Twenty-year Results of the Naples GUN Randomized Trial: Predictive Factors of Adjuvant Tamoxifen Efficacy in Early Breast Cancer

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ABSTRACT

Purpose: Tamoxifen (TAM) is increasingly administered to new early breast cancer patients. Because it is not devoid of toxic effects, we studied factors potentially predictive of its efficacy.

Experimental Design: From 1978 to 1983, 433 patients were enrolled in the GUN randomized trial: 206 were assigned to TAM versus 227 controls (no-TAM). Premenopausal patients with axillary lymph node involvement (65 TAM versus 65 no-TAM) also received nine CMF cycles. Eight biological markers were retrospectively assayed for most patients: estrogen; progesterone; prolactin receptors (PrlRs); microvessel count (MVC); S-phase fraction; tumor ploidy; epidermal growth factor receptor (EGFR); and HER2. We performed a multivariate test of the TAM/covariate interactions to establish whether these variables predicted for TAM efficacy. Estimates of the TAM effect were expressed as hazard ratio (HR) of death of TAM over no-TAM patients with 95% confidence intervals (95% CIs).

Results: At a median follow-up of 15 years, PrlRs, MVC, S-phase fraction, ploidy, and EGFR did not influence TAM efficacy. Differently, HER2 had an overall significant predictive effect: HR = 0.59 (95% CI: 0.40–0.87) in HER2-negative subjects versus HR = 1.09 (95% CI: 0.63–1.87) in HER2-positive subjects (interaction test: P = 0.04). The predictive effect of HER2 was also evident in the subgroup of patients with steroid receptor-positive tumors (HER2 positive: HR = 1.33, 95% CI: 0.70–2.51; HER2 negative: HR = 0.73, 95% CI: 0.47–1.14).

Conclusions: With the statistical power of the present randomized trial, S-phase, ploidy, EGFR, PrlR, and MVC do not seem to predict for TAM efficacy. Conversely, our data support the hypothesis that tumors overexpressing HER2 might not benefit from adjuvant TAM.

INTRODUCTION

The antiestrogen TAM is a mainstay of breast cancer treatment. Administered after local-regional treatment of early breast cancer (adjuvant therapy), it reduces the risk of relapse and death. The scientific evidence of its efficacy is more cogent than for any other anticancer drug because it derives from a systematic meta-analysis (overview) of all randomized trials worldwide (EBCTCG). According to the latest update of the overview (1), the magnitude of the effect of adjuvant TAM is directly correlated to duration of treatment, up to 5 years of administration; it is correlated to the expression of ERs in the primary tumor, having a scarce effect on ER-negative cancers. TAM is beneficial irrespective of age, nodal, and menopausal status, and it appears to be additive to the effect of chemotherapy (1). These findings have fostered an extension of the indications for long-term administration of adjuvant TAM in early breast cancer patients with ER-positive tumors (2).

In addition to classical steroid receptors, various predictive factors have been proposed: receptors or receptor-like proteins, including HER2, EGFR, prolactin receptor (3–6); indices of tumor cell proliferation such as S-phase and thymidine labeling index (7–9); and tumor neoangiogenesis (10). However, their validity has yet to be conclusively demonstrated.

In 1978, our group initiated a randomized trial in which TAM given for 2 years after local-regional treatment was compared with a control arm without TAM; all premenopausal...
patients with metastatic axillary nodes also received standard CMF. The 10-year results have been reported (11), and the trial has been included in all EBCTCG overviews. Because of its simple randomized design, monoinstitutional nature, and very long follow-up, the GUN trial represents a good model with which to look for interactions between TAM and some biological features of the disease. Preliminary data on a few predictive factors have already appeared (5, 12, 13).

In this study, we present survival data of the GUN trial at 20 years of follow-up, focusing our analyses on a panel of potential predictive markers such as HER2, EGFR, PrlR, angiogenesis, tumor ploidy, and SPF, that have been assayed retrospectively for the majority of these randomized patients. The aim was to investigate their effect on the efficacy of TAM in early breast cancer patients.

PATIENTS AND METHODS

Patients

All patients were enrolled at the Division of Medical Oncology of the University of Naples ‘Federico II’ (Naples, Italy). Inclusion criteria were: age ≤80 years; histologically confirmed noninflammatory, stages I, II, III (T3a) operable breast cancer.

Primary treatment consisted either of radical or modified mastectomy or of quadrantectomy followed by radiation therapy on the residual breast. Complete axillary lymph node dissection was performed in all patients with a minimum of six lymph nodes examined by the pathologists. Additional details are reported elsewhere (11).

Study Design

From February 1, 1978, to December 31, 1983, 433 patients were enrolled in the trial. Overall, 206 patients were assigned to the TAM arm versus 227 controls (no-TAM). Premenopausal patients with axillary lymph node involvement (60 TAM versus 65 no-TAM) also received nine cycles of CMF as a part of their adjuvant treatment. Adjuvant treatment was started 4–6 weeks after surgery. No patient was subsequently found to be ineligible and withdrawn from the study. The study profile is reported in Fig. 1.

Treatment Schedules

TAM was administered p.o. at 30 mg daily for 2 years. CMF for premenopausal patients with axillary lymph node involvement consisted of cyclophosphamide (100 mg/m² p.o.) from day 1–14, methotrexate (40 mg/m² i.v.), and fluorouracil (600 mg/m² i.v.) on days 1 and 8. The cycle was repeated every 28 days.

Predictive Factors Assay

Hormonal Receptors. Hormonal receptors were assayed from a fragment of the primary tumor removed during surgery. Tumor specimens were immediately frozen in liquid nitrogen and stored before analysis. ER and PgR levels were determined by the charcoal-coated-dextrane method, as reported previously (12). The cutoff value to classify tumors as ER positive was 10 fmol of specific binding sites/mg of cytosol protein. PrlR assays were performed on microsomal membrane preparations by a radio-binding method. Iodinated (125I) bovine prolactin was used as specific ligand (see Ref. 6 for additional details). Values of specific binding <1% were considered negative.

Ploidy and SPF. Tumor ploidy and the proportion of cells in S-phase (SPF) were evaluated by DNA flow cytometry. The distribution of tumor DNA content was analyzed on nuclear suspensions obtained from 50 μm of paraffin-embedded sections by a FACSscan flow-cytometer (Becton-Dickinson, San Jose, CA) as reported previously (14). Tumors with a DNA index equal to 1 were classified diploid, whereas all others were classified nondiploid. An SPF of 6% was the cutoff value between tumors with high or low proliferation, as previously determined in a larger series of breast cancer patients (14).

EGFR and HER2. EGFR and HER2 expression were determined by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections (5 μm) as described previously (13). The following antibodies were used: MAb-1, an anti-HER2 mouse monoclonal antibody against the extracellular domain of the p185HER2 protein (MAb-1); and C216, an anti-EGFR mouse monoclonal antibody, which was generated using human EGFR as an immunogen. Both antibodies were purchased from Triton (Alameda, CA). Only cell membrane staining was considered, and tumors were scored as positive when >10% of the cancer cells were stained. Slides were read independently by two pathologists who were blinded to the patient follow-up. Cases of disagreement were resolved by consultation.

Neoangiogenesis. The tumor neoangiogenesis was evaluated by immunohistochemical detection and count of microvessels within the tumor (MVC) as described by Weidner et al. (15). Briefly, 5-μm thick sections, representative of invasive carcinoma, were cut from formalin-fixed and paraffin-embedded tissues, pretreated with trypsin, incubated with a monoclonal antibody raised against the human factor VIII-related
antigen (Dako), and stained with a standard immunoperoxidase method (Vectastain ABC kit; Vector). Each slide was first scanned at low power (×10–100 magnification), and the area with the higher number of new vessels was identified (hot spot). This region was then scanned at a microscope magnification of ×250 (0.37 mm²). Five fields were analyzed, and the number of stained blood vessels in each was counted. For individual tumors, MVC was scored by averaging the count over the five fields analyzed. Slide reading policy was the same as for EGFR and HER2. MVC was considered a dichotomous variable, and the median observed value was the cutoff.

Statistical Methods

The end point of the present analysis is OS, defined as the time elapsed from randomization to death or the last follow-up date, for living patients. Termination date was January 31, 1999. All analyses were performed according to an intention-to-treat rule. OS curves were drawn by the Kaplan-Meier product-limit method and compared by a stratified Mantel-Haenszel test, using combined nodal and menopausal status as strata. Estimates of the effect of TAM are given in terms of HRs of death for TAM-treated over control subjects. HRs and 95% CIs for each stratum were estimated using observed and expected values from the Mantel-Haenszel test (16). The overall HR estimates of TAM efficacy were directly derived from a stratified Cox proportional hazard model using treatment as the only covariate.

All variables under study were analyzed as dichotomized but steroid receptor status. ER and PgR data were combined in a new three categories ER-PgR variable as in a previous study (12). The estrogen and progesterone receptor combination was defined as (a) negative, if both ER and PgR were <10 fmol/mg; (b) positive, if the concentration of at least one of the two receptors was >10 fmol/mg; or (c) highly positive, if ER and PgR were both positive with at least one of the two receptors having a concentration of ≥10 fmol/mg.

Treatment-covariate interactions were tested, using for each covariate a stratified Cox model adjusted for the ER-PgR combined variable where the first-order interaction was entered together with the main effects of the treatment and the covariate. Combined menopausal and nodal status categories were the strata.

Finally, HRs in subgroups of patients, defined according to the predictive factors examined, were calculated by a model with three dummy variables contrasting treated versus controls in negative subjects, treated versus controls in positive subjects, and positive versus negative in control subjects. HRs of death for TAM-treated over control patients are plotted along with 95% CIs. All analyses were performed with the BMDP statistical software.

RESULTS

Overall, 186 (43.0%) patients were premenopausal and 173 (40.0%) were node negative. Data on biological characteristics were available for most patients, ranging from 64.2% (ER/PgR)
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Effect of TAM and Biological Markers.

A multivariate Cox model (adjusting for hormonal receptor status, tumor size, menopausal status, and nodal status) was used to estimate the effect of TAM in relation to other biological variables. As shown in Fig. 4, HRs of death for TAM treated over controls are similar regardless of the status of PrlR, MVC, tumor ploidy, SPF, and EGFR, denoting that with the statistical power of this study, these biological markers did not significantly modify the effect of TAM.

Fig. 4 Effect of TAM according to biological markers status. Estimates are expressed as HR of death of TAM-treated patients over controls only in each subgroup and are corrected for menopausal, nodal, and ER/PgR status. *, test for interaction.

Differently, HER2 status showed a marked influence on the efficacy of TAM. Indeed, the HR of death for HER2-positive patients is significantly different from that of HER2-negative subjects (HR: 1.09 versus 0.59; test for interaction: P = 0.04). These figures imply that adjuvant TAM is effective in reducing the hazard of death only among HER2-negative patients. Such effect is clearly seen in Fig. 5, reporting Kaplan-Meier curves showing OS for treatment arms in relation to HER2 status: TAM-treated patients have a better survival versus controls only in case of HER2-tumors, whereas a nonsignificant trend to the opposite situation is evident for HER2-positive subjects.

A more detailed analysis of the predictive effect of HER2 is reported in Fig. 6, where it was taken into account the effect of two potential confounders, respectively, the concomitant administration of chemotherapy and ER status. As shown, the efficacy of TAM appears to be unrelated to HER2 status in patients who also received CMF chemotherapy. Conversely, there was a strong interaction in the chemotherapy-free subgroup of patients, with an apparent detrimental effect of TAM in case of HER2-tumors, whereas a nonsignificant trend to the opposite situation is evident for HER2-positive subjects.

DISCUSSION

In this article, we report the long-term results of the GUN randomized trial of adjuvant TAM for breast cancer. As expected, we confirm the positive effect of TAM on OS after a prolonged follow-up. We also performed interaction tests in a multivariate model to evaluate whether TAM efficacy could be affected by some biological features of the primary tumor. Neither angiogenesis, nor ploidy, nor S-phase, nor EGFR, nor
PrlR affected in any way the efficacy of adjuvant TAM. Therefore, with the limited power of our study, the results presented here do not lend support to the preliminary data upon which the predictive role of such factors was hypothesized. This is hardly surprising, however, given that experimental data in support of these hypotheses are scant, whereas clinical studies are scarce and unconvincing because of conflicting results and of flawed experimental design (5, 7–10).

The most impressive result of our study is the finding that TAM was not beneficial, if not detrimental, for patients whose tumors overexpressed HER2. This result confirms in the whole study sample our earlier finding in the node-negative subset of patients at a shorter follow-up time (13).

Interestingly, in our analysis, HER2 did not have a predictive effect in patients who received concurrent CMF-based chemotherapy. Although this may be attributable to a chance effect arising from an additional subset analysis, recent data from the Milan randomized trial (17) suggest that the efficacy of adjuvant CMF could be improved by HER2 overexpression. This result is in agreement with a previous report from Berns et al. (18), showing an increased response rate to CMF for advanced breast cancers overexpressing HER2. Given this effect, the positive HER2/CMF interaction may have, in our study, counterbalanced the effect of the negative HER2/TAM interaction in patients receiving chemoendocrine adjuvant therapy. A similar effect may occur with anthracyclin-based chemotherapy given that an improved efficacy of these drugs in HER2-positive patients has been described in a randomized trial (19). Incidentally, this argues against the simplistic interpretation of data on HER2/TAM interaction regarding series of patients who received chemoendocrine adjuvant treatment. In fact, the underlying HER2/chemointeraction may act as a confounding variable, and any analysis of the HER2/TAM interaction in such series of patients may be strongly biased and, thus, of scarce significance.

The predictive power of HER2 has been extensively studied in the literature (for a review of the issue see Ref. 6). Complex cross-talks and interactions between the HER2/tyrosine kinase pathway and the ER pathway have been reported (20–22). It has been demonstrated that transfection of HER2 into MCF-7 cells results in overexpression of p185 and promotes estrogen-independent growth of this breast cancer cell line (22). Moreover, HER2-transfected MCF-7 cells are insensitive to the addition of TAM to culture medium (23). A similar effect has also been observed in animal models (21). Lately, Kurokawa et al. (24) have demonstrated that the inhibitory effect of TAM on HER2-overexpressing MCF-7 cell proliferation is restored by the inhibition of HER2 and mitogen-activated protein kinase, both in vitro and in athymic mice xenografts. From a clinical standpoint, however, available data have reported contrasting results. In the metastatic setting, HER2 overexpression has generally been associated to lower response rates and worse prognosis for patients treated with hormonal agents (18, 25–33) and only few negative studies have been reported (34–36). Two of these negative reports, however, deserve comment. In a series of 92 patients, Archer et al. (34) reported a nonsignificant (P = 0.24) correlation between HER2 expression and the response rate to first-line hormonal therapy. Still, the response rate was much lower for HER2-positive than for HER2-negative patients (29 versus 43%); thus, the lack of statistical significance could be attributable to the low-power of the study (power = 0.33 for the detection of a 50% reduction of the response rate in HER2-positive patients at α = 0.05). The second negative report is a well-powered study by Elledge et al. (35), but the results are equivocal. In this study, the HER2 expression of 204 metastatic breast cancers was independently evaluated by two pathologists, and although the authors conclude for the absence of an interaction with TAM treatment, the data from one of the pathologists could indicate the opposite. This internal divergence undermines their conclusion about the lack of a predictive role of HER2 and raises the issue of the reproducibility of their HER2 scoring system.

Also in the adjuvant setting, contradictory results have been reported. Three nonrandomized studies have been pub-
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Among these reports, the role of HER2 overexpression in patients with endocrine therapy is given, which indirectly suggests a negative interaction between HER2 expression and TAM effect. However, randomized evidence is more conflicting.

In a randomized trial designed to compare two different duration of TAM treatment (2 versus 5 years), an advantage for the longer treatment was seen only among HER2-negative patients, although absent for HER2-positive patients (40). This suggests that the latter subgroup of patients may lack of sensitivity to the drug.

Recently, the Cancer and Leukemia Group B group (41) reanalyzed the data of a randomized trial, which had been designed to compare three different doses of an anthracycline-based regimen, and found no evidence of a TAM/HER2 interaction. However, the setting was not appropriate to study an interaction between HER2 and TAM. First, the results may be biased because of the nonrandom assignment of TAM. Second, and more important, the administration of anthracycline to all patients is a major confounding feature, which could have masked a potential interaction between HER2 and TAM. In fact, a trend toward a greater efficacy of anthracyclines for HER2-positive tumors has been previously reported for the same trial (19) and additionally demonstrated by many other reports (42). This interaction seems to act in the opposite direction as compared with the HER2/TAM interaction, and thus it may have offset this latter in the Cancer and Leukemia Group B trial. The authors tried to avoid this confounding effect by calculating subgroup results (univariate only) for each dose level of anthracycline. This procedure limits but does not eliminate the noise in the analysis, while it greatly reduces statistical power.

Lately, in a trial in which TAM was randomly compared with no treatment, there was no evidence of a TAM/HER2 interaction (43). However, it should be noted that in this trial, TAM was given for only 1 year, which is a barely effective treatment (1). Moreover, in the HER2-positive subgroup of patients, there was a clear unbalance of treatment assignment with a lower proportion of TAM-treated patients as compared with control patients (P < 0.001). Both these issues may have diluted any interaction effect. In addition, as stated by the authors themselves, the low statistical power of the study with regard to TAM/HER2 interaction does not allow to confirm or disconfirm such an interaction.

There are some feature of our trial that make it an appropriate setting to investigate for a TAM/HER2 interaction: (a) it is a monoinstitutional randomized clinical trial with a simple two-arm design (TAM versus no-TAM); (b) follow-up data are remarkably mature and have been independently verified in all editions of the EBCTCG overview; (c) a large amount of information on the biological characteristics of the primary tumors is available, and the biological marker assays were performed at single laboratory with well standardized methods; and (d) the use of an appropriate statistical analysis allowed a direct test for the TAM-covariate interactions.

Our study, yet, shares with other published reports a main limitation: the GUN trial was not prospectively designed to look for TAM’s effect modifiers; therefore, a multiple-testing bias may have occurred.

The potential therapeutic impact of our findings, should they be confirmed, is remarkable. For instance, classical hormonal therapeutic agents that directly or indirectly act through the ER pathway would no longer be appropriate for patients with HER2-overexpressing tumors or should be administered only in combination with hypothetical drugs targeting the HER2 pathway. This kind of strategy is already undergoing clinical evaluation and might be a valid therapeutic option in HER2-overexpressing cases (44). Alternatively, the development of hormonal agents whose activity is not impaired by HER2 expression may be beneficial. In this respect, based on a small randomized trial of neoadjuvant therapy (45), it has been suggested that letrozole, unlike TAM, may overcome resistance of HER2-positive tumors. However, this finding has not been confirmed in a large randomized trial of first line chemotherapy for metastatic breast cancer (31). Another potentially useful drug in this context is the pure antioestrogen ICI 182,780 whose antiproliferative action is not affected by HER2 expression (46).

In conclusion, our randomized study does not support the hypothesis of interaction between TAM treatment and S phase, ploidy, EGFR, PrIR, and MVC, which were proposed as predictive factors based on the results of a nonrandomized study (5, 7–10). Conversely, it supports the hypothesis that HER2-overexpressing tumors may be less responsive to adjuvant TAM. This coincides with various reports in the literature, nonetheless there are some discordant results that prevents us to reach any reasonable conclusion about this topic (6). Therefore, additional research must be done before to definitely clarify the role of HER2 evaluation and might be a valid therapeutic option in HER2-overexpressing cases (44).

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