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

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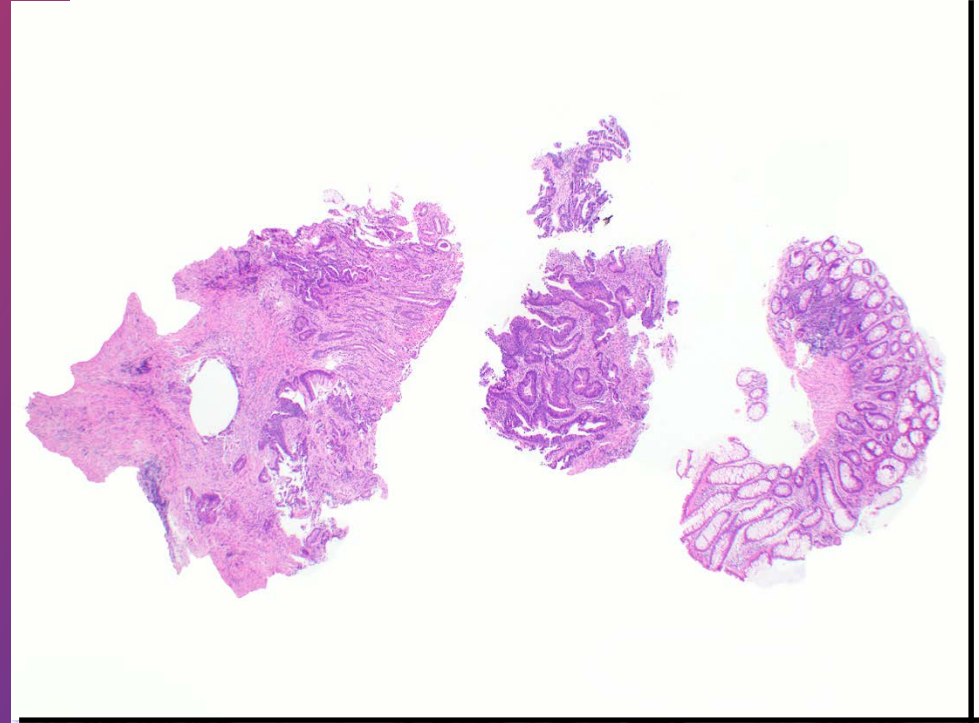
Disclosures

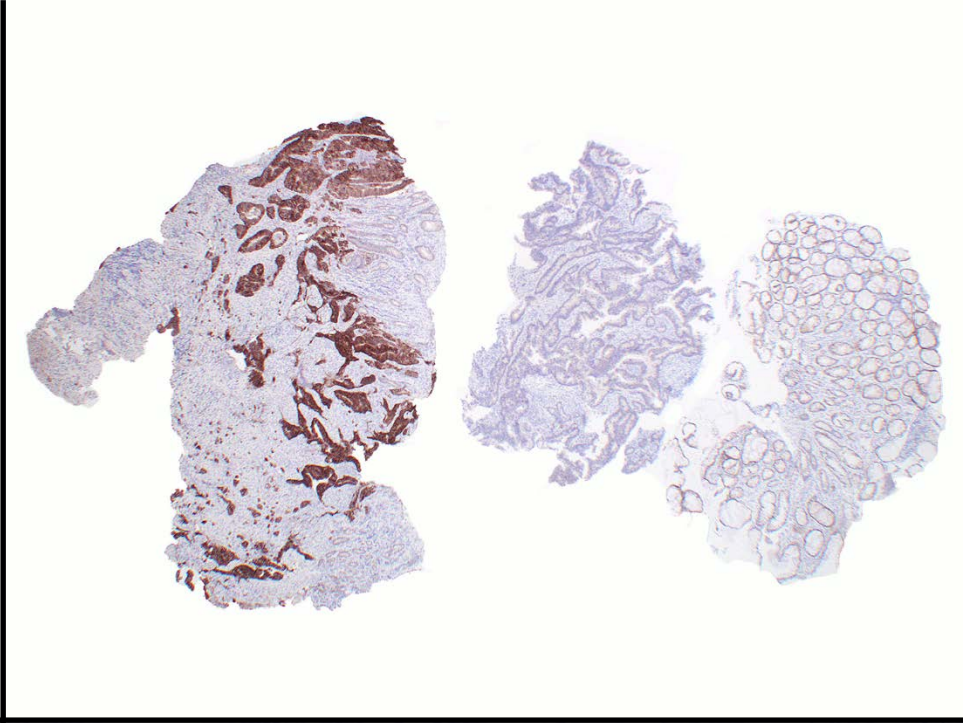
- None

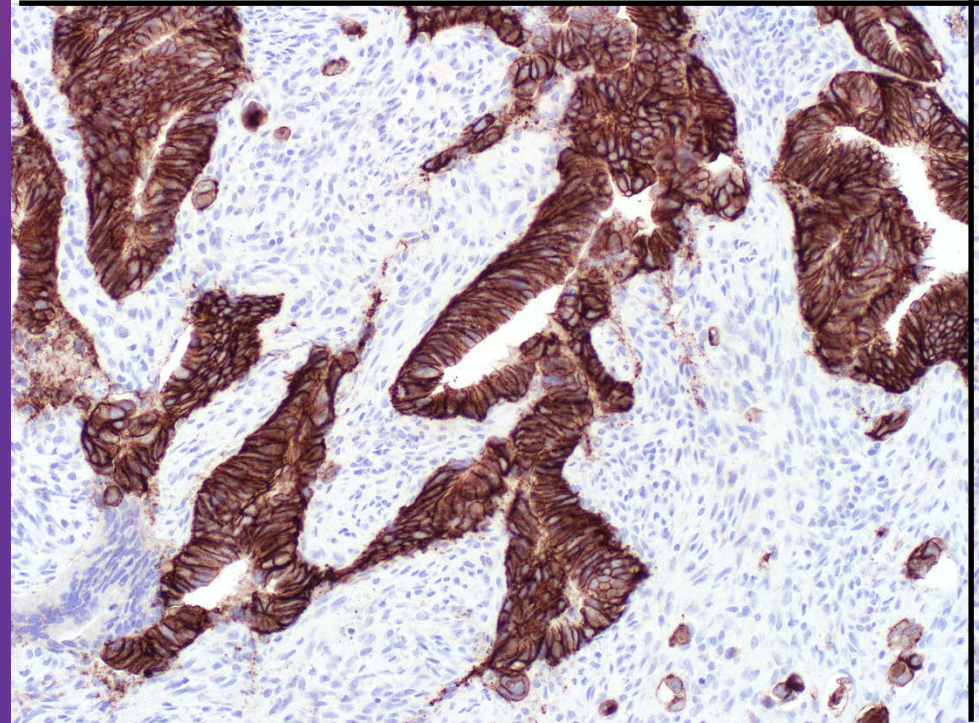
Funder Statement

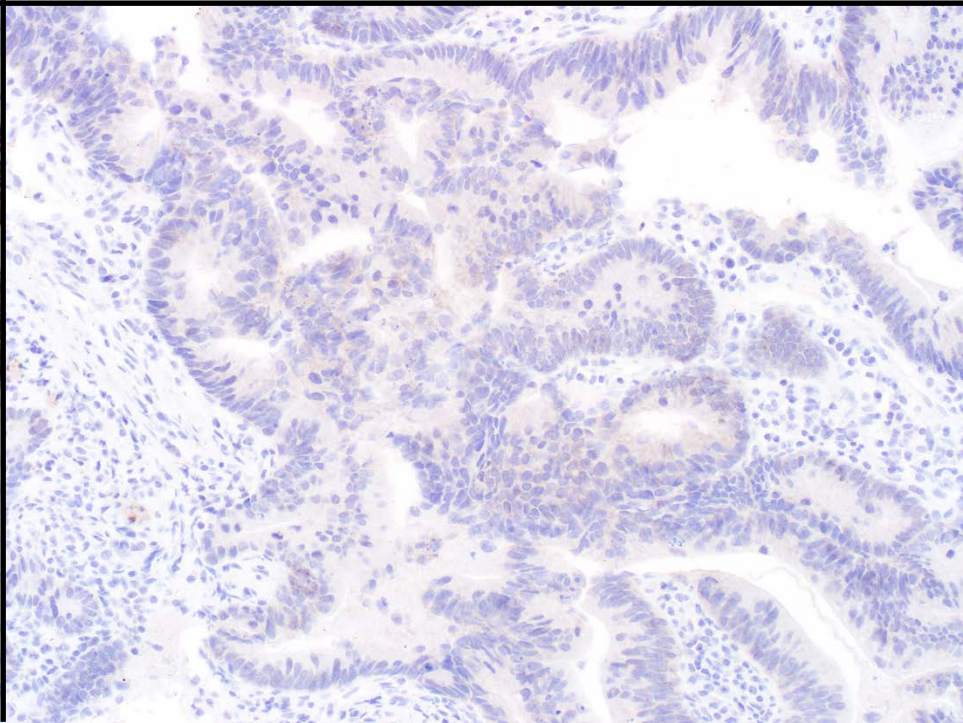
Funded by an independent educational grant from Merck Sharp and Dohme Corp.

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

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.

Q. 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.

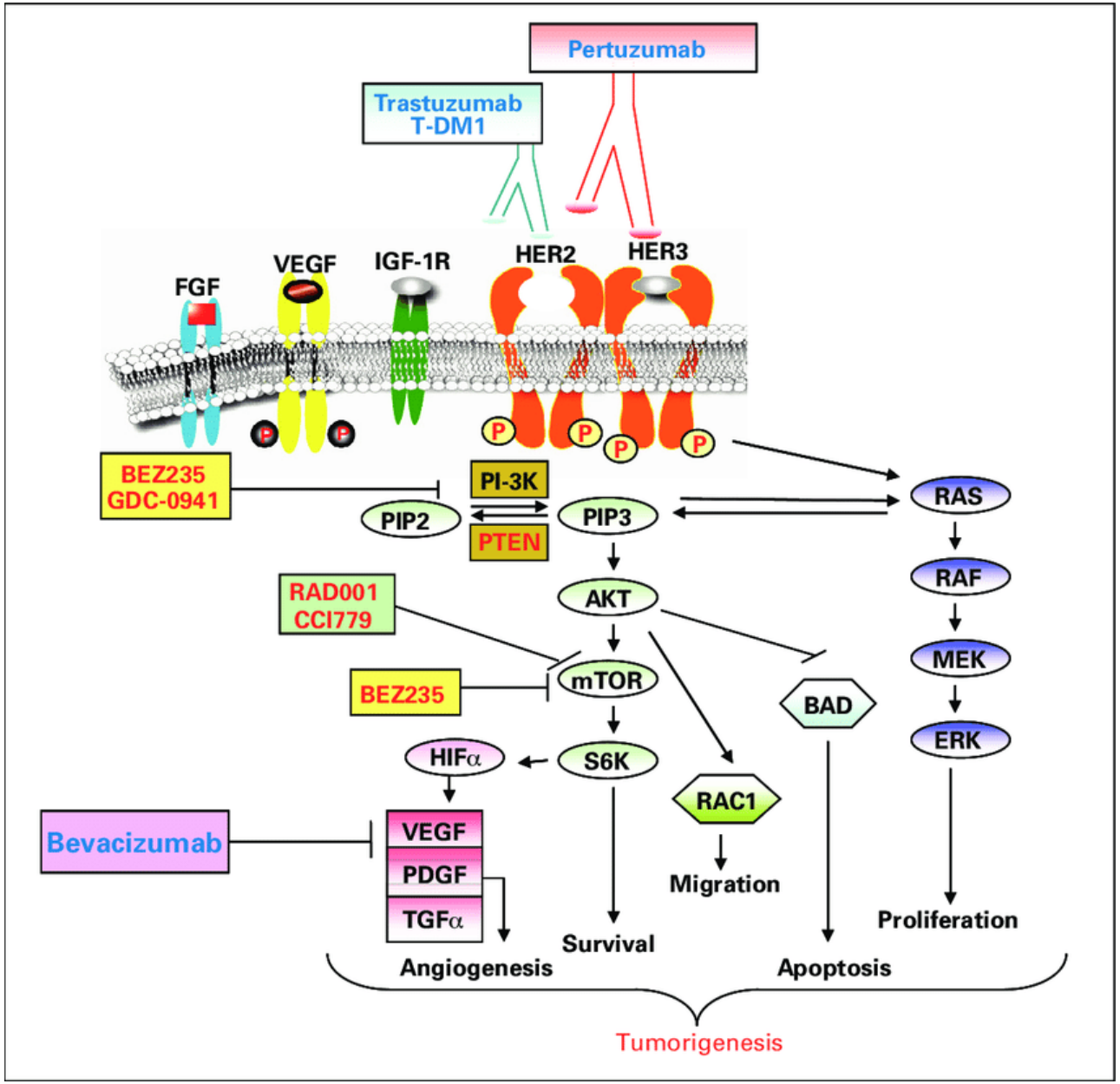
A. February 2020—The CAP has no official position on HER2 testing in colorectal

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

HER2 Signaling Cascade

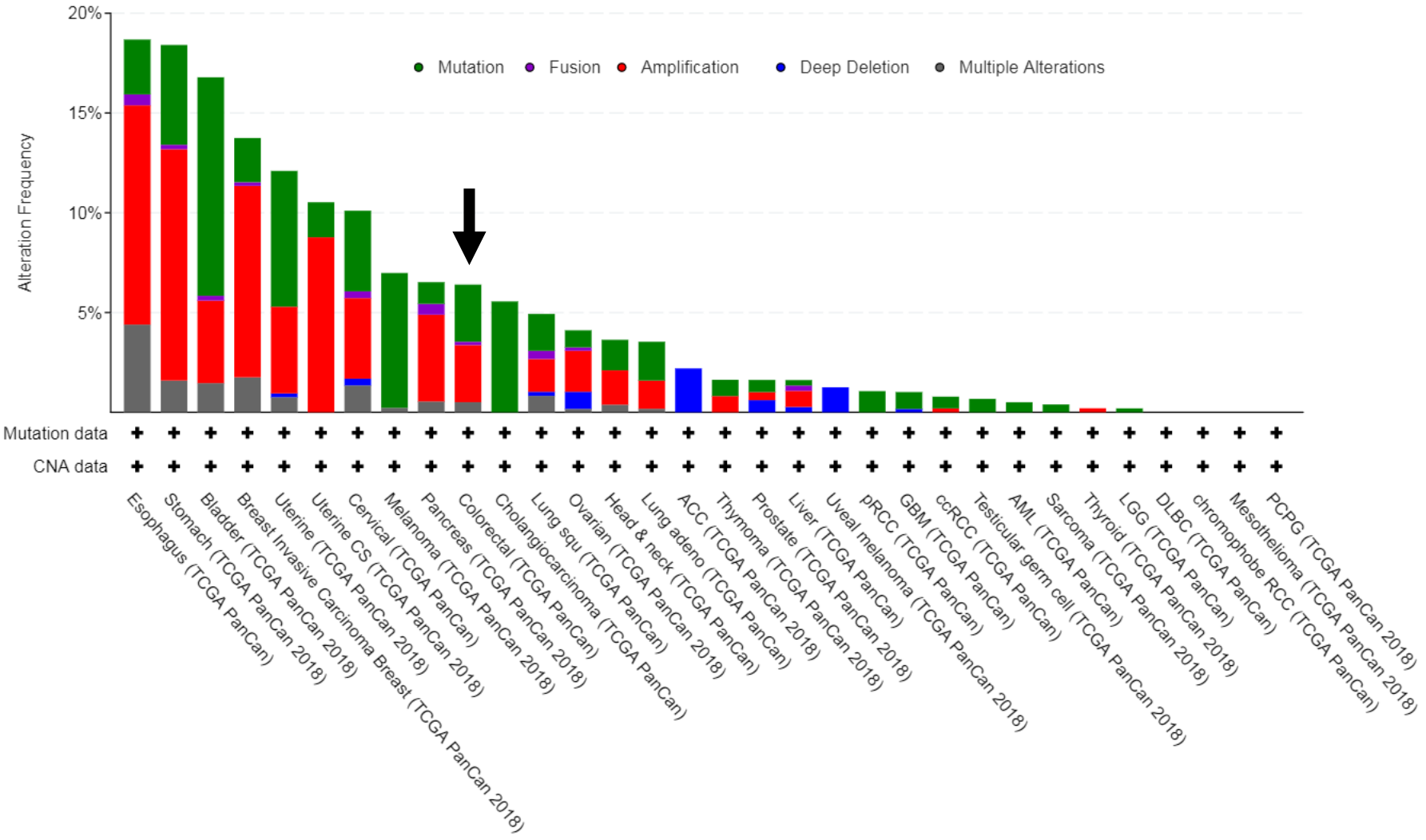


DOI:10.1200/JCO.2009.25.8624

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



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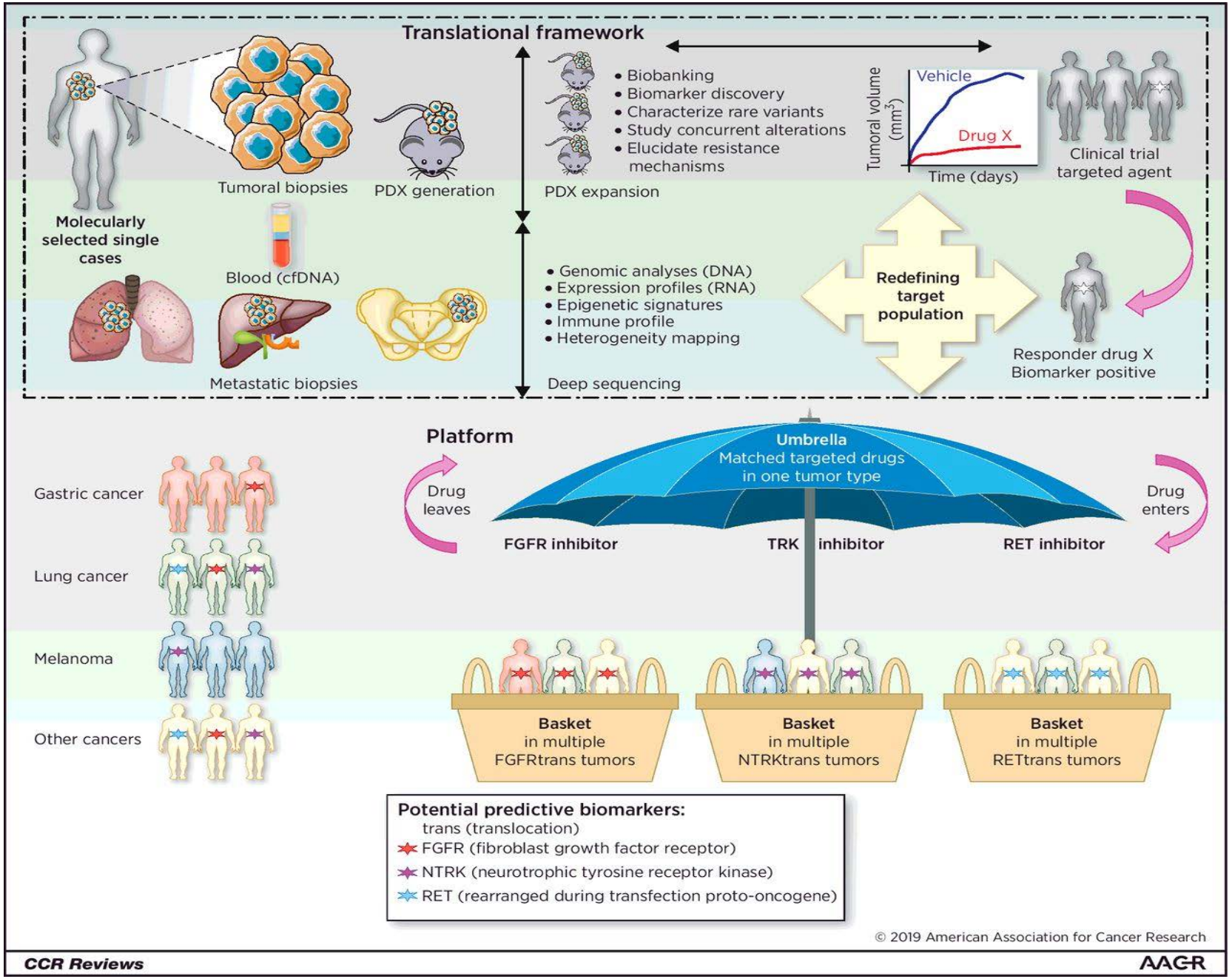
Precision Oncology Clinical Trial Designs



Webpage	Description	Date
FDA grants accelerated approval to pembrolizumab for HER2-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 approves fam-trastuzumab deruxtecan-nxki for HER2-positive gastric adenocarcinomas	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
FDA approves margetuximab for metastatic HER2-positive breast cancer	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
FDA approves combination of pertuzumab, trastuzumab, and hyaluronidase-zzxf for HER2-positive breast cancer	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
FDA approves tucatinib for patients with HER2-positive metastatic breast cancer	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
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

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NCCN Guidelines

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Colon Cancer

Version 3.2021 — September 10, 2021

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NCCN Guidelines: Who to Test

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Colon Cancer

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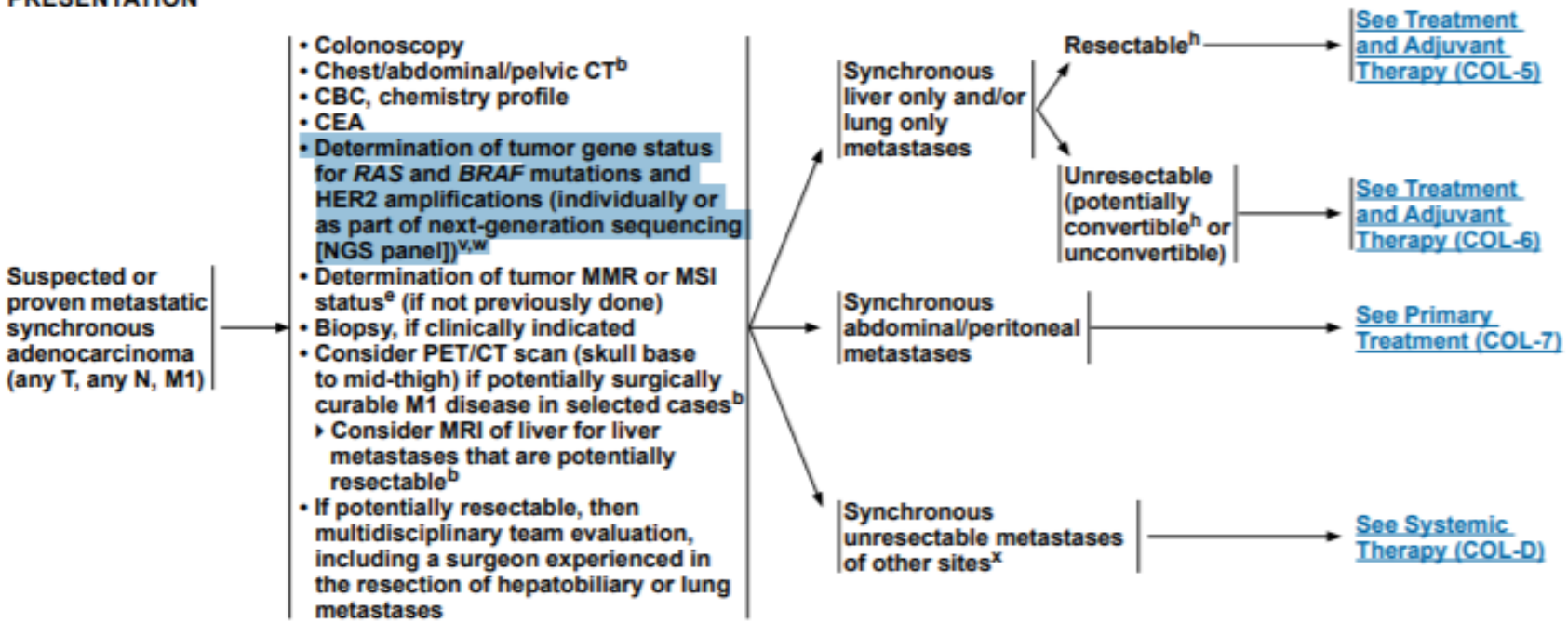

[b] See Principles of Imaging (COL-A).
[e] See Principles of Pathologic Review (COL-B 4 of 8) - MSI or MMR Testing.
[h] See Principles of Surgery (COL-C 2 of 3).
[v] See Principles of Pathologic Review (COL-B 4 of 8) - *KRAS*, *NRAS*, and *BRAF* Mutation Testing.
[w] If known *RAS/RAF* mutation, HER2 testing is not indicated. NGS panels have the ability to pick up rare and actionable mutations and fusions.
[x] 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

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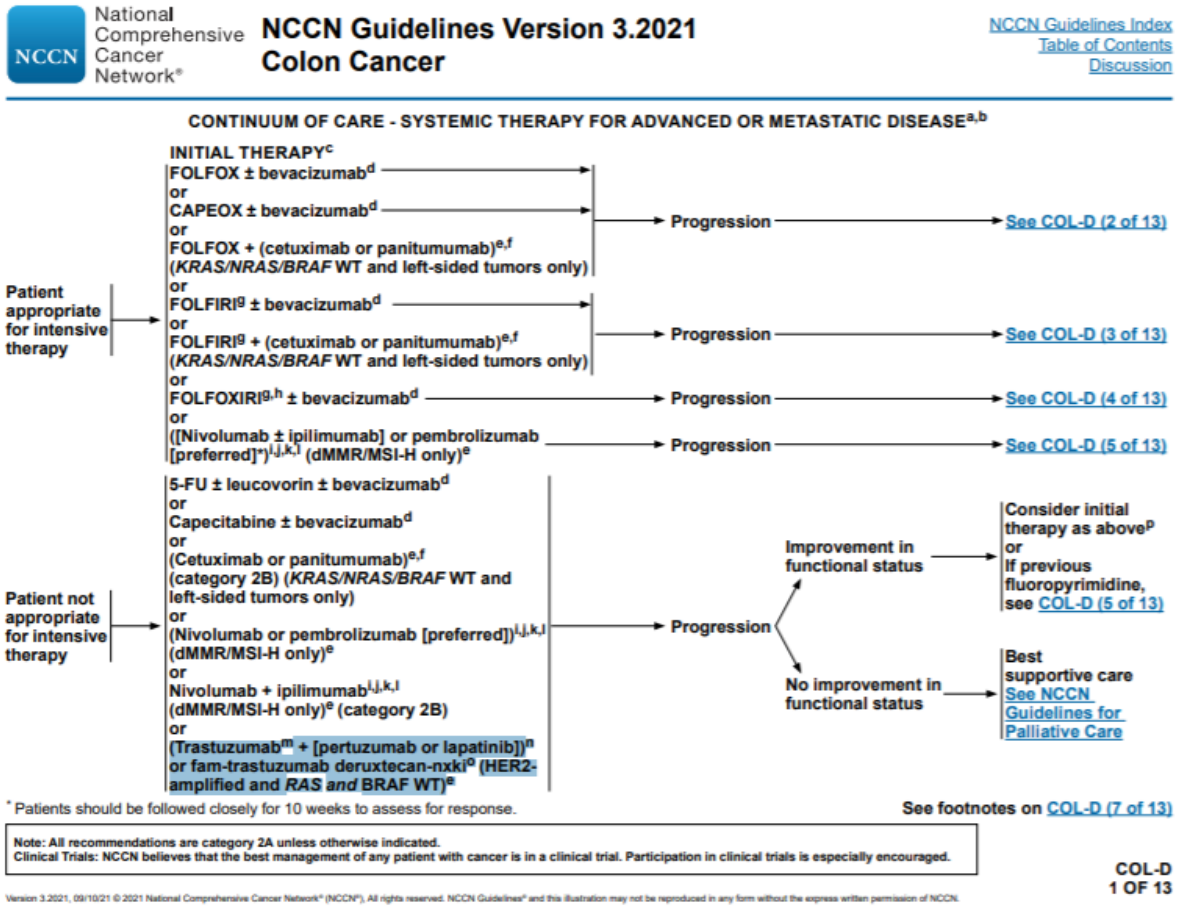
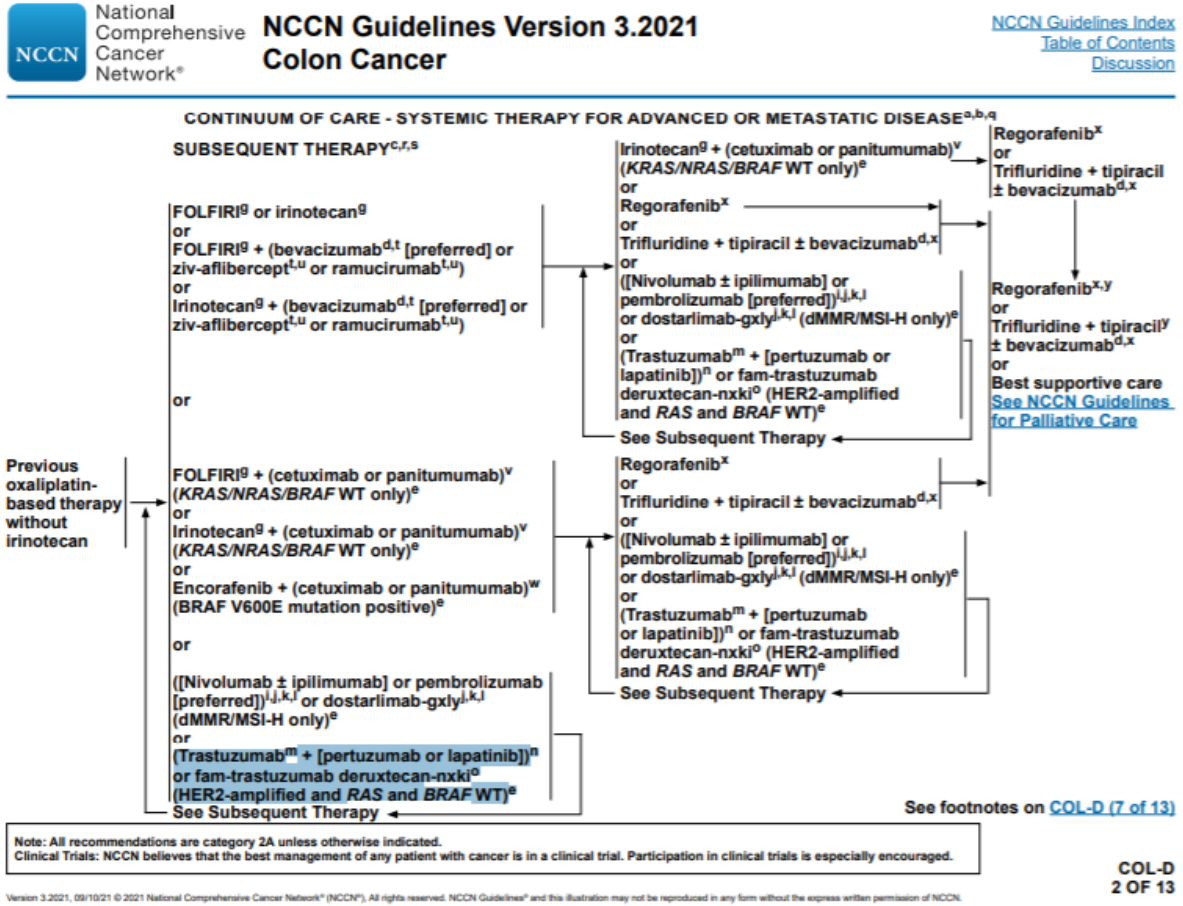
PRINCIPLES OF PATHOLOGIC REVIEW

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





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)
  - 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

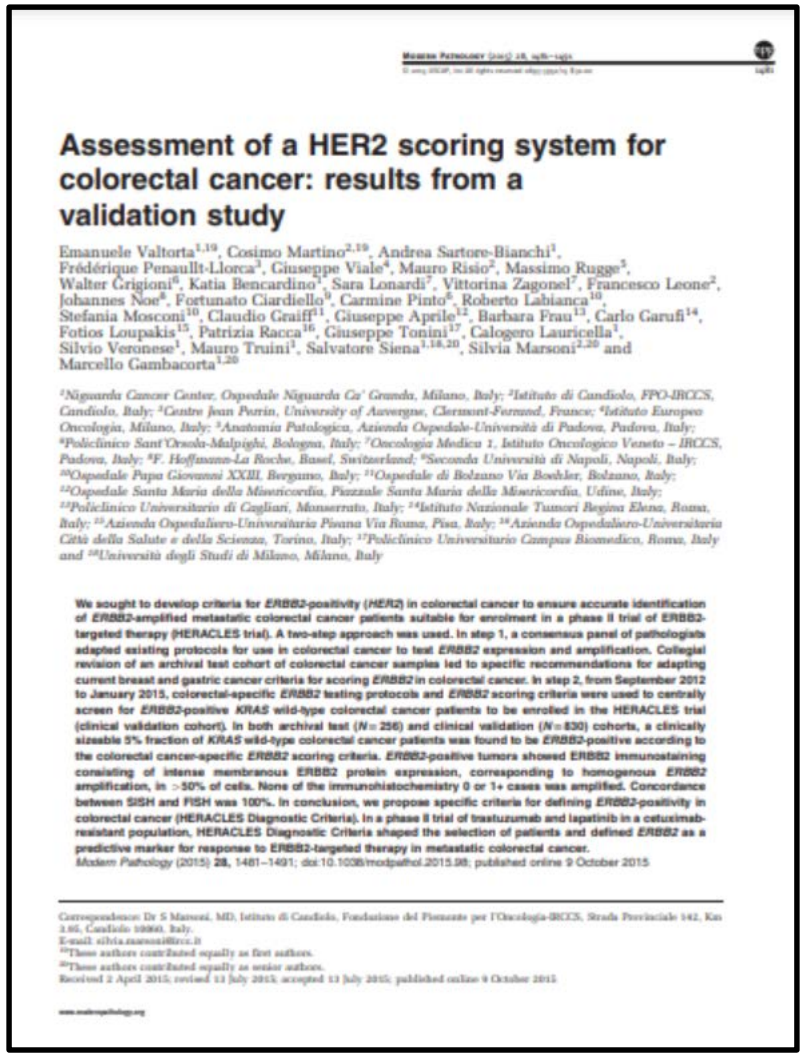
Ventana/ToGA/Ruschoff/Hoffman vs HERACLES IHC Criteria

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 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)	HER2:CEP17 ratio ≥2.0 in ≥50% of 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-Bianchi[1], Frédérique Penault-Llorca[3], Giuseppe Viale[4], Mauro Risio[2], Massimo Rugge[5], Walter Grigioni[6], Katia Bencardino[1], Sara Lonardi[7], Vittorina Zagonel[7], Francesco Leone[2], Johannes Noe[8], Fortunato Ciardiello[9], Carmine Pinto[6], Roberto Labianca[10], Stefania Mosconi[10], Claudio Graiff[11], Giuseppe Aprile[12], Barbara Frau[13], Carlo Garufi[14], Fotios Loupakis[15], Patrizia Racca[16], Giuseppe Tonini[17], Calogero Lauricella[1], Silvio Veronese[1], Mauro Truini[1], Salvatore Siena[1,18,20], Silvia Marsoni[2,20] and Marcello Gambacorta[1,20]

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We sought to develop criteria for *ERBB2*-positivity (*HER2*) in colorectal cancer to ensure accurate identification of *ERBB2*-amplified metastatic colorectal cancer patients suitable for enrolment in a phase II trial of ERBB2-targeted therapy (HERACLES trial). A two-step approach was used. In step 1, a consensus panel of pathologists adapted existing protocols for use in colorectal cancer to test *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 scoring *ERBB2* in colorectal cancer. In step 2, from September 2012 to January 2015, colorectal-specific *ERBB2* testing protocols and *ERBB2* scoring criteria were used to centrally screen 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 *KRAS* wild-type colorectal cancer patients was found to be *ERBB2*-positive according to the colorectal cancer-specific *ERBB2* scoring criteria. *ERBB2*-positive tumors showed ERBB2 immunostaining consisting of intense membranous ERBB2 protein expression, corresponding to homogenous *ERBB2* amplification, in >50% of cells. None of the immunohistochemistry 0 or 1+ cases was amplified. Concordance between SISH and FISH was 100%. In conclusion, we propose specific criteria for defining *ERBB2*-positivity in colorectal cancer (HERACLES Diagnostic Criteria). In a phase II trial of trastuzumab and lapatinib in a cetuximab-resistant 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

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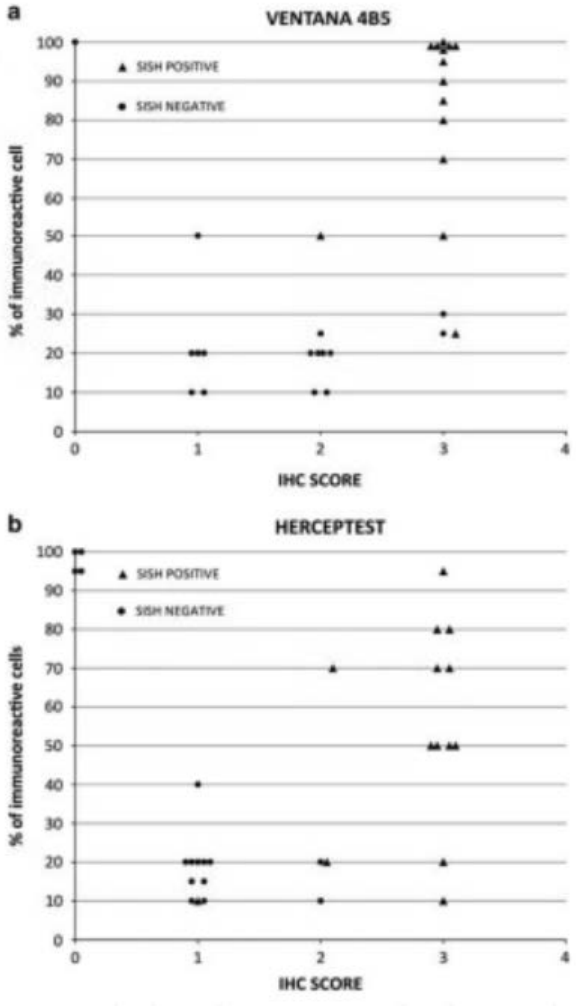
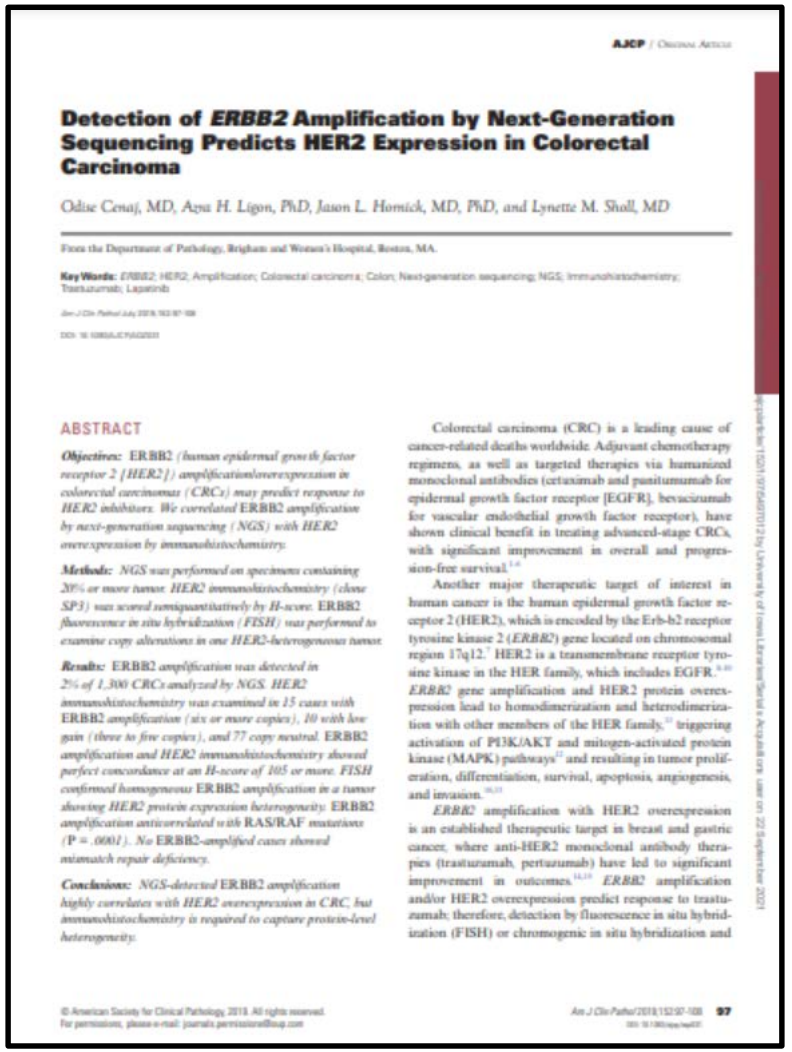


- Compared Ventana Pathway (4B5) FDA-approved kit to Dako A0485 polyclonal antibody to predict ISH positivity at the 50% ISH threshold
- Ventana assay most accurate at 50% IHC threshold, while Dako assay most accurate at 10% IHC threshold

NGS as a Surrogate for Overexpression/Amplification



AJCP / Original Article

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

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Key Words: *ERBB2*; HER2; Amplification; Colorectal carcinoma; Colon; Next-generation sequencing; NGS; Immunohistochemistry; Trastuzumab; Lapatinib

ABSTRACT

Objectives: ERBB2 *(human epidermal growth factor receptor 2 [HER2]) amplification/overexpression in colorectal carcinomas (CRCs) may predict response to HER2 inhibitors. We correlated* ERBB2 *amplification by next-generation sequencing (NGS) with HER2 overexpression by immunohistochemistry.*

Methods: *NGS was performed on specimens containing 20% or more tumor. HER2 immunohistochemistry (clone SP3) was scored semiquantitatively by H-score.* ERBB2 *fluorescence in situ hybridization (FISH) was performed to examine copy alterations in one HER2-heterogeneous tumor.*

Results: ERBB2 *amplification was detected in 2% of 1,300 CRCs analyzed by NGS. HER2 immunohistochemistry was examined in 15 cases with* ERBB2 *amplification (six or more copies), 10 with low gain (three to five copies), and 77 copy neutral.* ERBB2 *amplification and HER2 immunohistochemistry showed perfect concordance at an H-score of 105 or more. FISH confirmed homogeneous* ERBB2 *amplification in a tumor showing HER2 protein expression heterogeneity.* ERBB2 *amplification anticorrelated with* RAS/RAF *mutations ($P = .0001$). No* ERBB2*-amplified cases showed mismatch repair deficiency.*

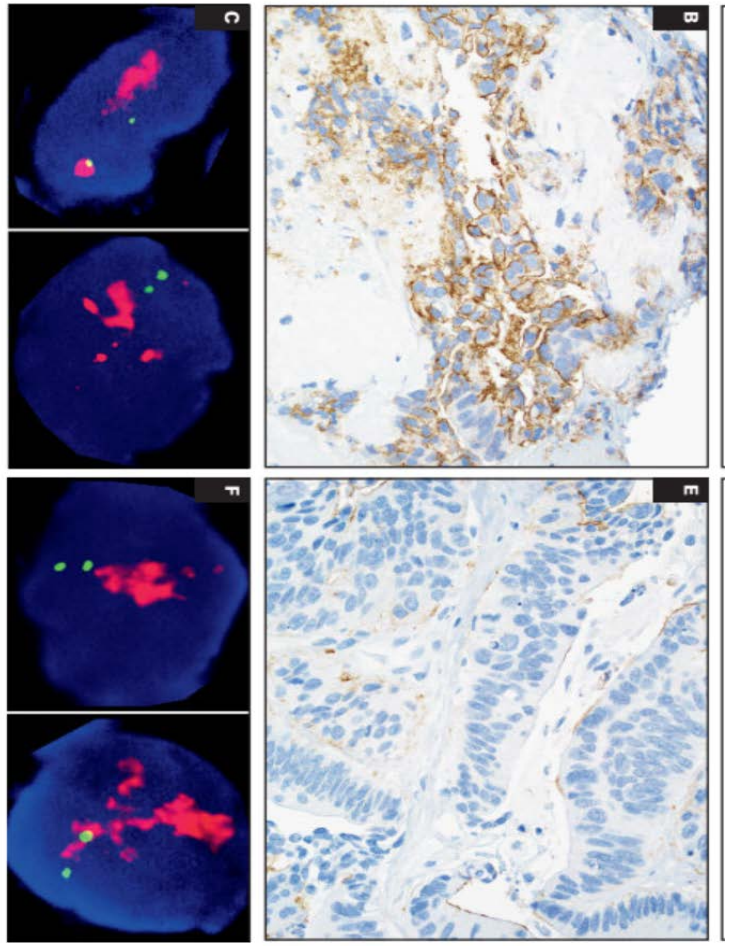
Conclusions: *NGS-detected* ERBB2 *amplification highly correlates with HER2 overexpression in CRC, but immunohistochemistry is required to capture protein-level heterogeneity.*

Colorectal carcinoma (CRC) is a leading cause of cancer-related deaths worldwide. Adjuvant chemotherapy regimens, as well as targeted therapies via humanized monoclonal antibodies (cetuximab and panitumumab for epidermal growth factor receptor [EGFR], bevacizumab for vascular endothelial growth factor receptor), have shown clinical benefit in treating advanced-stage CRCs, with significant improvement in overall and progression-free survival.[1-6]

Another major therapeutic target of interest in human cancer is the human epidermal growth factor receptor 2 (HER2), which is encoded by the Erb-b2 receptor tyrosine kinase 2 (*ERBB2*) gene located on chromosomal region 17q12.[7] HER2 is a transmembrane receptor tyrosine kinase in the HER family, which includes EGFR.[8-10] *ERBB2* gene amplification and HER2 protein overexpression lead to homodimerization and heterodimerization with other members of the HER family,[11] triggering activation of PI3K/AKT and mitogen-activated protein kinase (MAPK) pathways[12] and resulting in tumor proliferation, differentiation, survival, apoptosis, angiogenesis, and invasion.[10,13]

ERBB2 amplification with HER2 overexpression is an established therapeutic target in breast and gastric cancer, where anti-HER2 monoclonal antibody therapies (trastuzumab, pertuzumab) have led to significant improvement in outcomes.[14,15] *ERBB2* amplification and/or HER2 overexpression predict response to trastuzumab; 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



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

HER2 Immunohistochemistry and *ERBB2* Amplification by NGS

Characteristic	*ERBB2* Copy Number by NGS: Amplified	Low Gain	Neutral	Total Nonamplified	Sensitivity, % (95% CI)	Specificity, % (95% CI)
HER2 IHC result by HERACLES diagnostic criteria						
Positive	7	0	0	0	47 (0.21-0.73)	100 (0.96-1.00)
Equivocal	7	0	0	0		
Negative	1	10	77	87		
HER2 IHC result by HERACLES diagnostic criteria (including equivocal)						
Positive or equivocal	14	0	0	0	93 (0.68-1.00)	100 (0.96-1.00)
Negative	1	10	77	87		
HER2 IHC result by cutpoint proposed in this study (H-score ≥105)						
Positive	15	0	0	0	100 (0.78-1.00)	100 (0.96-1.00)
Negative	0	10	77	87		

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
- **Uterine Neoplasms: 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 updates

Bob T. Li, Ronglai Shen, Michael Offin, Darren J. Buonocore, Mackenzie L. Myers, Aishwarya Venkatesh, ...

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Abstract Disclosures

Abstract

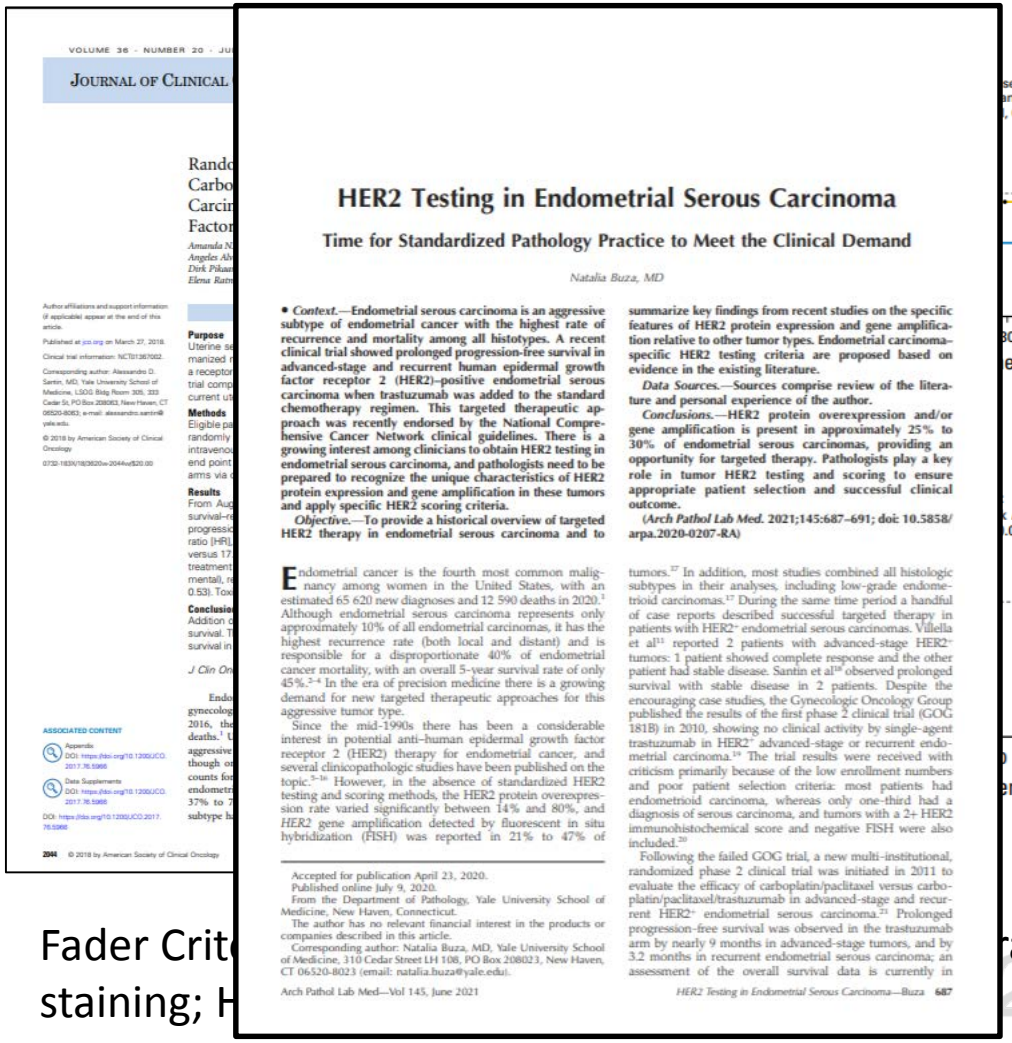
6001

Background: SGCs are rare tumors with no approved therapy for metastatic disease. *HER2* amplification occurs in 8% 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 Simon two-stage optimal design was applied with type I error rate under 2.7%, power of 89%, H0 10%, H1 40%; H0 will be rejected if 6 or more responses are observed in 24 patients. Other endpoints include duration of response (DOR), progression-free survival (PFS), and toxicity. *HER2* amplification was identified by next generation sequencing (NGS), and tumors were subsequently tested by 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 *HER2* amplified SGCs 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 reaction, thrombocytopenia and transaminitis; there were no treatment related deaths. *HER2* amplification by NGS (fold change 2.8 to 22.8) correlated with HER2/CEP17≥2 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 *HER2* 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.

- HER2 targeted therapy for HER2+ tumors[a]
  - Trastuzumab[b,6]
  - Ado-trastuzumab emtansine (TDM-1)[7]
  - Trastuzumab/pertuzumab[b,8]
  - Docetaxel/trastuzumab[b,9]

NCCN Guideline references 2018 ASCO/CAP Breast Criteria



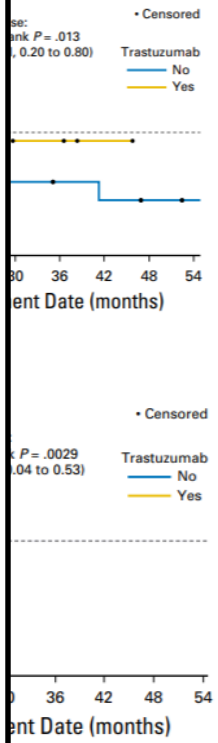
JOURNAL OF CLINICAL

HER2 Testing in Endometrial Serous Carcinoma

Time for Standardized Pathology Practice to Meet the Clinical Demand

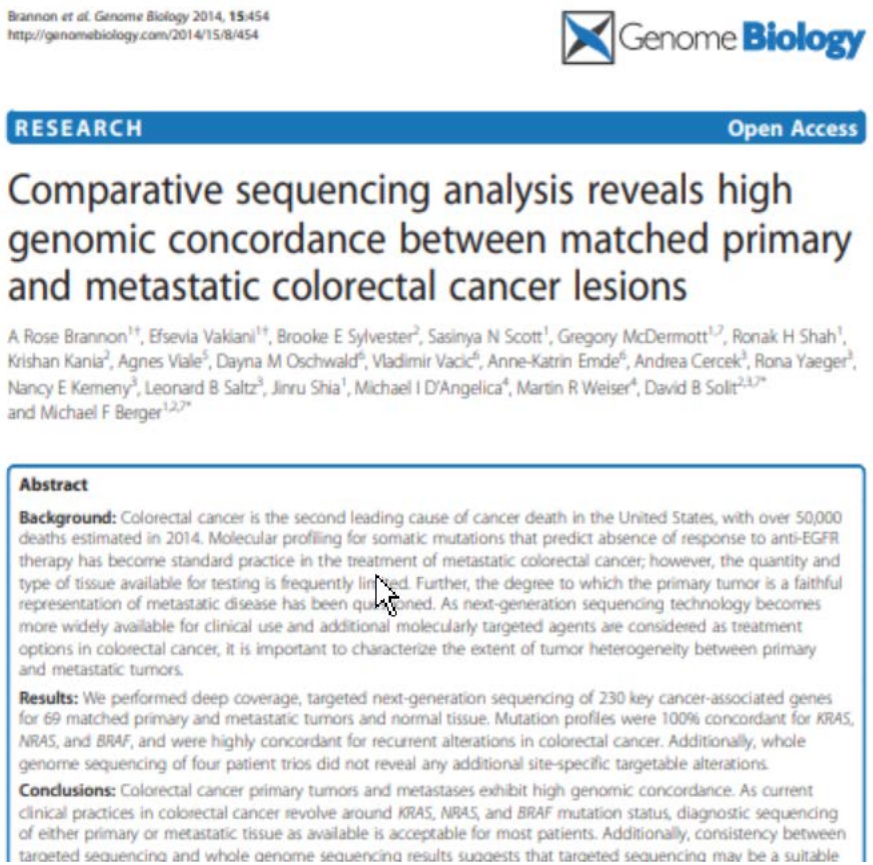
Natalia Buza, MD

Fader Crit staining; H



ral/lateral

What Tissue to Test (Primary vs Metastasis)



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

Genome Biology

RESEARCH Open Access

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

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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 questioned. 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 tumors 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 targeted sequencing and whole genome sequencing results suggests that targeted sequencing may be a suitable

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

- 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 primary-metastatic pairs IHC concordant (positive in 3, negative in 41, positive in primary-negative in metastasis in 1)

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

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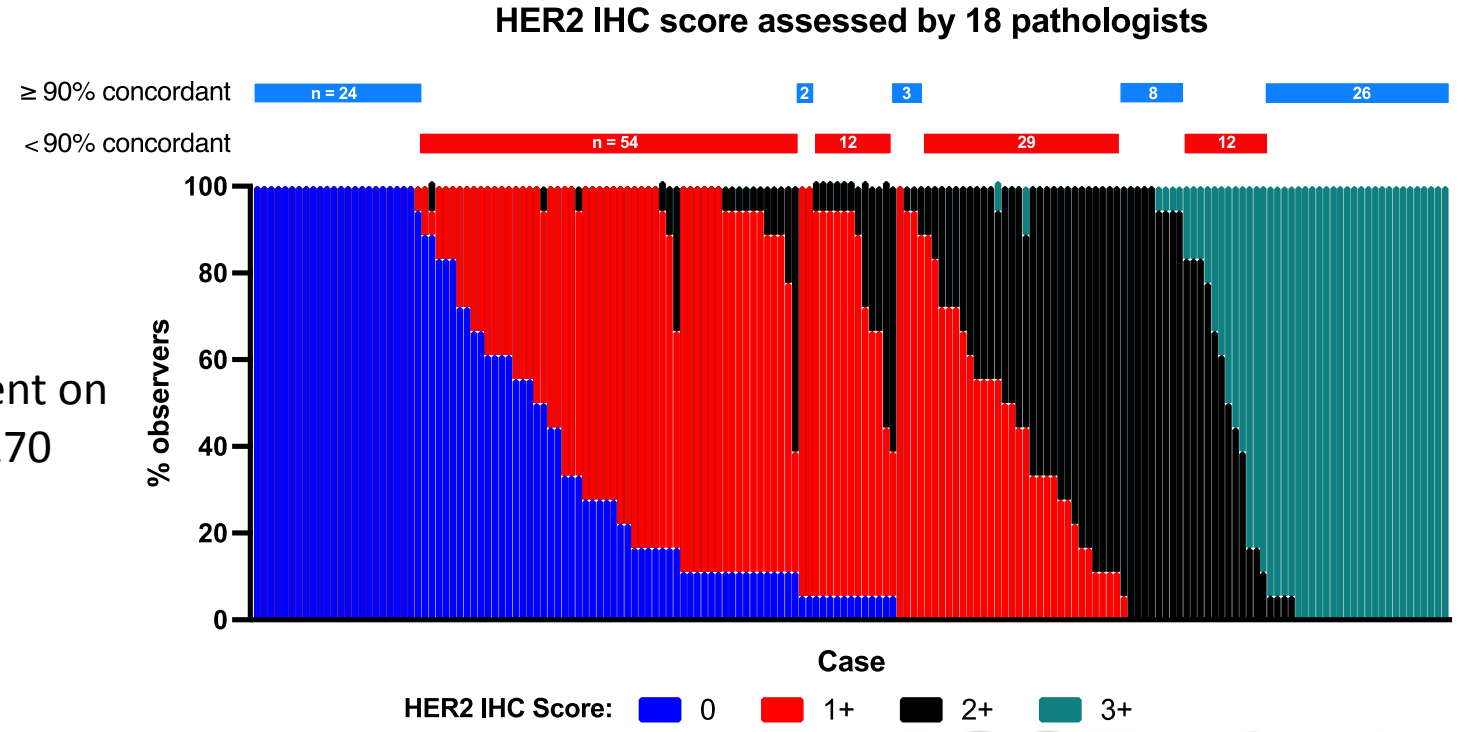
≥90% agreement on 37% of 170 cases



DOI https://doi.org/10.1200/JCO.19.02318

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) adenocarcinoma	MMR, Iowa Cancer Mutation Profiling (with fusion for 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

*repeat GHER2 on resection, if negative on the biopsy

- 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

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 ISH-positivity 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