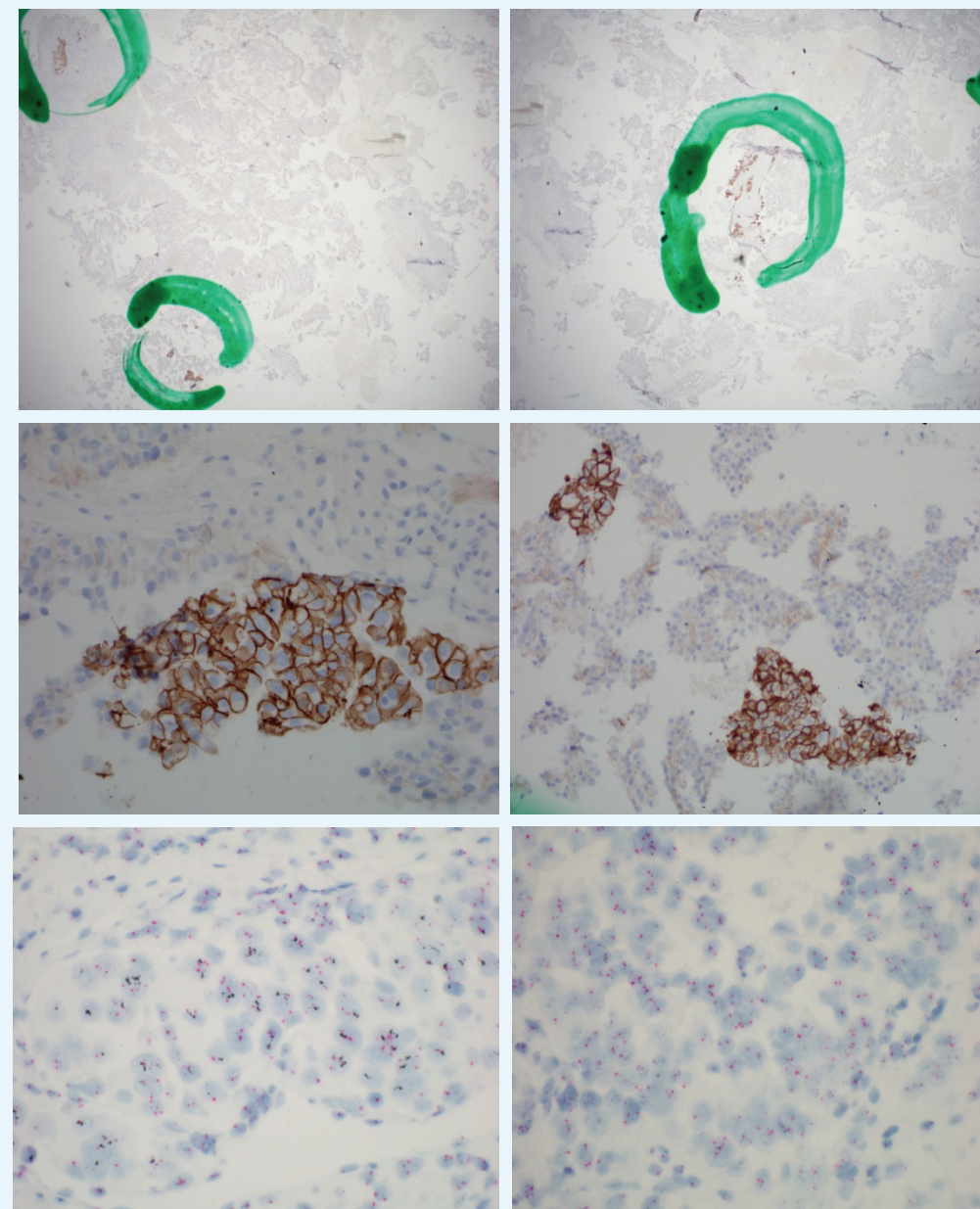


## CASE

The patient is a 75 year-old female with a history of stage 1 invasive ductal carcinoma of the left breast measuring 1.2 cm. Core biopsy of the mass demonstrated Nottingham grade 3 invasive ductal carcinoma; ER negative, PR negative, HER-2 negative (1+). The patient underwent mastectomy in April 2013. Mastectomy confirmed Nottingham grade 3 invasive ductal carcinoma pT1c/pN0/pMX with peritumoral lymphovascular invasion and invasive tumor less than 1 mm from the superoanterior margin. Due to comorbidities the patient was not considered a candidate for adjuvant chemotherapy.

In January 2014 the patient presented with a mastectomy site recurrence. Needle core biopsy confirmed recurrent Nottingham grade 3 ductal carcinoma with papillary differentiation. ER and PR remained negative. However, HER-2 immunohistochemistry showed clear intratumoral heterogeneity. The percentage of total tumor that the positive subclone represented was approximately 1%. The biopsy was vacuum-assisted, generous, and consisted essentially entirely of invasive carcinoma without an in situ component.



On low magnification the HER-2 IHC studies conducted on the core biopsy of the mastectomy site recurrence show a largely HER-2 negative tumor (score: 0/1+) with small islands of positive cells highlighted.

On higher magnification, it is evident that the focal proliferations of HER-2 positive cells are strongly stained on IHC (score: 3+).

In situ hybridization of the IHC positive subclones confirmed HER-2 amplification (ISH amplified areas shown on left, non-amplified on right)

## BACKGROUND

HER-2/neu (c-erbB-2) is a proto-oncogene located on chromosome 17 (17q12-21.32) that codes for a 185-kDa transmembrane protein of the epidermal growth factor receptor family of tyrosine kinases. Overexpression of HER-2 is present in around 25% of breast cancers. It promotes cell growth and division and is associated with increased disease recurrence and poor prognosis. Herceptin and related drugs are monoclonal antibodies that bind to the extracellular domain of the receptor altering its expression and effects by various mechanisms. When Herceptin was developed it was generally accepted that tumors either demonstrated HER-2 amplification or they did not. It was during the continuing evolution in methodologies for assessing HER-2 status that the issue of tumor heterogeneity arose.

## CURRENT ASCO/CAP GUIDELINES

- IHC: 10% complete circumferential membrane staining (3+)
- ISH (dual probe): HER-2:CEP17 ratio  $\geq 2$
- ISH (single probe): average HER-2 copy number  $\geq 6$ /nucleus

## DISCUSSION

Most breast cancer cases are uniform in their expression and amplification of HER-2. Intratumoral heterogeneity has been estimated to occur in 5-30% of HER-2 positive breast cancer cases. Recent papers suggest it may be even more frequent, and that a subset of these may benefit from HER-2 targeted therapy. This begs the question of why a certain tumor, such as that presented in this case, would be considered negative and ineligible for Herceptin therapy despite containing HER-2 positive cells. In analyzing the ASCO/CAP guidelines and modalities of determining HER-2 positivity (IHC and ISH), we discovered certain inconsistencies which demonstrate that neither technique is yet definitive.

The HER-2:CEP17 ratio method for accounting for chromosome 17 polysomy was largely implemented before the extreme rarity of this condition in breast cancer was appreciated. In cases where ISH is conducted alone, tumors with ratios less than 2 may be ruled as negative due to CEP17 co-amplification with HER-2. Additionally, traditional fluorescent in situ hybridization techniques

allow for good HER-2 signal discrimination but suffer from poor visualization of the cells themselves, thus making it difficult to discern tumor from non-tumor when counting amplified cells. ISH also requires high magnification (600x -1000x) which makes assessment of heterogeneity within an entire tumor prohibitively demanding and time consuming. The major limitation of IHC is that some tumors fall into the equivocal 2+ category and are indeterminate without reflex ISH testing. The 10% cutoff in the ASCO/CAP guidelines is similar to that used in most studies with Herceptins, so little is known about the efficacy of monoclonal antibody treatment against tumors with smaller HER-2 amplified populations.

## QUESTIONS FOR FURTHER STUDY

- Do treatment guidelines for Herceptin initiation need to be altered in order to better account for patients with HER-2 heterogeneity?
- Is treatment without Herceptin a selective pressure for HER-2 proliferation in a heterogeneous tumor?
- Are there identifiable characteristics or markers which indicate an increased likelihood of tumor evolution from HER-2 negativity to positivity?
- Should HER-2 be re-assessed in biopsy-negative excision specimens to more thoroughly search for heterogeneous subpopulations?
- Would the more inclusive guidelines for HER-2 heterogeneous gastric tumors be applicable in certain cases of breast cancer?

## CONCLUSIONS

HER-2 tumor heterogeneity is increasingly recognized as an unresolved issue in breast cancer that poses significant diagnostic and therapeutic challenges. Our understanding of the process is incomplete but continued work in this area holds promise not only for more effective personalization of therapy, but for increasing our understanding of tumor evolution in general.

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