HER2 Testing Revision Webinar Questions & Answer

Q: Finally we are saying that HER2 copy number is more important and HER2/CEP ratio is bringing all difficulties. Is it OK to count only HER2 copy number even though we use dual probe?

- Although some have advocated for getting rid of the CEP17 and just focusing on the HER2 copy #, if you are using a dual probe test, I think it is still prudent to count both HER2 and CEP17 and calculate a ratio. However, what the guidelines update is saying is that you need to look at both the ratio and the HER2 copy number in order to most accurately interpret the result.

Q: I am still difficult to understand HER2/17 Cen>2 and HER2 # is < 4 is deemed as positive? Is sufficient data is available? I saw the supplement, but is it convincing to declare as HER2 positive?

- Some members on the ASCO/CAP panel where in agreement with you on this. No one would expect a breast cancer with <4 copies of the HER2 gene to behave as HER2 positive breast cancer, and in the unlikely event if the ratio is >2 this is probably due to chromosome 17 monosomy. The challenge here is that these patient where eligible for enrollment on the adjuvant Herceptin trial and several members on the panel felt that we could not say that someone that was eligible for the trial was now not eligible for therapy in spite of the fact that there really is no good data to support benefit. In my view when this scenario is encountered one need to do a careful review of all of the data for the case (tumor grade, proliferative index, ER/PR) and then have a discussion with the patient’s medical oncologist. I think that the decision about therapy becomes a clinical decision at that point.

Q: Please comment on fine needle aspiration sample for HER2 diagnosis?

- A core is always better than an FNA because the test has been optimized and validated for formalin fixed paraffin embedded tissue. If an FNA is to be used, the sample should be collected in formalin and then fixed for a minimum of 6 hours before processing the cell block.

Q: Recent NIH study published in NIH journal states that more than 5% IHC0 or IHC+1 respond to transtuzumab? Also, highly amplied HER2 BC only 50% only respond to transtuzumab? Is this correct?

- There is some data that suggests that some patients that we would consider HER2 negative by clinical testing may respond to Herceptin. There is a current clinical trial (NSABP B47) that is looking at response in patients who are consider HER2 low/normal to try and address this question and the result from this trial are eagerly awaited. De novo and acquired resistance to HER2-targeted therapy is an important clinical issue that is an area of active investigation.

Q: Again it appears perhaps go only average HER # scoring may be a better alternative? The question is just follow FDA approved kit of Abbott. There are contradictions with guidelines. So which one to follow?

- There are several FDA approved testing kits for IHC and FISH/CISH and the guideline does not recommend one of these over another. The guideline does strongly encourage the use of an FDA approve test, however lab developed tests are acceptable if they have been properly and rigorously validated. As far as the CAP checklist requirements are concerned, if the manufacturer scoring criteria from an FDA-approved kit are different from the Guideline, the lab can choose to use either scoring criteria.

Q: Is there any scientific evidence that fixation greater than 72 hours affects results?
There is a prospective report from David Dabbs looking at out to 96 hours of fixation and there is no effect on ER, PR and HER2 testing. The guidelines committee settled on 72 hours because this would be in alignment with the time published in the ER guidelines. There are a few studies in the literature that suggest than long fixation times (weeks to months) can affect IHC, but this would not be clinically relevant.

Q: Is there any indication for HER2 testing on DCIS without invasive component?

There is a clinical trial looking at using a short course of Herceptin as a "radiosensitizer" in high grade HER2+ DCIS. The only reason to do HER2 testing on DCIS currently is in the context of a clinical trial. If the trial is a positive one leading to approval for Herceptin in high grade DCIS, we may be doing HER2 testing on DCIS in the future.

Q: Should the 10% neutral buffered formalin be buffered with phosphate or may other buffers be used?

There is a little bit of data coming from Steve Hewitt at the NIH that says that phosphate buffered formalin is better, but I do not know the details of this.

Q: What are your thoughts about performing FISH HER2 on decal specimens? Should it be performed with a disclaimer or should it be rejected altogether?

In my view, decal should be an exclusion criterion for HER2 FISH. We have tried numerous cases (the clinician called and said that this was the only site of metastatic disease) and they have all failed to hybridize. Even if you managed to get a result, I would include a disclaimer that said that the results might not be accurate because the test had not been validated for decalcified tissue. If HER2 is tested on decalcified tissue, CAP requires a disclaimer on the report (see ANP.22985).

Q: To say that you must have >10% of cells positive by FISH is misleading. If the sample is heterogeneous, we look for the area that shows positivity and count there. If we scored the entire sample, you would not necessarily find 10%. I think that the 10% rule should be removed for the FISH and it should be stressed to look at the entire specimen for amplification in pockets. If you find heterogeneity with some amplification, why is it important to report both ratios? Could you not just call this amplified?

You bring up a good point. The intention of the 10% of the tumor being positive was not intended in the literal sense (1 in 10 cells). For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires as you suggest a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important this here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important this here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+.

Q: Why was there a change from "weak" to "faint/barely perceptible"? Definitions in the article would be critical for reproducibility Score 0 - no staining or membranous staining. What do you do with cytoplasmic staining?
The language “faint/barely perceptible” I think is a little more specific than the descriptive term weak staining. In my experience, weak membrane staining is something that you only see at high magnification, moderate staining at intermediate magnification and strong staining is readily apparent at low power. Cytoplasmic staining is something that you occasionally will see, but only membrane staining has been shown to be clinically related to HER2-targeted therapy benefit. When I seen strong cytoplasmic staining in the absence of membrane staining I will usually send the case for FISH, but in my experience, the vast majority of these cases come back non-amplified.

Q: How did CAP inform its BPFT certificate holders of this change?
- I think that the BPFT certificate holders were emailed on the guideline update but I will have to check on this.

Q: The recommendation for testing metastases - does this mean lymph node staging at the time of definitive surgery? Micrometastases? Distant mets from different sites (patient with lung, bone and liver lesions)? Recurrences?
- The intention here with this recommendation was not to test synchronous lymph node metastases at the time of definitive surgery (the primary should be tested), rather, recurrent breast cancer metastases should be tested.

Q: Are there recommendations for testing different tumors of similar histology in the same breast?
- In my practice if we have multifocal breast cancer that shows a similar histologic appearance, I think that testing the largest focus of invasive cancer is all that is required, particularly if there is an extensive intraductal component or prominent LVI which is probably the explanation for multifocal disease. If the two or more foci of invasive tumor show different morphologies, then I think testing all of the tumors is warranted.

Q: Should we already being using these guidelines?
- I think that now that you are aware of the guideline changes, then you should begin taking steps to implement these changes in your lab. This of course may take some time, changes in SOP’s and education of all of the stake-holders (technical staff, clinicians, and administrators) in order to implement. As was discussed, many of these changes will find their way into the LAP checklist. The 2014 CAP checklist edition, which will reflect the revised guideline, will be published in April 2014; labs subject to these new accreditation requirements will be those with accreditation expiration dates in Sept. 2014 and later. Notwithstanding this, I agree that best practice would be to begin to transition to the new guideline as soon as practical.

Q: Regarding the definition of equivocal 2+ IHC - has the term "incomplete staining" been changed from "non-uniform staining"? If so don't these terms have different meanings?
- I agree with you that this is confusing. The equivocal category includes circumferential membrane staining that is weak to moderate in >10% of the invasive tumor (non-uniform or “incomplete staining”) and circumferential membrane staining that is intense in ≤10% of the invasive tumor.

Q: For HER2 FISH, if 2 cells among 20 cells scored has ration >2 but the total ratio is <2 should this case be called positive for amplification by FISH?
- The intention of the 10% of the tumor being positive was not intended in the literal sense (1 in 10 cells or 2 in 20 cells). For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule
should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important thing here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important thing here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+.

Q: with routine pre-operative MRI, we are seeing many more multifocal/multicentric breast tumor cases. It is not that infrequent to see 3-5 separate tumors in some cases. Considering testing costs and turnaround time for test results, it seems impractical to follow the "test each and every tumor" recommendation. Are there any guidelines or thoughts on how to best handle these cases?

- I will give you my personal perspective here. In my practice if we have multifocal breast cancer that shows a similar histologic appearance, I think that testing the largest focus of invasive cancer is all that is required, particularly if there is an extensive intraductal component or prominent LVI which is probably the explanation for multifocal disease. If the two or more foci of invasive tumor show different morphologies, then I think testing all of the tumors is warranted.

Q: How about HER2 PCR in cases of otherwise Equivocal results?

- The guideline panel felt that there was insufficient clinical data to warrant recommended PCR results for HER2 be used to make treatment recommendations.

Q: Is there a minimal tumor size (on core biopsy) that you would either not test for HER2 or recommend testing on resection specimen?

- This is an interesting and practical question that I have given some thought to. If the ER or the HER2 result is positive on a core biopsy with limited tissue then I think that you have your answer. It is when the result is negative that you need to think about whether or not you have excluded HER2+ disease and this is where looking at the clinical and morphologic feature for the case can help guide the need for additional testing.

Q: Will these new guidelines change when we are treating patients with trastuzumab vs T-DM1 or more sensitive methods like NGS will be needed

- Interesting question. Right now, pertuzumab and TDM1 are not being used for front line therapy of HER2+ disease but that could change with the next generation of adjuvant and neoadjuvant clinical trials. All of the trial for the next generation HER2 targeting agents used the same HER2 testing strategy that we use for qualifying patients for Herceptin so I suspect that things will not change. Good clinical data will be necessary before we can say that there is any role for NGS is making treatment decision for HER2-targeted agents.

Q: If a high grade tumor is triple negative, you would suggest HER2 FISH if ANY of the parameters you listed apply (ie under 50 years, Ki67 >20%, etc)

- The guidelines only state that reflex or repeat testing should be considered if the initial HER2 test is negative and the tumor is high grade. I think that decisions about repeat testing are best made after a discussion with the medical oncologist about the case and their level of clinical concern for the patient.
Q: New checklist items regarding repeat testing - how will lab show compliance? Do we need to have SOP’s? Something in the report?

- Compliance can be documented by having a policy that reflects that is in accordance with the checklist requirement, and simply having the records on file of multiple HER2 test reports on appropriate cases.

Q: We are already using the new guidelines but will be inspected between January and April next year. That will not result in a penalty on our inspection will it?

- No, there is no problem at all. The CAP inspection program allows labs to implement the checklist requirements in the future edition.

Q: New guidelines are confusing for a tumor that has weak to moderate membrane staining in <10%. Is this 1+ or 2+?

- Weak to moderate membrane staining in <10% of the invasive tumor would be considered negative (1+). Intense membrane staining in <10% of the invasive tumor would be considered equivocal.

The 2013 guideline update asks the pathologist to interpret the staining in the morphologic context of the case.

Weak to moderate membrane staining pattern in a patient with a grade 1 tumor which was strongly ER/PR+ and had a low proliferative index is consistent with a negative result.

The same staining pattern in a patient with a grade 3 tumor which had a high proliferative index should raise a concern about HER2+ disease and consideration should be given for additional, either reflex or repeat testing to make sure that you have ruled out a HER2-driven cancer. Decisions about repeat testing are probably best made after discussion with the patient’s medical oncologist.

Q: Were any digital image analysis tools used or considered in updating the HER2 guidelines? What are the implications for digital whole slide imaging vendors who have received FDA approval for utilizing their respective platforms and the 2007 guidelines in light of the updated guidelines this year? Given the potential problems raised that intratumoral heterogeneity pose, should an approach that incorporates more objective quantitative methods (with computational assistance) be considered?

- Digital image analysis was not really part of the discussion for the HER2 guidelines. My own personal bias is that we need to improve the quality of the clinical samples (standardize pre-analytic variable) and the quality of the assays in order for image analysis to make sense and I think we have made great strides in this. Like other laboratory procedures, image analysis will need to be validated and the CAP has published standards for this validation.

As far as image analysis providing help with intratumoral heterogeneity I think that this is an important research question that needs to be pursued.

I don’t think there are any implications for the vendors who have received FDA approval using the 2007 guidelines. The vendor labeling (ie, instructions to users) are still ok. (I have reviewed this point with Dr. Gerry Hoeltge, Chair of the CAP Checklist Committee, and he is in agreement.)

Q: If the complete and strong membranous staining for HER2 is not found in a contiguous cluster
but rather diffusely present throughout the tumor at a 10 % rate, how is that interpreted?

- In my experience, intratumoral heterogeneity is uncommon, and when I have encountered this I typically see discrete contiguous clusters of HER2+ cells as opposed to scattered individual positive cells. The panel felt that cases that showed discrete clusters of cells where more clinically relevant. In evaluating these sorts of cases I think that it is important to interpret what you are seeing in the morphologic context for the case, and if the tumor is high grade with unfavorable histologic features and I saw scattered single positive cells I think that FISH analysis would be appropriate regardless of the percentage.

Q: Any comment on running HER2 FISH on an automated counting system, which is analyzed by HER2/tile instead of HER2/cell? In our experience, a tile could be equivalent to 1.5-2 cells.

- I think that automated counting systems for FISH are appropriate as long as the system has been rigorously validated against manual counts and a pathologist is checking the result to make sure they make sense.

Q: When a positive HER2 FISH is observed in >50% of invasive tumors, is it considered as heterogeneity or straight positive

- The panel felt that this issue with intratumoral heterogeneity needed to be addressed because of the potential for discordant results between IHC and FISH, cores and excisions as well as primaries versus metastases. The bottom line from the panel’s perspective was that all of these cases should be considered HER2+ for the purpose of treatment planning if there was at least 10% of the invasive tumor that showed HER2 amplification/over-expression in contiguous clusters.

Q: Is there value in testing a primary breast cancer and a concurrent metastasis?

- The testing for all of the clinical trial for HER2-targeted therapy was done on the primary tumor. In the vast majority of cases the HER2 status for the primary and concurrent metastasis will match. If the primary is HER2+ I think you have your answer in terms of therapy. However, on occasion I have seen cases of high grade breast cancer with unfavorable histology where the initial block from the primary was negative and a lymph node met was strongly positive. We went back and tested additional blocks of the primary which showed positive cells and intratumoral heterogeneity. Therefore in certain clinical settings when the primary is negative and the tumor is high grade, there may be some value in testing lymph node metastases.

Q: Is decalcified bone sample as rejected sample?

- In my view, decal should be an exclusion criterion for HER2 testing by IHC and FISH. We have tried numerous cases (the clinician called and said that this was the only site of metastatic disease) and they have all failed to hybridize and I have no confidence in the IHC result. Even if you managed to get a result, I would include a disclaimer that said that the results might not be accurate because the test had not been validated for decalcified tissue If HER2 is tested on decalcified tissue, CAP requires a disclaimer on the report (see ANP.22985).

Q: Regarding tumor heterogeneity in HER2 FISH, is it necessary to score and report separate population tumor cells even though amplified cells are >50%?

- No I do not think that this is necessary

Q: can you comment on the effects of fixation at the upper limit of 72 hr and beyond? On FISH and IHC

- There is a prospective report from David Dabbs (I think in the AJCP) looking at out to 96
hours of fixation and there is no effect on ER, PR and HER2 testing. The guidelines committee settled on 72 hours because this would be in alignment with the time published in the ER guidelines. There are a few studies in the literature that suggest than long fixation times (weeks to months) can affect IHC, but this would not be clinically relevant.

Q: In the 2011 "update" benchmark values were given for expected rate of HER2 3+ in a population (12-18%). I assume that was based on the cut-off of 30% complete chickewire staining. With the lowering of this threshold back to 10%, would you expect the incidence of HER2 3+ to rise? Will a new benchmark be issued?

- This is an interesting question. One of the goals of this guideline update was to try and minimize false negative results by providing algorithms and interpretive guideline to help deal with unusual/complex HER2 cases including intratumor heterogeneity and aneusomy. This along with the language about repeat testing for negative results in certain clinical settings should lead to finding more HER2+ case. It remains to be seen how much of an effect this will have and what the new bench mark will be.

Q: What are the implications for patients who had HER2 testing before the 2013 modifications?

Some may now actually be considered positive

- This is an interesting and important question that is hard to answer. I think it would be impractical to go back and start retesting and hard to know who should be retested. The way that we have chosen to handle this at our institution is we discussed the guideline update changes at one of your multidisciplinary breast center meeting including the changes in the interpretation criterion and the language about repeat testing. The medical oncologists are in the best position to know which patients potentially could benefit from revisiting their HER2 status by doing some additional testing and this was a big part of the discussion. They have started to request repeat testing on some of their patients where there is a clinical concern.

Q: Do you do the three markers on the positive axillary lymph node sent at the same time with the breast core biopsy?

- No, we typically will do the three markers on the breast core, and if there is clinical concern, we will repeat the result on the excisional sample. The testing for all of the clinical trial for HER2-targeted therapy was done on the primary tumor. In the vast majority of cases the HER2 status for the primary and concurrent metastasis will match. If the primary is HER2+ I think you have your answer in terms of therapy. However, on occasion we have seen cases of high grade breast cancer with unfavorable histology where the initial block from the primary was negative and a lymph node met was strongly positive. We went back and tested additional blocks of the primary which showed positive cells and intratumoral heterogeneity. Therefore in certain clinical settings when the primary is negative and the tumor is high grade, there may be some value in testing lymph node metastases.

Q: If the initial breast cancer is HER2 positive (3+), should the metastasis be tested for HER2?

- No I do not think that this is necessary. If your initial breast cancer test is positive, and the result fit with the other histopathologic feature for the case, the patient is a candidate for treatment. Even if a met was negative, I think that most medical oncologists would recommend HER2-targeted therapy if they have one reliable HER2-positive result.

Q: can you review "homogeneous histology" for heterogeneous tumors one more time. In the data supplement, it states that we should be counting non-overlapping and contiguous invasive cancer cell nuclei for FISH. Does your IHC image qualify for that scenario or do the cells need to
be grouped more closely together?

- Intratumoral heterogeneity for HER2 is rare, it was seen in about 4% of the HER2+ cases that were enrolled on the 9831 clinical trial. The patterns of intratumoral heterogeneity that have been described are a mixed histology (low and high grade component to the tumor where the high grade is HER2+ and the low grade is HER2-negative) and homogeneous histology (invasive tumor cells of similar morphology that are a composite of HER2 positive and negative tumor cells). Most of the time when there is heterogeneity, the positive tumor cells are seen as discrete clusters of cell that are intermixed with negative cells. This is easiest to recognize with IHC but can also be detect with a careful low power scan of the FISH slide. The intention of the 10% of the tumor being positive was not intended in the literal sense (1 in 10 cells or 2 in 20 cells). For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important thing here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important thing here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+

Q: If 40 positive and 40 negative HER2 tests are required, what about the equivocal tests?

- This is currently under discussion by the Checklist Committee, but it is very probable that the revised CAP checklist will not require validation to include samples with equivocal results.

Q: Although the adopted guideline does not reference the MIB-1 proliferative index, Dr. Hicks discusses it as a motivator for repeating HER2 testing. Does he recommend doing MIB-1 on all invasive mammary carcinomas?

- Ki67 has been somewhat controversial because there are no real standards for how the assay is done and how this should be interpreted. Having said that, I think it can be helpful in assessing risk and other factor in breast cancer and we routinely do this as part of the initial breast cancer workup.

Ki-67 is a nuclear antigen expressed in G1, S, G2 and in the mitosis phase of the cell cycle. In breast cancer, high levels of Ki-67 expression are associated with carcinomas of higher grade and positive lymph node involvement and have been shown to have both prognostic and predictive values for breast cancer. HER2+ tumor will generally have a high Ki67 proliferative index, such that when I see this, I worry more about HER2-driven disease

Many studies have also demonstrated that the expression level of Ki-67 is predictive for benefit from chemotherapy and hormonal therapy. In 2009, Cheang et al. reported that expression of Ki-67 can be used to separate the original ER-positive luminal subtypes of breast cancer into luminal A with low Ki-67 (<14%) and luminal B with high Ki-67 (>14%). Luminal B group has a much worse prognosis than luminal A breast cancers. There is increasing evidence showing that proliferation rate is perhaps the strongest determinant of outcome in patients with ER-positive tumors. Interestingly, of the 16 cancer related
genes that are measured by the Oncotype Dx test, 5 represent proliferation related genes (including Ki67), and these genes are more heavily weighted than the other gene transcripts in the panel.

Although many major cancer centers have been testing and reporting the expression of Ki-67 in breast cancer at the request of clinicians, currently there are no guidelines, recommendations, international or national standards regarding how to test should be performed, interpreted and reported. Standardization of current methods and/or better ways of assessing proliferation in routine clinical samples is needed.

Q: How frequent is the HER2 tumor heterogeneity? How can you find HER2 tumor heterogeneity if you use only HER2 FISH test and count only 20 or 40 tumor cells?

- The incidence of intratumoral heterogeneity is dependent on how you define this, and in my view we still do not have a good working definition.

I think that the best data that we have is the retrospective review of the 9831 trial published by Dr. Perez where about 4% of the HER2+ cases enrolled on that trial have strong circumferential membrane staining seen in 10-30% of the invasive tumor cells (which I would interpret as intratumoral heterogeneity). The interesting thing here was these patient appeared to receive the same benefit as those who had strong circumferential membrane staining in >30% of their tumor.

Most of the time when there is heterogeneity, the positive tumor cells are seen as discrete clusters of cell that are intermixed with negative cells. This is easiest to recognize with IHC but can also be detect with a careful low power scan of the FISH slide. The intention of the 10% of the tumor being positive was not intended in the literal sense (1 in 10 cells or 2 in 20 cells). For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important thing here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important thing here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+.

Q: How does the lab that performs only interpretation for FISH handle proficiency testing of the pathologist’s interpretation of the FISH results?

- The lab must NOT participate in PT. Instead, it needs to do an alternative assessment twice a year. For example, send slides to another lab for review, or have several observers in the lab evaluate patient slides and compare results.

Q: Talking about heterogeneity, does the outcome of response to treatment is different as per percentage of HER2 positivity? In another word, does the tumor that show 15% + have the same response to treatment as of 70% +?

- This is a good question and there is not a lot of good clinical data to know what the clinical significance of this finding is. I think that the best data that we have is the retrospective review of the 9831 trial published by Dr. Perez where about 4% of the HER2+
cases enrolled on that trial have strong circumferential membrane staining seen in 10-30% of the invasive tumor cells (which I would interpret as intratumoral heterogeneity). The interesting thing here was these patient appeared to receive the same benefit as those who had strong circumferential membrane staining in >30% of their tumor.

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Q: Are there settings in which you would recommend testing on concurrent positive axillary lymph nodes, in addition to the primary tumor?

- The testing for all of the clinical trial for HER2-targeted therapy was done on the primary tumor. In the vast majority of cases the HER2 status for the primary and concurrent metastasis will match. If the primary is HER2+ I think you have your answer in terms of therapy. However, on occasion we have seen cases of high grade breast cancer with unfavorable histology where the initial block from the primary was negative and a lymph node met was strongly positive. We went back and tested additional blocks of the primary which showed positive cells and intratumoral heterogeneity. Therefore in certain clinical settings when the primary is negative and the tumor is high grade, there may be some value in testing lymph node metastases.

Q: The HER2 PT does not send out Equivocal IHC. How are we able to validate our equivocal IHC stains?

- At this time, most likely the revised CAP checklist will not require validation to include samples with equivocal results. Equivocal results may not be as reproducible as positive and negative results, but equivocal results are re-tested using the other method, so lessens the problem.

Q: How do you calculate the tumor percentage in multiple cores? Is it per core or average everything in the slide?

- My personal opinion is that you should look at all of the cores that contain tumor and then estimate the average over the entire slide.

Q: Does cold ischemic time end when an excision specimen is immersed in formalin or when the tumor is actually incised and exposed to formalin?

- Formalin penetrates tissue fairly slowly (~1mm per hour) and so simply placing a whole specimen into a container of formalin is not the same as fixation. The tissue, particularly the area with the tumor needs to be incised so that formalin can more easily penetrate
into the tissue and the chemical reaction of formalin can take place.

Q: 10% vs >10% appears to be a critical difference in the IHC interpretation. Any hints on how to count/estimate?
- I don’t have any good way of doing this other than looking at the entire slide and estimating what % of the tumor is positive. If you encounter a case that you feel is near the cut off, then I would show this case around and come up with a consensus opinion about what the best interpretation of the case should be.

Q: Can CAP proficiency testing results be used for validation of IHC and ISH HER2 in order to have a total of 20 positive and 20 negative?
- Yes, for a test previously validated under the old guidelines using fewer samples than the new requirement of 20 pos and 20 neg. However, I do not think it would be acceptable to use only PT samples for an initial validation since they do not reproduce all the factors affecting the tissue specimens in the laboratory (ex. fixation, test method variables, etc.).

Q: Given the HER2 heterogeneity in tumors, do you feel it would be more accurate to perform testing on the resection specimen instead of cores which may be separated into multiple blocks. In your example, what if you tested the block that had cores 2 and 5 in it instead of cores 1, 3 and 4.
- I think that the best you can do is to test the initial block and then interpret the results in the morphologic context for the case. If the initial core is negative but the tumor is high grade then some consideration needs to be given to whether or not it would be prudent to reflex test or repeat the test on a second block or a different specimen to make sure you are not missing a patient who could benefit from HER2-targeted therapy.

Q: IF a lab was considering doing ISH in house testing with a FDA approved test for HER2-neu, then only 20 positive and 20 negative validation set needs to be performed. What about equivocal cases? Should any and how many should be tested?
- This is currently under discussion by the Checklist Committee, but it is very probable that the revised CAP checklist will not require validation to include samples with equivocal results.

Q: What is the best way to deal with cases that are equivocal by both IHC and FISH? Would PCR be helpful?
- I think that an equivocal result by both IHC and FISH should trigger a review of all of the relevant clinical and morphologic features for the case and a discussion with the patient’s medical oncologist about the clinical significance of the finding and the need for any repeat testing.

The problem with PCR is that there is not any clinical data on whether or not you can use this information to make treatment decisions. The ASCO/CAP panel felt that it was premature to use PCR for this application and did not recommend this.

Q: Does this guideline also apply to gastric HER2 testing with ratio<2, but HER2 signal > 6?
- There currently are no guideline for HER2 testing in gastric cancer so the only thing we have to go by is the validation study for the ToGA trial which used a ration of >2 as the only criterion for HER2 amplification. I think that if you encounter a case like you describe (I have seen several) you need to look at the HER2 IHC and if this is clearly positive then the patient is a candidate for therapy.

Q: Does the ban on referral make it impossible for HER2 to be done in a TC/PC setting?
I have had the following QA re IHC with CAP5.

What is a reasonable standard for PT for laboratories that perform Professional only functions? CAP does offer an interlab comparison program. These programs are considered educational programs and therefore the rules for PT interlab communication do not apply.

- Question 1: No, it does not; just be absolutely certain not to participate in PT for (F)ISH HER2 testing if the interpretation is done in a different lab (ie, different CLIA number) than the lab that does the technical preparation.
- Question 2: for IHC, the professional only lab should participate in PT. There is no problem with PT referral for IHC. For (F)ISH, the lab must NOT participate in PT, but could use, as its alternative assessment, an educational program that is clearly not PT to evaluate its performance. The lab should NOT use the CAP PT program for this purpose.

Q: For Labs that perform IHC interpretation only, should they perform validation studies? 40 or 80 cases? Can you suggest a method for validation?
- The lab should use stained slides from the lab that will do the technical preparation. The technical prep lab should have already validated its method (whether FDA approved or an LDT) so the interpretation only lab can use 40 cases (20 + and 20 neg).

Q: we perform Ventana DUAL-ISH. If the ration is >2.0 after the first count of 20 cells should we count additional 20 cells as recommended by the manufacturer instruction
- The lab could either follow the manufacturer instructions or the guideline. If the lab follows the manufacturer instruction, it would count an additional 20 cells if the initial 20-cell count gives a HER2/CEP ratio of 1.8 – 2.2. Note that the manufacturer instructions do NOT have an “equivocal” category, so if the ratio is 1.8-2.2 after the total 40-cell count, the result will be positive if the ratio >=2 and negative if it is <2. Alternatively, the lab could follow the updated ASCO/CAP scoring guidelines. In this case, if the ratio on the initial 20-cell count is 1.8-2.2, then: if the ratio is >=2, the result is reported as positive; if the ratio is <2, the result is reported as positive if there is an average copy number of >= 6 signals/cell, while it is reported as negative if there are <4 signals / cell, and equivocal if there are >=4 and <6 signals / cell.

Q: Specimen in formalin within 1 hour, with tumor bisected is a guideline or a requirement
- I think that standardizing tissue handling is important and every effort should be made to minimize the time from removal of the tissue from the patient until it is incised and placed in an appropriate fixative so that the tissue is stabilized and degradation of clinically important molecules like ER and HER2 is stopped. Ideally this should be within one hour from removal from the patient to the start of tissue fixation.

The checklist states (ANP.22983) that since the lab may not have control over the clinical personnel who may be the ones putting the tumor in formalin, the requirement for the lab is that it communicates the guidelines to clinical personnel, and educate clinical personnel if the fixation guideline is not being met. See ANP.22983.

Q: How do you measure cold ischemic time for mastectomy and large partial mastectomy specimens?
- I will share with you what we do here. The collection time is recorded in the OR (time the tissue is handed to the circulating nurse from the operative field) and the tissue is then immediately transported to the laboratory for gross assessment. Once in the gross room the tissue is oriented, inked and then sliced to identify where the tumor is. This is usually guided by a review of the imaging studies so that one can focus on the area of the breast where the tumor is. If gross tumor is identified, thin section of the tumor and a
piece of adjacent fibrous normal breast tissue can be put into a cassette and placed immediately into formalin, and this time (fixation start time) is recorded. Paper towel can be placed between the slices and the specimen can then be placed in an adequate volume of formalin and the remainder of the grossing can be done at a later time after the tissue has had a chance to fix.

Q: is there suggestion on how to manage those EQUIVOCAL cases that with HER2 4-6 and ratio <2?

- This is a great question and there is no easy answer. If you remember with the old single probe FISH test, an average HER2# of <4 was considered negative, >6 was considered positive and 4-6 was considered equivocal. The thinking was that those cases that contained 4-6 gene copies likely represented cases of polysomy or heterogeneity and additional testing was required. This additional testing could include a dual probe test (ratio of < 2 would be considered negative), an IHC test or repeating the test on a second block to try and exclude heterogeneity. In my mind I think a FISH equivocal results according to the 2013 guideline require that you do some additional testing, but the guidelines leave this up to the discretion of the pathologist what the next steps should be. I think using an alternative chromosome 17 reference probe is reasonable if available, as well as repeating the test on another block from the tumor, to try and resolve the HER2 status for the purpose of treatment planning. If the IHC is negative (0 or 1+), and the repeat FISH has a ratio of <2 (but is still in the FISH equivocal range), then the patient would not have been eligible for the adjuvant clinical trials of Herceptin + chemotherapy and I suspect they would be unlikely to benefit from therapy, but at this point there is no clinical data to support this either way. Such patients fall into a gray zone, and would have been considered ineligible for the adjuvant clinical trial but perhaps should be left in an FISH equivocal category to reflect a degree of uncertainty or they could also be considered negative.

For such cases, I would lean to the HER2 IHC results to help guide treatment as the target of the drug is the overexpression of the protein receptor.

Q: Should we repeat all core test result that is HER2 negative but MBR is III on excision?

- I think that some consideration should be given to whether or not is would be helpful to repeat the HER2 results on the excision when the core is negative and the tumor is grade 3 to help ensure that a HER2-driven breast cancer is not missed. This decision is probably best made after consultation with the patient’s medical oncologist. As we said in the web-ex, the yield here will be low, but the magnitude of benefit from finding additional HER2+ patient would be worth doing some additional testing on those patients who are more likely to be HER2+ (high grade tumor with unfavorable histologic features).

Q: Are there any FDA approved test assay kits for HER2 testing in GASTRIC cancer besides DAKO HercepTest and DAKO FISH Pharmadx?

- I know that the FDA approved Dako kits are approved but I am not aware of the FDA status of other HER2-testing kits for gastric cancer. You may want to check with the vendor to see what the status is. I think that it is important to perform a separate validation for HER2 testing in gastric cancer specimens regardless of which vendor you use.

I believe that the Ventana IHC and dual probe FISH kits are approved for gastric cancer

Q: How many foci of tumor should we test for HER2 in multifocal cancer?

- I will give you my personal perspective here. In my practice if we have multifocal breast
cancer that shows a similar histologic appearance, I think that testing the largest focus of invasive cancer is all that is required, particularly if there is an extensive intraductal component or prominent LVI which is probably the explanation for multifocal disease. If the two or more foci of invasive tumor show different morphologies, then I think testing all of the tumors is warranted.

Q: Can HER2 FISH be interpreted by a pathologist based on few pictures only. Tech does the count.
   • For cases that are highly amplified, I think that the answer is yes, but the gene signals seen by FISH tend to be in different planes of section and this requires focusing up and down on the slide to make sure all of the gene signals are accurately counted. Because of this I think that static images would underestimate the number of gene signal and therefore I would think that for many cases such an approach would be unreliable and not advisable.

Q: The guideline states to scan the entire specimen on the slide. If it is obviously amplified, does the entire specimen need to be scanned? If areas of heterogeneity are observed does the entire specimen need to be scanned?
   • The reason for scanning the entire slide on low power for FISH analysis is to try and pick up on intratumoral heterogeneity. This is most important if primary FISH testing is being used for HER2 analysis. If you have an IHC slide that show heterogeneity in protein expression this can be used to target areas for analysis by FISH.

So if the entire slide is obviously amplified, then no a careful low power scan will not be that helpful. If areas of heterogeneity are seen, I think that the low power scan can be helpful in estimating the percentage of invasive tumor cells that demonstrate this finding.

Q: What type of proficiency is needed for a pathologist who reads FISH her-2 remotely (tech provided by a different lab)?
   • The lab must NOT participate in PT. Instead, it needs to do an alternative assessment twice a year. For example, send slides to another lab for review, or have several observers in the lab evaluate patient slides and compare results.

Q: How are TC/PC HER2 FISH testing with online interpretation by the pathologists going to comply with a low power scan of the slides? Currently, the only images sent are high power, single cell images
   • You will need to get low power images of the slides. The new checklist edition may not state this explicitly, but it will require a low power scan.

Q: If you are using an FDA validated Kit and have been using the ASCO 2007 guidelines, how do you validate the new interpretation guidelines with the same staining method
   • This is currently under discussion by the Checklist Committee prior to finalizing the revisions to the 2014 checklist, but the feeling now is that a formal validation study is not required if only the scoring system is changed. Changing the scoring system is analogous to changing the reference range for a clinical laboratory test. It would be good for the lab to run an initial limited comparison study among pathologists who sign out HER2 cases, to confirm that all pathologists are reading the slides in a consistent way. Note that annual comparison of pathologists’ interpretations is required by ANP.22970.

Q: How are you handling requests for retrospective review of cases which may have been negative using 2007 guidelines, but would be equivocal or positive with the new guidelines?
   • This is an interesting and important question that is hard to answer. I think it would be
impractical to go back and start retesting and hard to know who should be retested. The way that we have chosen to handle this at our institution is we discussed the guideline update changes at one of our multidisciplinary breast center meeting including the changes in the interpretation criterion and the language about repeat testing. The medical oncologists are in the best position to know which patients potentially could benefit from revisiting their HER2 status by doing some additional testing and this was a big part of the discussion. They have started to request repeat testing on some of their patients where there is a clinical concern and we have been providing repeat testing on these select cases.

Q: we use new guidelines already. First observation - we have increased number of equivocal ISH cases with >4<6 signals. Obviously our oncologists are not happy - what would you suggest to do? I count more cells, scan the slide, etc

- This is a great question and there is no easy answer. If you remember with the old single probe FISH test, an average HER2# of <4 was considered negative, >6 was considered positive and 4-6 was considered equivocal. The thinking was that those cases that contained 4-6 gene copies likely represented cases of polysomy or heterogeneity and additional testing was required. This additional testing could include a dual probe test (ratio of < 2 would be considered negative), an IHC test or repeating the test on a second block to try and exclude heterogeneity. In my mind I think a FISH equivocal results according to the 2013 guideline require that you do some additional testing, but the guidelines leaves this up to the discretion of the pathologist what the next steps should be. I think using an alternative chromosome 17 reference probe is reasonable if available, as well as repeating the test on another block from the tumor, to try and resolve the HER2 status for the purpose of treatment planning. If the IHC is negative (0 or 1+), and the repeat FISH has a ratio of <2 (but is still in the FISH equivocal range), then the patient would not have been eligible for the adjuvant clinical trials of Herceptin + chemotherapy and I suspect they would be unlikely to benefit from therapy, but at this point there is no clinical data to support this either way. Such patients fall into a gray-zone, and would have been considered ineligible for the adjuvant clinical trial but perhaps should be left in an FISH equivocal category to reflect a degree of uncertainty or they could also be considered negative.

For such cases, I would lean to the HER2 IHC results to help guide treatment as the target of the drug is the overexpression of the protein receptor.

Q: Is it true that, for validation, we do not have to compare IHC results with a second method (such as FISH/ISH) but can instead compare the IHC results with IHC done at another lab?

- Yes, this is OK.

Q: Could you please comment on performing HER2 FISH on decalcified specimens with metastatic breast cancer, is it necessary to validate 40 additional decalcified cases?

- In my view, decal should be an exclusion criterion for HER2 testing by IHC and FISH. We have tried numerous cases (the clinician called and said that this was the only site of metastatic disease) and they have all failed to hybridize and I have no confidence in the IHC result. Even if you managed to get a result, I would include a disclaimer that said that the results might not be accurate because the test had not been validated for decalcified tissue. We have not attempted to validate decalcified specimens, but I suppose you could although I would still worry about the accuracy of the result. It would be necessary to use 40 specimens. Whether such a validation study would be successful is unknown but I think it is unlikely. In any case, since most labs won’t have enough samples to validate decalcified specimens, if decalcified patient specimens are
performed, there should be a disclaimer on the report. See ANP.22985.

Q: Any comment about updating microscopic computer assisted scoring and manual percentage estimation of positive cells for HER2. How many cells one needs to assess before reporting HER2 by IHC

- Digital image analysis was not really part of the discussion for the HER2 guidelines. My own personal bias is that we need to improve the quality of the clinical samples (standardize pre-analytic variable) and the quality of the assays in order for image analysis to make sense and I think we have made great strides in this. Like other laboratory procedures, image analysis will need to be validated and the CAP has published standards for this validation.

Because IHC is bright field, you can assess all of the areas of invasive tumor rather easily to see what if any part of the tumor demonstrates immuno-reactivity. If there is a limited amount of invasive tumor present on a needle core biopsy, one needs to consider if repeat testing on the excision may be warranted and this decision depends somewhat on the tumor morphology.

Q: Do you recommend CISH over FISH in order to find tumor heterogeneity better

- The 2013 guideline recognized CISH for evaluating HER2 status because of published concordance studies between FISH and CISH and because CISH evaluated an analyte (HER2 gene amplification) that had clinical data the predicted benefit from HER2-targeted therapy. There are clear advantage and disadvantages when comparing CISH versus FISH and I do not recommend one over the other at this point.

Q: We have been doing FISH up front on our cases at request of our clinicians. Should we be doing IHC routinely now? What combo is best?

- I think that primary FISH testing is fine as an upfront test as long and there is an algorithm that includes IHC testing in a proportion of cases where it makes sense to have this additional information. Intratumoral heterogeneity is rare and it is easier to see by IHC versus FISH, however doing a careful low power scan when evaluating a FISH slide will enable one to detect this in most cases. If intratumoral heterogeneity is suspected by FISH, it may be prudent to confirm this finding by IHC. I also think that the discussion about repeat testing when you have a negative HER2 results in a patient with a high grade tumor applies to FISH as well as IHC.

Q: Before 2007 our Lab performed HER2 IHC on the cores of breast tissue. We switched into performing this test on excisional specimens as per 2007 recommendations. Now again a test on biopsy is recommended. Is going back to cores now a requirement as the recommendations are considered standard of care sooner or later?

- I have always felt that the excision was the preferred sample and allow for the evaluation of more of the patient’s tumor (as long as the pre-analytic variables were within the guidelines). The 2013 guidelines state that the core is an acceptable for the initial HER2 test but points out that there are situations where the is less than ideal and consideration for repeat testing on the excision should be discussed. I see no problem with continuing to perform HER2 testing on the cores if your clinicians are comfortable with this. The one exception would be for a patient who is a candidate for neoadjuvant chemotherapy.

Q: If FISH is done at a reference lab and IHC is done in house, do the 2013 changes need to be made simultaneously or can the IHC changes be made first

- The in-house and reference lab should communicate on this point. Ideally both labs
should make the changes as soon as practical and at about the same time, but neither lab is required to do so until it is inspected using the 2014 edition of the checklist. If there is a period of time when the 2 labs are using different guidelines, this should be communicated in the lab report.

Q: Tumor heterogeneity. If we have two areas, one with amplification (15%), other no amplification (85%), do we need to report results separately? Since the result is considered as positive for amplification, do we need to report the score from the non-amplified area, as long as we are saying that 15% of tumor is amplified, why would it not be sufficient? I do not see any benefit for reporting the negative result.

- I think that it is most important that one is able to recognize that heterogeneity is present in a case as these patient will are candidates for treatment and heterogeneity can lead to discordant results. Most of the time when there is heterogeneity, the positive tumor cells are seen as discrete clusters of cell that are intermixed with negative cells. This is easiest to recognize with IHC but can also be detect with a careful low power scan of the FISH slide. For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important thing here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important thing here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+.

Whether or not it is useful to report ratios for both the amplified and the non-amplified component of the tumor is debatable but I think having this information available may make it possible to go back later and try and understand the clinical significance of the finding.

Q: In cases where both breast primary and positive node is present, should we test both, or only the node?

- The testing for all of the clinical trial for HER2-targeted therapy was done on the primary tumor. In the vast majority of cases the HER2 status for the primary and concurrent lymph node metastasis will match. If the primary is HER2+ I think you have your answer in terms of therapy. However, on occasion I have seen cases of high grade breast cancer with unfavorable histology where the initial block from the primary was negative and a lymph node met was strongly positive. We went back and tested additional blocks of the primary which showed positive cells and intratumoral heterogeneity. Therefore in certain clinical settings when the primary is negative and the tumor is high grade, there may be some value in testing lymph node metastases.

Q: CAP currently requires revalidation of HER2 with major method changes. If we change to ASCO recommendations, will a full revalidation be required?

- This is currently under discussion by the Checklist Committee prior to finalizing the revisions to the 2014 checklist, but the feeling now is that a formal validation study is not required if only the scoring system is changed. Changing the scoring system is analogous to changing the reference range for a clinical laboratory test. It would be good for the lab
to run an initial limited comparison study among pathologists who sign out HER2 cases, to confirm that all pathologists are reading the slides in a consistent way. Note that annual comparison of pathologists’ interpretations is required by ANP.22970.

Q: Is there good evidence that using the same criteria for interpreting ER/PR/HER2 in primary breast cancer is adequate for metastatic breast cancer?

- This is a great question and the simple answer is no, there is no good clinical evidence to support this. The reason for recommending repeating the biomarker on metastatic disease is that the literature says that roughly 10% of the time, the HER2 status can change between the primary and the metastatic recurrence. Most of the time the change will be from negative in the primary to positive in the metastases and this is important because it give the oncologist another option for treatment that they wouldn’t otherwise have. Tumor that are initially positive that turn negative with the recurrence have also been described, and this is more problematic in terms of what to do clinically.

I think that discordant results between the primary and recurrent disease should always be thoroughly investigated to try and determine if this is a technical issue or is related to tumor biology.

Q: In my experience (ca 1400 cases analyzed per year, all of them both by IHK and FISH), most cases with ratio < 2 and HER2 copy nr > 6 are IHC 2 +. With the new guidelines, these cases should be considered HER2 amplified, and thus are committed to treatment with tranquuzumab. Is there any clinical evidence to justify this new classification of these cases? If not, why is CAP recommending to treat this cases without evidence of efficiency? Should not we stick to the principle of evidence based criterion?

- The reason for this recommendation was based on evidence from publications like the one listed below which suggested that cases with an abnormally high number of copies of the HER2 gene and CEP17 with a ratio <2 represented cases with amplification of both HER2 and the centromere where the ratio was misleading as to the true HER2 status for the patient. In this and other studies, repeating the FISH with a different chromosome 17 reference probe usually gave a ratio that was in the amplified range (>2), which would have meant that the patient would have been eligible for the adjuvant Herceptin trial. In addition, if you remember with the old single probe FISH test which only had a probe for HER2, an average HER2# of <4 was considered negative, >6 was considered positive and 4-6 was considered equivocal.

These cases that you bring up are rare and it is unlikely that we will ever have good clinical data but the panel felt that there was enough evidence that was compelling to help support this recommendation.


Q: Can we report HER2 FISH on diff quik, if no other tissue available

- This is an interesting question and one that I had not heard before. I would probably validate this before trying to use this clinically. You could do touch preps of resected breast cancer specimens and then FISH the diff quick touch preps and compare with the results of the FISH from your FFPE tissue sample.

Q: More than 6 signal per cell in 10 % of the tumor by FISH, is it equivalent to 2 of 20 cells with
more than 6 signals?

- The intention of the 10% of the tumor being positive was not intended in the literal sense (1 in 10 cells). For IHC it has been accepted that if >10% of the invasive tumor cells are show strong circumferential membrane staining, the case should be considered positive for eligibility for treatment and this is well accepted. The same rule should apply for FISH, if >10% of the invasive tumor show gene amplification then the case is considered positive. This requires as you suggest a careful low power scan of the slide looking for pockets or homogeneous/contiguous clustered on amplified cells, and if this constitutes at least 10% of the invasive tumor then the case should be reported as amplified. The amplified ratio and counts should be reported separately from the non-amplified component of the tumor. I think that the important this here is that you do not combine the ratio between the amplified and non-amplified component of the tumor, because this may dilute out the average numbers for the amplified component of the tumor and be misleading. The important this here is that if there is intratumoral heterogeneity and an amplified clone of the tumor that we recognize it and call the breast cancer HER2+.

Q: I do not think there is a need to document special training or need for proving competency in HER2 interpretation through PT (which are expensive) by a pathologist with the new scoring system. It is simple & very easy. Besides you can check with FISH or ISH or Molecular results in case of doubt and self correct with other methodologies. I have never been wrong on my IHC interpretation. All you need is an atlas, like that of DAKO. A pathologist interprets a lot difficult things than scoring of HER2

- I think that it is important that pathologists interpreting HER2 results understand the criteria for interpretation as well as artifacts and technical issue that can lead to difficulties with scoring given how this test results is used clinically and the implications for treatment and outcome.

I agree with you that checking yourself against FISH is very helpful. Proficiency testing is a requirement for CAP accreditation and so is documentation of inter-pathologist agreement on interpretation. I agree with you that this is expensive, but it is part of an ongoing QA/QC program for testing that I think is useful and important.

Note that ANP.22970 (see below) applies to HER2 testing. There should be an annual comparison among pathologists doing HER2 interpretations to be sure that everyone is on the same page.

**ANP.22970 Annual Result Comparison**

For immunohistochemical and FISH/ISH tests that provide independent predictive information, the laboratory at least annually compares its patient results with published benchmarks, and evaluates interobserver variability among the pathologists in the laboratory.

**NOTE:** Individuals interpreting the assay must also have their concordance compared with each other and this concordance should also be at least 95%.

With specific reference to estrogen and progesterone receptor studies: in general, the overall proportion of ER-negative breast cancers (invasive and DCIS) should not exceed 30%. The average is somewhat lower in postmenopausal than premenopausal women (approximately 20% vs. 35%). The average is considerably lower in well-differentiated carcinomas (<10%) and certain special types of invasive carcinomas (<10% in lobular, tubular, and mucinous types). The proportion of PgR-negative cases is 10-15% higher than for ER in each of these settings. Investigation is warranted if the proportion of negative cases is significantly higher in any of these settings.
Q: Specimen in formalin within 1 hour, with tumor bisected is a guideline or a requirement

- I think that standardizing tissue handling is important and every effort should be made to minimize the time from removal of the tissue from the patient until it is incised and placed in an appropriate fixative so that the tissue is stabilized and degradation of clinically important molecules like ER and HER2 is stopped. Ideally this should be within one hour from removal from the patient to the start of tissue fixation.

The relevant checklist requirement is ANP.22983 (see below, highlighted text). We recognize that laboratories may not have authority over the clinical personnel, so the requirement is that the laboratory communicates the fixation requirement to clinical personnel; and if the guideline is not met, the lab should contact clients to educate them on the importance of adhering to the fixation guidelines.

Also, note that this is the current checklist item; in the 2014 checklist revision, the maximum fixation time will be revised to 72 hours.

**ANP.22983**  
**HER2; ER/PgR by IHC - Fixation**

If the laboratory assesses HER2 protein over-expression by immunohistochemistry, HER2 gene amplification by in situ hybridization, or estrogen/progesterone receptor expression by immunohistochemistry, there is a documented procedure to ensure appropriate specimen fixation time.

**NOTE:** Specimens subject to these tests should be fixed in 10% neutral buffered formalin for at least 6 hours, up to a maximum of 48 hours for HER2 testing and 72 hours for estrogen receptor and progesterone receptor testing. The volume of formalin should be at least 10 times the volume of the specimen. Decalcification solutions with strong acids should not be used. For cases with negative HER2 results by IHC that were fixed outside these limits, consideration should be given to performing confirmatory analysis by in-situ hybridization.

**Laboratories should communicate the following fixation guidelines to clinical services:**

1. Specimens should be immersed in fixative within 1 hour of the biopsy or resection procedure
2. If delivery of a resection specimen to the pathology department is delayed (e.g. specimens from remote sites), the tumor should be bisected prior to immersion in fixative. In such cases, it is important that the surgeon ensure that the identity of the resection margins is retained in the bisected specimen; alternatively, the margins may be separately submitted.
3. The time of removal of the tissue and the time of immersion of the tissue in fixative should be recorded and submitted to the laboratory

**Communication may be through memoranda, website, phone, face-to-face meetings, or other means. The laboratory should consider monitoring compliance and contacting clients when these guidelines are not met.**

If specimens are fixed in a medium other than 10% neutral buffered formalin, the laboratory must perform a validation study showing that results are concordant with results from formalin-fixed tissues.

Laboratories testing specimens obtained from another institution should have a policy
that addresses time of fixation. Information on time of fixation may be obtained by appropriate questions on the laboratory’s requisition form.

Laboratories should qualify any negative results for specimens not meeting the above guidelines.

Q: Is that true the 2013 new guideline is mostly based on dark field FISH methodology? Can this also perfectly apply to bright field methodology such as SISH? For example, the new guideline suggests “counting at least 20 homogenous and contiguous cells” while current Ventana SISH protocol says “counting the peak HER2 and CEP17 signal (cherry picking)”. We found that in a bright field setting like SISH, when one can clearly see most of the nuclei in a field, it is difficult to count a cell with only 2 dots within a field that composed of cells mostly with 4-5 dots, just because it is a “contiguous cell”. It seems to us “cherry picking” will produce more HER2 copy 4-6 (equivocal) cases than counting “contiguous cells”. Do you think in bright field situation, counting “representative” rather than “contiguous” is more reasonable?

“Cherry picking” is in the Ventana protocol and we asked if they are going to modify it in accordance with the new 2013 CAP guideline? The answer was no, the cherry picking in their SISH protocol was validated that way and approved by FDA, they cannot change their protocol. If we change to “contiguous” or “representative” with current kit, is it legally ok?

- I do not think that the “cherry-picking” concept is too far off from what the 2013 guidelines is saying related to doing a careful low power scan looking for clustered or contiguous cells with amplification and then counting two ratios, one in the amplified region (as long as this represents >10% of the invasive tumor cell population and another in the non-amplified population.

In general, the requirements in the CAP checklists do not go down to the level of detail involved in determining exactly which cells to count. This is a decision that is left up to the laboratory director. One principle to remember, however, is that as far as the checklists are concerned, it is acceptable to follow either the ASCO/CAP guideline, or manufacturer instructions for an FDA cleared/approved kit.

Most Checklist requirements frame the goal, not the process. That is certainly true for these predictive marker requirements. Good laboratory practice is to construct a specific validation plan that is in accord with regulatory and accreditation criteria and then to follow that plan. An onsite inspector can be confident that your chosen process is reasonable, that it takes into consideration your particular circumstances, and that you have executed the validation successfully when she/he can compare your data with a written plan.

Q: In the past we always use CAP BPFT Benchmarks for our HER2 test QA monitoring program. Is CAP going to put out new BPFT Benchmarks in accordance with the new guideline? E.g. what are the target % ranges for POSITIVE, NEGATIVE or EQUVICAL cases?

- It remains to be seen how much if any the benchmarks will change. I suspect with the new algorithm and interpretative guidelines that a few more HER2 positive cases will be detected and the positive rate may increase a bit but probably not that much.

The number for HER2 positive cases should still be in the 12-18% range in the general breast cancer population.