HER2 Testing in Gynecological Cancer

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Making Cancer History®

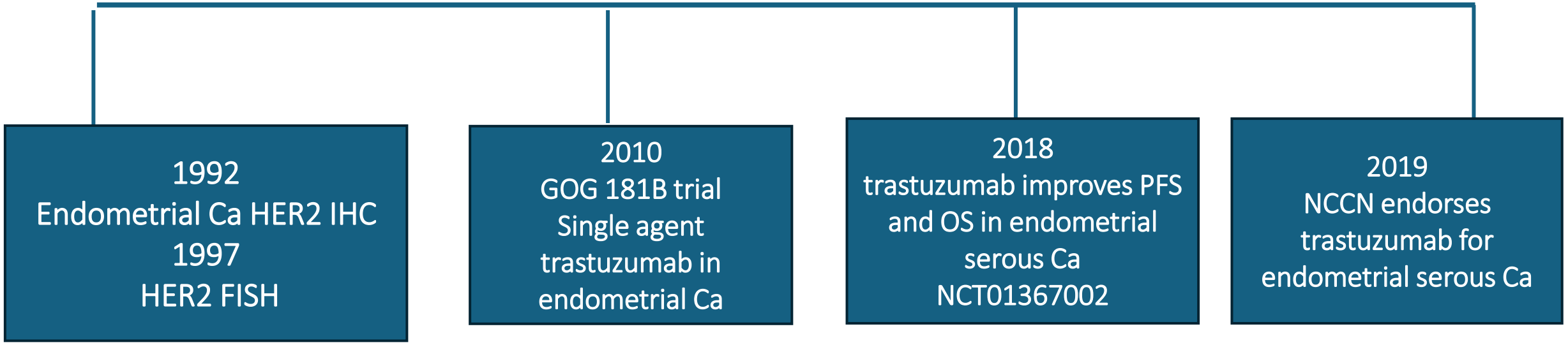
HER2, What Is It?

- Human epidermal growth factor receptor 2 (HER2)
  - A member of the EGFR family of transmembrane tyrosine kinases [HER1 (ERBB1), HER2 (ERBB2), HER3 (ERBB3), and HER4(ERBB4)]
    - Activation occurs as a result of dimerization of receptors (homo or heterodimerization)
      - Phosphorylation of the intracellular domains
        - Activation of various pathways involved in proliferation, survival, migration and differentiation

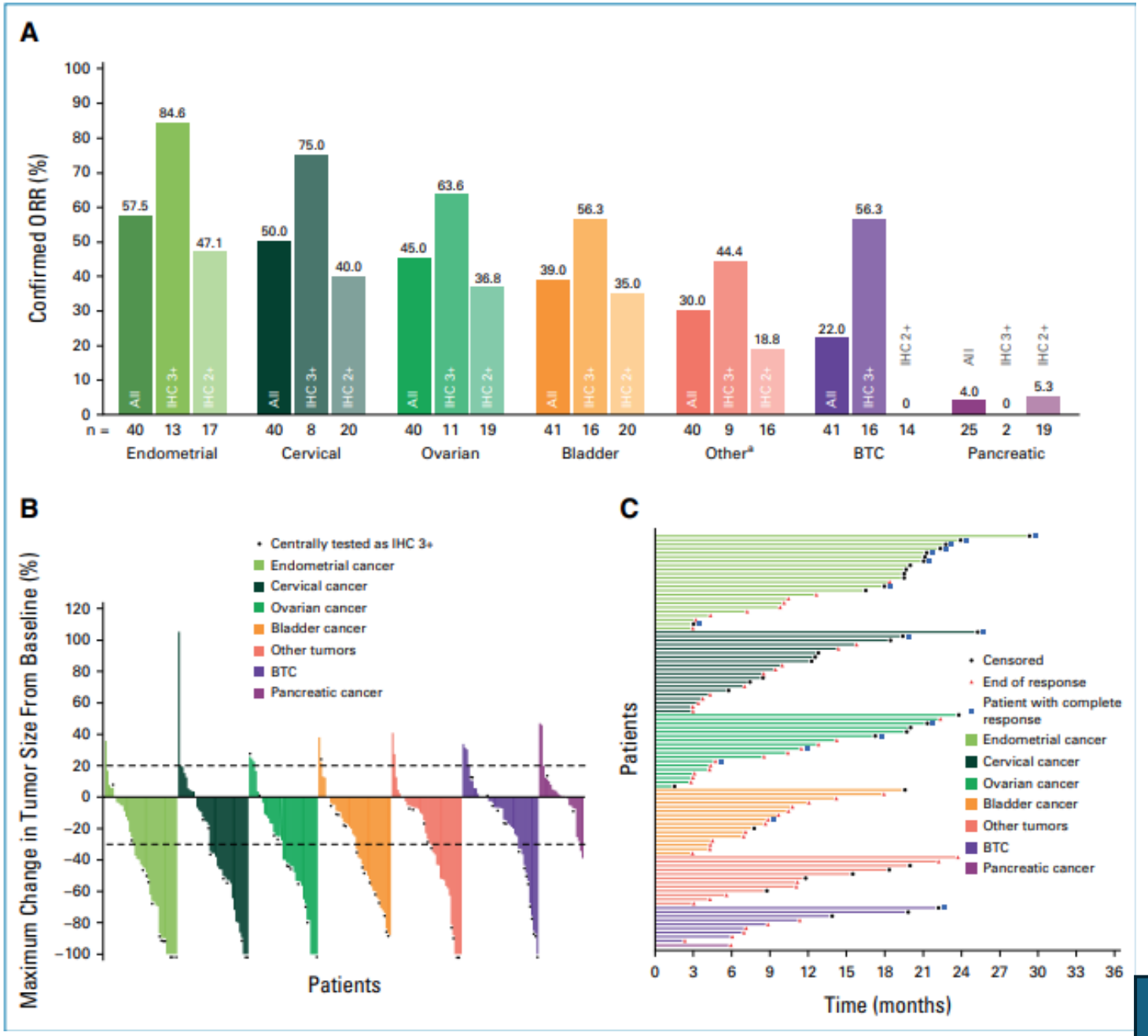
HER2, What Is It?

- Human epidermal growth factor receptor 2 (HER2)
  - HER2 is a preferred partner for heterodimer formation with HER1, HER3 and HER4
  - Heterodimers containing HER2 have a stronger and more diverse signaling potential than other receptor dimers
  - HER2 may apply its oncogenic capacity through
    - Overexpression of the wild-type form due to gene amplification
    - Transcriptional deregulation
    - Activating mutations

Her-2 and anti-Her2 agents in GYN Oncology, Timeline

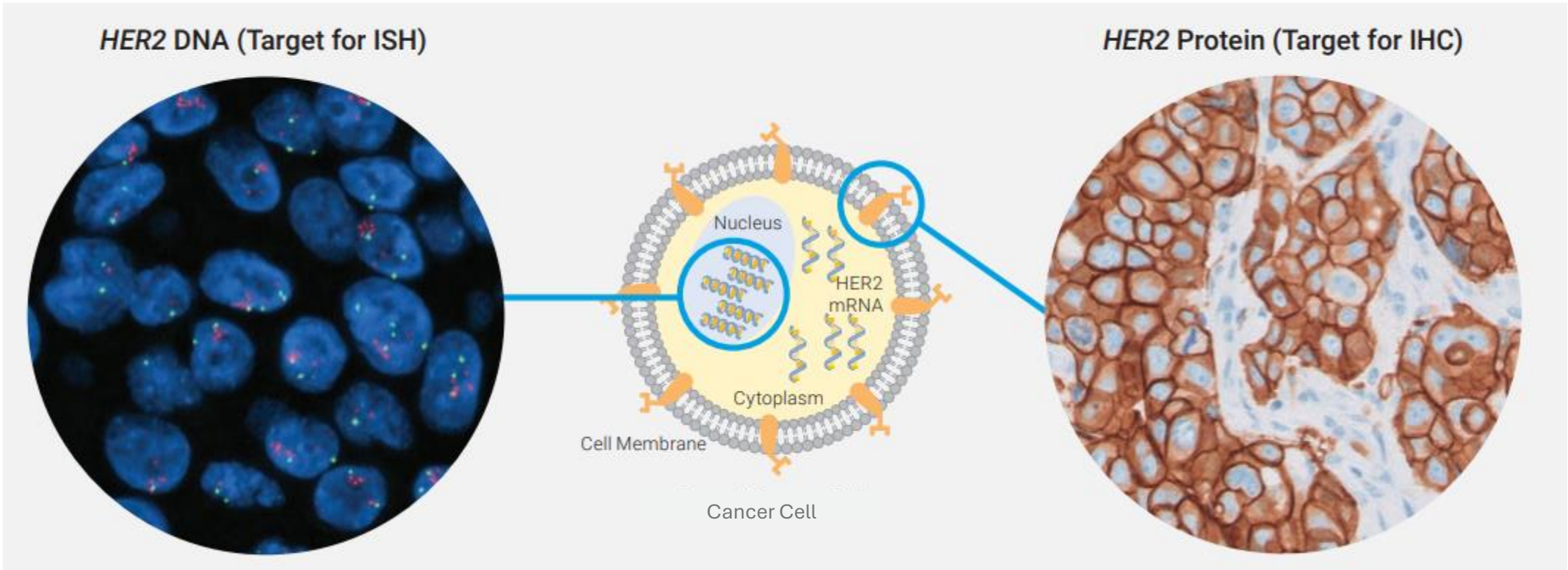


Her-2 and anti-Her2 agents in GYN Oncology, Timeline



Meric-Bernstam F, et al. 2024

HER2, What Can We Test?



Modified from Hercep Test manual (Agilent)

HER2, What Can We Test ?

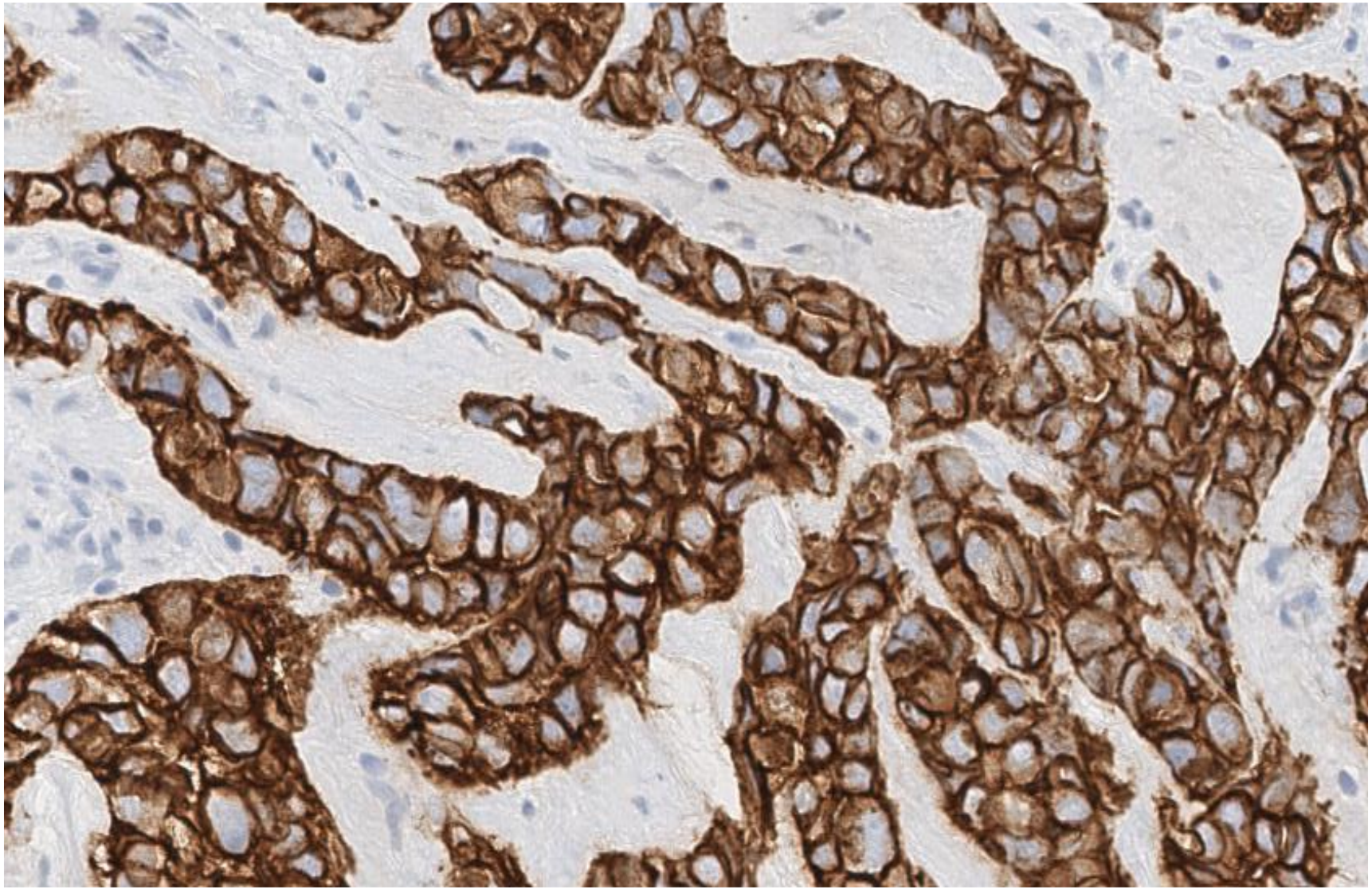
- Next generation sequencing (NGS)
  - Not used as part of the guidelines
  - Activating HER2 mutations in endometrial Ca: 3.5 %, Cervical Ca: 3.3%, ovarian epithelial tumors: 0.7%

  - Endometrial Ca
    - Concordance rate between NGS-IHC/ISH: 91.6% and 100%
      - Highest concordance rate seen in cohorts composed of HER2 negative tumors
      - Most discordant results in HER2 positive tumors most likely due to intratumoral heterogeneity

HER2, What tissue to test ?

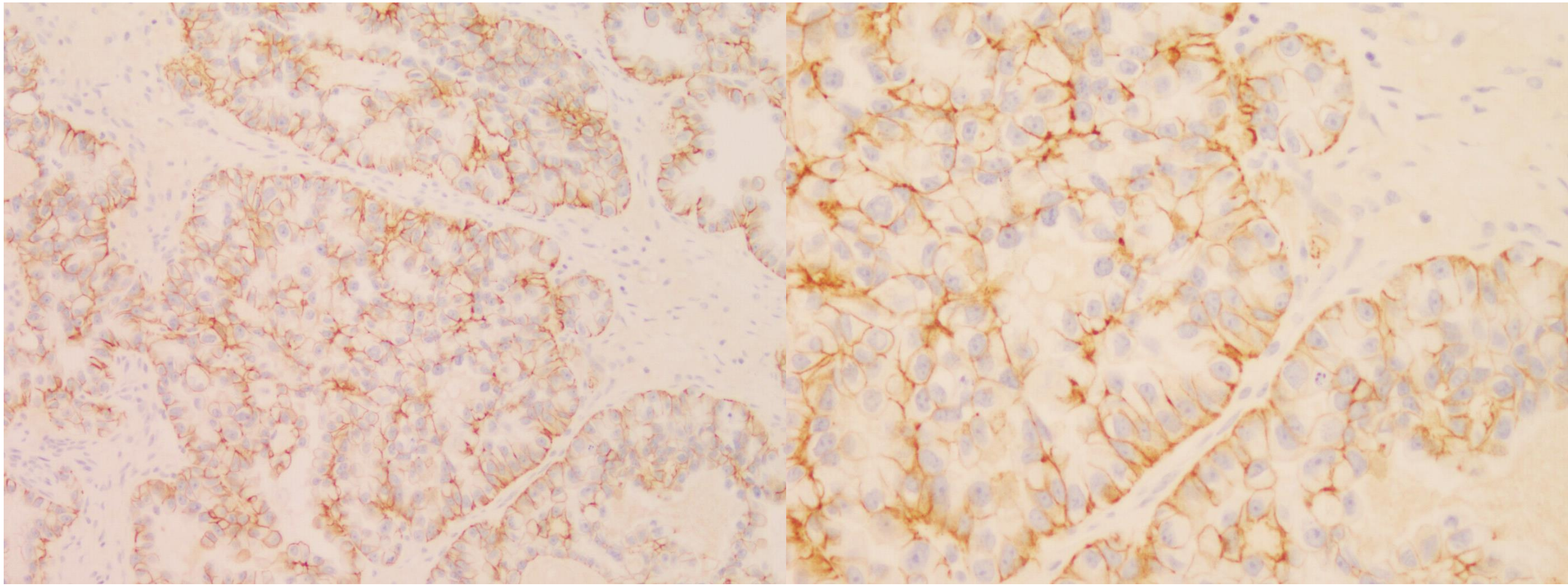
Bx or surgical specimen

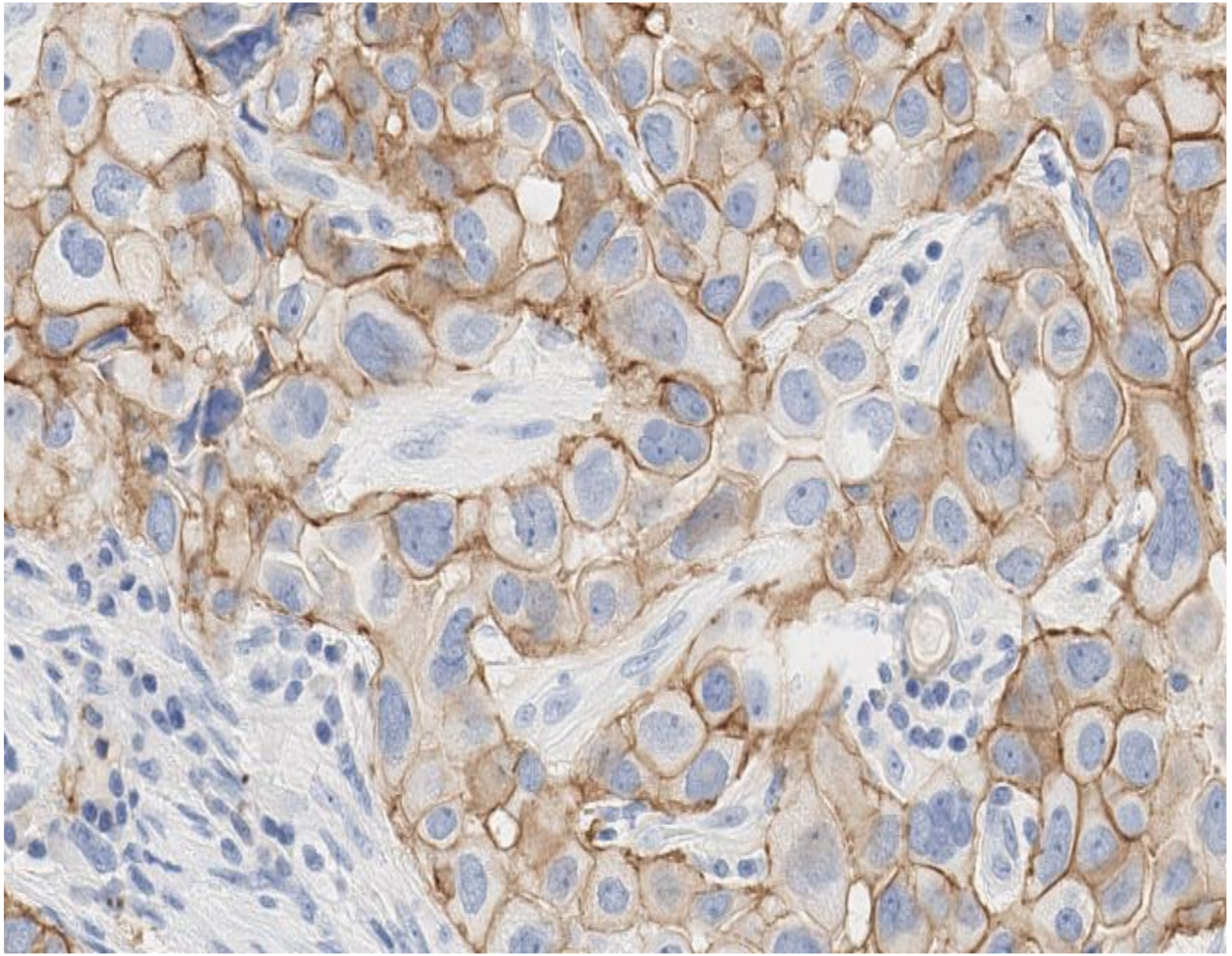
Testing multiple specimens is encouraged



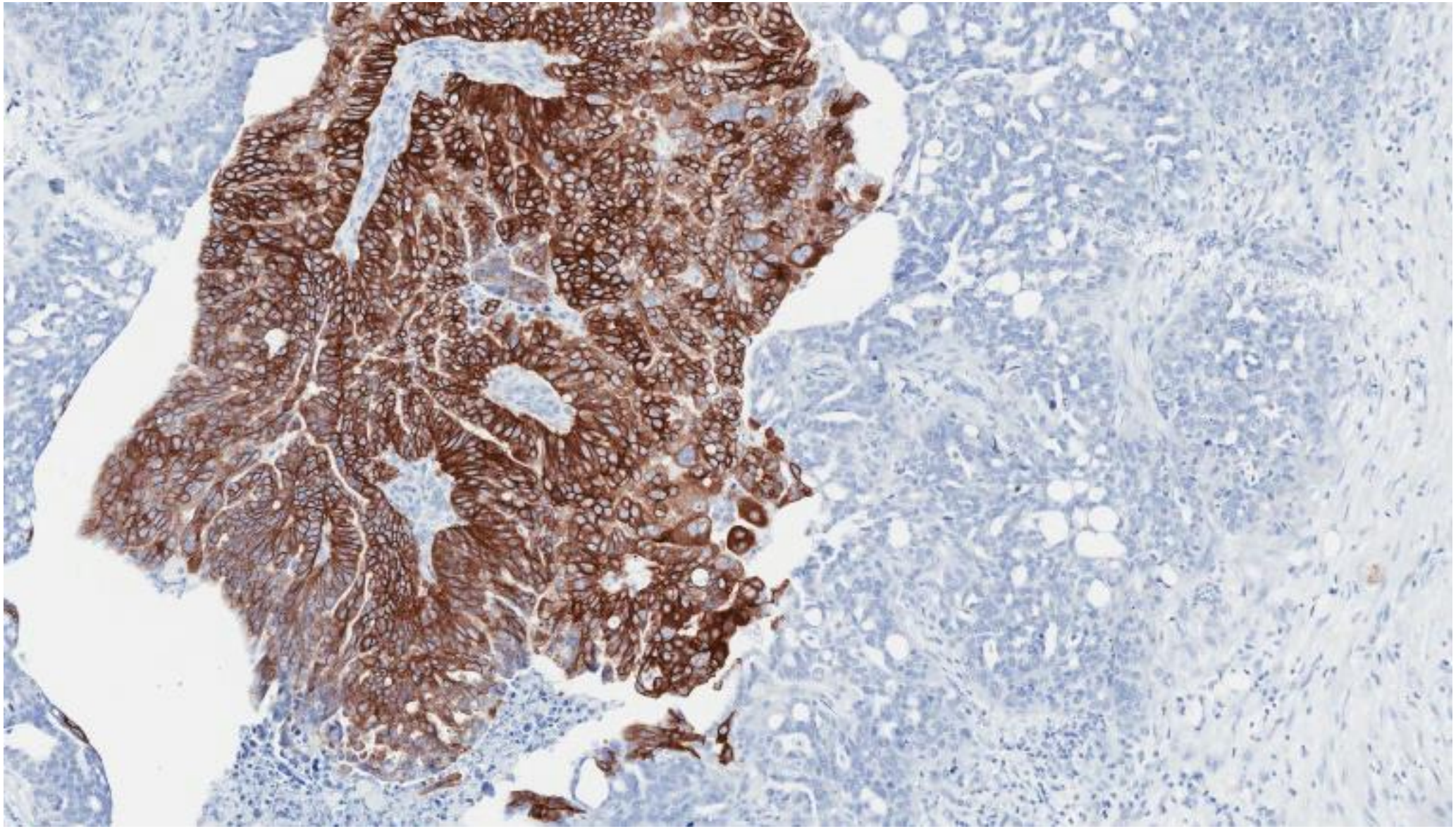
Her2 complete membranous, ring shape staining

Clear cell carcinoma of the ovary
HER2 basolateral staining





Her2 membranous, combination of complete and basolateral staining



Her2 heterogeneous staining

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Version: 1.2.0.0
Protocol Posting Date: December 2024

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Version Contributors
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HER2 Status for Trastuzumab Use

- Negative (score 0) for protein overexpression
  - No staining in tumor cells

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HER2 Status for Trastuzumab Use

- Negative (score 1+) for protein overexpression *
  - Incomplete membrane and faint/barely perceptible staining in any proportion of cells
  - Complete and weak staining in <10 % of tumor cells

*Clinicians may ask you to sign the case as Her2 score 1+

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HER2 Status for Trastuzumab Use

- Equivocal (score 2+) for protein overexpression*
  - Strong complete or basolateral/lateral membrane staining in ≤ 30% of the cells
  - Weak to moderate staining in ≥ 10% of tumor cells

*Reflex Her2 FISH

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HER2 Status for Trastuzumab Use

- Positive (score 3+) for protein overexpression
  - Strong complete or basolateral/lateral membrane staining in > 30% of the tumor cells

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HER2 Status for Trastuzumab-Deruxtecan Use

- Different assessment criteria for biopsies and surgical specimens
- Tumor cell cluster: 5 or more tumor cells
- No HER2 FISH for equivocal cases (score 2+)

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HER2 Status for Trastuzumab-Deruxtecan Use

- Negative (score 0) for protein overexpression
  - Bx
    - no staining in any tumor cells
  - Surgical specimen
    - no staining in any tumor cells
    - membrane staining in <10 % of tumor cells

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HER2 Status for Trastuzumab-Deruxtecan Use

- Negative (score 1+) for protein overexpression*
  - Bx
    - Tumor cell cluster with a faint/barely perceptible membrane staining irrespective of percentage of positive tumor cells
  - Surgical specimen
    - Faint/barely perceptible, incomplete membrane staining in ≥ 10 % of tumor cells

*Clinicians may ask you to sign the case as Her2 score 1+

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HER2 Status for Trastuzumab-Deruxtecan Use

- Equivocal (score 2+) for protein overexpression
  - Bx
    - Tumor cell cluster with a weak to moderate, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells
  - Surgical specimen
    - Weak to moderate, complete, basolateral or lateral membrane staining ≥ 10 % of tumor cells

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HER2 Status for Trastuzumab-Deruxtecan Use

- Positive (score 3+) for protein overexpression
  - Bx
    - Tumor cell cluster with a strong, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells
  - Surgical specimen
    - Strong, complete, basolateral or lateral membrane staining in ≥ 10 % of tumor cells

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Information to be included in the report

Test Type

- FDA cleared (specify test/ vendor)
- Laboratory-developed test
  - Non-US-based health system
    - Health Canada approved (specify test/vendor)
    - Other (specify)

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Primary Antibody

- 4B5
- HercepTest
- A0485
- SP3
- EP3
- CB11
- Other (specify)

Reporting Results of HER2 Testing by Dual-Probe Fluorescence In Situ Hybridization (FISH) for Trastuzumab Use

Result	Criteria (Dual-probe Assay)
Negative for *HER2* gene amplification	FISH *HER2/CEP17* ratio <2.0 **and** average *HER2* copy number < 6 per nucleus
Positive for *HER2* gene amplification	FISH *HER2/CEP17* ratio ≥ 2.0 **or** FISH *HER2/CEP17* ratio <2.0 **and** average *HER2* copy number ≥ 6 per nucleus

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HER2 testing by FISH

- Heterogeneity at the genetic level may be encountered
  - A subset of tumor cells with gene amplification may be seen either as:
    - Group(s) of 20 or more cells (cluster amplification)
    - Isolated tumor cells (mosaic amplification)

- FISH signals should be counted in at least 20 contiguous tumor cells in conjunction with the highest protein expression by IHC

- Heterogeneity in *HER2* amplification should be reported when present

NCCN Guidelines Version 1.2025, Ovarian/Fallopian Tube/Peritoneal Cancer

Upfront setting

*BRCA*1/2 testing

Loss of heterozygosity (LOH)

Homologous recombination deficiency (HRD) status in the absence of a germline *BRCA* mutation

Recurrences

HER 2 status (by IHC)
*BRCA*1/2 testing
HRD status
Microsatellite instability
Mismatch repair proteins (MMR)
Tumor mutation burden (TMB)
BRAF
FOLR1
RET
NTRK

ESGO-ESMO-ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease

Ledermann JA, et al. 2024

*BRCA*1/2 testing for high grade non-mucinous tubo-ovarian Ca

Routine testing for homologous recombination deficiency (HRD) is not required, but encouraged in the research setting

Genomic instability tests are recommended in pts with *BRCA* wild-type high grade non-mucinous tubo-ovarian Ca, FIGO stages III-IV

TCGA-based molecular classification can be considered to stratify ovarian endometrioid Ca

MMR IHC and/or MSI testing is recommended in ovarian endometrioid and clear cell Ca

HER2 status in mucinous Ca can be considered to identify pts who may benefit from HER2-targeted strategies

KRAS and *BRAF* mutational status in low grade serous Ca may be considered to identify pts who may benefit from targeted therapy

Ovarian Carcinoma, Her2 +

- Mucinous Ca, 20-38%
- Ca Endometrioid Ca, 9%
- Clear Cell Ca , 9%
- Carcinosarcoma, 7%
- High grade serous Ca, 1-6%
- Low grade serous Ca, 0%

NCCN Guidelines Version 3.2025, Endometrial Carcinoma

Initial Dx

- MMR
  - IHC
    - MLH1 Loss
      - MLH1 promoter methylation
- p53 IHC
- POLE status
- HER2, advanced stage:
  - Serous Ca
  - Carcinosarcoma
  - p53 aberrant Ca
- ER, PR stages III or IV

Recurrence

- ER, PR
- HER2:
  - Serous Ca
  - Carcinosarcoma
  - p53 aberrant Ca
- Tumor mutation burden (TMB)
- *NTRK* fusion

HER2 in Endometrial Carcinoma, Carcinosarcoma

- Serous Ca, 25%-30% of cases are HER2 + using the endometrial scoring system for IHC
  - Some cases with heterogeneity, show HER2 overexpression or amplification in the myoinvasive component or intravascular tumor
  - >90% of HER2 amplified endometrial carcinomas have *TP53* mutations
  - Carcinosarcoma, 15% to 18% of cases are HER2 +

NCCN Guidelines Version 4.2025, Cervical Cancer

Initial Dx

- HPV status in AdenoCas
  - ISH or molecular testing
    - p16 is acceptable
- HER 2, advanced stage

Progressive or recurrent disease

- PD-L1
- HER 2
- NGS
- MMR
- TMB
- *NTRK* fusion
- *RET* fusion

HER2 in Cervical Carcinoma

- Squamous Ca, 4.1 % to 5.5% of cases HER2 + IHC
- AdenoCa, 12.5 % of cases (HPV-associated) HER2 + IHC
  - HER2 overexpression in 3.8% of gastric type adenocarcinoma
- TCGA report, HER2 amplification in cervical Ca , 17% of cases
  - More common in adenoCa than in squamous Ca

NCCN Guidelines Version 1.2025, Vulvar Cancer

Initial Dx

- HPV status
  - ISH or molecular testing
    - p16 is acceptable
- p53
- HER2, advanced stage

Progressive or recurrent disease

- PD-L1
- HER2
- NGS
- MMR
- TMB
- *NTRK* fusion

HER2 in Vulvar Carcinoma

Squamous Ca, 1% to 2% of the cases HER2 +

Paget's disease, 15% to 58% of the cases HER2+

Invasive >non-invasive


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