BREAST CANCER IN FOCUS

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Intratumoral HER2 Heterogeneity in Breast Cancer

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H&O How is intratumoral HER2 heterogeneity defined?

MP No general consensus exists regarding the definition of human epidermal growth factor receptor 2 (HER2) heterogeneity. That being said, Dr Otto Metzger Filho and colleagues at the Dana-Farber Cancer Institute presented a definition of HER2 heterogeneity, used in a phase 2 trial of neoadjuvant ado-trastuzumab emtansine (T-DM1; Kadcyla, Genentech) plus pertuzumab (Perjeta, Genentech) in patients with HER2-positive breast cancer, at the 2019 annual meeting of the American Society of Clinical Oncology (ASCO). The trial investigators defined intratumoral HER2 heterogeneity as at least 1 of 6 areas of a biopsied tumor demonstrating either (1) HER2 positivity by fluorescence in situ hybridization (FISH) in more than 5% and fewer than 50% of tumor cells or (2) an area of tumor testing negative for HER2. As is frequently the case when attempts are made to define cutoffs for variables that are continuous rather than dichotomous, a concern regarding the first part of the definition is that in a rapidly proliferating tumor (eg, with a high Ki67 level), some percentage of the cells may be undergoing DNA synthesis in S phase in which the DNA at the *HER2* locus has been copied but the chromosome 17 centromere (control probe sequences) DNA replication is incomplete. In this situation, one might see a fraction of cells (perhaps >5% and <50%) with *HER2*/CEP17 ratios that are slightly greater than 2, and approximately 4 copies of *HER2* by FISH. This finding is clearly not an example of HER2 heterogeneity, but rather of pathophysiologic DNA synthesis during S phase in non–*HER2*-amplified tumor cells that are proliferating rapidly. A precise definition of intratumoral HER2 heterogeneity probably must await further technological breakthroughs. One example of such a breakthrough would be the application of single-cell sequencing technologies when or if this breaks the cost threshold.

Nearly all oncologists who treat patients with breast cancer have seen cases in which the *HER2* gene is focally (geographically) amplified in some of the tumor cells but not all—a classic example of heterogeneity in HER2 alteration. This more general descriptive definition that we see on pathology reports from time to time, however infrequently, falls within the second part of the definition of HER2 heterogeneity used in the Dana-Farber study.

H&O Is this form of breast cancer recognized as a distinct subset of HER2-positive breast cancer?

MP I was fortunate to be asked to be the discussant after a series of presentations on the issue of intratumoral HER2 heterogeneity at the 2019 ASCO annual meeting. At that time, I declared it to be a "distinct clinical entity." These tumors tend to have lower levels of HER2 expression on average than other HER2-positive tumors. They certainly have lower rates of a pathologic complete response (pCR) to T-DM1 alone or to T-DM1/pertuzumab. The I-SPY2 trial, presented by Dr Julia Wulfkuhle of George Mason University at the 2019 ASCO meeting, also found similarly low pCR rates among patients with HER2-heterogeneous tumors. So, emerging evidence from the clinic suggests that HER2 heterogeneity is a distinct clinical entity.

H&O How common is HER2 heterogeneity?

MP In the Dana-Farber study, intratumoral HER2 heterogeneity was detected in 16 of 157 samples—approximately 10% of viable cases. In another trial presented at the 2019

ASCO annual meeting and published on the same day, the phase 3 KRISTINE trial of 444 patients, the rate of locoregional progression events before surgery was 6.7% for those who were randomly assigned to neoadjuvant T-DM1 and pertuzumab and was 0% for those who were assigned to docetaxel, carboplatin, trastuzumab, and pertuzumab. The researchers concluded that HER2 heterogeneity probably accounted for this difference because the HER2-positive clones of the patients whose disease progressed would have been wiped out while the HER2-negative clones continued to grow despite treatment. So I would put the rate at somewhere between 6% and 10% on the basis of the results of these 2 trials. However, if one looks at the purely geographic definition of intratumoral HER2 heterogeneity by FISH, in which a definite boundary exists between areas that are clearly amplified and areas that are clearly diploid, the frequency is very low, perhaps 1% or 2%

H&O How do oncologists go about assessing intratumoral HER2 heterogeneity?

MP FISH is already routinely done in the pathology laboratory to assess HER2 status. Although the pathology reports do not routinely contain information on HER2 heterogeneity as defined by the Dana-Farber group, if precise definitions and cutoffs could be agreed on by consensus, then such information could be added to future pathology reports.

In routine FISH pathology reports for the *HER2* gene amplicon, approximately 20 tumor cell nuclei are counted to determine the *HER2* gene copy number. To detect heterogeneity, more than 20 nuclei would have to be counted to identify cases with a lower number of cells in which *HER2* is not amplified. Detection would also require examining multiple biopsy samples or multiple parts of a tumor to look for geographic differences in *HER2* amplification across the face of the tumor, which requires a lot more time. Both steps would have to be taken in the pathology laboratory on a regular basis to identify these cases prospectively.

H&O How does HER2 heterogeneity affect response to treatment?

MP None of the patients in the Dana-Farber study who were classified as having breast cancer with HER2 heterogeneity had a pCR to T-DM1/pertuzumab. The study met its primary endpoint by demonstrating a significant association between intratumoral HER2 heterogeneity and pCR rate, stratified by estrogen receptor (ER) status. As this and other studies have shown, patients who are HER2-positive have a lower pCR rate if they are also ER-positive. However, even after control for ER status, the pCR rate of patients with HER2-heterogeneous samples was still lower than the pCR rate of those with HER2-homogeneous samples. In a secondary analysis, the Dana-Farber group also demonstrated a statistically significant association between HER2 heterogeneity and pathologic response, defined as a residual cancer burden of 0 or 1. Finally, the statistically significant association between HER2 heterogeneity and pCR was maintained when patients were stratified by ER status and by HER2 immunohistochemistry (IHC) status (2+ vs 3+).

As I mentioned earlier, in the KRISTINE trial, 6.7% of the patients in the T-DM1/pertuzumab group had locoregional progression events, compared with none of the patients in the control arm (chemotherapy plus dual-antibody therapy). These findings suggest that a high level of HER2 heterogeneity can lead to a drug-resistant phenotype. Clinicians ultimately have the ability to define HER2 heterogeneity as the situation in which an obviously non–*HER2*-amplified clone or clones emerge following the selection pressure of treatment of a clearly *HER2*-amplified tumor with HER2-targeting agents. However, this definition obviously can be applied only in hindsight, not prospectively at the time of primary diagnosis.

H&O Should HER2 heterogeneity affect the choice of treatment?

MP Yes, it should. As we saw in both the KRISTINE trial and the nonrandomized Dana-Farber trial, with a chemotherapy-based treatment option, such as chemotherapy plus dual-antibody therapy with pertuzumab and trastuzumab, the chemotherapy can "cover" the HER2-negative clone in patients with a heterogeneous admixture of HER2-positive plus HER2-negative tumor cells. The standard of care remains a taxane-based chemotherapy combination, along with trastuzumab and pertuzumab. Some people still use anthracycline-based regimens followed by taxanes plus the 2 antibodies, which can be effective, but the chemotherapy backbone is important because it can directly address tumor heterogeneity.

Other approaches may become available in the future, such as the use of newer HER2 antibody-drug conjugates (ADCs) with soluble payloads and bystander effects. In contrast, T-DM1 does not have a soluble payload (DM1) and cannot diffuse into neighboring tumor cells that are HER2-negative. Without a bystander effect, the HER2-negative cells survive intact, and only the HER2-positive cells are killed by T-DM1.

We also know that the ADC trastuzumab deruxtecan (Enhertu, Daiichi Sankyo/AstraZeneca) is different from T-DM1 in that the topoisomerase 1 payload on the newer ADC does have activity against tumors with low levels of HER2. A phase 2 trial that Dr Hiroji Iwata of Aichi Cancer Center Hospital in Nagoya, Japan, presented at the ASCO annual meeting in 2018 established that trastuzumab deruxtecan has clinical activity even in patients with an HER2 status of 1+ or 2+ on IHC. Published

studies have also shown that HER2 transcript levels are detectable in HER2 IHC 0 tumors, and that the transcript levels in IHC 0 tumors are similar to those in 1+ or 2+ samples, suggesting that ADCs like trastuzumab deruxtecan may work for nearly all patients because HER2 is expressed at least at some level in most breast cancers. Dr Cristina Saura, of Vall d´Hebron University Hospital in Barcelona, Spain, presented a phase 1 study of the ADC trastuzumab duocarmazine, also known as SYD985, at the 2018 ASCO annual meeting. This study also found responders among patients with HER2-low, FISH-negative breast cancers treated with SYD985.

Another possible alternative for the future would be to use 2 different ADCs: one that targets HER2 in HER2-positive clones and one that covers a different target that is present on HER2-negative cells. For example, tumor-associated calcium signal transducer 2 (TROP2) is a target on triple-negative breast cancer cells, so if you had an admixture of triple-negative plus HER2-positive cancer, you could use that ADC combination to get responses, at least in theory. These approaches represent future opportunities for clinical trials, although the overlapping toxicities of the ADCs in some of the combinations could be challenging.

H&O What other studies have looked at HER2 heterogeneity?

MP A 2019 study by Dr Jennifer L. Caswell-Jin and colleagues from the laboratory of Christina Curtis at Stanford University analyzed multiple-region whole-exome sequencing data from 15 primary breast tumors across clinical subtypes. The investigators measured the HER2-negative population over time following the use of trastuzumab and lapatinib (Tykerb, Novartis) and showed the gradual replacement of HER2-positive tumors by HER2-negative clones in a modeled fashion. So you can see how the use of HER2-targeted therapy in HER2-heterogeneous tumors actually selects for a HER2-negative population.

One possible explanation for the emergence of HER2-negative clones following treatment with HER2-targeted therapy is loss of the *HER2* amplicon by genetic deletion. However, with all of the emerging new data on heterogeneity, this theoretical mechanism is no longer believed to be a viable model. It seems counterintuitive for an oncogenic driver gene amplification event to be lost because of a subsequent deletion event. Now we know that the HER2-negative clones are there from the start, in some cases on a microscopic level, and can be missed even by a good pathologist. When we see treatment failures during HER2-targeted therapy, we have evidence that the cause may be HER2 heterogeneity.

H&O Are any ongoing or planned studies looking at HER2 heterogeneity?

MP The phase 3 DESTINY-Breast04 trial of trastuzumab deruxtecan is enrolling patients with HER2-low breast cancer that has spread or cannot be surgically removed (NCT03734029). Although this trial is not directly aimed at the heterogeneity problem, if successful it could provide a scientific rationale for the deliberate use of HER2-directed ADCs to target cell populations in heterogeneous tumors that are non–HER2-amplified.

H&O What other questions remain to be answered regarding intratumoral HER2 heterogeneity?

MP We need to understand the biology better. So far, we do not have a sufficient number of experimental tumor samples from human clinical trials to be able to characterize these novel and interesting HER2-negative clones that emerge after HER2-targeted therapy. Do they tend to be ER-positive? Do they have distinct clinical pathologic biomarker features, or do they have completely different molecular alterations that are yet to be discovered? If so, we need to learn about them so we can think about future molecularly targeted therapeutic approaches or immunotherapeutic approaches. This research is still in its infancy because we do not have an in-depth understanding of the molecular characteristics of HER2-negative cells that can outgrow a HER2-positive tumor.

Disclosure

Dr Pegram has consulting agreements with Roche/Genentech, AstraZeneca, and Novartis.

Suggested Readings

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