In this issue of the Journal, O’Malley et al. (1) present important, critical, and compelling new evidence regarding the association between alterations in the human epidermal growth factor receptor type 2 (HER2) amplicon and incremental sensitivity to anthracycline-based adjuvant therapy for breast cancers. This report is the latest in a series of publications that question the generalized assumption that there is an incremental benefit to all breast cancer patients who receive anthracycline-based adjuvant therapy as opposed to non-anthracycline-containing adjuvant therapy. This premise has dominated the design of studies and clinical practice utilization for the vast majority of adjuvant regimens worldwide over the past 30 years. The widespread use of anthracyclines is almost entirely based on the well-known meta-analysis published by the Early Breast Cancer Trialists’ Collaborative Group, frequently referred to as the Oxford “Overview” (2), rather than results from any single study. Indeed, the majority of individual studies have failed to show a substantial benefit from anthracycline-based adjuvant regimens over non-anthracycline-containing adjuvant regimens in breast cancer. The “Overview” used data derived from more than 15,000 women enrolled in 17 separate trials to show that breast cancer patients who received an anthracycline as part of their adjuvant treatment who had an absolute 3 to 4.5 percentage point improvement in relapse-free survival (RFS) and overall survival (OS) compared with women who received non-anthracycline regimens (2). The magnitude of these benefits has generally been considered to outweigh the well-known, long-term, and life-threatening problems associated with anthracyclines, that is, cardiac toxicity including congestive heart failure (3,4) and bone marrow dysfunction including acute leukemia and myelodysplasia (5). However, we are now beginning to appreciate that our previous estimates of these major long-term safety issues were frequently assessed using follow-up times designed to measure differences in efficacy between various regimens rather than the very late toxicities that might be caused by them. Longer follow-up from registry databases of breast cancer patients who received adjuvant anthracyclines for their breast cancers indicates that our initial assessments may have underestimated these long-term toxicities (6–8). This is true for both cardiac (6,8) and marrow (7) toxicities, making the judicious and appropriate use of anthracyclines even more critical.

While there is little doubt that the “Overview” findings regarding the relative benefits of anthracyclines are correct, it must be noted that they are based on the incorrect assumption that all breast cancer patients requiring adjuvant chemotherapy have the same or similar disease. We now know that the molecular diversity of human breast cancers is much more complex and that clinical benefits derived from various systemic therapeutic interventions can be profoundly affected by the molecular subtype of the disease (9,10). Before our appreciation of this molecular complexity, the use of meta-analyses that did not take such diversity into account was rational. In the post-genomic era, however, it is appropriate to assess the benefits of various therapeutics, including anthracycline-based chemotherapy, in the context of relevant breast cancer molecular subtypes. This phenomenon is best illustrated by the unique responsiveness of ER positive disease to therapies directed at the estrogen/estrogen receptor pathway or the ER or of benefits from antagonists to the HER2 pathway being restricted to HER2-positive disease. In each case, only those breast cancers driven by these pathways benefit from these respective “targeted” therapies (11–14). We are now coming to appreciate that the same is true for many standard cytotoxic agents. The article by O’Malley et al. (1) underscores this fact and supplies further evidence indicating that this is a realistic objective for chemotherapeutic as well as biologic therapies.

Over the past 15 years, a substantial amount of clinical data from multiple individual studies has indicated that the incremental benefit from adjuvant anthracycline-based therapies is largely restricted to the HER2-positive subgroup of human breast cancers (15–19). This fact could not have been appreciated prior to identification of the HER2 subgroup (20,21), making the “Overview” relevant for that era. However, in another and more recent meta-analysis of some 5354 women for whom HER2 status was determined and who had been enrolled in eight separate large trials comparing anthracycline- with non-anthracycline-based therapies, there was consistent evidence that the incremental benefit from adjuvant anthracyclines is largely restricted to the HER2-positive subgroup (22). Indeed, benefits of anthracyclines in this breast cancer subgroup were substantially greater than those previously indicated by the Oxford “Overview” for the overall breast cancer population. Moreover, this more recent, classification driven meta-analysis demonstrated that little or no benefit accrued to patients whose cancers were HER2 normal (22), although these patients remain at risk for all the attendant toxicities associated with anthracyclines. Importantly, HER2-normal patients constitute some 75%–80% of the global breast cancer population. These data led Gennari et al. (22) to note, “The added benefits of adjuvant...
chemotherapy with anthracyclines appear to be confined to women who have HER2 overexpressed or amplified breast tumors … The absence, in our study, of any effect of anthracyclines observed in patients with HER2-negative disease suggests that this group of patients could be spared unnecessary toxic effects related to the use of this class of agent and raises questions as to the appropriateness of control arms in randomized clinical trials in which anthracycline-based regimens are used in unselected patient populations” (22). Despite these data, the vast majority of currently used adjuvant regimens for almost all breast cancer patients worldwide, both on and off study, are anthracycline based.

What is unique about HER2 amplification that might explain the enhanced efficacy of anthracyclines seen in this breast cancer subgroup? HER2 overexpression alone has been studied in preclinical models both in vitro and in vivo and appears to confer no incremental sensitivity to anthracyclines in the absence of HER2 gene amplification (23). How do we reconcile these preclinical findings with the clinical data? Although it is generally accepted that the HER2 gene is the “driver” of the amplification event on chromosome 17q12–q21, it is now known that this amplicon is variable in size, frequently extending telomerically and/or centromerically from the HER2 gene to include other genes. In addition, the amplicon, like others in the human genome, is sometimes flanked by deletions of immediately adjacent regions of DNA. The result of these amplicon-related phenomena is that a number of flanking genes are frequently either coamplified or deleted in breast cancers containing HER2 amplification. One such gene is the topoisomerase II alpha (TOP2A) gene. This gene is more than 700 kb telomeric to HER2 and encodes a cell cycle–regulated protein that is critical to several functions involving cell division including transcription of mRNA as well as DNA replication. In addition, the topoisomerase II alpha protein is a known major target of anthracycline activity (24,25). In their study, O’Malley et al. focus on alterations occurring in this gene using an analysis of 438 of the 710 premenopausal patient tissue samples obtained from the National Cancer Institute of Canada Mammary 5 Trial that compared two adjuvant regimens: cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) with cyclophosphamide, epirubicin, and 5-fluorouracil (CEF). The authors convincingly show that major improvements in RFS (hazard ratio [HR] = 0.53) as well as overall survival OS (HR = 0.38) in favor of anthracycline-based treatment are restricted to patients whose cancers contain TOP2A alterations. Conversely, anthracyclines conferred little or no improvement (HR = 0.90 for RFS and HR = 1.09 for OS) among patients with TOP2A-normal tumors, who constitute 358/438 (78%) of the breast cancer patients in their study. It was initially reported some 9 years ago (25,26) that TOP2A alterations, rather than HER2 amplification alone, might be responsible for conferring the incremental anthracycline sensitivity seen in HER2-positive patients. These older data indicated that there may be a subset of the HER2-amplified malignancies that were responsible for the observed markedly improved efficacy seen in HER2-positive breast cancers. Several publications since that time have made the same or similar observations, but many were limited by analysis of small numbers of patients. However, eight separate studies containing at least 100 subjects or more have now evaluated and reported or published on the association between TOP2A alterations and HER2 amplification (Table 1) (27–34). The overwhelming majority of these reports indicate that TOP2A changes rarely occur in the HER2-normal population (25, 28, 31–33, 35). In aggregate, tumor tissues from women in these larger clinical trials demonstrated that approximately 88% (184/210) of the reported

Table 1. Prevalence of TOP2A alterations (amplification and deletion) by HER2 status in published studies*

<table>
<thead>
<tr>
<th>Number in study</th>
<th>TOP2A ampl, No. (%)</th>
<th>TOP2A normal, No. (%)</th>
<th>TOP2A deleted, No. (%)</th>
<th>TOP2A ampl, No. (%)</th>
<th>TOP2A normal, No. (%)</th>
<th>TOP2A deleted, No. (%)</th>
<th>First author, year (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>354</td>
<td>23/61 (38)</td>
<td>73/354 (21)</td>
<td>8/61 (13)</td>
<td>ND</td>
<td>281/354 (89)</td>
<td>ND</td>
<td>Di Leo, 2002 (27)</td>
</tr>
<tr>
<td>120</td>
<td>10/30 (33)</td>
<td>30/120 (25)</td>
<td>6/30 (20)</td>
<td>1/90 (1.1)</td>
<td>90/120 (75)†</td>
<td>3/90 (3.3)</td>
<td>Olsen, 2004 (28)</td>
</tr>
<tr>
<td>805</td>
<td>79/263 (30)</td>
<td>263/605 (42.6)†</td>
<td>60/263 (22.8)</td>
<td>14/542 (2.6)</td>
<td>542/805 (67.3)†</td>
<td>26/542 (4.7)</td>
<td>Knoop, 2005 (29)</td>
</tr>
<tr>
<td>391</td>
<td>48/128 (37.5)</td>
<td>128/391 (32.7)†</td>
<td>ND</td>
<td>ND</td>
<td>263/391 (67.3)†</td>
<td>ND</td>
<td>Tanner, 2006 (30)</td>
</tr>
<tr>
<td>284</td>
<td>18/74 (24.3)</td>
<td>74/284 (26)</td>
<td>6/74 (8.1)</td>
<td>2/210 (1)</td>
<td>210/284 (74)</td>
<td>0/210 (0)</td>
<td>Park, 2006 (31)</td>
</tr>
<tr>
<td>351</td>
<td>40/94 (42.6)</td>
<td>94/251 (37.9)</td>
<td>17/94 (18.0)</td>
<td>0/257 (0)</td>
<td>257/351 (73.2)</td>
<td>257/257 (100)</td>
<td>Koncny, 2006 (32)</td>
</tr>
<tr>
<td>245</td>
<td>20/37 (54)</td>
<td>37/233 (15.9)†</td>
<td>ND</td>
<td>0/196 (0)</td>
<td>196/233 (84.1)†</td>
<td>ND</td>
<td>Arriola, 2007 (33)</td>
</tr>
<tr>
<td>303</td>
<td>17/63 (27)</td>
<td>63/303 (20.8)</td>
<td>22/63 (35)</td>
<td>9/240 (3.8)</td>
<td>240/303 (79.2)</td>
<td>28/240 (11.7)</td>
<td>Bartlett, 2008 (34)</td>
</tr>
<tr>
<td>2853</td>
<td>255/750 (34)</td>
<td>762/2841 (26.8)</td>
<td>119/585 (20.3)</td>
<td>26/1535 (1.6)</td>
<td>2079/2841 (73.2)</td>
<td>57/1339 (4.2)</td>
<td>Totals</td>
</tr>
<tr>
<td>438</td>
<td>33/116 (28.4)</td>
<td>116/438 (26.5)</td>
<td>17/116 (14.7)</td>
<td>20/314 (6.4)</td>
<td>314/438 (71.7)</td>
<td>9/314 (2.9)</td>
<td>O’Malley, 2009 (1)</td>
</tr>
</tbody>
</table>

* Study populations of ≥100 women. TOP2A = topoisomerase II alpha; HER2 = human epidermal growth factor receptor type 2.
† HER2 status determined by immunohistochemistry.
‡ HER2 status determined by chromogenic in situ hybridization. ND = Not Determined.
TOP2A-amplified cases occur within the HER2-positive subgroup of breast cancers (28,29,31–33). Two other publications from this group of larger studies did not report on HER2-normal cases (27,30) likely because their earlier efforts failed to demonstrate TOP2A amplification in this population (25,35). With regard to TOP2A deletion rates in HER2-normal breast cancers, these larger studies reported a prevalence that ranges from 0% to 12%. One notable discrepancy between the O’Malley report and previous studies is the higher (≥6%) TOP2A amplification rate found in HER2-normal patients (Table 1). O’Malley et al. report the largest number of TOP2A alterations to date in these HER2-normal breast cancers. Only one other study besides the current one found a TOP2A amplification rate greater than 1%–2% in the HER2-normal cancers (Table 1) (34). Indeed, the majority of the published data from large studies indicate that the bulk of the TOP2A amplification events occur in a subset of the HER2-amplified breast cancers and represent a co-amplification phenomenon (Table 1). In addition, the association of TOP2A deletions with anthracycline responsiveness conflicts with in vivo and in vitro findings that TOP2A deletions are associated with anthracycline resistance (25,35,36). Further research will undoubtedly be required to resolve this latter issue.

It is likely that most of the various discrepancies between studies concerning altered TOP2A gene prevalence are due to technical differences in measuring those alterations, that is, different ratios and/or cutoffs used for calling a tumor amplified or deleted as well as very different technologies and/or reagents used to determine alteration rates including use of non-cell-based assays (25,27,28,35,37–39). For example, one recent report claimed that coamplification of TOP2A is very rare in human breast cancers using a methodology that does not score malignant tissue on a cell-by-cell basis. Instead, this study used polymerase chain reaction amplification of DNA extracted from whole tissues to assess alteration rates that are specific to tumor cells (39) and thus likely underestimated rates of TOP2A alteration in a malignant cell population that was diluted by the presence of normal cell DNA. This is especially likely because the TOP2A amplification level, when present, is invariably lower than that of the HER2 gene. Such discrepancies can best be resolved by exchange and retesting of annotated samples using appropriate and validated methods, cutoffs, reagents, and analytes.

Currently, the overwhelming bulk of the published and/or reported data indicate that TOP2A alterations are the important predictive factors for determining the likelihood of incremental benefits from anthracyclines in the adjuvant treatment of human breast cancers. These same data also show that TOP2A alterations most often occur in the context of HER2 amplification. Given that the majority (75%–80%) of breast cancer patients are HER2 normal, the question can now reasonably be asked: what benefits do these patients receive from anthracycline-based therapies? In the current report, O’Malley et al. restate the case as clearly and unequivocally as possible: “Patients whose tumors do not have TOP2A alterations or do not amplify HER2 appear to receive virtually no benefit from CEF as compared with CMF.” A question remains as to whether patients with HER2 amplification and TOP2A co-amplification will still benefit incrementally from anthracyclines now that we can use drugs like trastuzumab or lapatinib which target the HER2 alteration directly. This will require analysis of recently completed and ongoing large adjuvant studies that compare anthracycline-based regimens with non-anthracycline regimens in combination with these HER2 antagonists. It would seem, however, that based on the current data as well as most of the published literature, the “verdict” is in for HER2-normal breast cancers; they should not receive anthracyclines as part of their adjuvant treatment.

References


**Note**

The authors take full responsibility for the writing of this editorial and report no conflicts of interest.