Cancer Care Ontario

June 2016

Summary Statement: 2013 ASCO/CAP HER2 Guidelines: Building a Consensus for Ontario

Introduction

The most recent updates to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations for HER2 testing in breast cancer (Wolff et al., 2013) were released in October 2013.

CCO convened an expert panel in April 2014 (see Appendix A) to discuss how these changes impact practice in Ontario. The panel focused their attention on difficulties arising from changes in the guidelines and not on issues that continue to be a challenge from previous guidelines (e.g. pre-analytic handling). The following points were addressed:

- Interpretation of HER2 immunohistochemistry (IHC): 1+ vs. 2+
- Testing on core biopsies and re-testing on the excision
- Using ISH when the IHC is clearly negative
- Approach to take when both IHC and ISH are equivocal
- How to report “monosomy”
- Implications for treatment of “equivocal” and unusual cases
- Heterogeneity

The CCO panel reached a consensus concerning some of the more common difficulties encountered in interpreting the 2013 ASCO/CAP HER2 Guidelines, which was presented by Dr. Martin Chang in May and June 2014 as a web conference. This document summarizes the recommendations made concerning these points.

Background

The most significant changes made to HER2 testing by the 2013 ASCO/CAP HER2 guidelines are shown in Table 1. These changes reflect the consensus that the criteria set for the bulk of the trastuzumab clinical trials in the adjuvant setting should be used to define “HER2 Positive”. This approach is similar to that taken prior to the 2007 Guidelines and by the U.S. FDA in listing the indication for trastuzumab for breast carcinoma. The guidelines also re-define “equivocal”, to create a category for tumours *not fully satisfying the indication to treat*. This is to be contrasted to the previous approach, which was intended to express a “coefficient of variation” near the threshold. The revised approach should simplify the interpretation of an “Equivocal” report by the treating oncologist(s).

Table 1: Major Changes in 2013 Breast HER2 Guidelines

2007 Criteria	2013 Criteria
• IHC: 3+ defined as > *30%* strong, membranous staining	• 3+ defined as > *10%* strong, circumferential membranous staining
• ISH: Positive defined as having a HER2/Chr17 *Ratio > 2.2*	• ISH: Positive defined as having a HER2/Chr17 ***Ratio ≥ 2.0 or HER2 ≥ 6***
• ISH Equivocal: Defined as *HER2/Chr17 Ratio of 1.8 to 2.2 Ratio of 2.0 and above are eligible for trastuzumab*	• ISH Equivocal: Defined as ***HER2 copy number ≥ 4 but < 6 None of this range eligible for trastuzumab***

Recommendations

Interpretation of HER2 IHC: 1+ vs. 2+

1. **Interpretation of IHC 1+ and 2+ have not changed with the release of the 2013 ASCO/CAP Guidelines; the new Guidelines should *not* be interpreted as lowering the threshold between 1+ and 2+.**
2. **To classify IHC as Equivocal (2+), "circumferential" pattern, defined as having at least a cluster of cells with "rim-like" or "honeycomb" membranous staining that comprise >10% of the tumour, is required.**
3. **"360-degree" staining throughout every cell present is not required, but it is recommended that enough staining be present using 10x objective to appreciate this pattern.**

Discussion

The panel notes that the 2013 ASCO/CAP wording of the IHC 1+ and 2+ interpretation categories is a potential source of confusion. It gives the impression that many cases previously considered *negative (1+)* would now be considered *equivocal (2+)*. For example, the 2013 definition of *equivocal (2+)* includes this description:

> *Circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of tumor cells.*

The phrase "incomplete and/or weak…" is also implied in previous definitions of *negative (1+)*. The 2013 ASCO Guideline Data Supplement (#7: IHC Interpretation Criteria) includes the recommendation to "Ignore incomplete or pale membrane staining." It should be emphasized that the evidence base concerning the interpretation of IHC has not changed with the release of the 2013 guidelines. As such, the guidelines should not be interpreted as lowering the threshold between 1+ and 2+.

The definition cited above begins with "circumferential", and the presence of the circumferential pattern is required to classify IHC as *equivocal (2+)*. "Circumferential" should be defined as having at least a cluster of cells with rim-like or "honeycomb" membranous staining. Taken together, the clusters of cells with this pattern should comprise >10% of the tumor to be classified as 2+.

To qualify as IHC 2+, it is not necessary to observe "360-degree" staining throughout every cell present, but enough staining should be present to appreciate this pattern using the 10x objective (the magnification used to appreciate circumferential staining can be used to estimate overall intensity). Because of this, the term "circumferential" may be considered more precise than "complete", the latter term being widely used previously.

Testing on core biopsies and re-testing on the excision

4. **It is recommended that the first test for HER2 should be performed on the core biopsy as routine practice, upon diagnosis of invasive breast carcinoma, if available.**
5. **Repeat testing on the subsequent excisional specimen is recommended and should be viewed as medically necessary where:**

    a) **Test results from core needle biopsies are equivocal**
    b) **Core needle biopsy specimens are insufficient for evaluation**
    c) **Core needle biopsy specimens cannot be evaluated owing to a pre-analytic or technical problem**
    d) **Core needle biopsy result is unexpected with respect to other histopathologic features of the tumour (see Table 2 of 2013 ASCO/CAP Guidelines for the common discordances)**
    e) **Other circumstances in which the pathologist deems repeat testing to be appropriate based on their clinical judgment**
6. **Repeat testing should continue to be performed for cases after neo-adjuvant treatment**
7. **An ER-negative core biopsy (regardless of PgR or HER2 result) should also allow ER and PgR to be reassessed on the excision specimen. In this instance, HER2 may also be retested, if HER2 was negative or equivocal.**

Discussion

The 2013 ASCO/CAP guidelines state that the first test for HER2 should be performed on the core biopsy, upon diagnosis of invasive carcinoma, if available. ER/PgR/HER2 testing on breast cancer core biopsies is routine in the United States, and in numerous Canadian labs. The panel acknowledges that this is not a universal practice in Ontario, either because of resource constraints or because of lower neoadjuvant-treatment rates in some regions. Nevertheless, receptor status on the core biopsy is useful for planning of pre-operative management, including deciding suitability for breast-conserving surgery and neoadjuvant chemotherapy.

In some cases, additional testing on a subsequent excisional specimen will be indicated. The main indication for repeat testing is if the first result is not clearly positive or negative. This includes core biopsies that are equivocal (by both IHC and ISH), core biopsies that have insufficient tumour for evaluation, or core biopsies that cannot be evaluated owing to a preanalytic or technical problem. In addition, there are cases in which the result on the core biopsy is unexpected with respect to other histopathologic features of the tumour. The 2013 ASCO/CAP guidelines has tabulated the most common discordances—the panel supports the consultation of this guideline in re-testing decisions, but notes that there may be circumstances not covered in this table in which the pathologist deems repeat testing to be appropriate based on their clinical judgment. In

all of the above situations, the panel recommends adding a statement to the core biopsy biomarker report recommending re-testing on the excisional specimen.

Both the 2012 CAP Cancer Protocol and the 2013 guidelines continue to recommend that cases be re-tested after neoadjuvant treatment. An ER-negative core biopsy should also be reassessed at excision with respect to hormone receptors; however, it is common practice to also reassess HER2 at this time if the HER2 was also negative on the core. Finally, the panel emphasizes that testing on core biopsies does not remove the need to standardize and report the pre-analytic handling of excisional specimens.

In summary, routine breast biomarker testing on core biopsies with invasive breast carcinoma is a reasonable practice and recommended by the 2013 ASCO/CAP guidelines. Compared to testing the excision alone, this practice involves a higher number of tests per patient because of the need for repeat testing. These repeat tests are to be viewed as medically necessary, and should be supported by CCO reimbursement.

When to perform ISH when HER2 IHC is clearly negative?

8. **ISH should be performed only if IHC is equivocal (2+). ISH should *not* be routinely performed on specific sets of negative IHC cases (0/1+). When IHC result is clearly negative, this should be reported as HER2-negative based on the IHC result.**

Discussion

Canadian laboratories perform HER2 IHC as the first test, with additional testing by ISH if IHC is equivocal. This is accepted as being the most cost effective; ISH is a complementary test and should not be considered a "gold standard" relative to IHC. Other algorithms are accepted by the 2013 ASCO/CAP guidelines, including performing ISH first, and performing both IHC and ISH on all cases. As such, the 2013 ASCO/CAP guidelines have no specific guidance for performing ISH on HER2-negative IHCs. Some major US centres use their own criteria to re-test IHC-negative cases using ISH, as presented by Hicks and Sarewitz in a College of American Pathologists web presentation on the HER2 update. Some of these criteria include high tumour grade and patient age under 50. This and similar approaches are not supported by the overall evidence.

The Canadian experience has been that IHC negative cases are overwhelmingly also ISH Negative (Hanna et al., J Clin Oncol 2014). This is attributed to the relatively high degree of standardization between laboratories performing HER2 IHC. Therefore, the panel does not support routinely performing ISH on specific sets of negative IHC. When an IHC result is clearly negative, this should be reported as HER2-negative based on the IHC result.

Testing of additional blocks and/or specimens on cases that are equivocal by both IHC and ISH

9. **Testing of additional blocks and/or specimen on equivocal cases may depend on pathologist judgment.**
10. **On core biopsies, an equivocal result can be reported with a recommendation to perform repeat testing on an excision.**
11. **On excisional specimens, most cases of low-level increase in HER2 copy number (i.e. 4 to 5) is not recommended for repeat testing. If the HER2 copy number is between 5**

and 6 on an excisional specimen, or if there are other clinical concerns, testing of one additional block may be recommended.

12. **If the disease is node-positive, testing of the lymph node metastasis may be considered if the primary tumour is equivocal.**

Discussion

In contrast to the 2007 version, the 2013 ASCO/CAP guidelines define the "Equivocal" category to include only cases that do not meet the major clinical trials and FDA criteria for HER2-positive. In order to report a case as HER2-equivocal, both IHC and ISH should be performed. When performing ISH, assessment of additional nuclei or by a second observer is recommended. (The number of nuclei recommended varies according to testing platform; it is common practice to count at least double the usual number of cells in equivocal cases.)

In some cases, it may be a concern that the block selected for testing may not be representative of the larger tumour. The recommendation to test additional blocks on equivocal cases may depend on pathologist judgment. On a core biopsy, an equivocal result can be reported with a recommendation to retest on an excision.

On excisional specimens, the panel agrees that most cases of low-level increase in HER2 copy number (i.e. 4 to 5) are not worth re-testing. If the HER2 copy number is between 5 and 6 on an excisional specimen, there may be a role for testing 1 additional block. This may also be considered based on clinical concern. The current evidence does not provide specific guidance on how to select this additional block.

If upon re-testing the case remains equivocal, it should be reported as Equivocal. A comment stating that the tumour is not considered eligible for trastuzumab treatment may be included, to clarify the significance of "Equivocal" to clinicians.

How to report monosomy

13. **Monosomy of Chr17 may lead to a HER2/Chr17 ratio > 2.0, but without a high HER2 copy number. In the literature, true "monosomy" is rare and likely corresponds to those cases with >90% of cells having only 1 Chr17 signal. However, there are cases in which a smaller subset of tumour cells have monosomy. When cells with both 1 and 2 Chr17 signals are present in an ISH study, it is recommended to selectively count the cells with two Chr17 signals.**
14. **For cases of monosomy classified as "HER2-positive", clinical judgment must be applied to determine the most appropriate management. As it is acknowledged that monosomy remains controversial and that there is a lack of definitive evidence for decision-making in these cases, a disclaimer may be included in the interpretation and/or report.**
15. **Terminology equating "monosomy" with "HER2-negative" should be avoided.**

Discussion

When the copy number of the control (chromosome 17) probe is low, the HER2/Chr17 ratio may fall in the Positive range, sometimes without HER2 being sufficiently amplified to present a target for therapy. Although true loss of the entire chromosome 17 is rare, these cases are referred to

as “Monosomy”. It is the panel’s opinion that these cases of true Monosomy likely correspond to those tumours presenting with >90% of cells having 1 signal for Chr17. However, there are cases in which a smaller subset of tumour cells have monosomy. To avoid underestimating the Chr17 copy number, pathologists and designates interpreting ISH are encouraged to selectively count cells with two Chr17 signals when cells with both 1 and 2 Chr17 signals are present. The group acknowledges that Monosomy remains controversial and that there is a lack of definitive evidence for decision-making in these cases.

The 2013 ASCO/CAP HER2 Guidelines are strongly worded to consider such cases as “HER2-positive”. This is based principally on subset analysis from the HERA trial (Dowsett et al, 2009) showing that the treatment group defined as HER2-positive but with monosomy did not have a significantly reduced survival compared to the rest of the HER2-positive arm. This finding is limited by a relatively small sample size. One other study (retrospective design in the metastatic setting) found a lower response rate to trastuzumab in some cases of Monosomy (Risio et al, 2005).

In general, cases of Monosomy have an average HER2 copy number that falls between 2 and 6. Cases in which the Ratio is >2.0 and the HER2 copy number is ≥4 are widely considered to be HER2-positive. However, some cases considered HER2-positive based on Ratio >2.0 may have a copy number less than 4. As a result, there will be cases classified as “HER2-positive” in which some clinicians would not be inclined to treat with HER2-targeted agents. Clinical judgment must be applied to consider the most appropriate management in such cases. To reconcile this difficulty with the 2013 ASCO/CAP Guideline, a disclaimer may be added to the interpretation and/or report, for example: “HER2 Interpretation: Positive based on Ratio. (HER2 copy number <4. See Comment.) Comment: Although this case is considered Positive by 2013 ASCO/CAP guidelines, the predictive significance of this finding is not entirely clear.”

To avoid direct contradiction of the 2013 ASCO/CAP Guidelines (Supplement 2E), terminology equating “Monosomy” with “HER2-Negative” is to be avoided. Because clinical correlation is needed, CCO should evaluate funding these on a case-by-case basis when a treating oncologist wishes to consider targeted treatment.

Eligibility of “equivocal” cases for targeted therapies

16. **Cases that are “HER2-Equivocal” after IHC and ISH evaluation (i.e. those that have HER2 copy number between 4 and 6) are generally *not* eligible for targeted treatment.**
17. **Cases that remain difficult to resolve (i.e. intratumoural heterogeneity), should be considered on a case-by-case basis.**

Discussion

The changes implemented in the 2013 ASCO/CAP Guidelines should simplify decisions regarding eligibility for HER2-targeted treatment in most cases. The “HER2-positive” category is now defined to include all patients who should be considered eligible for trastuzumab treatment, notwithstanding the inclusion of some controversial cases of Monosomy (see above). Therefore, cases that are “HER2-equivocal” after IHC and ISH evaluation (i.e. those that have HER2 copy number between 4 and 6) are generally not eligible for targeted treatment. Some cases will remain difficult to resolve (e.g. heterogeneity, with a small but clearly amplified population), and should continue to be considered case-by-case by CCO.

Intratumoural heterogeneity

Intratumoural heterogeneity for HER2 expression/amplification can be difficult to report, owing both to complexity and to lack of clear evidence with respect to treatment. Based on the 2013 ASCO/CAP Guidelines, two distinct patterns of heterogeneous HER2 expression that do not meet the criteria for HER2-Positive can be identified:

Regional heterogeneity

Regional heterogeneity is defined as a small region (contiguous cells) of strong HER2 overexpression in less than 10% of the invasive tumour area.

18. **ISH should be performed in such cases as the pattern is considered "IHC equivocal (2+)". When interpreting ISH results, the ISH ratio should be computed separately in each region, the amplified focus and the non-amplified background area.**
19. **Reporting recommendation (ISH): Heterogeneous or "Focally amplified", with the percentage and size of the surface area amplified.**
20. **These cases may be considered for targeted therapy under the Evidence Building Program at CCO.**

Discussion

This reflects the presence of a distinct localized clone. This pattern is considered "IHC equivocal (2+)" by the 2013 ASCO/CAP criteria, and ISH is usually performed. However, interpretation of ISH is difficult, because the small region of HER2 overexpression is usually strongly amplified whereas the bulk of the tumour is not. In these cases, ISH ratio should be computed separately in each region, the amplified focus and the non amplified background area.

Scattered heterogeneity

Scattered heterogeneity is defined as IHC results that show scattered single cells with "360-degree" strong staining that comprise <10% of the overall population.

21. **ISH should be performed for such cases as this pattern is also considered "HER2-equivocal (2+)". When interpreting ISH results, the ISH score should include areas where these cells are present.**
22. **Negative/equivocal/positive based on the average ratio and HER2 copy number, according to the 2013 ASCO/CAP criteria. A comment describing the scattered calls can be provided.**

Discussion

If the IHC shows scattered single cells with "360-degree" strong staining that comprise <10% of the overall population, it should be considered Equivocal (2+) and further studied by ISH. In practice this pattern is rare. In these cases, ISH scoring needs to include areas where these cells are present. The average HER2 and Chr17 copy numbers are derived from all the cells counted in this field (representative of the tumour overall, and not only of the amplified subpopulation). There is currently no evidence that this pattern of heterogeneity has any predictive or prognostic significance independent of the actual HER2/Chr17 Ratio.

Appendix A – Expert panel members

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