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CORRESPONDENCE

# Is There Any Benefit From Hypofractionation in External-Beam Irradiation for Prostate Cancer?

**TO THE EDITOR:** Pollack et al[1] recently reported the results of a large randomized trial of hypofractionated external-beam irradiation for prostate cancer; the article was accompanied by an editorial by Lee.[2] In the study, more than 300 men with favorable- to high-risk prostate cancer were randomly assigned to receive either conventionally fractionated intensity-modulated radiation therapy (IMRT; 38 fractions of 2.0 Gy each to a cumulative dose of 76 Gy), or a higher, biologically equivalent dose by hypofractionated IMRT (26 fractions of 2.7 Gy each to a cumulative dose of 70.2 Gy). The study's goals were to compare the relative efficacy of the two regimens in preventing biochemical (ie, prostate-specific antigen [PSA]) and/or clinical failure (biochemical and/or clinical disease failure [BCDF]), and to compare their relative toxicities—specifically, to assess the theoretical superiority of hypofractionation over standard fractionation. The results of that study showed no significant difference in disease-free survival between the two arms at a median follow-up of over 5.5 years, and although overall toxicity of the two regimens was also comparable, men whose baseline urinary function was already compromised before treatment had significantly worse obstructive symptoms after receiving the hypofractionated regimen. The authors concluded that the primary advantage of the hypofractionated regimen lies in its shorter time course (by 2.5 weeks), but that this benefit must be weighed against the increased risk of significant urinary toxicity. The study was powered to observe a 30% versus 15% BCDF at 4.0 years, but the control group's BCDF at 5.0 years was 21%, suggesting that the study was underpowered to test the authors' hypothesis. Additionally, as the authors state, the increased urinary toxicity with the hypofractionated regimen was derived from an unplanned subgroup analysis, but obviously it is of concern.

We do not dispute the results or the conclusions drawn from the study by Pollack et al,[1] but there are growing data for both modest hypofractionation (25 to 30 fractions) and accelerated hypofractionation (four to five fractions), the latter using stereotactic body radiotherapy (SBRT) techniques. The Radiation Therapy Oncology Group (RTOG) has recently completed accrual for a large prospective study of 1,115 patients randomly assigned to either 28- or 41-fraction regimens (RTOG 0415), and a randomized phase II trial comparing five- and 12-fraction accelerated hypofractionation is nearing accrual completion (RTOG 0938). Beginning approximately 10 years ago, radiation oncologists at several institutions began treating prostate cancer with accelerated SBRT using the CyberKnife—generally 36.25 Gy in five fractions—and the results have been favorable, both in terms of maintained biochemical response and in terms of toxicity, culminating in two major multi-institution studies being presented at the 2012 American Society for Therapeutic Radiology and Oncology conference. One was a pooled 5-year retrospective series of 1,100 patients treated at eight institutions, 60% with favorable-risk disease (Gleason grade < 6 and PSA < 10), 30% with intermediate-risk disease, and 10% with high-risk disease (Gleason grade 7 to 10 and PSA 10 to 20).[3] At a median follow-up of 36 months, the 5-year biochemical relapse–free actuarial survival (bRFS) for all patients was 93%, and for the 335 patients with at least 4 years of follow-up, the bRFS was 97% for low risk and 89% for intermediate risk. The other study was a prospective multi-institution trial wherein 129 patents with intermediate-risk disease (Gleason grade 7 with PSA < 10, or Gleason grade 6 with PSA 10 to 20) received 40 Gy in five fractions (and 36.25 Gy to the seminal vesicles).[4] At a median of 30 months of follow-up, the 3-year bRFS was 99.2%, and GI and urinary toxicities were minimal and in line with external-beam data, as was the preservation of sexual potency. Most recently, Katz et al[5] presented their 6-year single-institution series of more than 300 patients, and the data are still holding up, both with regard to efficacy and toxicity.

We therefore believe that there are in fact good data to support the advantage of accelerated hypofractionated irradiation in the treatment of prostate cancer. In addition to making it feasible to administer an entire course of treatment in only five fractions—far less than the standard 8+ weeks, which is now the norm for IMRT, yet with both equal efficacy and morbidity—SBRT also carries with it the added benefit of substantially lower costs to both patients and insurers.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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# Hypofractionated Radiation Therapy for Prostate Cancer: More Food for Thought From Recent Trial

**To the Editor:** Pollack et al[1] compared a standard fractionation (SFX) versus a hypofractionated (HFX) schedule in men with intermediate- or high-risk prostate cancer in a moderately sized (N = 303), randomized controlled trial with up to 10 years of follow-up. No statistically significant difference was seen in terms of biochemical no-evidence-of-disease (bNED) status between the SFX arm, which received 76 Gy in 38 fractions (five fractions per week), and the HFX arm, which received 70.2 Gy in 26 fractions (five fractions per week). The analysis found a hazard ratio (HR) of 1.43 (95% CI, 0.8 to 2.58), with a higher HR in the HFX arm when using the Nadir + 2 definition of bNED. This outcome is unexpected, both because of the knowledge that was available at the time that this trial was designed and because of the evidence that has accumulated since then. From a biostatistical point of view, the question is whether the outcome of the trial by Pollack et al is statistically significantly different from the outcome that would be expected on the basis of other published trials. Or could the sample in the trial by Pollack et al just be a spurious sample from the same underlying population?

In clinical radiobiology, the sensitivity of a tumor or normal-tissue end point to a change in fraction size is quantified by the $\alpha/\beta$-value of the linear-quadratic bioeffect model.[2] In a recent meta-analysis of evidence from other two-arm dose-fractionation trials in prostate cancer,[3] we estimated an $\alpha/\beta$ of 1.9 Gy (95% CI, −0.3 to 4.1 Gy). It turns out that the simple method to extract a formal $\alpha/\beta$ estimate that was proposed in our article does not allow a point estimate to be made from the data in the trial by Pollack et al[1] because of the results being near a singularity, as discussed in our report. However, we can use the steepness of the dose-response curve to compare the equi-effective doses in 2-Gy fractions, EQD2, corresponding to the observed outcome, $D_{obs}$, with that expected from the linear-quadratic model, $D_{exp}$.

In a previous meta-analysis, the steepness of the dose-response curve[3,4] was estimated at $\gamma_{50} = 1.0$, which together with the observed bNED rates after SFX and HFX, leads us to a $D_{obs}$ of 71 Gy when a time factor is included in the analysis.

The expected effective dose ($D_{exp}$) from the results of our meta-analysis of previous data is 88 Gy for the HFX arm of the trial by Pollack et al,[1] much larger than the observed dose of 71 Gy. The result of the meta-analysis can furthermore be used to derive an expected HR for the study by Pollack et al, where the CI is estimated by propagation of the uncertainty originating from the steepness of the dose-response function, the $\alpha/\beta$ estimate, and the position of the dose-response function. This yields an expected HR of 0.43 (95% CI, 0.39 to 0.48) favoring hypofractionation. This is in stark contrast to the estimate by Pollack et al; a two-sided test for difference in HR yields $P < .001$ (Fig 1). The outcome of this comparison is virtually unchanged if the time factor is set to zero, given that the benefit from shortening the schedule cancels the effect of the larger $\alpha/\beta$ estimate (cf, Table 2 of our article[3]).

Thus, the outcome of the trial by Pollack et al[1] is in fact statistically significantly different from what would be expected on the basis of previously published trials. The cause of this difference is not clear. One issue could be the difference in margins used in the SFX and HFX arms, as discussed by Pollack et al. The application of dose constraints at the same effective dose level toward the rectum in both arms could also lead to a loss of the theoretical dose-escalation effect of the HFX schedule. However, this would assume that the outcome in terms of bNED was critically dependent on the dose to the most dorsal part of the prostate. It seems incredible that these minor differences between the target dose distributions in the two arms should have such a large impact. Another speculation relates to the possible impact of the short- or long-term androgen deprivation used as adjuvant therapy in this trial.

In any case, the results of the trial by Pollack et al[1] are intriguing. Arguably, we learn most from the clinical trials that do not in any simple way confirm the beliefs we held when designing the trial. Additional evidence from further analysis of this trial as well as from ongoing trials is eagerly awaited.

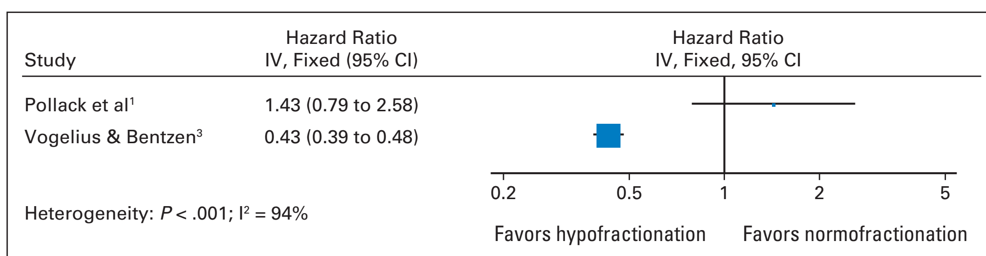


Fig 1.

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**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

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# Reply to M.J. Brenner et al and I.R. Vogelius et al

We very much appreciate the comments of Brenner and Kaplan[1] as well as those of Vogelius and Bentzen[2] regarding our report of a randomized dose-escalation trial using hypofractionation.[3] Brenner and Kaplan point out that the control arm patients in our trial had a lower biochemical and/or clinical disease failure (BCDF) rate than expected, making the study underpowered. Although the patients in the control arm did better than expected, the patients receiving moderate hypofractionation treatment did worse than expected. The original BCDF estimates from conventional fractionation and moderate hypofractionation–calculated 2.0-Gy equivalent doses using an $\alpha/\beta$ ratio of 1.5 were based on a large Fox Chase Cancer Center database. Although there were no significant differences in BCDF between the arms, a subgroup analysis suggested that patients with worse urinary function at the outset had more late urinary toxicity when treated with moderate hypofractionation. These data are a warning to those adopting hypofractionation, whether it be moderate or extreme, that long-term follow-up and randomized comparisons are essential to evaluate altered fractionation strategies. Although pooled extreme hypofractionation analyses of late adverse effects and quality of life are encouraging,[4] few patients, relatively speaking, have been followed for more than 5 years. As we have learned from the long-term follow-up of men treated for prostate cancer[5] and women treated for cervix cancer,[6] late urinary adverse effects increase over time, even 10 or 15 years after treatment. A randomized comparison of extreme hypofractionation (ie, 36.25 Gy in five fractions) to either standard fractionation or a moderate hypofractionation regimen that has been characterized previously (ie, 70.2 Gy in 26 fractions) is being implemented (Radiation Hypofractionation via Extended Versus Accelerated Therapy [HEAT] for Prostate Cancer) and is of paramount importance.

Vogelius and Bentzen[2] have thoughtfully compared our results with those compiled in their meta-analysis.[7] They calculated a 2.0-Gy-per-fraction equivalent $D_{obs}$ to be 71 Gy for both study arms, when the $D_{exp}$ would be 88 Gy for the patients undergoing hypofractionated therapy. One of the benefits of conducting a randomized trial is that the $D_{obs}$ is known to be the conventional fractionation dose (76 Gy at 2.0 Gy per fraction) for the conditions and population under study. There are many assumptions in such meta-analyses, as pointed out by Strigari et al,[8] that are of concern. The approach used by Vogelius and Bentzen does attempt to minimize the effects of heterogeneity of conditions and populations, but notable compromises were made. Acknowledging that there is no better way at present to calculate an $\alpha/\beta$ ratio for prostate cancer from existing data and that the burden of the data supports a low value, the use of a biochemical end point as applied to different risk groups in whom treatment was varied (ie, use of androgen deprivation therapy [ADT], length of ADT, treatment of the pelvic lymph nodes, 3D conformal radiation therapy *v* intensity-modulated radiation therapy) is problematic, in our view. Biochemical failure at 5 years is being used as an estimate of local control, which likely will be variable depending on patient risk features (favorable $>$ intermediate $>$ high risk). As shown in Figure 1 and in Table 1, the data from our trial suggest that high-risk patients had a worse prognosis when treated using moderate prostate hypofractionation, pelvic lymph node coverage, and long-term ADT when the nadir + 2 definition of biochemical failure was incorporated into BCDF. Notably, there were no statistical differences between the arms by risk group when the protocol definition of BCDF incorporated a modified American Society for Therapeutic Radiology and Oncology definition of biochemical failure. The data on hyperfractionation by Valdagni et al[9] also suggest a higher $\alpha/\beta$ ratio for high-risk patients, whereas high-risk patients did well when treated with moderate hypofractionation in the Italian study.[10] The heterogeneity of treatment and patient factors, along with the inherent statistical error from multiple comparisons in


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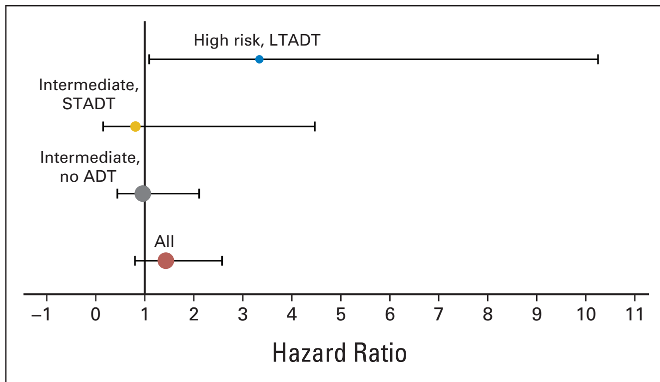


**Fig 1.** Hazard ratios (HRs) comparing hypofractionation with conventional fractionation by risk groups and overall, calculated incorporating the nadir + 2 definition of biochemical failure into the end point of biochemical and/or clinical disease failure. The bars indicate 95% CIs. Size of filled circles indicates precision. HR for the intermediate plus short-term androgen deprivation therapy (STADT) group is unadjusted because of small sample size (n = 35); other risk-group HRs are adjusted for T category, Gleason score, and pretreatment prostate-specific antigen. The only significant group was the high-risk plus long-term androgen deprivation therapy (LTADT; *P* = .035, Fine and Gray test) group. The overall HR was also adjusted for duration of androgen deprivation therapy (ADT).

**Table 1.** Cumulative Incidence Estimates of Nadir + 2 Biochemical and/or Clinical Disease Failure by Treatment Arm Stratified by Protocol Risk Classification and Use of ADT

| | Arm I: CIMRT | | Arm II: HIMRT | | |
|---|---|---|---|---|---|
| Risk Group per Protocol | 5-Year Rate | 95% CI | 5-Year Rate | 95% CI | *P* |
| Intermediate, no ADT | 16.0 | 8.4 to 25.8 | 9.1 | 4.0 to 16.9 | .684 |
| Intermediate with STADT | 15.3 | 3.6 to 34.6 | 13.9 | 2.0 to 36.6 | .817 |
| High with LTADT | 12.5 | 5.0 to 23.6 | 37.0 | 21.8 to 52.2 | .018 |

Abbreviations: ADT, androgen deprivation therapy; CIMRT, conventionally fractionated intensity-modulated radiation therapy; HIMRT, hypofractionated intensity-modulated radiation therapy; LTADT, long-term ADT; STADT, short-term ADT.

subgroup analyses, contributes to the uncertainty of drawing conclusions on whether moderate hypofractionation should be used in high-risk patients.

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# Interpretation of Human Epidermal Growth Factor Receptor 2 (*HER2*) In Situ Hybridization Assays Using 2013 Update of American Society of Clinical Oncology/College of American Pathologists *HER2* Guidelines

**To the Editor:** We would like to congratulate the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) human epidermal growth factor receptor 2 (*HER2*) testing guidelines committee for their meticulous work and for clarifying several issues that have needed to be addressed since 2007.[1] They have tried to minimize the number of equivocal cases, which will make it easier for oncologists to make treatment decisions.[2] Recommending only morphology-based assays for *HER2* evaluation is another important step that will result in more accurate reporting of *HER2* status. However, we would like to bring two specific issues to the attention of committee members.

First, the classification of cases with a HER2:CEP17 ratio ≥ 2 and average *HER2* gene copies < 4 as amplification does not make biologic sense.[2] Given that amplification literally means an increase in the number of gene copies, how can one accept the case to be amplified when *HER2* copies are not increased beyond what is normally observed in the S phase of the cell cycle? The authors' Data Supplement 2E (http://www.asco.org/sites/www.asco.org/files/final_her2_testing_ds_10-3-13.pdf),[2] which is supposed to provide clarification on this issue, clearly mentions that some committee members were against this stratification. The example of 48 patients (mentioned in Data Supplement 2E) from the Herceptin Adjuvant (HERA) trial[3] with a ratio ≥ 2 and *HER2* copies < 4 is insufficient to classify these cases as amplified. Data Supplement 2E mentions the favorable outcome of 453 patients in the HERA trial with ratios ≥ 2 but ≤ 4 in response to trastuzumab-containing therapy, which likely included these 48 patients, but does not specifically mention the separate outcome data on these 48 patients. Data Supplement 2E also mentions that "this recommendation [ie, considering cases with ratio ≥ 2 and *HER2* copies < 4 as amplified] was made easier in view of the favorable safety profile of trastuzumab."[2] However, there was no mention of the increased cost of treatment; an allusion to increased cost seems especially necessary given that there is lot of skepticism about the efficacy of treatment in such cases.

The other issue we would like to mention regards the recommendation for patients with a HER2:CEP17 ratio < 2 and average *HER2* copies ≥ 4 and < 6.[2] The committee recommends reflex testing on the same specimen using immunohistochemistry (IHC), testing with an alternative chromosome 17 probe via in situ hybridization (ISH), or testing another specimen (if available) via ISH or IHC. Using an alternate probe to CEP17 may or may not change the ratio, but clinical outcome data that are based on these alternate probes is lacking.[4] The best available evidence is from the Phase III Trial of Doxorubicin and Cyclophosphamide (AC) Followed by Weekly Paclitaxel With or Without Trastuzumab as Adjuvant Treatment for Women With HER2 Overexpressing Node Positive or High-Risk Node Negative Breast Cancer (N9831), which showed that trastuzumab benefit is independent of the HER2:CEP17 ratio or CEP17 copy number.[5] We believe that treatment decisions in such cases should be made on the basis of average *HER2* gene copies only, and additional testing using alternate probes will confuse the treating physician and will only add cost to ISH testing, without any actual clinical benefit.

We sincerely hope that the committee members will revisit these issues, and we would appreciate their response and clarification as we implement the updated HER2 guidelines in our routine clinical practice.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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# 2013 Update of the American Society of Clinical Oncology/College of American Pathologists Guideline for Human Epidermal Growth Factor Receptor 2 Testing: Impact on Immunohistochemistry-Negative Breast Cancers

To the Editor: The 2013 update of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline for human epidermal growth factor receptor 2 (HER2) testing in breast cancer reverts to the US Food and Drug Administration criterion regarding the definition of immunohistochemistry (IHC) -positive breast cancer.[1] Several studies have shown that switching from the US Food and Drug Administration to the 2007 ASCO/CAP guideline regarding an IHC-positive score resulted in a lower percentage of positive cases.[2-4] The effect of reverting to the pre-2007 guideline situation can thus be deduced, to a certain extent. However, the 2013 update also introduces new definitions of IHC scores 0 and 1+, as well as a new delineation of negative versus equivocal IHC results. The impact of these changes is currently unclear.

We recently studied IHC-negative breast cancer and found that, using the 2007 guideline, score 0 and 1+ tumors emerged as two distinct and clinically relevant populations.[5] Given the design and nature of our study, reassessment of this data set using the 2013 guideline could shed some light on the effects to be expected regarding HER2 testing at the negative and equivocal end of the spectrum.

We retrieved 126 of 129 IHC slides that were scored in our central laboratory as negative in the original analysis.[5] These slides were reassessed by the same pathologist (K.L.), according to the 2013 guideline update. The new IHC scores were compared with the former scores and with the original fluorescent in situ hybridization (FISH) results, interpreted according to the 2013 guideline update, using both the algorithm for single- and dual-probe in situ hybridization (ISH). Reassessment of FISH slides was not feasible because of signal fading. For three of the tumors, no score could be assigned because of weak to moderate incomplete membrane staining within less than 10% of the invasive tumor cells, a combination of intensity and pattern not included in the guideline algorithm. All three tumors were scored as 1+ according to the 2007 guideline, and two were FISH negative according to all algorithms of both versions of the guideline; one tumor showed an equivocal copy number–based and a negative ratio–based result according to the 2007 guideline and an equivocal result according to both algorithms of the 2013 version of the guideline. These three tumors were omitted from the remainder of the analysis. The 2013 guideline does not cover all staining patterns and intensities. It is probably preferable to withhold an IHC score in such situations and to perform an ISH assay, with the caveat that the IHC assay cannot serve as a reflex test if the ISH result is equivocal.

As illustrated in Table 1, we found that 16% of tumors (20 of 123) changed from IHC negative according to the 2007 version of the guideline to IHC equivocal according to the 2013 version, implying that the proportion of tumors needing reflex testing by ISH will increase considerably. Within the now smaller IHC-negative population, score 0 tumors account for 52% of cases (64 of 123), whereas these accounted for only 13% (16 of 123) according to the 2007 version. Moreover, as shown in Table 2, the mean *HER2* copy number showed no difference between score 0 and score 1+ tumors ($P = .1219$, Mann-Whitney test), whereas the mean copy number was significantly lower in score 0 than in score 1+ tumors in our original study.[5] Table 3 shows that, as in our original study, the so-called new IHC-negative group still contains a small number of FISH-positive tumors. Moreover, Table 3 also shows that, depending on whether a single- or dual-probe assay is used, 5% to 10% (1 to 2 of 20) of the new IHC 2+ tumors would yield an equivocal FISH result, implying an increase in double-equivocal results. Although more studies are needed, it is doubtful that the increased number of ISH tests and double-equivocal results at the negative end of the spectrum will be compensated for at the positive end of the HER2 spectrum as a result of reverting to the US Food and Drug Administration criteria for a positive HER2 IHC result. Lack of reassessment of the FISH slides according to the 2013 ISH interpretation modifications can be considered a weakness of our study. Nevertheless, our methodology of IHC reassessment of slides from a well-defined patient population by the same pathologist is not hindered by preanalytic, analytic, and postanalytic caveats of other studies[4] and enables us to draw the previously mentioned preliminary conclusions regarding the probable impact of the 2013 ASCO/CAP HER2 guideline update at the negative and equivocal end of the IHC spectrum. Whereas the rationale for returning to the US Food and Drug Administration criteria for HER2 IHC- and ISH-positive results as inclusion criteria in the original clinical trials is evidence based, the evidence for the new definitions of HER2 IHC-negative results remains unclear.

**Table 1.** Comparison of IHC Scores According to the 2007 and 2013 Versions of the Guideline

| IHC 2007 | IHC 2013 | | | |
|---|---|---|---|---|
| | Score 0 | Score 1+ | Score 2+ | Total |
| Score 0 | 16 | 0 | 0 | 16 |
| Score 1+ | 48 | 39 | 20 | 107 |
| Total | 64 | 39 | 20 | 123 |

Abbreviation: IHC, immunohistochemistry.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

*Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a*

**Table 2.** Mean *HER2* Copy Number in Nonamplified Tumors According to Single Assay Algorithm

| IHC Score | No. of Cases | *HER2* Signals/Cell |
|---|---|---|
| 0 | 64 | 2.48 ± 0.73 |
| 1+ | 38 | 2.37 ± 0.67 |

Abbreviation: IHC, immunohistochemistry.

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**Table 3.** Comparison of IHC Scores and FISH Results According to the 2013 Guideline Update

| IHC 2013 | Total | FISH Result: Single Probe Negative | Single Probe Equivocal | Single Probe Positive | Dual Probe Negative | Dual Probe Equivocal | Dual Probe Positive |
|---|---|---|---|---|---|---|---|
| Score 0 | 64 | 63 | 1 | 0 | 63 | 1 | 0 |
| Score 1+ | 39 | 37 | 1 | 1 | 36 | 1 | 2 |
| Score 2+ | 20 | 18 | 2 | 0 | 18 | 1 | 1 |
| Total | 123 | 118 | 4 | 1 | 117 | 3 | 3 |

Abbreviations: FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

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# Reply to R. Bhargava et al and K. Lambein et al

On the basis of reanalysis of a small group of highly selected patients, Lambein et al,[1] in their correspondence to *Journal of Clinical Oncology*, assert that approximately 16% of breast cancers previously deemed to be human epidermal growth factor receptor 2 (HER2) negative by immunohistochemistry (IHC) using the 2007 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 testing guideline would now require reflex testing by in situ hybridization (ISH), according to the criteria of the recently published 2013 HER2 testing guideline update.[2,3] Their data suggest that approximately one in six patients with an IHC 0 and 1+ test result, if using 2007 guideline criteria, would now be reclassified as IHC 2+ equivocal, and consequently require reflex ISH testing.

Although provocative, this assumption is influenced by the nature of the 150 consecutive patients they initially studied.[4] More specifically, the IHC performed on the 129 cases that were centrally confirmed as IHC 0 or 1+ had previously been identified as IHC negative at nonaccredited local laboratories. Consequently, the observed distribution of HER2 test results in their study set does not represent what is expected when testing patients from a general population, and we maintain that the latter is preferable for testing the accuracy of biomarker test results. Thus, we conclude that the findings of Lambein et al[1] do not apply to the general population of patients with newly diagnosed invasive breast cancer. The number of cases that will need reflex testing under the 2013 ASCO/CAP HER2 testing guideline update for the general population of patients with breast cancer is expected to be less than the estimate suggested by Lambein et al,[1] because the estimate must also consider patients who will no longer require confirmatory reflex fluorescent ISH (FISH) testing because of reclassification of their tumor as IHC 3+.

We also wish to correct a misinterpretation by Lambein et al[1] regarding the 2013 guideline update. They state that the update introduces new definitions for IHC scores 0 and 1+. In fact, the 2013 update provides clearer descriptive definitions of IHC 0 and 1+ test results (Fig 1 of our article[2,3]). Although Lambein et al chose not to rescore three of the 129 centrally confirmed IHC-negative cases because the combination of intensity and pattern they observed ("weak to moderate incomplete membrane staining within $<$ 10% of the invasive tumor cells") had not been included in the 2013 guideline algorithm, the Panel would not have considered these staining characteristics sufficient to classify them as IHC 2+ equivocal and trigger reflex ISH testing. We also dispute one of their main conclusions that "although more studies are needed, *it is doubtful* [emphasis ours] that that the increased number of ISH tests and double-equivocal results at the negative end of the spectrum will be compensated for at the positive end of the HER2 spectrum as a result of reverting to the US Food and Drug Administration criteria for a positive HER IHC result." In fact, the analyses by Lambein et al yield no information about


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the impact from the 2013 guideline update on reflex ISH testing at the equivocal or positive end of the IHC spectrum.

We thank Bhargava and Dabbs[5] for their separate correspondence and comments regarding reconsideration of what will be deemed positive HER2 testing and when to consider reflex or new testing, especially in the less common subsets. Indeed, a primary intent of the 2013 Guideline Update Panel was to provide greater clarity to guide pathologists and oncologists in routine clinical practice regarding less common cases, while emphasizing the importance of morphology-based assays.

The Panel is aware of the limitations of efforts to retrospectively assess clinical benefit of HER2-targeted therapy in various subsets within the prospective clinical trials. However, we wished to provide physicians and patients with actionable guidance, especially in regard to when to order a reflex test or a new test. Overall, we elected to err on the side of sensitivity rather than specificity, given the enormous benefit and low level of toxicities of anti-HER2 therapy, especially trastuzumab.

For example, in the 2007 guideline, the Panel called for reflex testing for tumors that exhibit complete and intense circumferential membrane staining in $> 10\%$ but $\leq 30\%$ of invasive tumor cells, although the previous clinical trials had generally enrolled patients if staining was seen in $> 10\%$ of cells. Subsequent studies demonstrated that the frequency of an ISH amplification ratio $\geq 2.0$ but $< 2.2$ in cases with $> 10\%$ but $\leq 30\%$ IHC staining is small (0.15% of all new cases).[6] However, given that reanalysis of the previously published clinical trials suggested that this group experienced a similar benefit to those with $\geq 30\%$ staining, the updated guidelines now considers these cases HER2 positive, and reflex ISH testing for these patients has been eliminated.

Bhargava and Dabbs[5] express concern that tumors with a *HER2*/CEP17 ratio $\geq 2.0$ with an average *HER2* copy number $< 4.0$ signals/cell now require reflex IHC testing. The Panel clearly marked the "ISH positive" gold diamond in our Figure 3[2,3] with a symbol that directs the reader to review Data Supplement 2E for a detailed summary of the data as reviewed and interpreted by the Panel. For example, we acknowledge in Data Supplement 2E (2) that "an analysis of the 48 cases alone is inappropriate because of its small size, [but that] there is no trend in these data to suggest they are nonresponsive to trastuzumab."[2,3] We further stated that "several members of the Update Committee expressed concern about describing an invasive breast cancer as HER2 positive on the basis of on a single HER2 test showing a HER2/CEP17 ratio $\geq 2.0$ and an average HER2 copy number $< 4.0$ signals/cell and recommended further testing of cases of this type."[2,3] Consequently, it is entirely appropriate for the attending pathologist in charge of such cases to recommend (and for the oncologist to suggest) additional HER2 testing using the same specimen or, if available, another specimen. However, if additional HER2 testing is not possible or not ordered, the Panel concluded that such patients should not be excluded from HER2-targeted therapy, given that these rare cases were included in the first generation of clinical trials.[2,3]

Bhargava and Dabbs[5] also question the recommendation to order a reflex test or a new test in tumors with an average *HER2* copy number $\geq 4.0$ and $< 6.0$ signals/cell (if using a single-signal ISH or if *HER2*/CEP17 ratio is $< 2.0$ when using a dual-signal ISH assay). This recommendation was guided by the intent to match the decision tree in Figure 2 (single-signal ISH) and Figure 3 (dual-signal ISH), by Panel concerns about this *HER2* signal range when using single-signal assays, and by the prospective use of FISH ratio $\geq 2.0$ (not *HER2* copy number) for clinical trial eligibility. The recommendation to use an alternative chromosome 17 probe is an option, not a mandate. This is exemplified by the statements in Data Supplement 2E (3) indicating that the Panel believed that ". . . coamplification of CEP17 region is occasionally observed in some ISH assays and may lead to a *HER2*/CEP17 ratio less than 2.0" [2,3] and that "if coamplification of CEP17 is suspected, laboratories *may* [emphasis added] pursue one of two options," with one of them being to "repeat HER2 testing in the same specimen using an alternative probe for CEP17 or for another gene in chromosome 17 not expected to coamplify with HER2."[2,3]

Although we elected to favor sensitivity over specificity in the 2013 Guideline Update, the Panel is fully cognizant that accuracy in HER2 testing will become ever more critical as the financial costs associated with HER2-targeted drugs continue to escalate and as regimens containing HER2-targeted drugs without chemotherapy are now being tested. Since the publication of the 2007 guideline, greater standardization of tissue handling, improved laboratory performance of HER2 testing, and more careful reporting of test results in the United States and elsewhere have been observed in clinical practice. Greater insight offered by further analysis of various prospective clinical trials has allowed the HER2 Testing Guideline Update Panel to expand its focus beyond earlier concerns about false-positive tests results. Greater clinical experience and new data have permitted the Panel to be more specific about less common clinical scenarios to better discriminate between HER2-positive and -negative results, thereby reducing the frequency of equivocal or inconclusive test results. The ASCO/CAP HER2 testing guideline will continue to evolve as greater experience and new data become available. In the meantime, the Panel expresses its appreciation for all the comments and suggestions it has thus far received.

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# T-Cell Characterization Using Multicolor Flow Cytometry After Allogeneic Hematopoietic Stem-Cell Transplantation

**To the Editor:** A recent article reported the results of a phase II trial evaluating the safety and efficacy of atorvastatin administration for graft-versus-host disease (GVHD) prophylaxis in both adult donors and recipients of matched-sibling allogeneic hematopoietic stem-cell transplantation (HSCT).[1] In this study, the incidence of acute GVHD in enrolled patients was promisingly low, and it was significantly lower than that of historical controls, without increasing the risk of relapse. These results were consistent with the previous findings of murine studies and retrospective studies, as reviewed previously.[2] We strongly agree with the authors that validation of their study by a phase III trial is warranted.

We do have a concern regarding a secondary end point of T-cell immune reconstitution, and request clarification from the authors.[1] The authors performed immunophenotypic analyses of T-cell recovery in transplantation recipients and reported that reconstitution of the T-cell compartment was prompt in the setting of atorvastatin therapy. Our primary concern relates to the stated definitions of naive and memory T-cell subsets, which defined $CD4^+$ memory T cells as $CD3^+CD27^+CD45RO^+CD4^+$, $CD8^+$ memory T cells as $CD3^+CD27^+CD45RO^+CD8^+$, $CD4^+$ naive T cells as $CD3^+CD45RA^+CD45RO^-CD4^+$, and $CD8^+$-naive T cells as $CD3^+CD45RA^+CD45RO^-CD8^+$. These definitions are consistent with early and classical descriptions using the CD45 isoforms (CD45RA and CD45RO) alone to distinguish naive and memory T cells. However, we now understand that there is greater diversity within the memory T-cell pool in humans, and that $CD3^+CD27^-CD45RO^+CD4^+$ T cells are also memory T cells, usually defined as effector memory T cells. Because the authors excluded $CD45RO^+$ T cells lacking coexpression of CD27 from their definition of memory cells, it is likely that the authors underestimated the number of memory T cells in recipients.

In addition, when assessing the number of naive T cells in recipients, the authors[1] did not include additional naive markers such as CD62L, CCR7, and CD27. It is now well known that a subset of late effector memory T cells re-express CD45RA ($T_{EMRA}$),[3,4] and the combination of CD45RA and CD45RO itself is not able to discriminate naive T cells from the $T_{EMRA}$ cells. The characteristics of $T_{EMRA}$ are completely different from those of naive T cells with respect to the

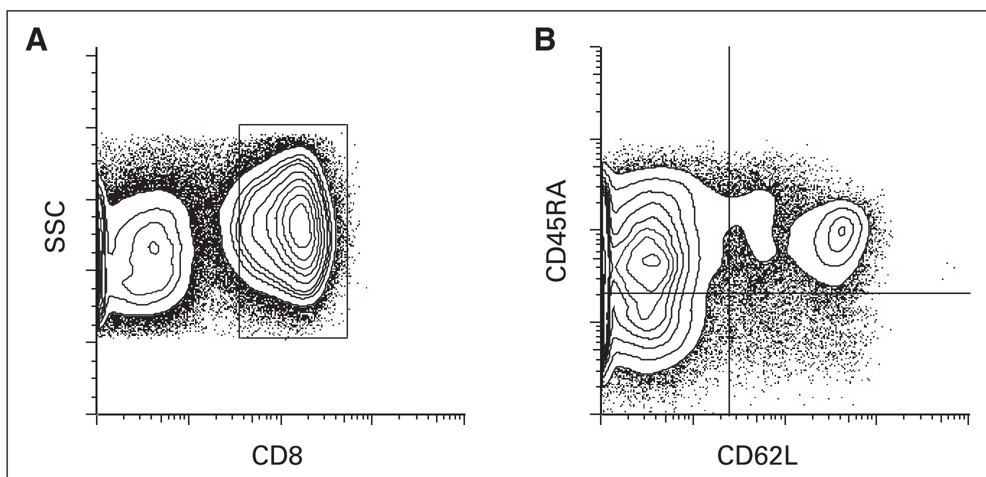


**Fig 1.** A representative result of flow cytometric analysis using peripheral blood mononuclear cells from a patient after allogeneic hematopoietic stem-cell transplantation. Figure shows the (A) CD8-gated lymphocytes and (B) their CD45RA/CD62L profile. SSC, side scatter.

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production of cytokines and cytotoxic molecules. Previous reports have already demonstrated the presence of such $T_{EMRA}$ in patients after allogeneic HSCT,[5,6] and it has been confirmed that $T_{EMRA}$ cells, which were determined by the combination of CD45RA and CD62L (Figs 1A and 1B), comprised a major population in peripheral blood after allogeneic HSCT.[7] Because of their inclusion of late effector memory cells as a result of the broad categorization of all CD45RA$^+$ cells as naive, it seems likely that the authors overestimated naive T-cell recovery by their phenotypic definitions.

We strongly agree that it is important and meaningful to correlate naive and memory T-cell reconstitution with GVHD after HSCT, and to examine these end points in correlative studies of novel GVHD strategies, such as studied here.[1] Although we are impressed with this clinical study of atorvastatin and the potential of this approach to reduce the incidence of GVHD, we find it difficult to conclude that reconstitution of the naive T-cell compartment in this setting was prompt, given the possibility that the apparent increase of naive T cells may actually have represented an increased number of $T_{EMRA}$ cells in the peripheral blood.

In conclusion, we applaud the novel clinical findings of the authors,[1] and we support their conclusion that additional studies of the clinical potential of this approach are warranted. In parallel, more precise phenotypic studies of T-cell immune reconstitution will be needed to better define how this intervention influences the recovery of naive and memory T cells.

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# Reply to S. Fuji et al

We thank Fuji and Shindo[1] for their helpful comments regarding the methodology of immune-reconstitution analysis used in our recent study.[2] Immune-reconstitution represented one of the secondary end points for our protocol. The definition of memory and naive T cells used in our study is largely a reflection of clinical feasibility, given that we performed these correlative assays in our Clinical Laboratory Improvement Amendments–certified clinical laboratory, as opposed to a research flow cytometry core facility. At our transplantation program at West Virginia University, we have historically assessed immune reconstitution after allografting by clinically adopting a previously validated flow-cytometric assay that our institution had used in pediatric patients with immune deficiencies (J.A. Vos, personal communication, January 2014).

We do agree with Fuji and Shindo[1] that classical markers such as CD45RO for memory T cells and CD45RA for naive T cells are not always reliable.[3] It is indeed possible that our definition of memory T cells (that required coexpression of CD27) would have slightly underestimated the memory T-cell compartment by excluding the small population of the so-called effector memory T cells. Keeping this limitation in mind, it is actually reassuring to see the absolute numbers of memory T cells detectable at all time points after transplantation in our study (Data Supplement Table 1S).[2] Fuji and Shindo further comment in their letter that they "find it difficult to conclude that reconstitution of the naive T-cell compartment in this setting was prompt, given the possibility that the apparent increase of naive T cells may actually have represented an increased number of $T_{EMRA}$ cells in the peripheral blood." Here we would like to clarify that in our article,[2] although we indicated that the reconstitution of the T-cell compartment (as a whole) was prompt, we did not assert that this applied to the naive T-cell compartment, specifically. We acknowledge the limitations of any flow cytometric panel in definitively delineating a bona fide naive T-cell reconstitution. It would certainly be desirable to estimate naive T-cell compartment recovery in the context of atorvastatin-based graft-versus-host disease prophylaxis using more reliable techniques, for example, T-cell receptor rearrangement excision DNA circle (sjTREC) assays, preferably adjusted for peripheral blood T-cell proliferation (ie, sjTREC calculated as copy numbers/CD3$^+$ T-cell counts or as absolute numbers of sjTREC/mL of peripheral blood),[3-6] or assaying circulating T-cell repertoires by determining T-cell receptor $\beta$-chain gene complexity with spectratyping.

We appreciate the authors'[1] enthusiasm for our encouraging results with atorvastatin-based prophylaxis in sibling transplantation donors and their corresponding recipients. We eagerly anticipate the results of other ongoing prospective studies using designs identical to our protocol (eg, the Safety and Efficacy of Atorvastatin for Prophylaxis of Acute Graft Versus Host Disease in Patients With Hematological Malignancies [and] HLA-Donor Hematopoietic Stem Cell Transplantation trial being conducted at Ohio State University). We also expectantly await the results of studies using atorvastatin prophylaxis only in sibling donors (Donor Atorvastatin Treatment for Prevention of Severe Acute GVHD After Nonmyeloablative Peripheral Blood Stem Cell Transplantation, and Donor Atorvastatin Treatment for Preventing Severe Acute Graft-Versus-Host Disease in Patients Undergoing Myeloablative Peripheral Blood Stem Cell Transplantation, both being conducted at Fred Hutchinson Cancer

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Center). Finally, we look forward to the results of studies using atorvastatin prophylaxis in recipients of matched sibling or unrelated donor transplantations (eg, Atorvastatin As GVHD Prophylaxis for Allogeneic Hematopoietic Cell Transplantation, which is being conducted at our programs at the Medical College of Wisconsin and West Virginia University). It is hoped that these ongoing trials will clarify the optimal strategy of atorvastatin administration to both transplantation donor and recipients versus prophylaxis in donors alone or prophylaxis in recipients alone. Needless to say, these mostly single-center study results will then require confirmatory, multicenter phase III trials.

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