

BTC Biopsy Reference Images

HER2 IHC Score 1+

These cases exhibit a faint/barely perceptible HER2 IHC membranous staining of tumor cells, requiring higher magnification (40x objective) for clear visualization.

START HERE

HER2/neu (4B5) membrane staining seen in ≥5 contiguous TC at **2-4x**?

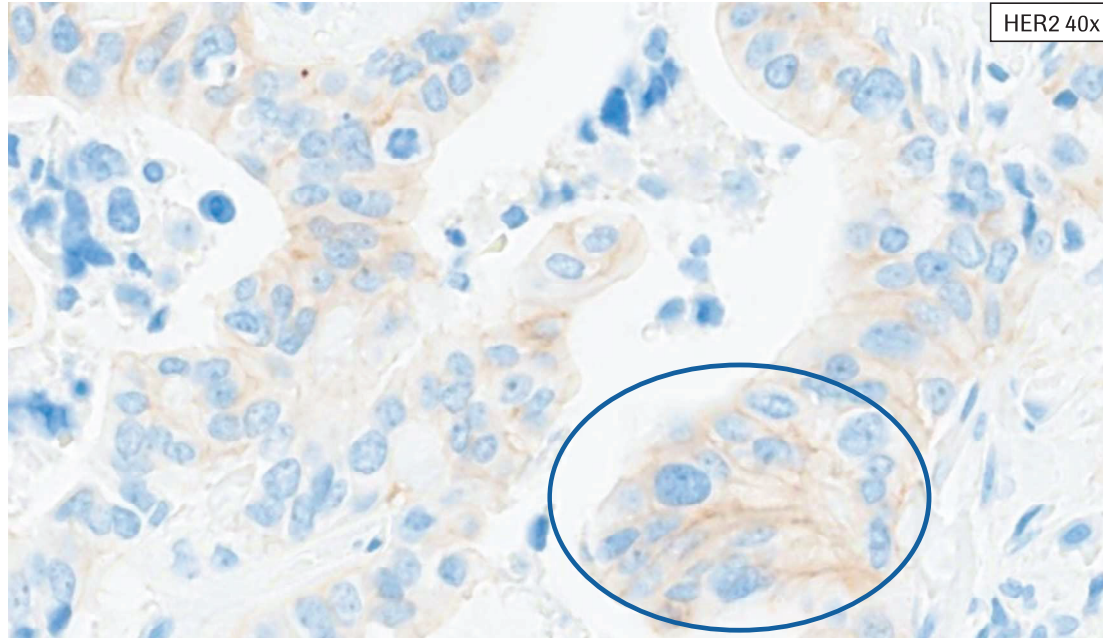
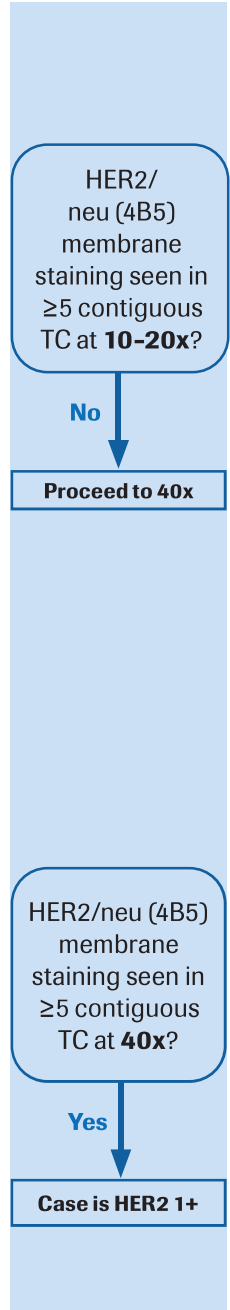
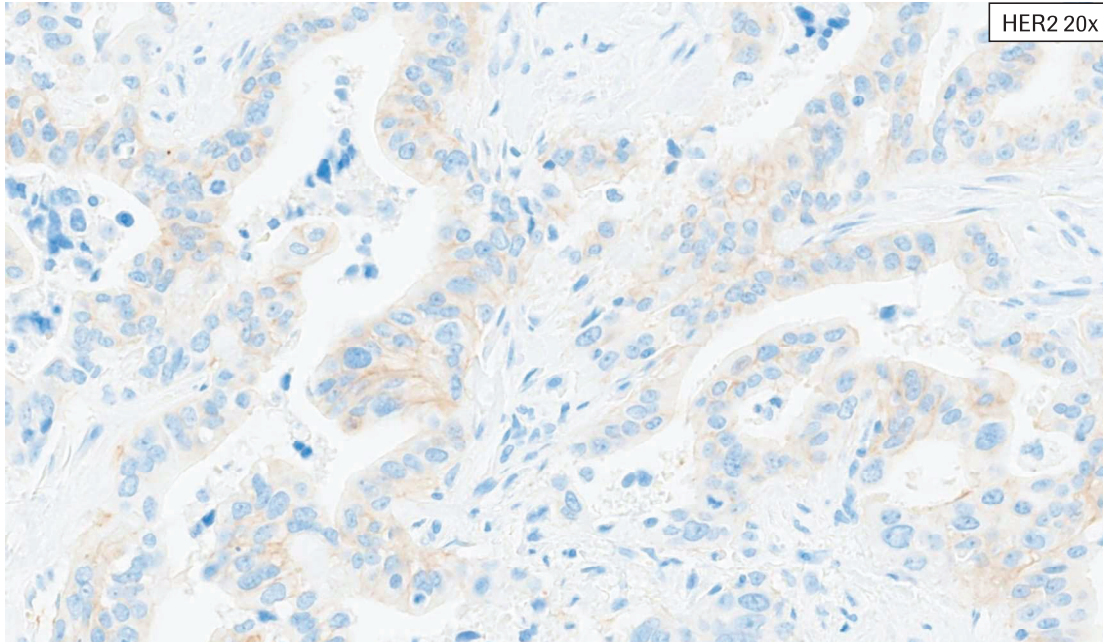
No

Proceed to 10-20x

H&E 20x

HER2 4x

Cholangiocarcinoma Biopsy Case: IHC score 1+. At 4x, no discrete membrane staining is seen, excluding IHC score 3+ for this case.



Cholangiocarcinoma Biopsy Case: IHC score 1+. At 20x, fewer than five (5) contiguous tumor cells demonstrate partial membrane staining, excluding IHC score of 2+. At 40x, membrane staining is seen in at least five contiguous tumor cells (oval).

BTC Biopsy Reference Images

HER2 IHC Score 2+

These cases exhibit HER2 IHC signal of weak to moderate membranous staining where tumor cell membrane staining can be visualized with 10x and/or 20x objectives.

START HERE

HER2/neu (4B5) membrane staining seen in ≥5 contiguous TC at **2-4x**?

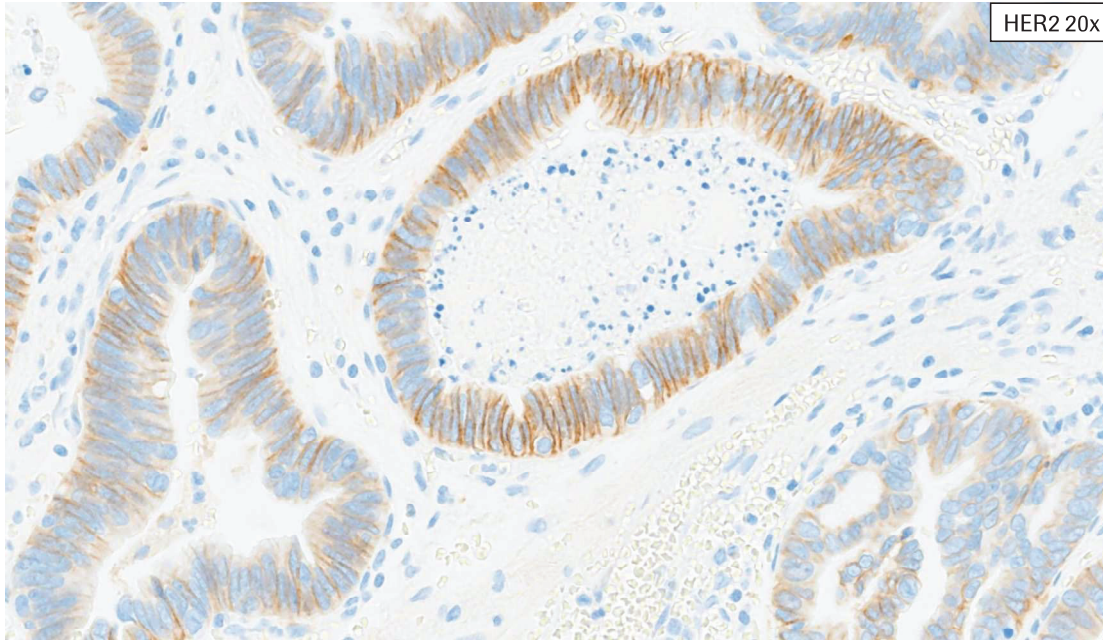
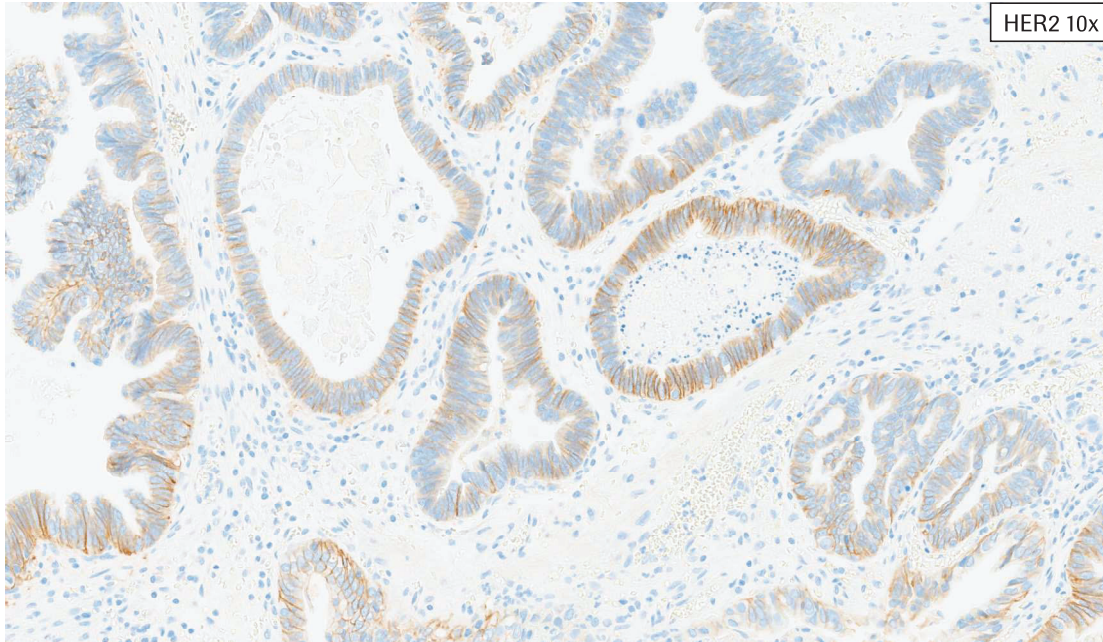
No

Proceed to 10-20x

H&E 20x

HER2 4x

Cholangiocarcinoma Biopsy Case: IHC score 2+. At 4x, membrane staining can be visualized/resolved but in < 5 contiguous tumor cells, so 3+ IHC score is excluded for this case.



HER2/
neu (4B5)
membrane
staining seen in
≥5 contiguous
TC at **10-20x**?

Yes

Case is HER2 2+

Cholangiocarcinoma Biopsy Case: IHC score 2+. At 10-20x, basolateral membrane staining can be seen >5 contiguous tumor cells, consistent with IHC 2+.

BTC Biopsy Reference Images

HER2 IHC Score 3+

These cases exhibit HER2 IHC signal of strong membranous staining where tumor cell membrane staining can be visualized with 2x and/or 4x objectives.

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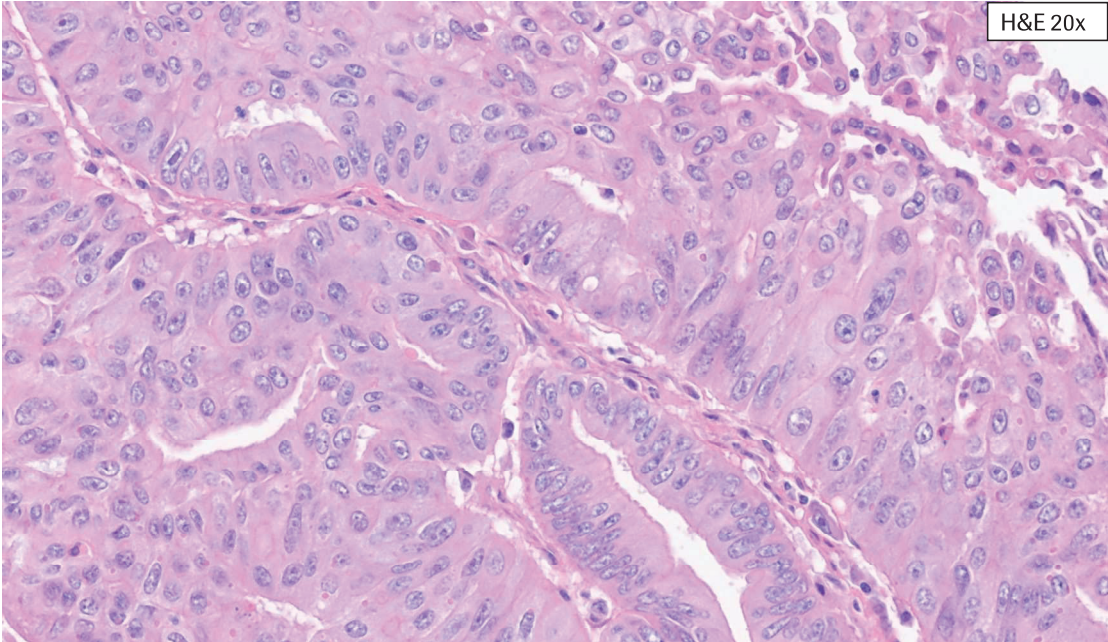
HER2/neu (4B5)
membrane
staining seen in
≥5 contiguous
TC at **2-4x**?

↓

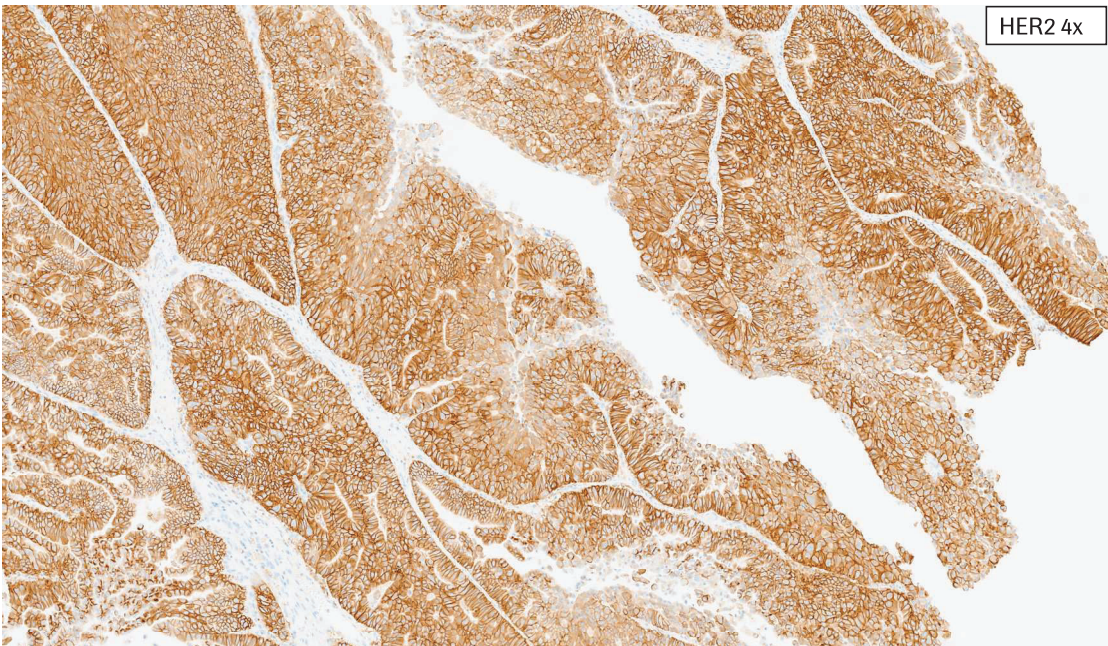
Yes

↓

Case is HER2 3+

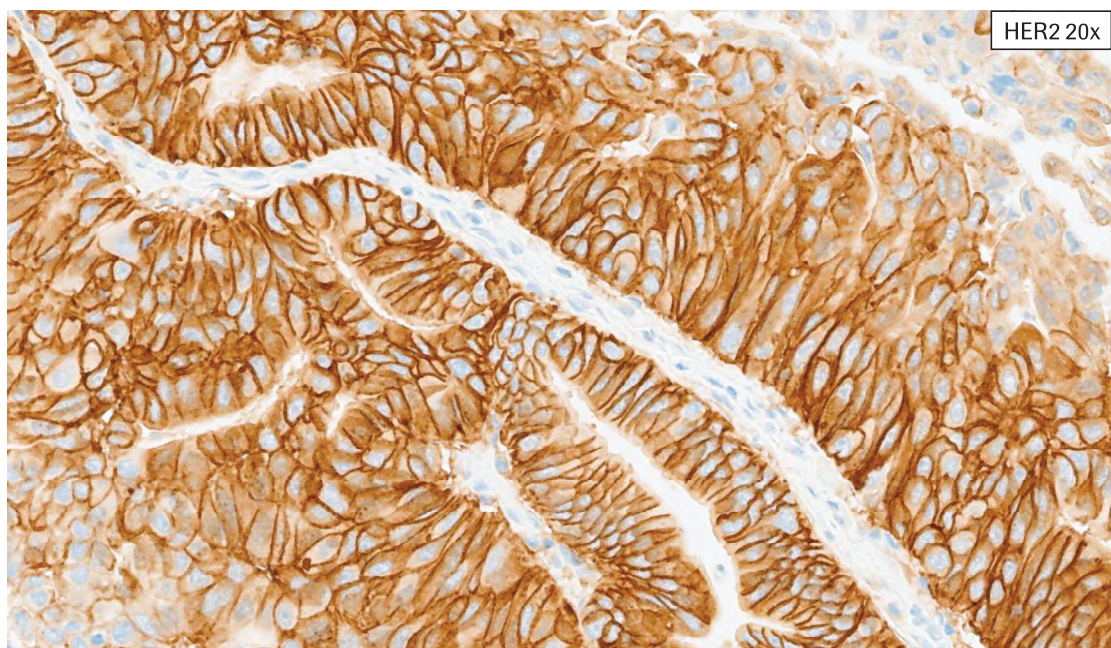
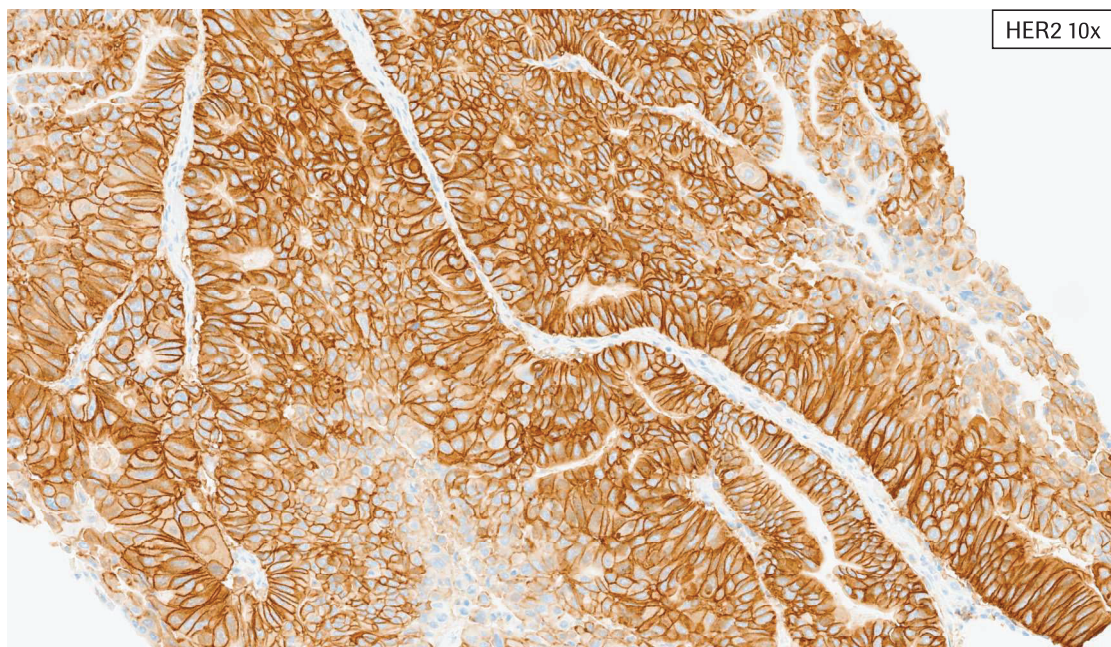


H&E 20x



HER2 4x

Gallbladder Adenocarcinoma Biopsy Case: IHC score 3+. At 4x, membrane staining is easily visualized in ≥ 5 contiguous tumor cells, consistent with IHC score 3+.



Gallbladder Adenocarcinoma Biopsy Case: IHC score 3+. At 20x, homogeneous strong basolateral and circumferential membrane staining is seen. Accompanying weak to moderate cytoplasmic staining is also present, but does not obscure membrane staining.

BTC Borderline Reference Images

The most difficult area of interpretation is cases that fall on the borderline between an intensity level of “1+” and “2+”, or where there is a mixture of different expression levels. Here are some tips for handling these cases:

1. Evaluate the borderline case within the context of unambiguous “1+” and “2+” cases to regain perspective.
2. Use the 20x and 40x objectives to help differentiate between 1+ and 2+ staining intensities. If the question is between a 3+ or 2+ then rely on the differences seen with the 4x and 20x objectives.
3. Remember that pattern plays a primary role in the score. Both complete and incomplete membranous staining can be considered in BTC, refer to [Table 1](#).
4. Scan complete tissue section to ensure scoring in well-preserved and well stained areas only.
5. Consider repeating the staining on another section or repeat staining on sections from a different block if none of the above suggestions resolve the diagnosis.

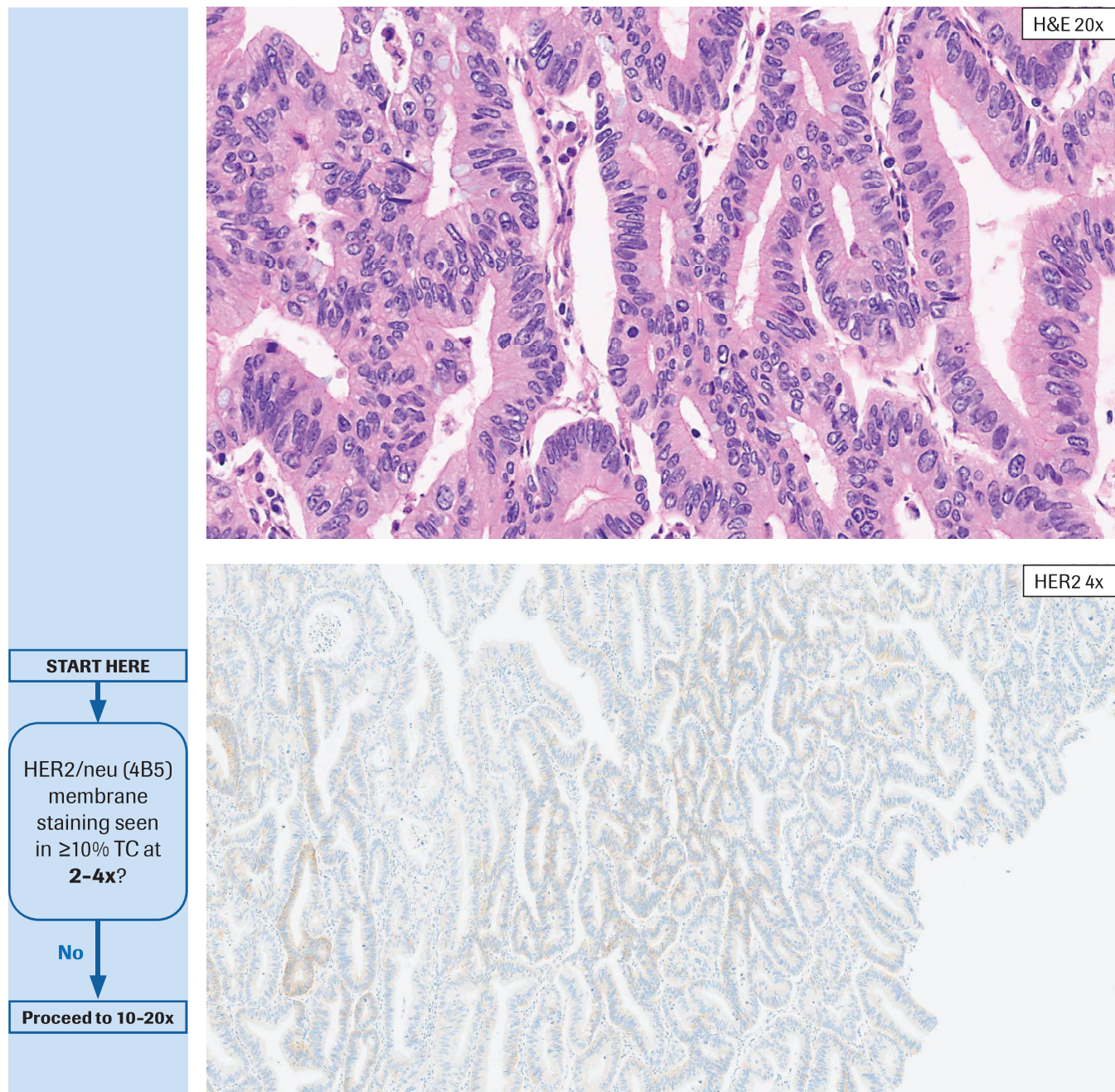
Examples of borderline cases are shown on the following pages.

- **HER2 1+, Borderline with 2+**
- **HER2 2+, Borderline with 1+**
- **HER2 2+, Borderline with 3+**
- **HER2 3+, Borderline with 2+**
- **HER2 3+, Borderline with 0**

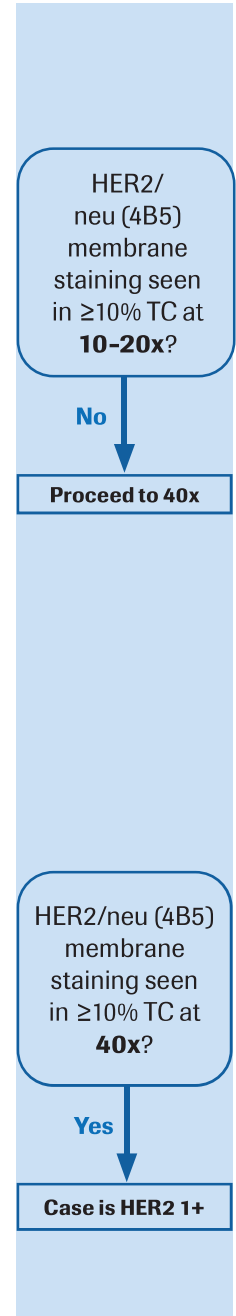
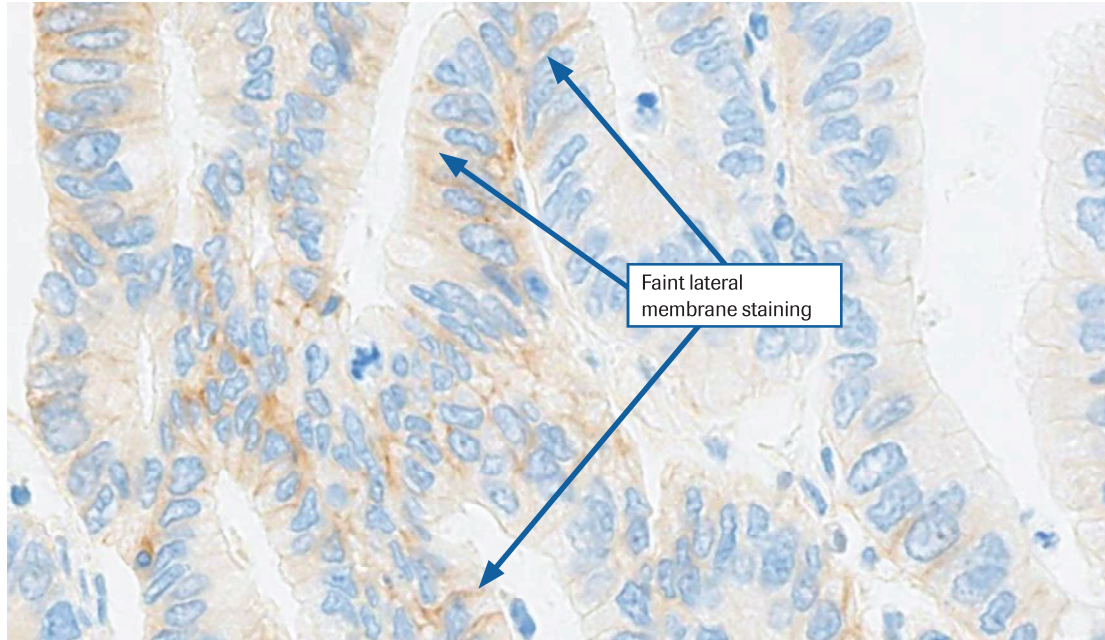
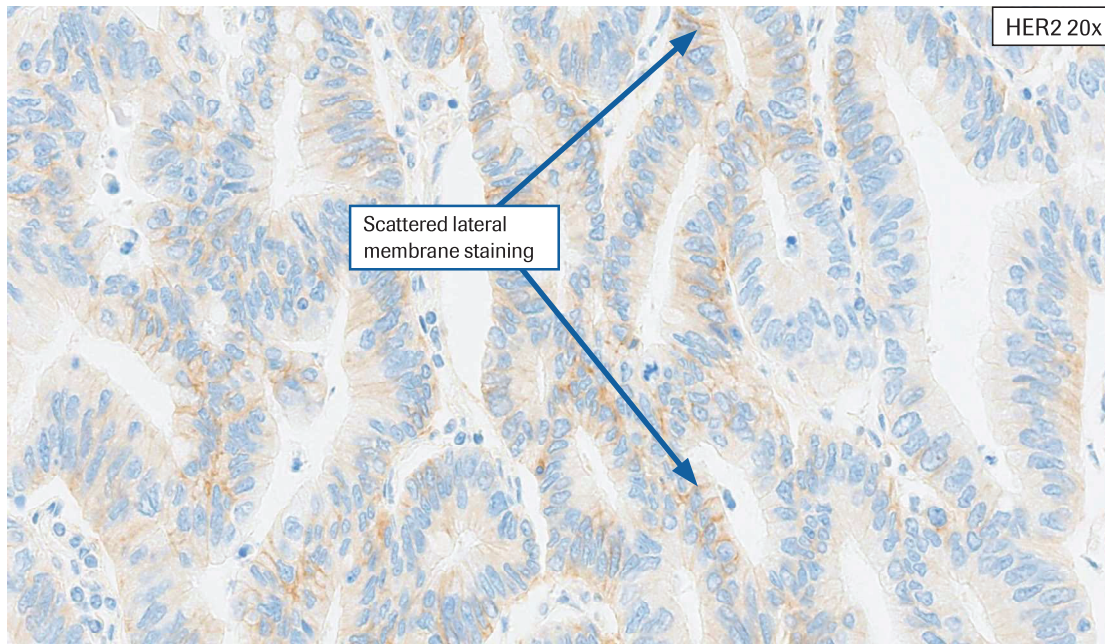
BTC Borderline Reference Images

HER2 1+, Borderline with 2+

These cases demonstrate some HER2 membrane staining that can be seen at 10x to 20x, close to, but <10% of tumor cells (resections) or in less than 5 contiguous tumor cells (biopsies).



Borderline 1+ (near 2+) Cholangiocarcinoma Resection Case: IHC score 1+ (borderline with 2+). Although vague, ill-defined staining can be observed at 4x, no discrete membrane staining is seen, thus excluding IHC score 3+ for this case.



Borderline 1+ (near 2+) Cholangiocarcinoma Resection Case: IHC score 1+ (borderline with 2+). Scattered lateral membranes can be seen with 20x objective, accounting for $\sim 5\%$ (but $<10\%$) of tumor cells in this field (arrows), thus excluding IHC score 2+ for this case. At 40x, faint lateral membranes are seen in $\geq 10\%$ of tumor cells (arrows), consistent with IHC score of 1+.

BTC Borderline Reference Images

HER2 2+, Borderline with 1+

These cases demonstrate HER2 membrane staining that can be seen at 10x to 20x, in 10% or just over 10% of tumor cells (resections) or in 5 or just over 5 contiguous tumor cells (biopsies).

START HERE

HER2/neu (4B5) membrane staining seen in ≥10% TC at **2-4x**?

No

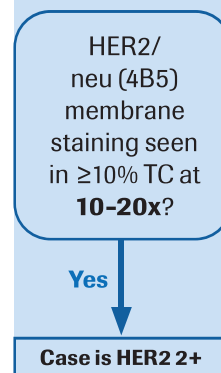
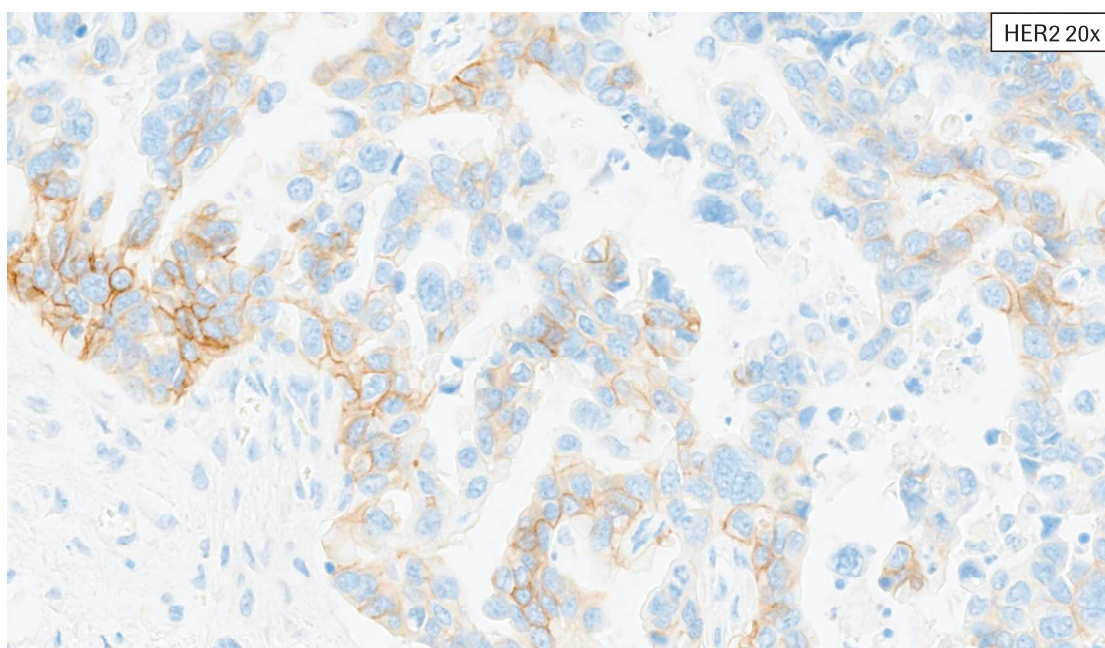
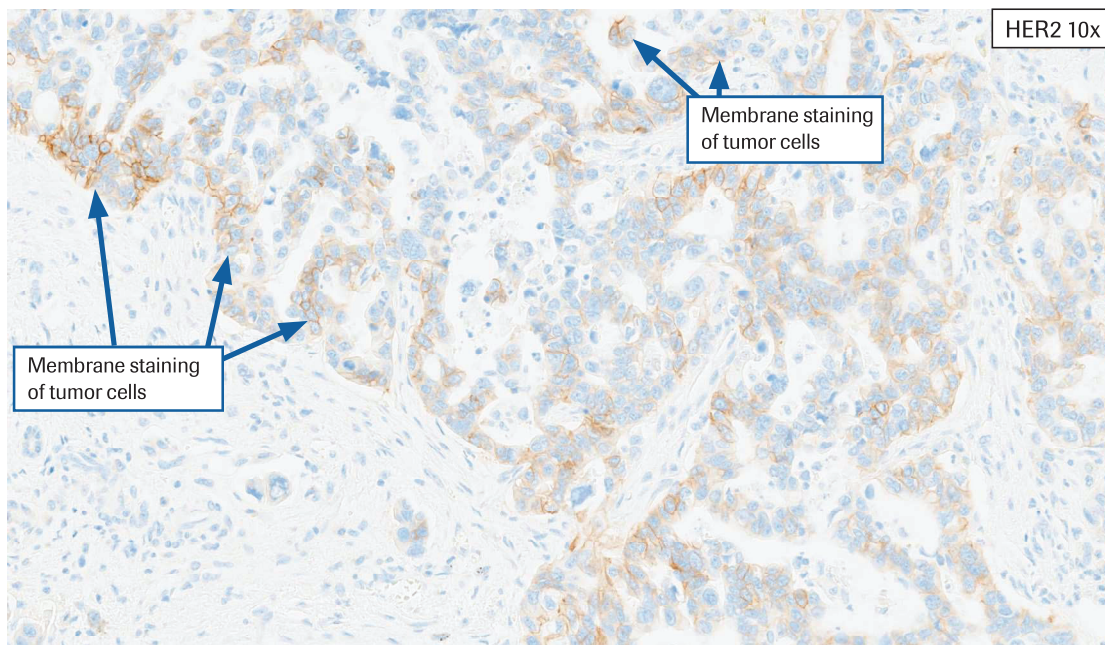
Proceed to 10-20x

H&E 20x

HER2 4x

Membrane staining of tumor cells

Borderline 2+ Cholangiocarcinoma Resection Case: IHC score 2+ (borderline with 1+). At 4x, membrane staining is seen in <1% of tumor cells (arrow), so IHC score 3+ is excluded for this case.

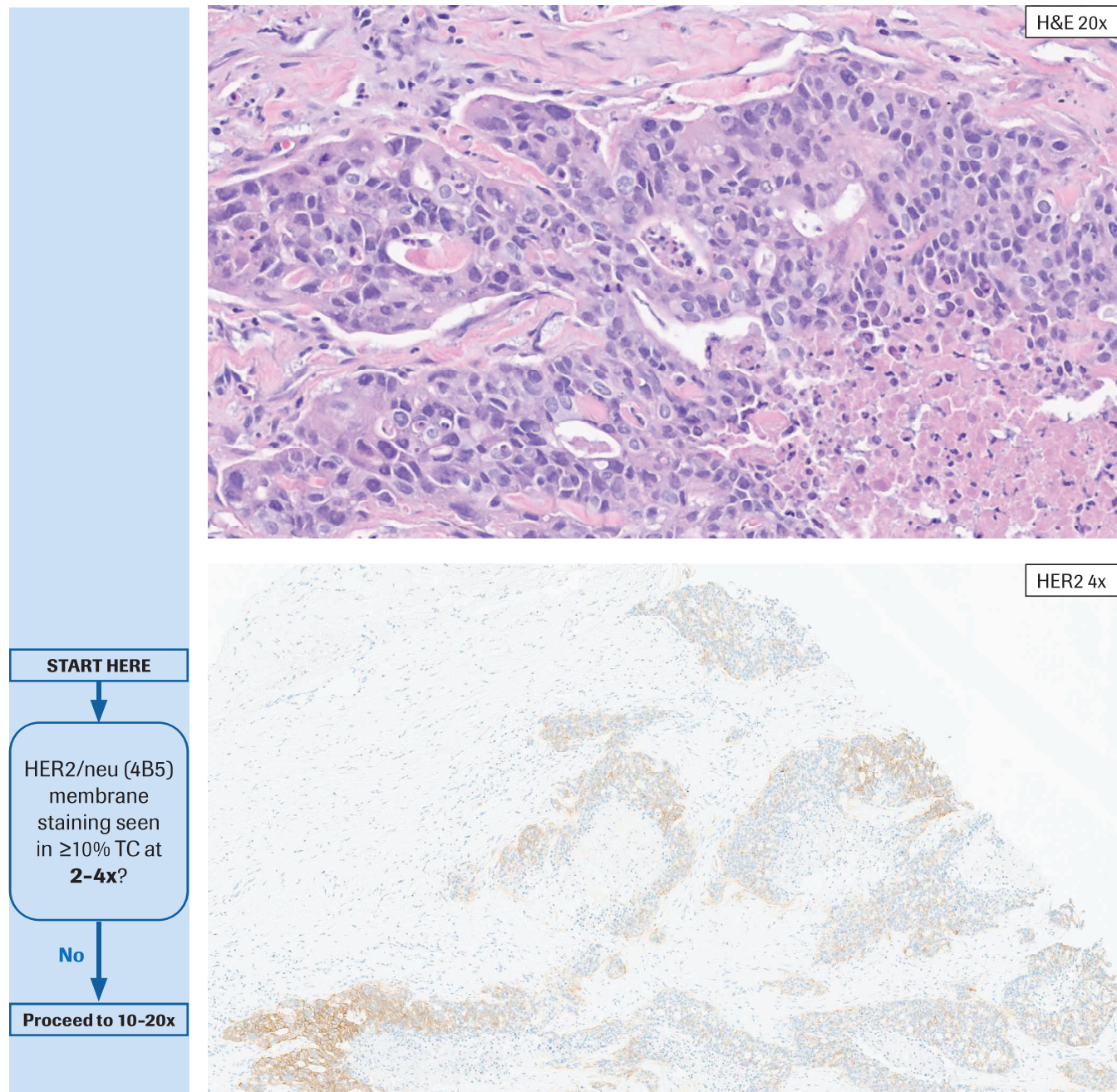


Borderline 2+ Cholangiocarcinoma Resection Case: IHC score 2+ (borderline with 1+). At 10-20x, 15% of tumor cells (arrows) demonstrate membrane staining, consistent with IHC score of 2+ (borderline with 1+).

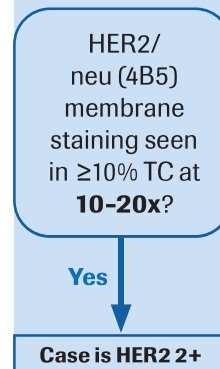
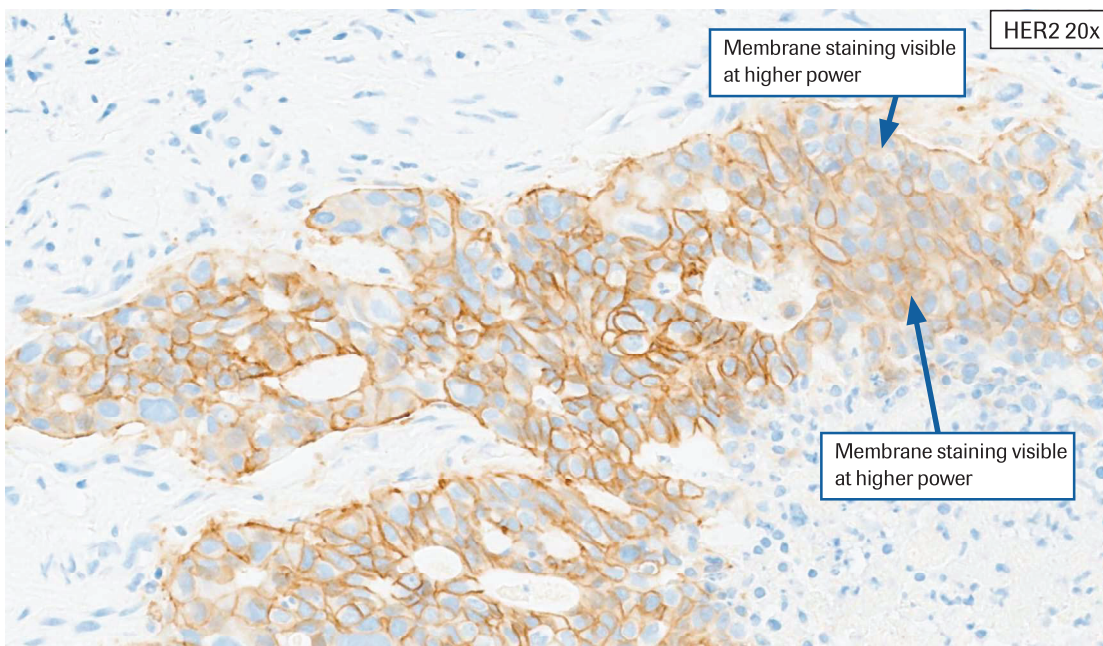
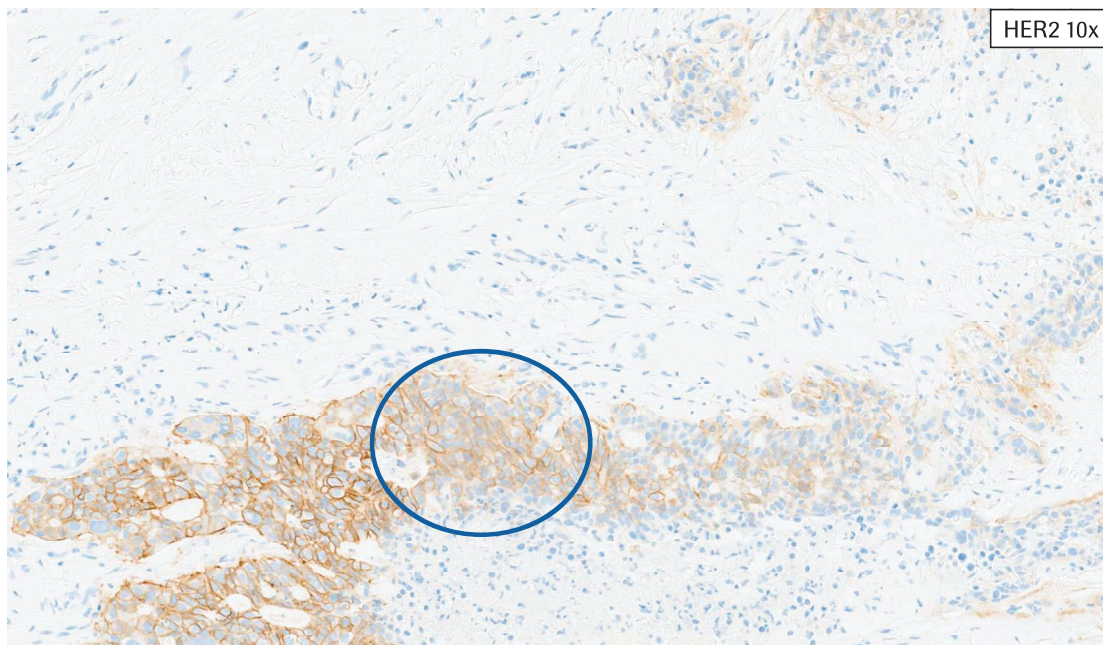
BTC Borderline Reference Images

HER2 2+, Borderline with 3+

These cases exhibit some membrane staining at 4x, but in <10% of tumor cells (resections) or in <5 contiguous tumor cells (biopsies).



Borderline Gallbladder Adenocarcinoma Resection Case: IHC score 2+. At 4x, possible hints of membrane staining are noted, but in <10% of tumor cells, (lower left of image), thus excluding IHC score of 3+ for this case.



Borderline Gallbladder Adenocarcinoma Resection Case: IHC score 2+. At 10x and at 20x, membrane staining can be visualized in $\geq 10\%$ of tumor cells, consistent with IHC score of 2+. Note: some membrane staining that was not as clearly resolved/visualized at 10x are definitely visible at 20x (oval and arrows). This is a good example of the variation of intensities that fall within the 2+ IHC score.

BTC Borderline Reference Images

HER2 3+, Borderline with 2+

These cases exhibit membrane staining at 4x in 10% or just over 10% (resections) or 5 or just over 5 contiguous tumor cells (biopsies).

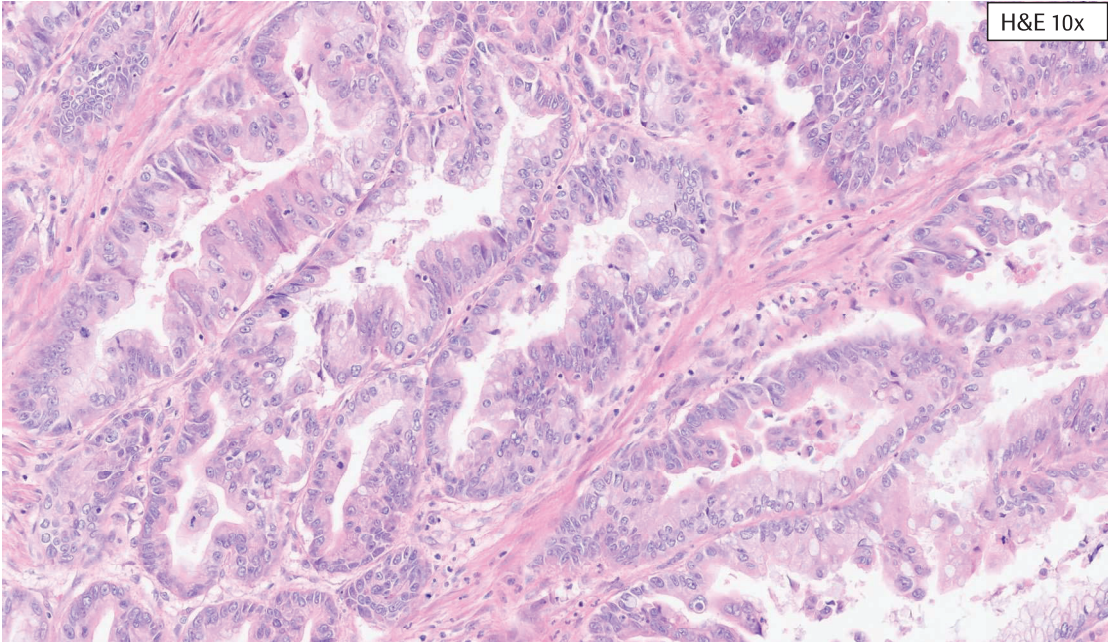
START HERE

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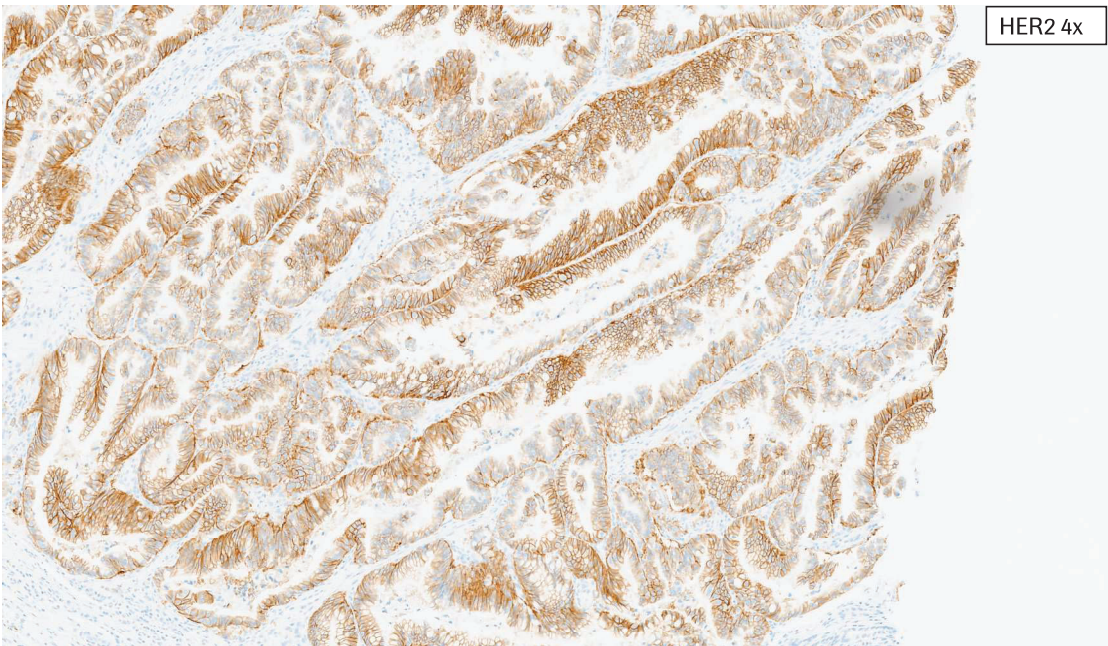
HER2/neu (4B5)
membrane
staining seen
in ≥10% TC at
2-4x?

Yes ↓

Case is HER2 3+

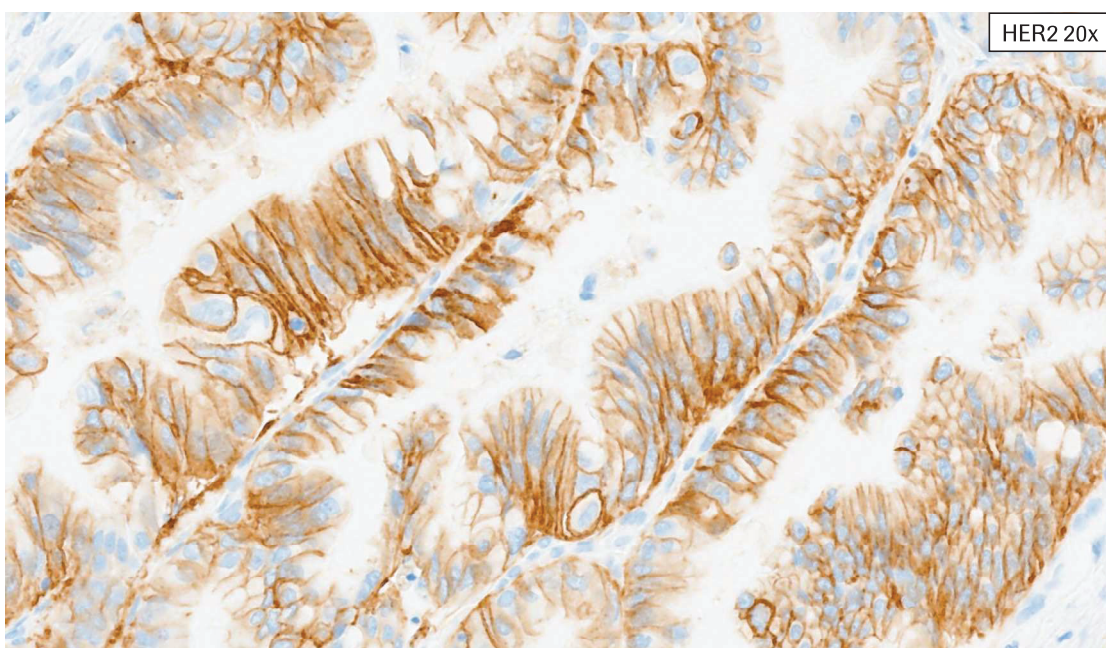
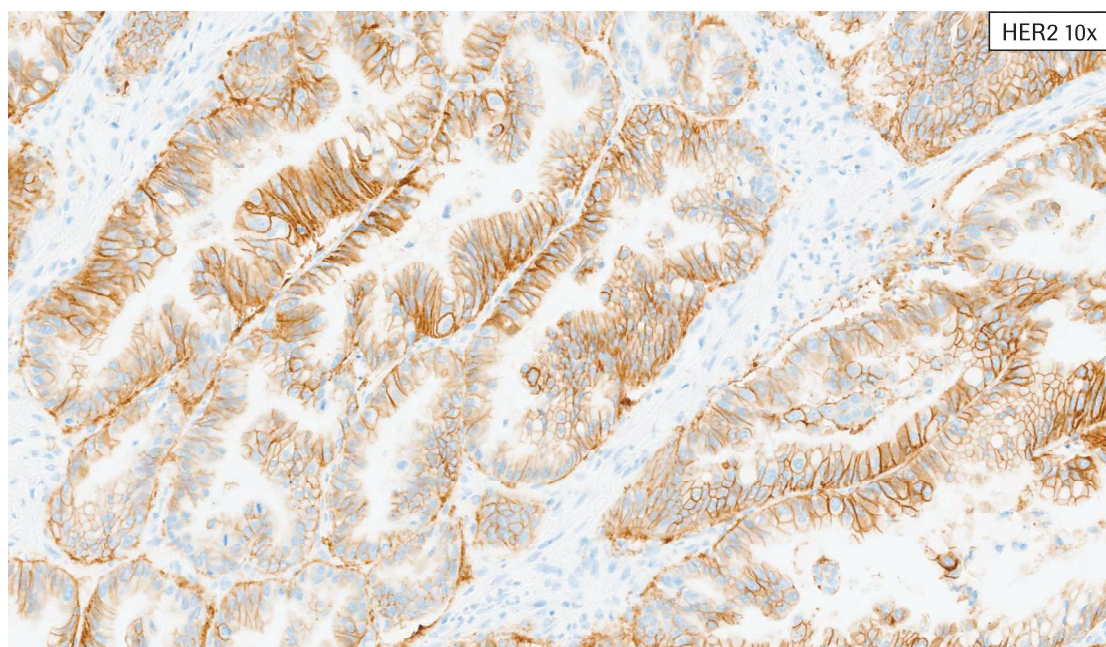


H&E 10x



HER2 4x

Borderline 3+ Cholangiocarcinoma Resection Case: IHC score 3+ (borderline with 2+). At 4x, membrane staining is seen in 15% of tumor cells, consistent with IHC score 3+.



Borderline 3+ Cholangiocarcinoma Resection Case: IHC score 3+ (borderline with 2+). At 10x and 20x images demonstrate clearly resolved basolateral membrane staining consistent with 2+ staining.

BTC Borderline Reference Images

HER2 3+, Borderline with 0

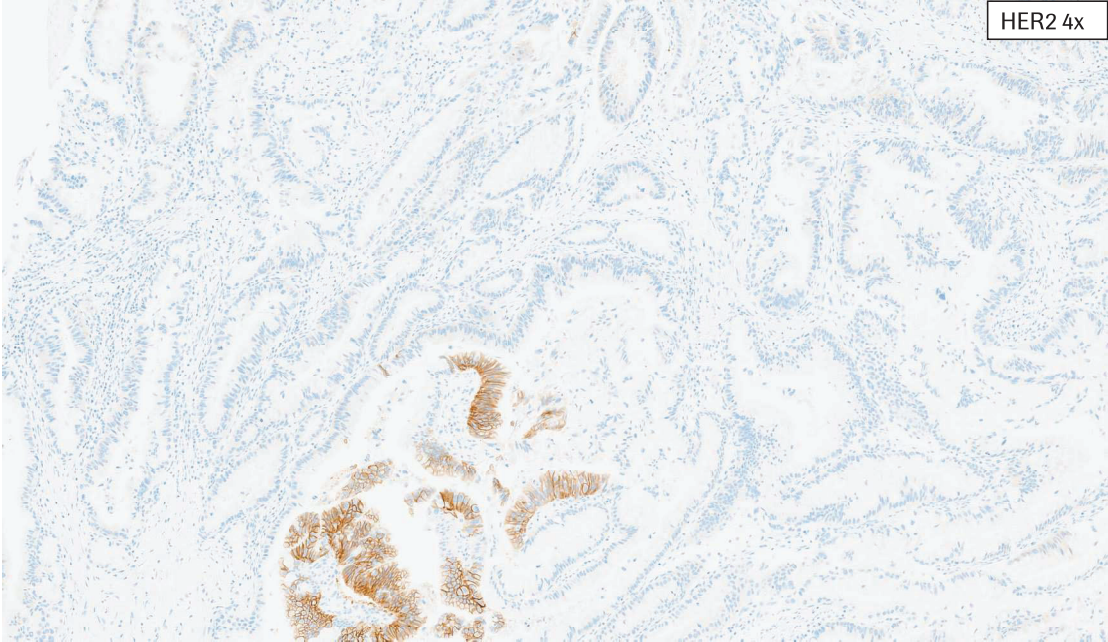
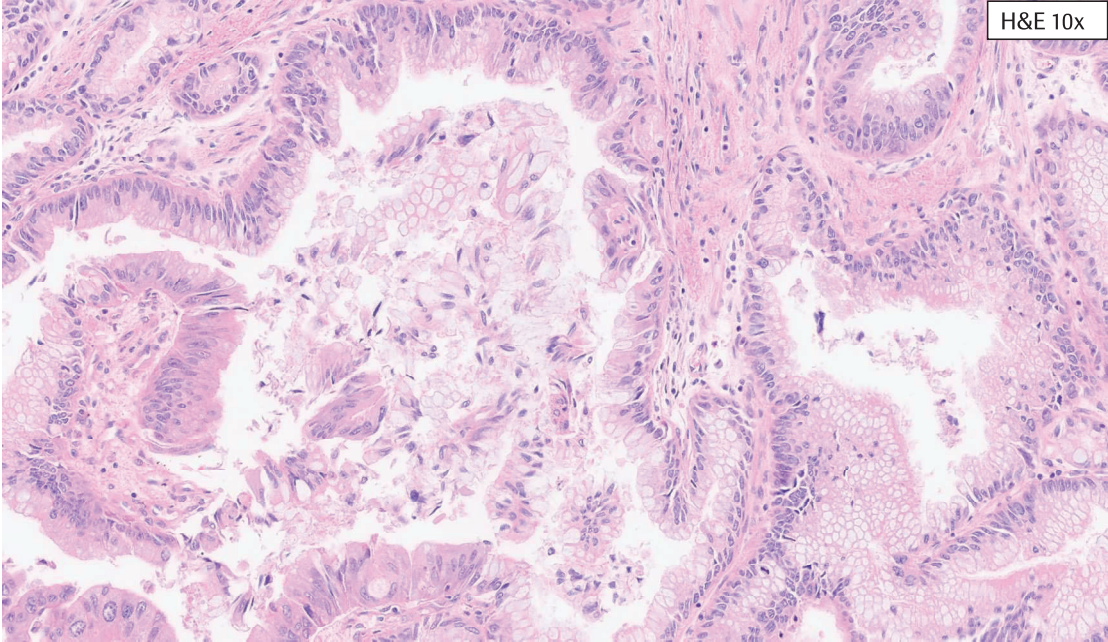
These cases exhibit membrane staining at 4x that is seen in 10% or just over 10% of tumor cells (resections) or 5 or just over 5 contiguous tumor cells (biopsies).

START HERE

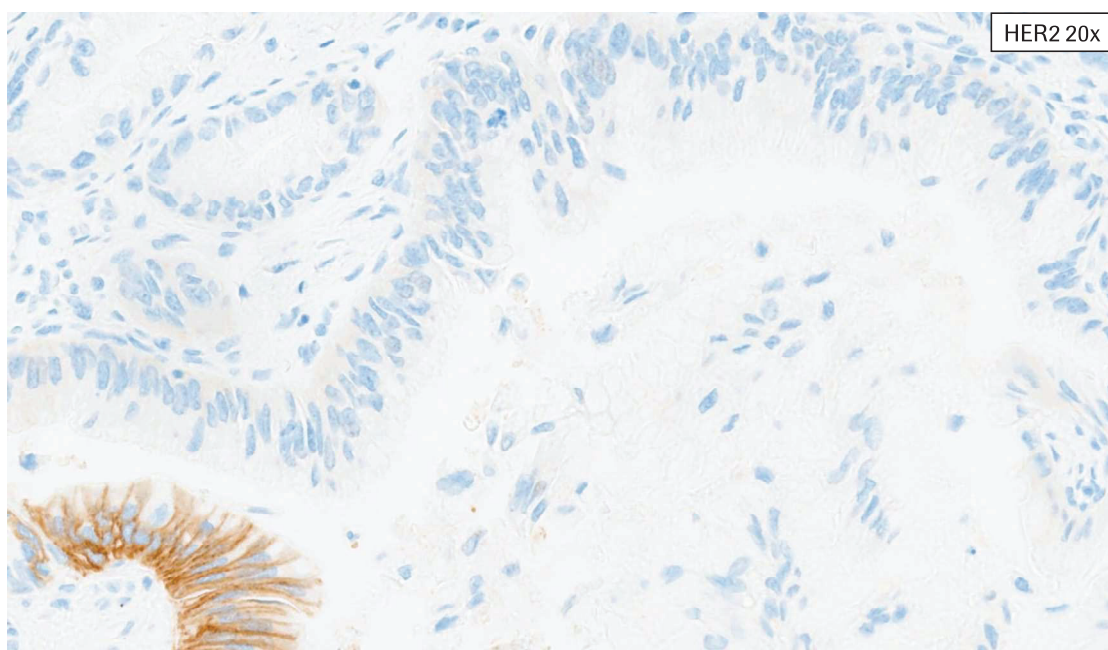
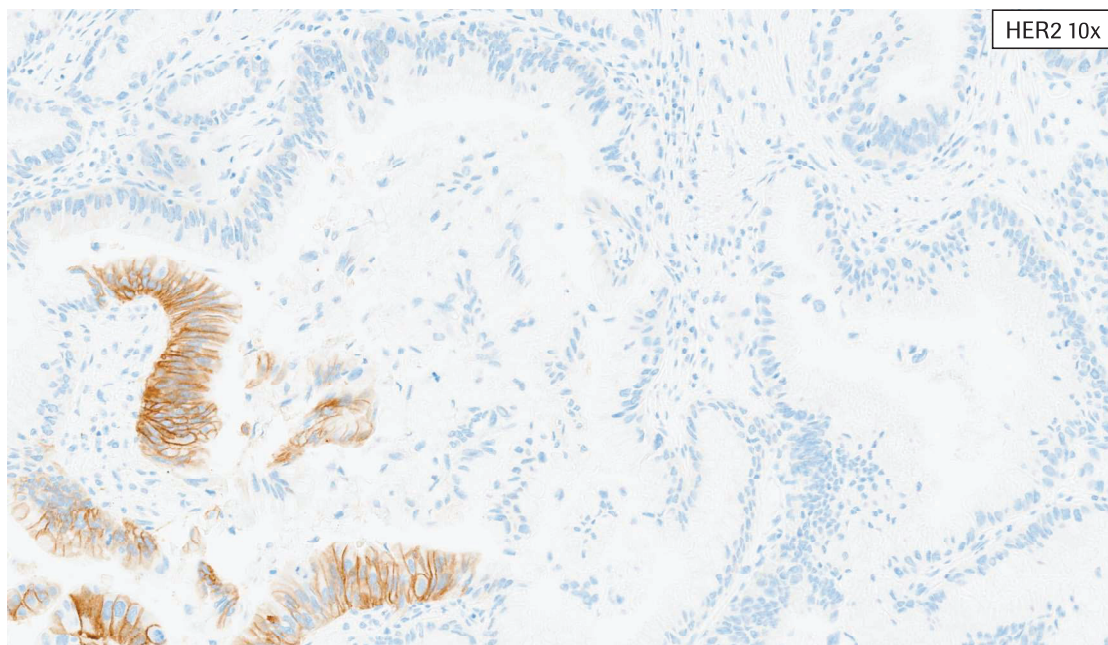
HER2/neu (4B5) membrane staining seen in ≥10% TC at 2-4x?

Yes

Case is HER2 3+



Borderline 3+ Cholangiocarcinoma Resection Case: IHC score 3+ borderline with zero. At 4x, membrane staining is present in 10% of tumor cells, consistent with IHC score 3+. Note that this case is also an example of heterogeneous HER2 over-expression, with the majority of the tumor showing no membrane staining.



Borderline 3+ Cholangiocarcinoma Resection Case: IHC score 3+ borderline with zero. Images at 10x and 20x demonstrate heterogeneity of HER2 overexpression that is localized to tumor cells in the left aspect of the image.

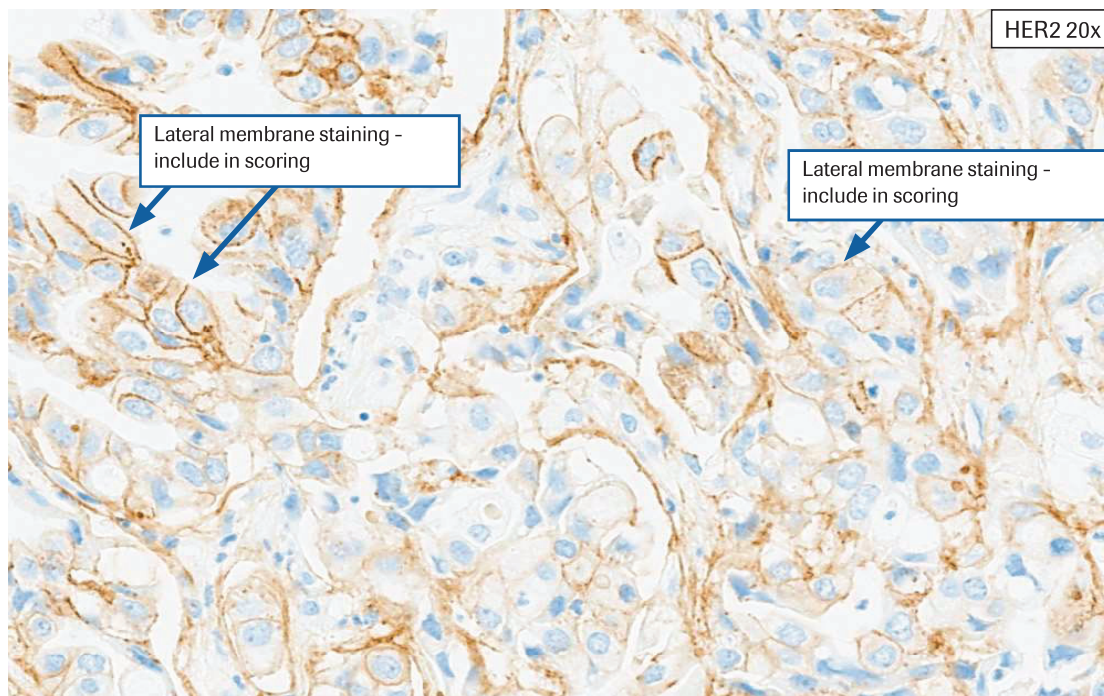
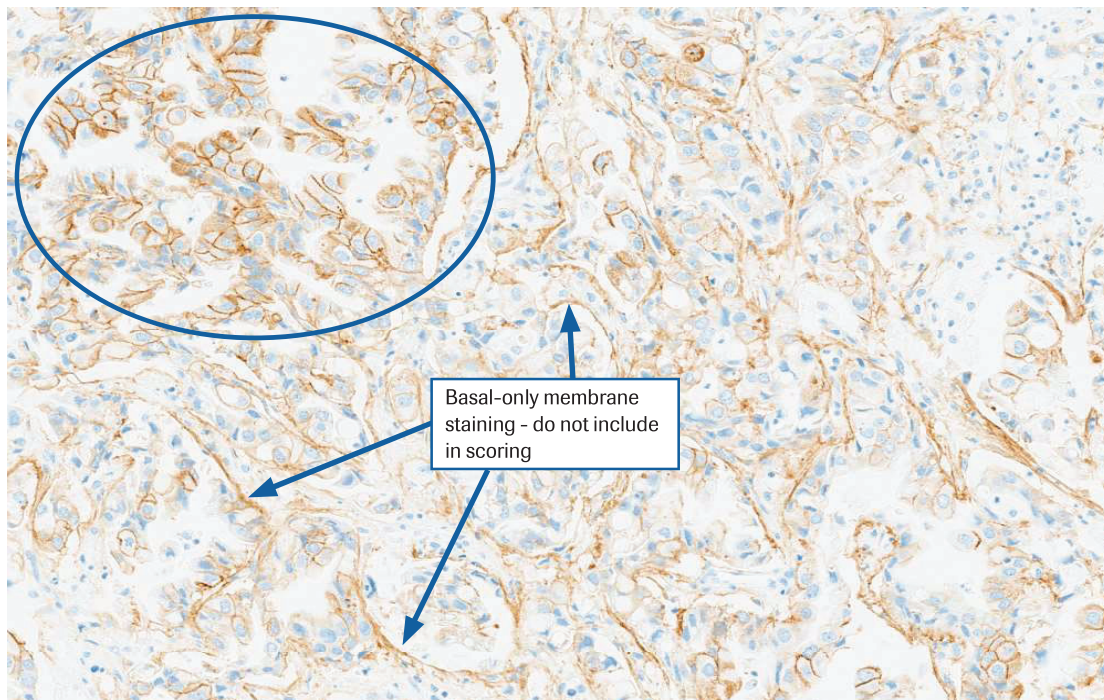
Challenging Cases

Atypical Staining Patterns

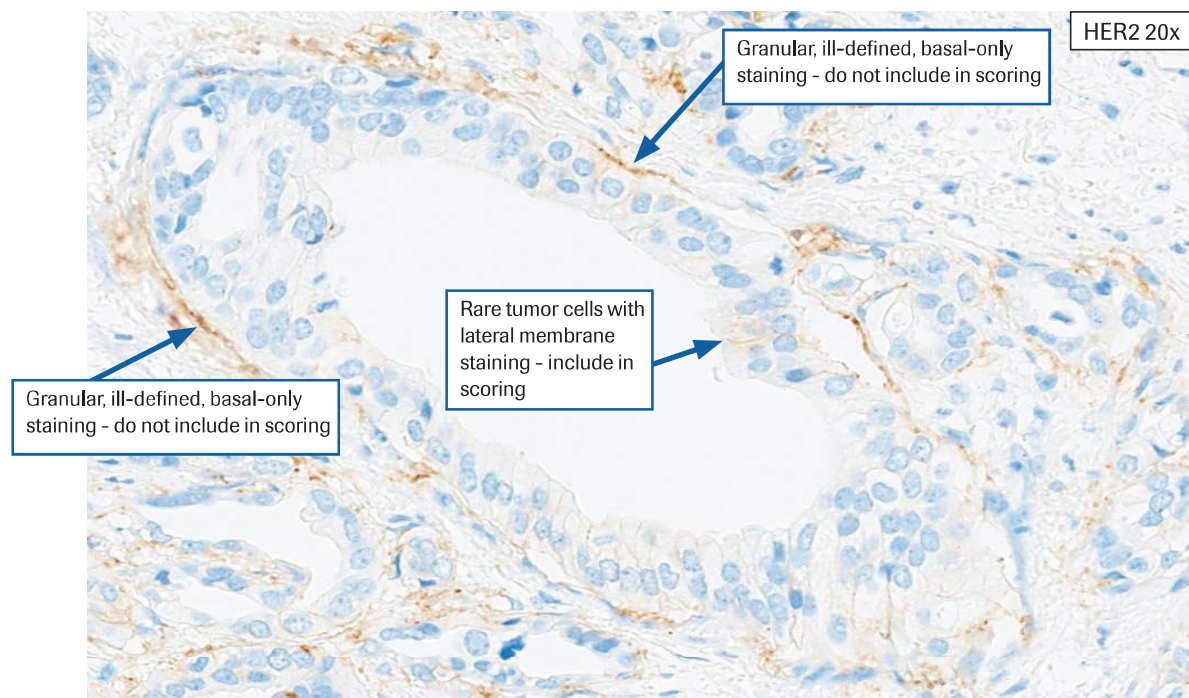
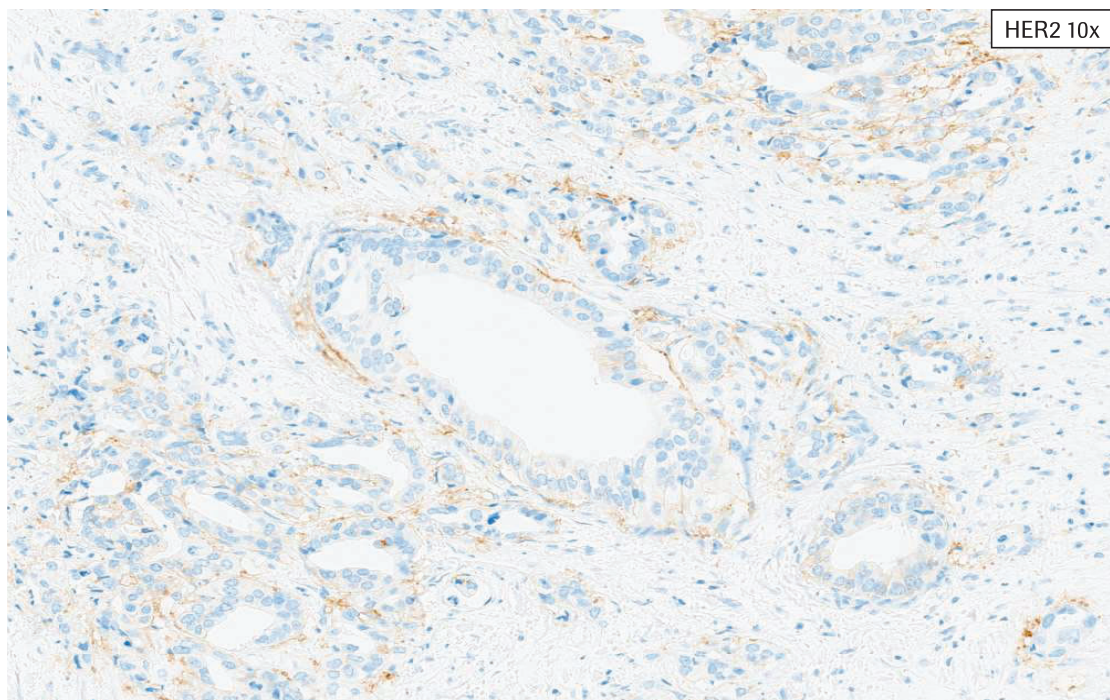
While the vast majority of cases stained with HER2 (4B5) assay have clear staining results, a few cases have been observed that present a challenge in interpretation.

- **Basal-only staining**
- **Apical-only staining**

Challenging Cases: Basal-only staining

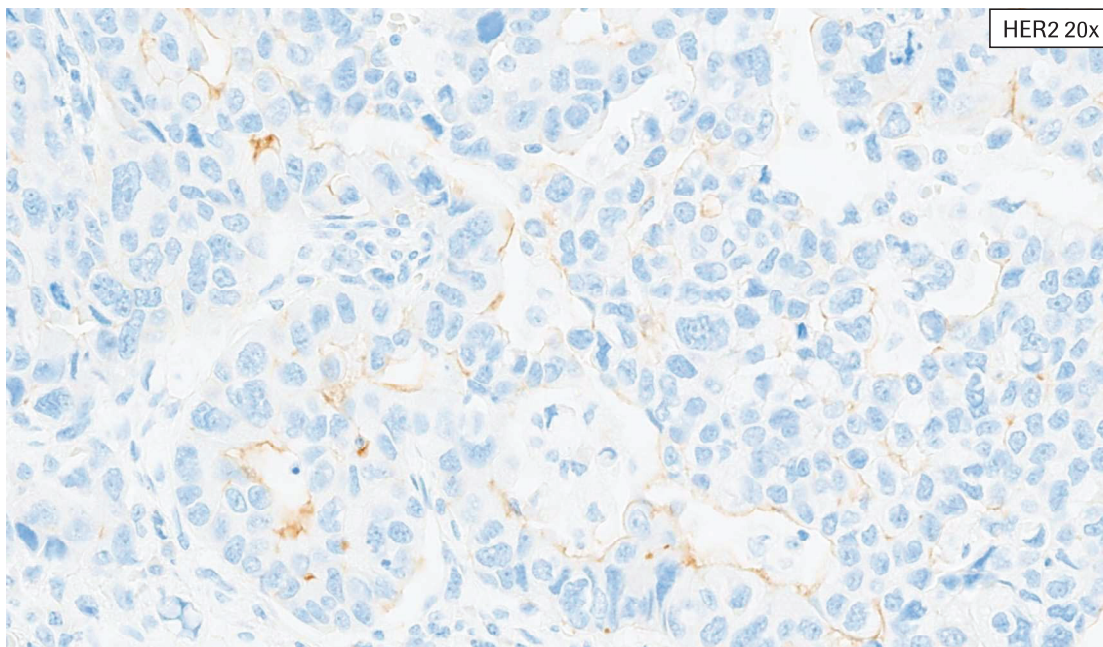
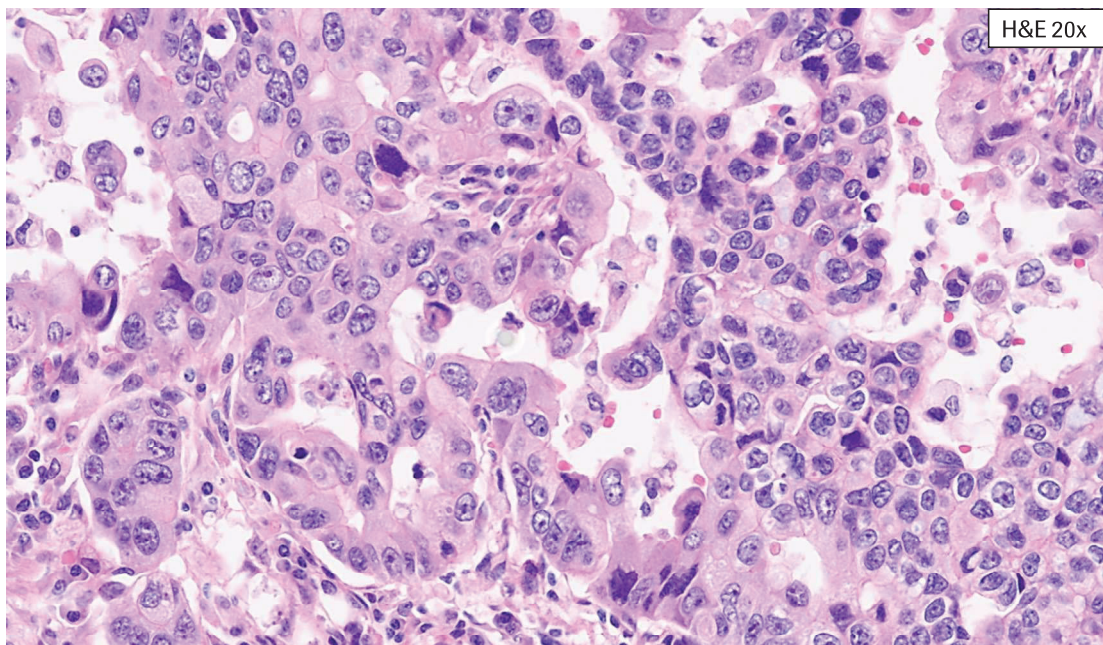


Challenging Gallbladder Adenocarcinoma Case: Images at 10x and at 20x demonstrate a region of moderate to strong basolateral membrane staining (oval). Prominent basal-only staining is seen in the remainder of the field and should not be included when determining IHC score (arrows - top panel). There are scattered tumor cells (arrows - bottom panel) where lateral membrane staining is present, that should be included in determining the IHC score.



Challenging Gallbladder Adenocarcinoma Case: These images demonstrate granular and ill-defined basal-only staining that should not be included as part of the HER2 IHC score (top and left arrows). There are rare tumor cells with lateral membrane staining (center arrow), which should be included as part of the HER2 IHC score.

Challenging Cases: Apical-only staining



Apical-only Esophageal Adenocarcinoma Case: In this image, the tumor cells demonstrate DAB along the apical surface of the tumor cells. In the absence of basolateral or lateral membrane staining, apical-only staining should not be included in the HER2 IHC score. Note that the cases used to demonstrate specific artifacts may include tumor indications other than BTC, based on tissue availability.

Staining Artifacts

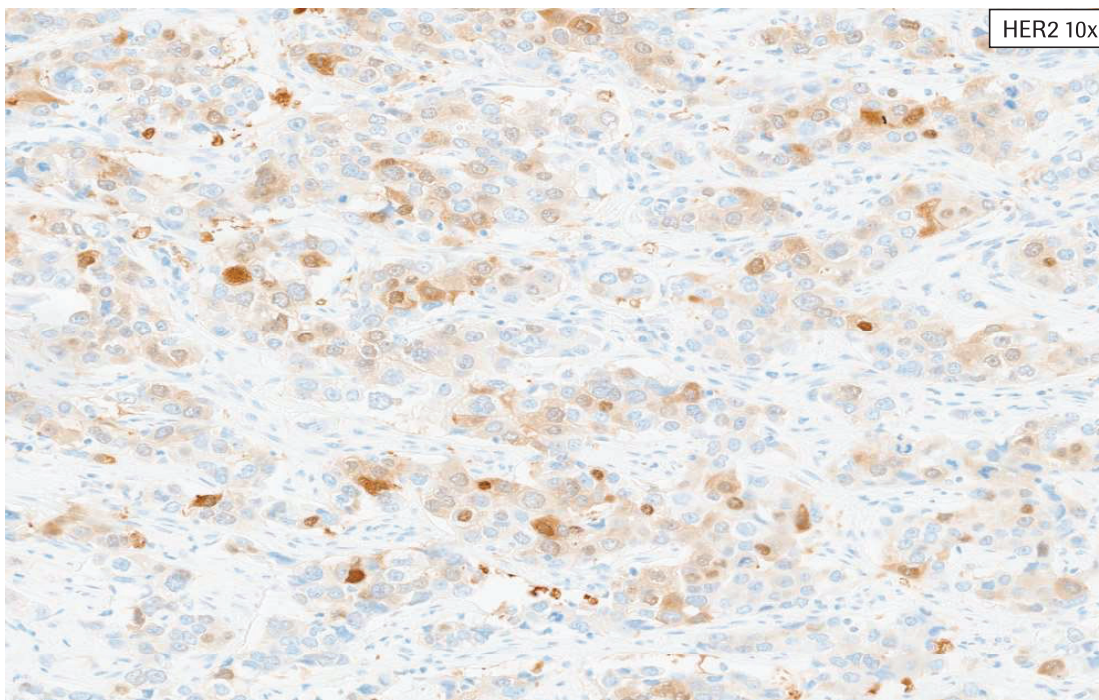
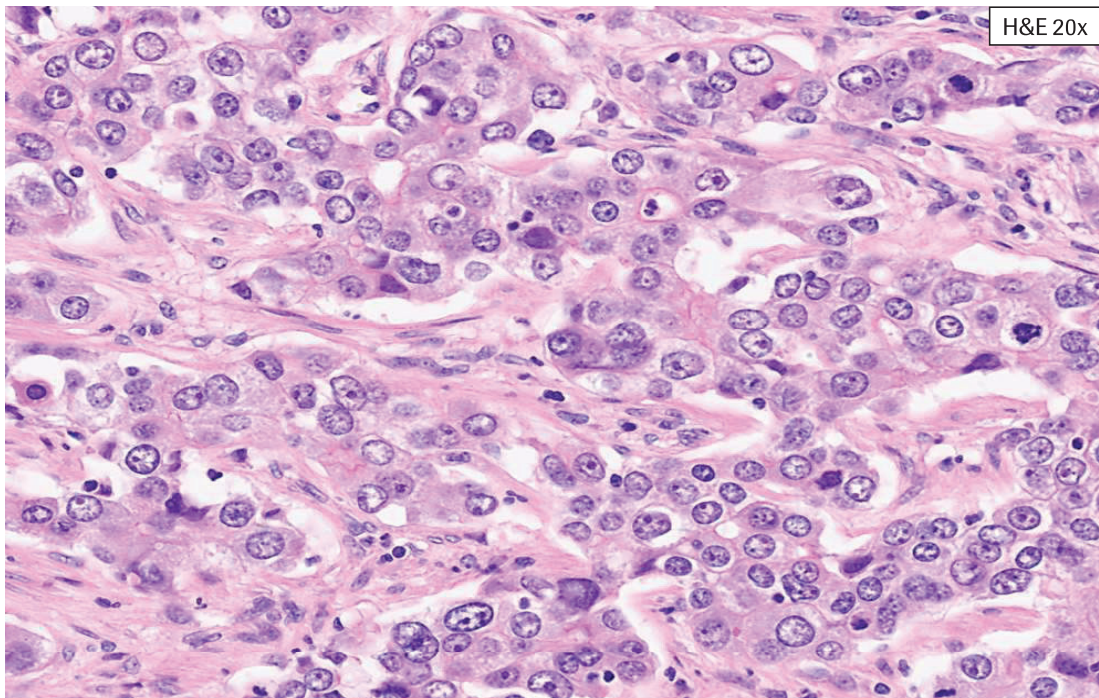
Artifacts noted in this section may be observed on HER2 (4B5) Assay-stained slides. The presence of these artifacts may require repeat staining if they interfere with interpretation of HER2 (4B5) Assay staining. Always review the corresponding Negative Control-stained slide to ensure that non-specific background staining is within acceptable limits.

Examples of staining artifacts are shown on the following pages.

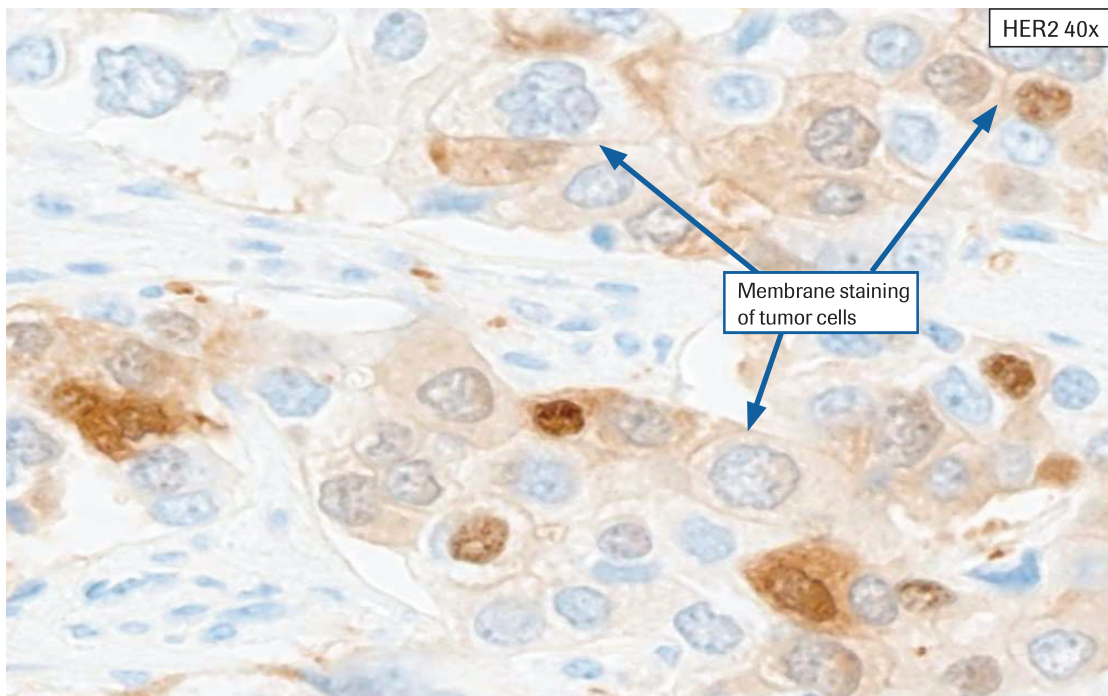
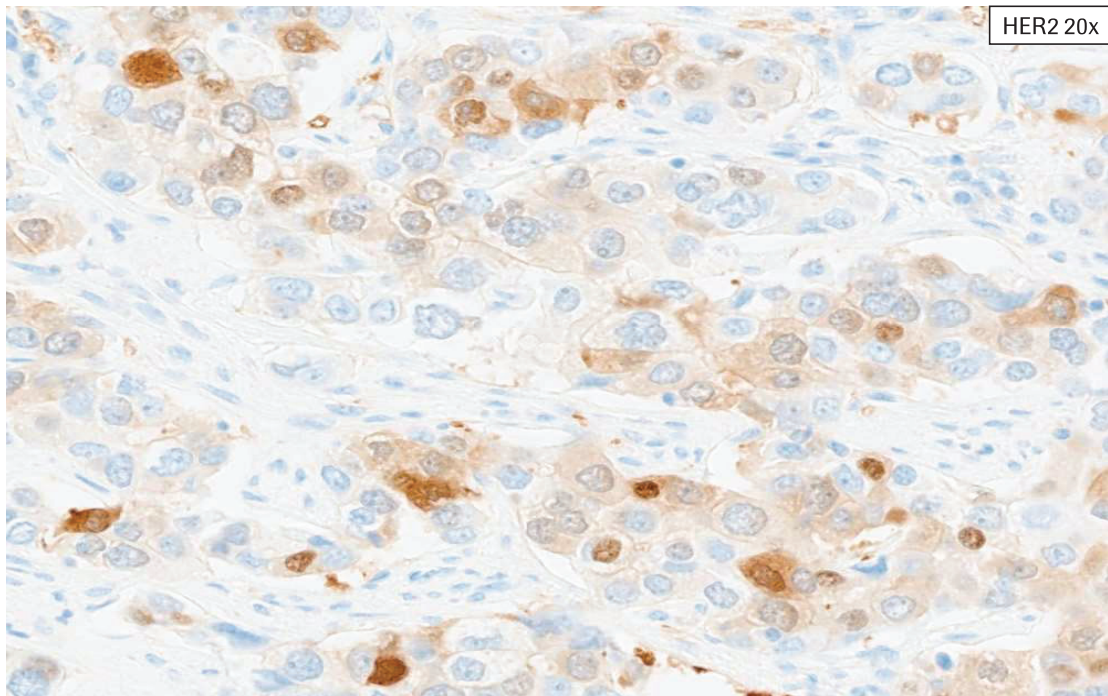
- **Cytonuclear staining in Carcinoma Cells,
Gallbladder Adenocarcinoma Resection Case 1**
- **Cytonuclear staining in Carcinoma Cells,
Gallbladder Adenocarcinoma Resection Case 2**
- **Cytonuclear staining in Non-Neoplastic Cells (Hepatocytes),
Cholangiocarcinoma Resection Case**
- **Cytonuclear staining in Non-Neoplastic Cells (Epithelium),
Cholangiocarcinoma Resection Case**

Staining Artifacts: Cytonuclear Staining in Carcinoma Cells

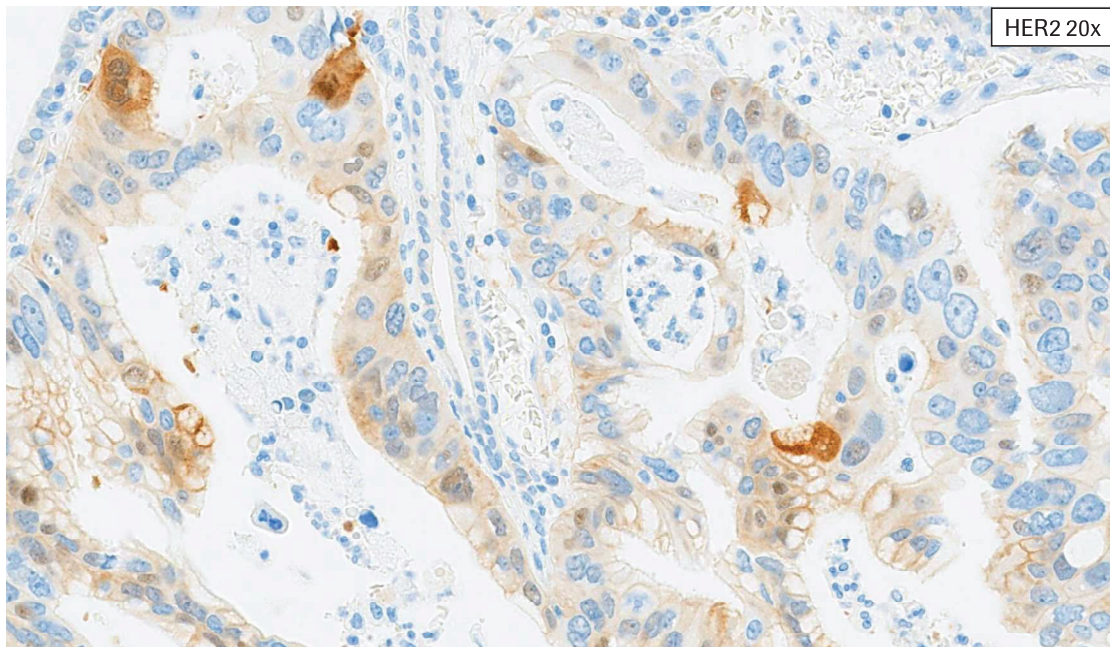
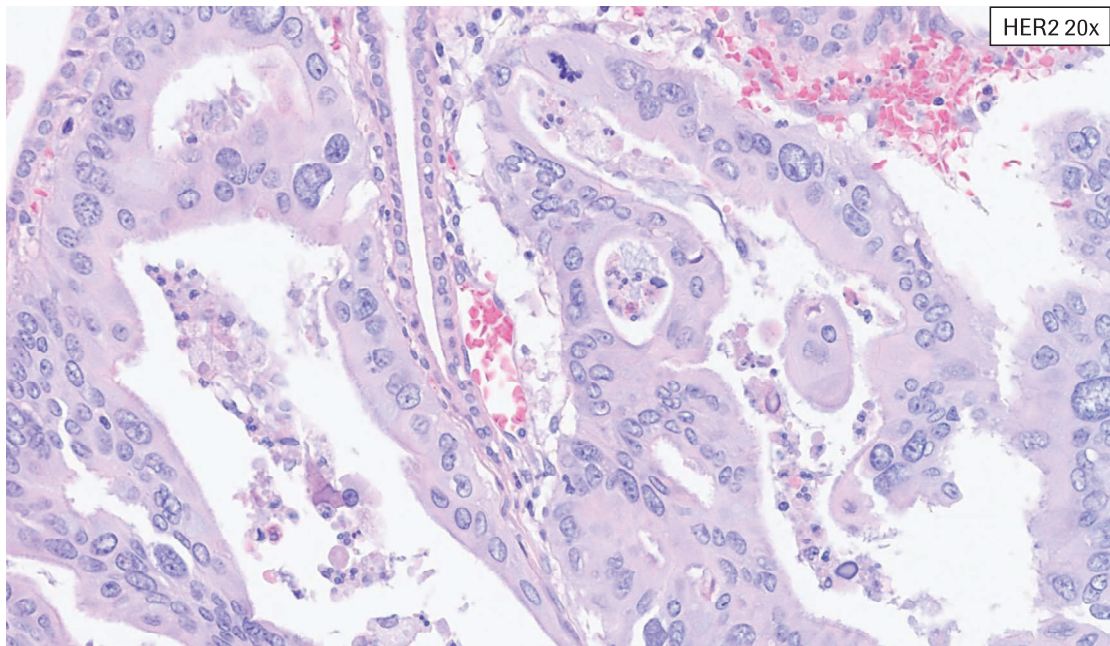
These cases exhibit staining in the cytoplasmic and/or nuclear compartments. Cytonuclear staining may be present with or without membrane staining. **HER2 IHC score should include ONLY membrane staining; cytonuclear staining should not contribute to the IHC score.**



Cytonuclear Gallbladder Adenocarcinoma Resection Case 1: IHC Score 0. The 10x image demonstrates tumor cells with faint to strong cytoplasmic and cytonuclear staining. Less than 10% of tumor cells demonstrate faint/weak membrane staining, consistent with IHC score 0. Only tumor cells demonstrating membrane staining should be evaluated to determine the HER2 IHC score. Those cells with only cytonuclear staining should be excluded.



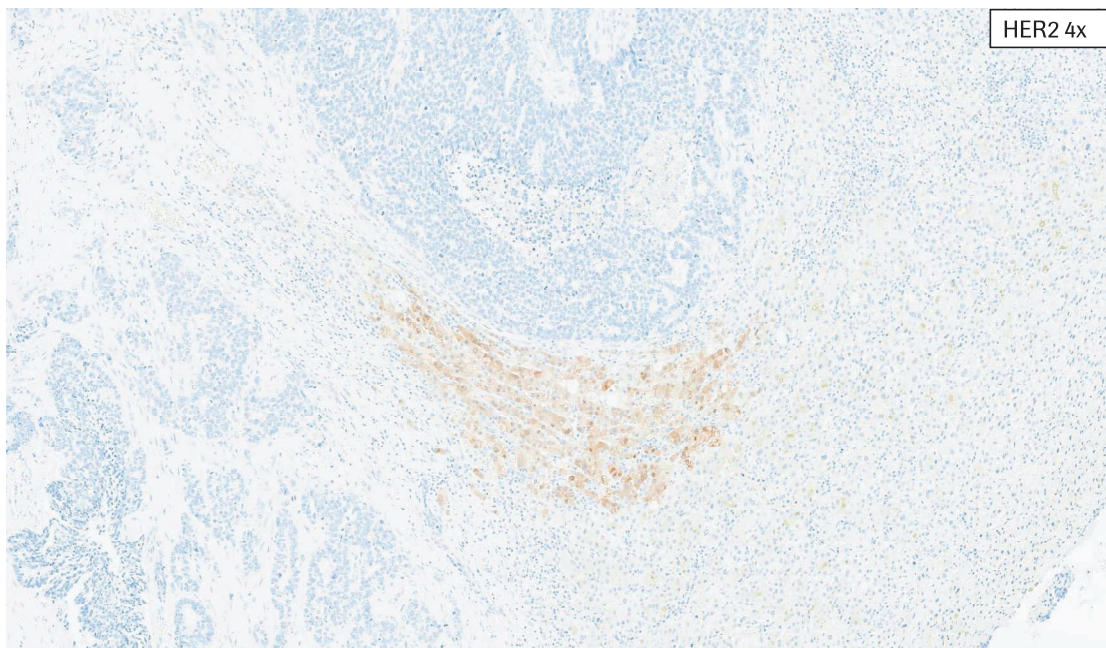
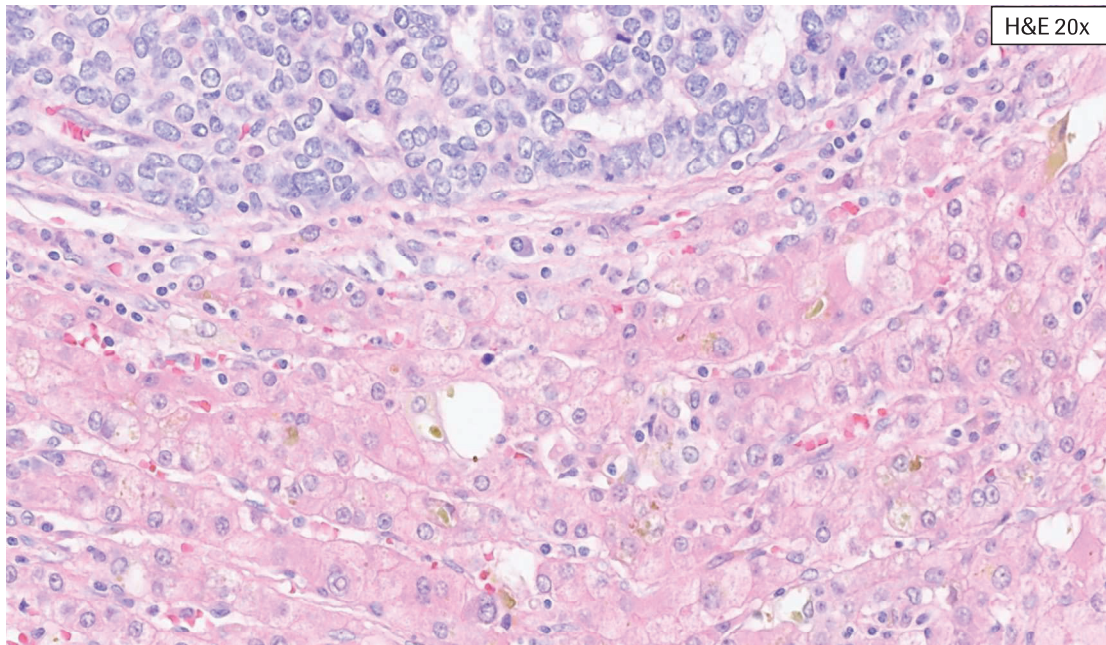
Cytonuclear Gallbladder Adenocarcinoma Resection Case 1: IHC Score 0. Higher magnification images demonstrate the range of intensity of cytonuclear staining. 20x and 40x images demonstrate examples of tumor cells with membrane staining (arrows) which should be included in determining HER2 IHC score.



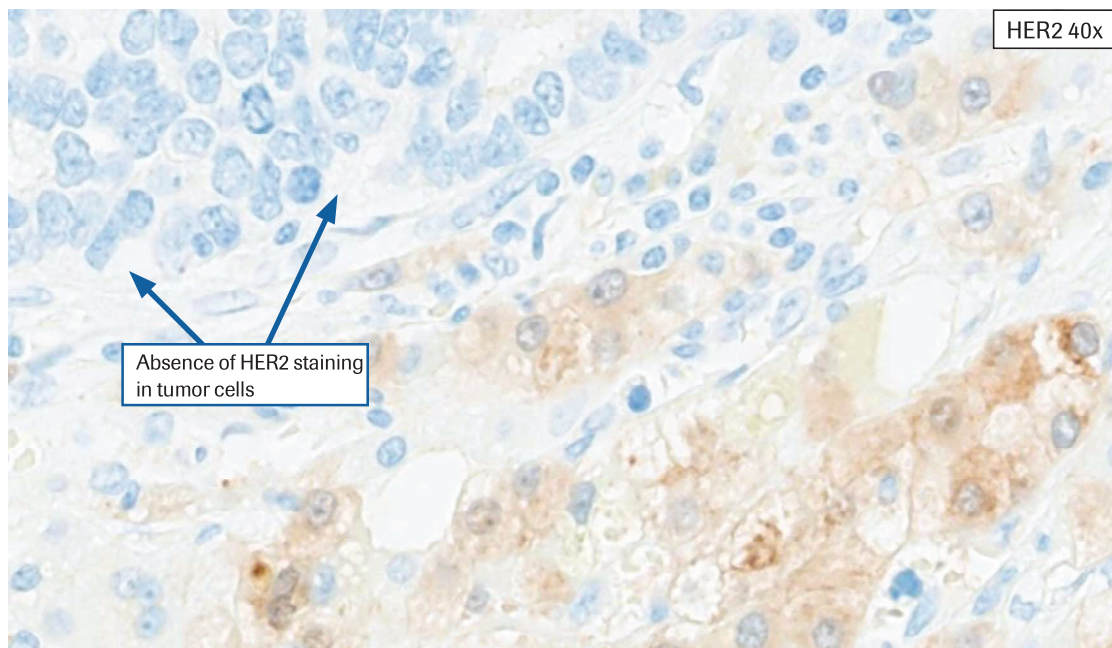
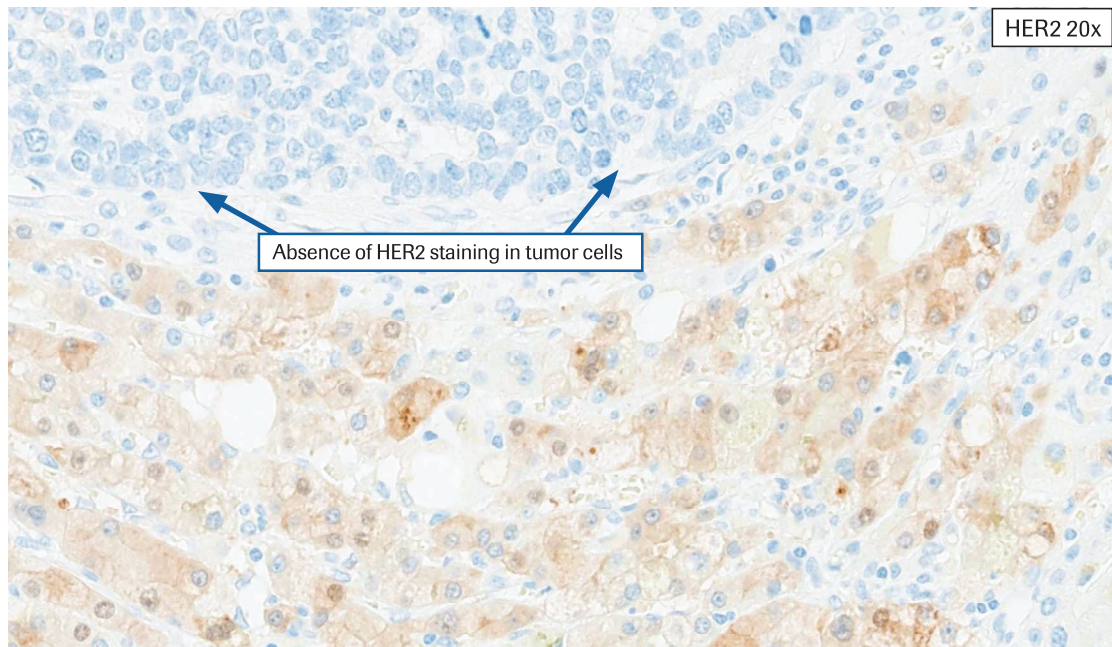
Cytonuclear Gallbladder Adenocarcinoma Resection Case 2: IHC Score 0. The 20x image demonstrates tumor cells with faint to strong cytoplasmic and cytonuclear staining. Less than 10% of tumor cells demonstrate weak to moderate membrane staining, consistent with IHC score 0. Only tumor cells demonstrating membrane staining should be evaluated to determine the HER2 IHC score. Those cells with only cytonuclear staining should be excluded.

Staining Artifacts: Cytonuclear Staining in Non-Neoplastic Cells

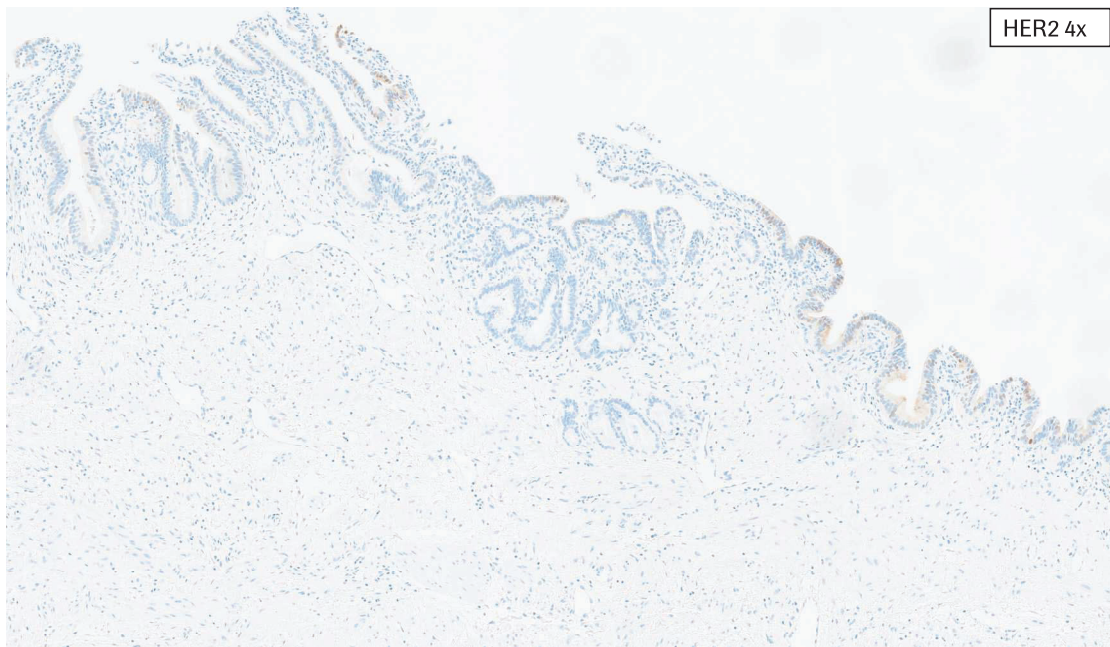
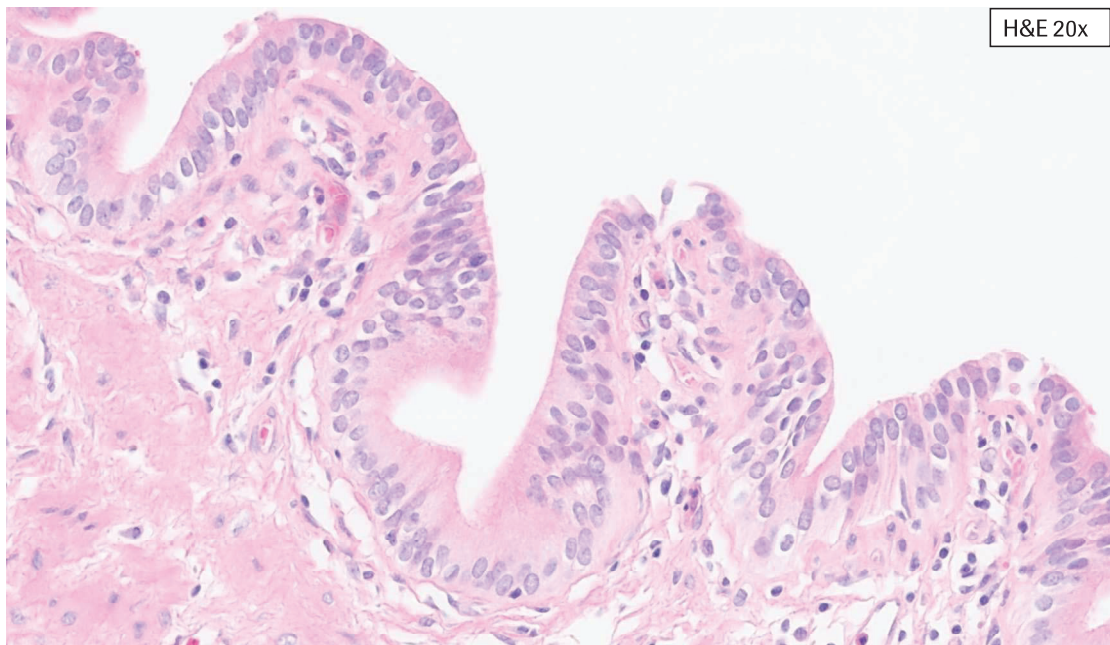
These cases exhibit HER2 IHC signal in the cytoplasmic and/or nuclear compartments of non-neoplastic cells that are adjacent to tumor cells.



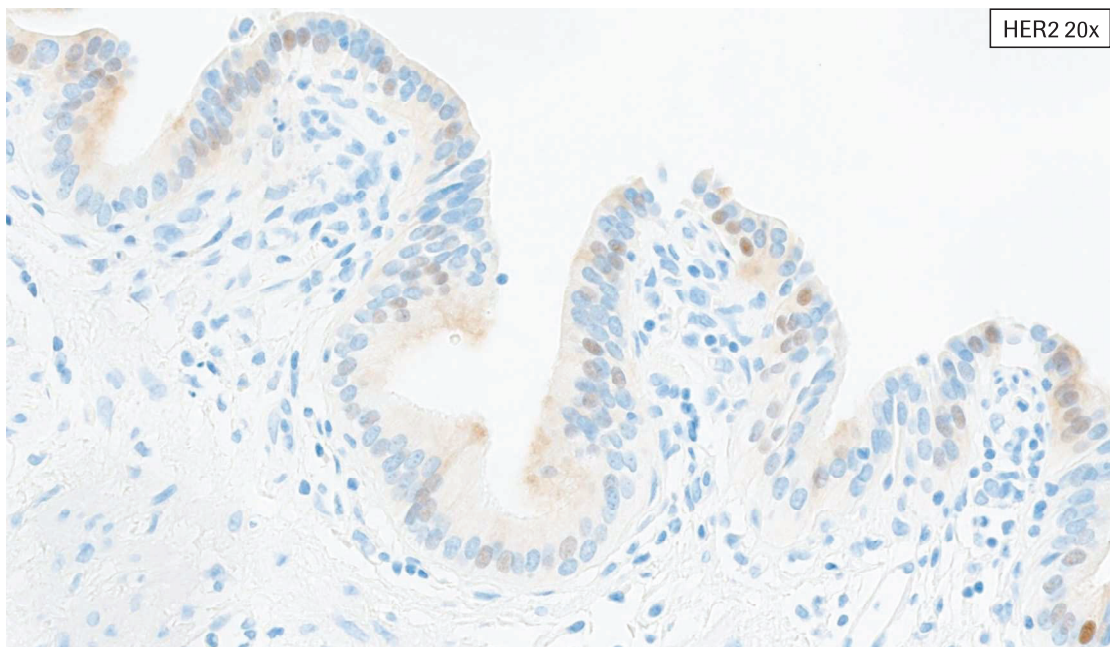
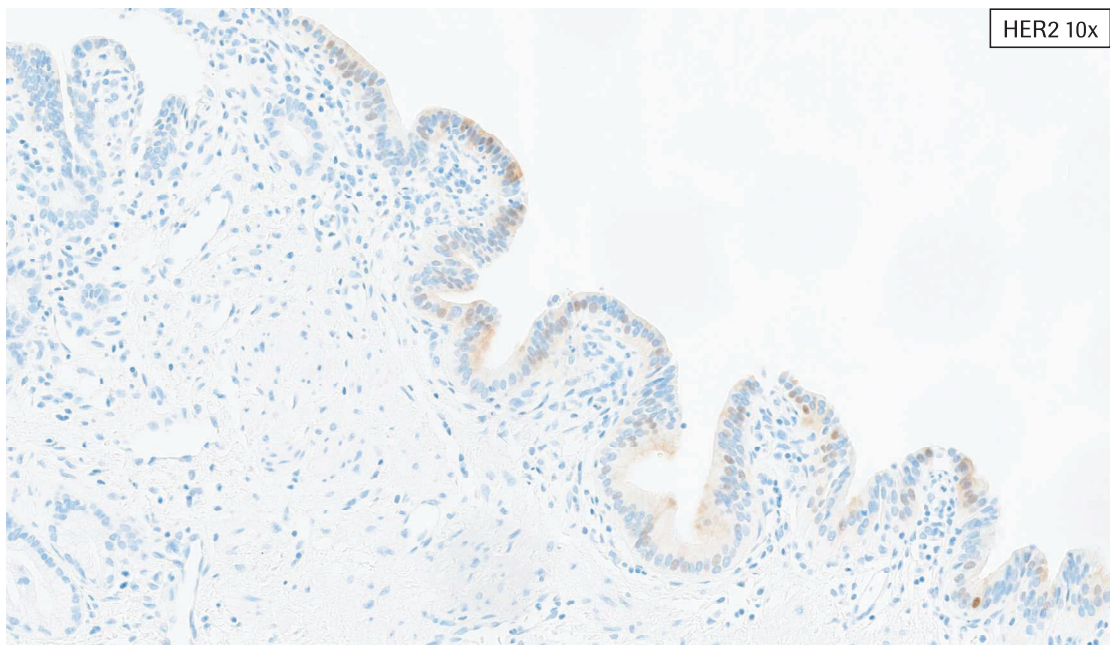
Non-neoplastic Hepatocytes - Cholangiocarcinoma Resection Case: Images demonstrate an intrahepatic cholangiocarcinoma without HER2 expression. The surrounding non-neoplastic hepatocytes, however, exhibit focal weak to moderate cytonuclear staining.



Non-neoplastic Hepatocytes - Cholangiocarcinoma Resection Case: Images at higher magnifications highlight the cytonuclear staining within hepatocytes and absence of HER2 staining within tumor cells (arrows).



Non-neoplastic Epithelium - Cholangiocarcinoma Resection Case: Images show cytonuclear staining within non-neoplastic biliary epithelium.



Non-neoplastic Epithelium - Cholangiocarcinoma Resection Case: Images at higher magnification demonstrate weak to moderate cytonuclear staining within non-neoplastic biliary epithelium.

Summary: Important Points to Consider

- The HER2 IHC scoring algorithm in BTC tissues is different from that in breast carcinoma
- Lateral, basolateral and complete membrane staining all count towards IHC score
- Staining that is limited to the basal or apical region (without lateral membrane staining) should not be included in HER2 IHC score*
- Staining in the basal region of tumor cells may be more intense than in the corresponding lateral membranes; the intensity of the lateral membranes drives the IHC score*
- Scoring method for biopsies and resections are the same, but cut-points are different:
 - $\geq 10\%$ for resections
 - ≥ 5 contiguous tumor cells for biopsies
- The objective method is used to evaluate intensity:
 - 4x and 20x are most important objectives for decision-making
- 2+ cases: include cases with membrane staining of weak to moderate intensity, (not just moderate intensity)

*Rationale: Staining in the basal region cannot be confirmed either as specific for HER2, or specifically localized to the tumor cell membrane.

References

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10. Neyaz A, Husain N, Gupta S, et al. Investigation of targetable predictive and prognostic markers in gallbladder carcinoma *J Gastrointest. Oncol*. 2018;9:111-25.
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