with the highest rate of subtype one andometrial cancer with the highest rate of initial trial showed prolonged progression-free survival in subtype one and prolonged progression-free survival initial trial showed prolonged progression-free survival factor receptor 2 (HER2)-positive endometrial serous carcinoma when traatruumah was added to the standard premotherapy regimen. This targeted therapeutic ap- nensive Cancer Network clinical suidelines. There is a indometrial serous carcinoma, and pathologists need to big protein expression and gene amplification in mese tumors. Metro and the statistical overview of targeted HER2 therapy in endometrial serous carcinoma and the	Buzz, MD summarize key findings from recent studies on the specific features of HER2 protein expression and gene amplifica- tion relative to other tumor types. Endometrial carcinoma- specific HER2 testing criteria are proposed based on evidence in the existing literature. Data Sources – Sources comprise review of the litera- ture and personal experience of the autor. Conclusions – HER2 protein overexpression and/or gene amplification is present in approximately 25% to opportunity for targeted therapy. Pathologist play a key prole in tumor HER2 testing and scoring to ensure autore and second score and successful clinical outcome. Arch Pathol Lab Med. 2021;145:687–691; doi: 10.5858/ arpa.2020-0207-RA)	se: nk P = .013 , 0.20 to 0.80) 	
imon two-stage optimal design was applied with type I error rate under 2.7%, ower of 89%, H0 10%, H1 40%; H0 will be rejected if 6 or more responses are bserved in 24 patients. Other endpoints include duration of response (DOR), rogression-free survival (PFS), and toxicity. <i>HER2</i> amplification was identified y next generation sequencing (NGS), and tumors were subsequently tested by	NCCN Guideline	treatment rental), re 0.53), Tox Addition c survival in survival in	Endometrial cancer is the fourth most common malig- nancy among women in the United States, with an estimated 65 620 new diagnoses and 12 590 deaths in 2020. ¹ Although endometrial serous carcinoma represents only approximately 10% of all endometrial carcinomas, it has the highest recurrence rate (both local and distant) and is	tumors. ¹⁷ In addition, most studies combined all histologic subtypes in their analyses, including low-grade endome- troid carcinomas. ¹⁷ During the same time period a bandful of case reports described successful targeted therapy in patients with HER2 ⁻ endometrial serous carcinomas. Villella et al ¹¹⁷ reported 2 patients with advanced-stage HER2 ⁻		
fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). Fluorescence lifetime imaging microscopy - Förster resonance energy transfer (FLIM-FRET) assessed the propensity for HER2-HER3 heterodimerization, which leads to receptor internalization. Results: 10 patients with <i>HER2</i> amplified SGCs	references 2018 ASCO/CAP Breast Criteria	J Clin On Endo gynecolog Associated content destrik 1 U	responsible for a disproportionate 40% of endometrial cancer mortality, with an overal 3-year survival rate of only 45%. ²⁻¹ In the era of precision medicine there is a growing demand for new targeted therapeutic approaches for this aggressive tumor type. Since the mid-1990s there has been a considerable interest in potential anti-human epidermal growth factor	tumors: 1 patient showed complete response and the other patient had stable disease. Sanite et al ¹⁰ observed prolonged survival with stable disease in 2 patients. Despite the encouraging case studies, the Gynecologic Oncology Group published the results of the first phase 2 diricial trial (GOG 181B) in 2010, showing no clinical activity by single-agent trastazumab in HER22 advanced-stage or recurrent endo-		
were treated. The median age was 65 (range 36-90 years), 90% were male. The median lines of prior systemic therapy was 2 (range 0-3). ORR was 90% (9/10, 95% CI 56-100%) including 5 complete responses after prior trastuzumab, pertuzumab and anti-androgen therapy. After a median follow up period of 12 months (range 4-20 months), median DOR (range 2-19+) and median PFS (95% CI 4-22+ months) were not reached. Toxicities included grade 1 or 2 infusion capacities. Therapeutical and therapeutical programs are up to the second sec		Aggregative Aggregative Data A for the approximation of the approximation Aggregative Data approximation Data approximat	receptor 2 (HER2) therapy for endometrial cancer, and several clinicopathologic studies have been published on the topic. ²⁻³⁶ However, in the absence of standardized HER2 testing and scoring methods, the HER2 protein overexpres- sion rate varied significantly between 14% and 80%, and <i>HER2</i> gene amplification detected by fluorescent in situ hybridization (FISH) was reported in 21% to 47% of	metrial carcinoma. ¹⁹ The trial results were received with criticism primarily because of the low enrollment numbers and poor patient selection criteria: most patients had endometrioid carcinoma, whereas only one-third had a diagnosis of serous carcinoma, and tumos with a 2+HER2 immunohistochemical score and negative FISH were also included. ²⁰ Following the failed GOG trial, a new multi-institutional,	o 36 4 ent Date (n	
reaction, thrombocytopenia and transaminitis; there were no treatment related deaths. <i>HER2</i> amplification by NGS (fold change 2.8 to 22.8) correlated with HER2/CEP1722 by FISH (8)8 tested) or IHC3+ (10/10 tested). FLIM-FRET tested positive in 3/3. Conclusions : Ado-trastuzumab emtansine is highly efficacious in patients with <i>HER2</i> amplified SGCs as identified by NGS. This study has met its primary endpoint, and cohort expansion is warranted to confirm these results. Clinical trial information: NCT02675829. [2]		Fader Crit	Accepted for publication April 23, 2020. Published online July 9, 2020. From the Department of Pathology, Yale University School of Medicine, New Haven, Connecticut. The author has no relevant financial interest in the products or comparise described in this article. Corresponding author. Natalia Buza, MD, Yale University School of Medicine, 310 Cedar Street H1 108, PO Rox 208023, New Haven, CT 05520-8023 (email: natalia.huza@yale.edu).	randomized phase 2 clinical trial was initiated in 2011 to evaluate the efficacy of craboplatin/paclitaxed versus carbo- platin/paclitaxel/trasturumab in advanced-stage and recur- rent HER2 ⁻ endometrial serous carcinoma. ²¹ Prolonged progression-free survival was observed in the trasturumab arm by nearly 9 months in advanced-stage tumoes, and by 3.2 months in recurrent endometrial serous carcinoma, an assessment of the overall survival data is currently in	ral/la	teral
© 2019 by American Society of Clinical Oncology		staining; H	Arch Pathol Lab MedVol 145, June 2021	HER2 Testing in Endometrial Serous Carcinoma—Buza 687		

2019 by American Society of Clinical Onco

What Tissue to Test (Primary vs Metastasis)

Brannon et al. Genome Biology 2014, 15:454 http://genomebiology.com/2014/15/8/454



RESEARCH

Open Access

Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions

A Rose Brannon¹¹, Efsevia Vakian¹¹, Brooke E Sylvester², Sasinya N Scott¹, Gregory McDermott^{1,2}, Ronak H Shah¹, Krishan Kania², Agnes Viale⁵, Dayna M Oschwald⁶, Vladimir Vacic⁶, Anne-Katrin Emde⁶, Andrea Cercek³, Rona Yaeger³, Nancy E Kemeny³, Leonard B Saltz³, Jinru Shia¹, Michael I D'Angelica⁴, Martin R Weiser⁴, David B Solit^{2,1,2*} and Michael F Berger^{1,2,7*}

Abstract

Background: Colorectal cancer is the second leading cause of cancer death in the United States, with over 50,000 deaths estimated in 2014. Molecular profiling for somatic mutations that predict absence of response to anti-EGFR therapy has become standard practice in the treatment of metastatic colorectal cancer, however, the quantity and type of tissue available for testing is frequently limited. Further, the degree to which the primary tumor is a faithful representation of metastatic disease has been quick oned. As next-generation sequencing technology becomes more widely available for clinical use and additional molecularly targeted agents are considered as treatment options in colorectal cancer, it is important to characterize the extent of tumor heterogeneity between primary and metastatic tumors.

Results: We performed deep coverage, targeted next-generation sequencing of 230 key cancer-associated genes for 69 matched primary and metastatic turnors and normal tissue. Mutation profiles were 100% concordant for KRAS, NRAS, and BRAF, and were highly concordant for recurrent alterations in colorectal cancer. Additionally, whole genome sequencing of four patient trios did not reveal any additional site-specific targetable alterations.

Conclusions: Colorectal cancer primary tumors and metastases exhibit high genomic concordance. As current clinical practices in colorectal cancer revolve around KRAS, NRAS, and BRAF mutation status, diagnostic sequencing of either primary or metastatic tissue as available is acceptable for most patients. Additionally, consistency between taroeted sequencing and whole genome sequencing results suggests that targeted sequencing may be a suitable.

Valtorta (HERACLES validation study): 100% concordance in matched primary-metastatic pairs assessed by Pathway and ISH (4/47 3+ and amplified)

 Shimada et al (PMID: 28235632): 44/45 matched primarymetastatic pairs IHC concordant (positive in 3, negative in 41, positive in primary-negative in metastasis in 1)

100% concordance for KRAS/NRAS/BRAF in 69 matched primary-metastatic pairs



My Approach to Non-Breast/Non-GEJ Adenocarcinoma

- At present, I report the intensity (0-3+, based on the magnification rule) and extent (0-100%) of lateral membrane, basolateral, or complete membrane staining. I reflexively FISH cases at the "2+, ≥10%" IHC staining threshold.
- To satisfy CAP requirements regarding predictive marker reporting (ANP.22969), I composed the following templated language for HER2 IHC:

HER2 immunohistochemistry is performed on formalin-fixed, paraffin-embedded tissue sections from non-breast/non-gastroesophageal carcinoma tissue using the rabbit monoclonal antibody SP3 and a polymer-based detection system.

There are no uniformly agreed on criteria in these tumor types. Recent clinical trials of anti-HER2 therapy have employed different selection criteria. Anti-HER2 therapy is currently only FDA-approved in breast and gastric/gastroesophageal junction carcinomas.

MyPathway is a multiple basket trial that has enrolled patients with diverse solid tumors for dual anti-HER2 therapy based on HER2-activiation detected by IHC, ISH, or NGS. IHC criteria were borrowed from breast, with the caveat that lateral membrane and basolateral staining were also considered. There are no separate biopsy criteria:

Score 0: No staining or membrane staining in <10% of tumor cells

Score 1+: Membrane staining in ≥10% of tumor cells of faint/barely perceptible intensity

Score 2+: Complete, basolateral, or lateral membrane staining in ≥10% of tumor cells of weak to moderate intensity

Score 3+: Complete, basolateral, or lateral membrane staining in ≥10% of tumor cells of strong intensity

Reference: Hainsworth JD, Meric-Bernstam F, Swanton C, Hurwitz H, Spigel DR, Sweeney C, et al. Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(6):536-42.

Low HER2: HER2 1+, HER2 2+/ISH-

Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low–Expressing Advanced Breast Cancer: Results From a Phase Ib Study

Shanu Modi, MD¹; Haeseong Park, MD, MPH²; Rashmi K. Murthy, MD, MBE³; Hiroji Iwata, PhD, MD⁴; Kenji Tamura, MD, PhD⁵; Junji Tsurutani, MD, PhD⁶; Alvaro Moreno-Aspitia, PhD⁷; Toshihiko Doi, MD, PhD⁸; Yasuaki Sagara, MD⁹; Charles Redfern, MD¹⁰; Ian E. Krop, MD, PhD¹¹; Caleb Lee, MD, PhD¹²; Yoshihiko Fujisaki, MS¹³; Masahiro Sugihara, PhD¹³; Lin Zhang, MD, PhD¹²; Javad Shahidi, MD¹²; and Shunji Takahashi, MD¹⁴



HER2 IHC score assessed by 18 pathologists



GI Reflex Biomarker Testing at UIHC

Reflex IHC Biomarker Testing on GI Service

	· · · · · · · · · · · · · · · · · · ·
Esophageal/GEJ adenocarcinoma	GHER2*, PD-L1, MMR
Esophageal squamous cell carcinoma	PD-L1
Gastric adenocarcinoma	GHER2*, PD-L1, MMR, EBER (if lymphoepithelioma-like)
Small intestinal adenocarcinoma	MMR
Pancreatic ductal (and acinar)	MMR, Iowa Cancer Mutation Profiling (with fusion for
adenocarcinoma	acinar cell carcinoma)
Cholangiocarcinoma (all)	MMR, Iowa Cancer Mutation Profiling with Fusion
Gallbladder cancer	MMR, HER2
Colon cancer	MMR, CDX2 (if pT3 N0), BRAFV600E IHC and BRAF
	mutation testing (if MLH1D)
	We're discussing adding RAS/BRAF mutation testing
Neuroendocrine tumor	Ki-67 (on bx; on 1 block each 1°, regional, distant dz on
	resections), SSTR2A (on bx; repeat on resection in 1 Part if
	weak-to-negative on bx), CXCR4 (atypical carcinoid tumor
	of lung origin), ATRX (on pancreatic NET-new diagnosis
	and disease progression)
Neuroendocrine tumor G3	add p53, Rb, Iowa Cancer Mutation Profiling
Neuroendocrine carcinoma	Ki-67 (not mandatory), SSTR2A, CXCR4, MMR,
	Iowa Cancer Mutation Profiling

 We recently considered reflex HER2 IHC in mCRC, but held back based on low frequency of positivity and difficulty for pathologist in identifying metastatic cases

 Local solutions based on multidisciplinary discussion



*repeat GHER2 on resection, if negative on the biopsy

Summary/Take Home Points

- Precision oncology is increasingly focused on low-incidence but highly clinically actionable pan-cancer targets
- HER2 is broadly activated in cancer, though much less so in colon cancer (2%) than in breast, GEA, salivary duct carcinoma, and uterine serous carcinoma
- NCCN Guidelines are dynamic and have outpaced FDA-approvals
- The first positive phase II anti-HER2 trial in CRC (HERACLES) defined IHC and ISHpositivity at an unprecedented 50% threshold; subsequent positive clinical trials in CRC have used a more permissive 10% threshold and/or molecular methods
- NGS is a promising tool but must be validated
- In non-breast/non-GEA adenocarcinoma, I test best available tumor (metastatic or primary), report intensity and extent of staining, and interpret based on MyPathway/CAP/ASCP/ASCO GEA resection criteria (unless explicitly told otherwise)
- Given low rate of positivity, we have not gone to reflexive HER2 testing in CRC

Thank you