CA125 Response Is Associated with Estrogen Receptor Expression in a Phase II Trial of Letrozole in Ovarian Cancer: Identification of an Endocrine-sensitive Subgroup

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ABSTRACT

Purpose: This study was an open-label Phase II trial of the aromatase inhibitor letrozole (Femara) in patients with relapsed ovarian cancer with evaluation of possible biological markers for response.

Experimental Design: 60 patients were treated with letrozole (2.5 mg daily) at the time of CA125 relapse. Disease response was assessed by Union International Contre Cancer (UICC) criteria and by CA125 measurement. Estrogen receptor (ER), progesterone receptor, epidermal growth factor receptor, erbB2, and HSP27 were measured by immunohistochemistry in paraffin-fixed material obtained from the primary tumors at initial surgery.

Results: 50 patients were evaluable by UICC criteria, and although no complete or partial responses were obtained, 10 patients had stable disease on scan for at least 12 weeks. CA125 responses were evaluable in 54 patients. A partial marker response (>50% decrease) was seen in 5, and the marker remained stable in an additional 14 patients (25% increase). Tumors from the UICC stable disease group had significantly higher ER (P = 0.027) and progesterone receptor (P = 0.0066) values than the progressive disease group, and a combination of these was strongly associated with stable disease (P < 0.0001). Using CA125 criteria, comparison of the CA125 stable/responding disease with progressive disease indicated that tumors with higher ER (P = 0.013), lower erbB2 (P = 0.026), and higher epidermal growth factor receptor (P = 0.009) were associated with CA125 stable/responsive disease.

Conclusions: These results imply that letrozole treatment can produce disease stabilization and CA125 responses that in turn are linked to higher levels of ER expression.

These data suggest the presence of an endocrine-sensitive group that could be targeted in future studies.

INTRODUCTION

Ovarian cancer is the fourth most common cause of cancer death in women. Most patients present at an advanced stage, and the disease commonly relapses after primary surgery and chemotherapy. Relapsed ovarian cancer is not curable in the majority of women, and responses to salvage chemotherapy are often sustained for <1 year at the expense of toxicities that may diminish quality of life.

Endocrine therapies have provided effective palliation with relatively little toxicity in other hormone-sensitive cancers. The response to endocrine therapy in breast cancer is determined by the expression levels and interplay of steroid hormone and growth factor receptors, which can be measured in the primary tumor (1, 2). In experimental models of ovarian cancer, we have demonstrated that moderate-high expression of ER is associated with a growth response to estrogen, and these models are growth-inhibited by antiestrogen strategies both in vitro and in vivo (3–5). In addition, a number of proteins are estrogen regulated, and these include the PR, erbB2, and HSP27 (6–9). Clinical studies of tamoxifen in chemoresistant ovarian cancer have suggested that a subset of unselected patients respond to tamoxifen treatment, but the characteristics of responding tumors have not been defined. In the largest published study, 105 patients in first relapse received tamoxifen, and an overall response rate of 17% was reported (10). This was later reanalyzed to give a 13% response rate in cisplatin-resistant disease and 15% in cisplatin-sensitive disease (11). Another trial of tamoxifen reported a 17% response rate (5 of 29 evaluable patients) in chemoresistant disease (12). ER has been suggested to be linked with clinical response, but to date no significant association has been demonstrated (10).

Aromatase is the catalyst for the final rate-limiting step in estrogen biosynthesis. Aromatase inhibitors such as letrozole produce reversible, nonsteroidal inhibition of peripheral and intratumoral aromatase and suppress circulating estradiol levels by >95% in postmenopausal women (13). This class of agents is now established as second-line treatment of postmenopausal hormone-dependent breast cancer and may displace tamoxifen as first-line treatment (14, 15).

In this study, we have sought to evaluate the antitumor activity of letrozole in the setting of relapsed ovarian cancer.

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2 The abbreviations used are: PR, progesterone receptor; ER, estrogen receptor; EGF, epidermal growth factor; HSP, heat shock protein; UICC, Union International Contre Cancer; ULN, upper limit of normal; CT, computed tomographic.
using UICC and CA125 marker criteria. A secondary objective was to relate endocrine sensitivity to steroid hormone and growth factor receptor status that in experimental models was associated with estrogen regulation of growth.

PATIENTS AND METHODS

Trial Population and Patient Eligibility. This was an open-label, nonrandomized Phase II study in patients with previously treated relapsed ovarian cancer. Letrozole (supplied by Novartis) was administered p.o. at a dose of 2.5 mg/day until disease progression occurred. The study was open to patients with relapsed ovarian cancer of any ER level, and the ER status was not known at the time of recruitment. Clinicians who performed response assessments remained unaware of the results of biomarker measurements until the patients had completed treatment. Patients were eligible for the study if all of the following criteria were met: histologically proven ovarian cancer, progressive disease following the most recent treatment, at least one prior systemic chemotherapy regime, postmenopausal with relapsed ovarian cancer of any ER level, and the ER status was not known at the time of recruitment.

Clinical Evaluation. Clinical and pelvic examinations, toxicity assessments, and measurement of CA125 were performed every 4 weeks during letrozole treatment. CT scans of abdomen and pelvis were performed in all patients before study entry and then every 12 weeks. Letrozole treatment was continued until there was evidence of progressive disease, either clinically or on CT scan. Tumor measurements on CT scans were assessed for response using standard UICC criteria, and Rustin’s criteria were used to classify CA125 responses (16, 17). In those patients with no evaluable disease on CT scan, treatment was withdrawn when the CA125 change met Rustin’s criteria for progression (16, 17). Duration of response or stable disease was measured from the first day of treatment until the date of clinical, CT, or marker progression, whichever was earliest. For the purpose of this study, stable disease was defined as the absence of clinical, CT, or marker evidence of progression over at least 12 weeks in patients with evaluable disease at study entry. An additional definition, “failure-free at 12 weeks,” was introduced to incorporate the results of those patients with marker relapse who had no evaluable disease on CT scan at study entry and could not, therefore, be classified as having stable disease by UICC criteria.

RESULTS

Antitumor Efficacy. Sixty patients entered the study, and the median follow-up was 24 months (range, 11–35 months). Two patients remained on therapy. Patient and tumor characteristics are described in Table 1. Fifty patients were evaluable for response by UICC criteria. Nine patients had no evidence of disease by scan, and 1 had an unsatisfactory baseline scan. No complete or partial responses were seen in the 50 patients with evaluable disease on scan. Ten patients (17%) had stable disease on scan for at least 12 weeks, and the median time to progression in this group was 35 weeks (range, 22–87 weeks). The disease progressed in 59 patients, and the median time to progression in all patients was 12 weeks. The median time to failure was 49 weeks (range, 4–123 weeks) in the 9 patients who entered the study with no evaluable disease. CA125 responses were evaluable in 54 patients, including eight of those with no clinical evidence of disease, but response could not be assessed in 6 because there were insufficient
CA125 samples to meet Rustin's criteria (Table 2). A partial marker response was seen in 5 of 60 patients (8%) whose CA125 measurements fell to 6, 9, 9, 29, and 32% of the pretreatment value (Fig. 1). The marker fell by <50% and then remained stable in another 14 patients. The overall rate of marker response plus stabilization was 19 of 60 (32%), and this figure included 4 patients with no clinical evidence of disease at study entry. In the 19 patients whose marker fell, CA125 reached its lowest level after 10 weeks (range, 4–28 weeks) of letrozole and progressed (a confirmed 25% increase over the lowest value) after 21 weeks (range, 8–63+ weeks). When objective and marker responses were evaluated together, 13 of 60 (22%) patients were free of progressive disease by both criteria for at least 12 weeks and 7 of 60 (12%) for 24 weeks. Forty-five patients have died. The median survival was 14 months (range, 1–35 months) with 10 patients (17%) alive at 2 years.

Toxicity. Toxicities were assessed for 328 cycles in 54 patients. Letrozole was generally well tolerated, but 2 patients withdrew from treatment for nausea (1 patient) and rash (1 patient). Toxicities reported by other patients which may have been related to letrozole were mild to moderate flushing, nausea, weight gain, dyspepsia, skin rash, and fatigue.

Biomarkers. The expression of ER, PR, EGF receptor, erbB2, and HSP27 were assessed in primary tumor blocks that had been obtained at initial surgery; these were available for 59 of 60 patients. ER and PR expression was measured by a scoring system consisting of the product of the percentage of positive cells and intensity of staining (0–3), producing a histoscore ranging from 0 to 300. These slides were also scored using a category score (resulting in scores ranging from 0 to 8). Both assessments produced similar results, and the histoscore values were used in further analyses. A similar but less complex scoring system was used for the other markers ranging from 0 to 12, and this measure combined the product of intensity with quartiles demonstrating a particular level of expression. The distribution of ER and PR scores and their relationship to clinical and marker response are illustrated in Fig. 2. The group that had UICC stable disease had significantly higher ER (P = 0.027) and PR (P = 0.0066) values than the progressive disease group, and the combination of ER score ≥150 with PR ≥70 was associated strongly with clinical stable disease; 9 of 14 patients with these scores had stable disease in contrast to 1 of 35 patients with lower scores (P < 0.0001; Fisher's exact test). Using CA125 criteria, comparison of the marker stable or responding group with the progressive disease group indicated that higher ER (P = 0.013), lower erbB2 (P = 0.026), and higher EGF receptor (P = 0.009) were associated with a CA125 stable or responsive disease (Fig. 3). A highly significant trend was also observed between the probability of a CA125 stabilization or response and the ER expression level (P = 0.0087; χ² for trend; Fig. 4). Analysis of the percentage of likelihood of a CA125 response or stabilization versus CA125 progression indicated that the former group was more likely to have ER ≥150 with PR ≥70 than those with progressive disease (P = 0.0046, Fisher’s exact test). In this high ER/high PR group, 4 of 16 patients (25%) had a CA125 response. No association was found between HSP27 expression and response.

DISCUSSION

This was an open-label Phase II study designed to test the association between putative biochemical markers of endocrine sensitivity and clinical response to letrozole therapy in ovarian cancer. The activity of letrozole was lower than expected, but a number of patients had stable disease on treatment. In breast cancer trials, stabilization of disease for at least 24 weeks is considered a valid end point for endocrine therapy, and ~49% of ER-positive breast cancer patients treated with letrozole have either an objective response or stable disease (15). Seven of 60 (12%) patients in this study had stable disease for 24 weeks, but it seemed reasonable to accept a shorter definition of stable disease, 12 weeks or more, for the purpose of the study, given the relative indolence of ER-positive breast cancer compared with unselected relapsed ovarian cancers. Ten of 50 (20%) evaluable patients had stable disease by this definition. Although these cases may simply reflect relatively slowly growing tumors rather than any effect of letrozole, this seemed unlikely because the median time to progression in the UICC stable group was more than 35 weeks. Furthermore, the CA125 results support the interpretation that letrozole delayed tumor progres-
All of the 19 patients classified as having a CA125 response or stable marker had had a rising CA125 before study entry, and in each case, the marker decreased to below the pretreatment value for at least 12 weeks. Once on letrozole treatment, a strict definition of marker progression was applied, and the date of progression was the first sample that was 25% higher than the previous lowest level.

Analysis of biomarkers indicated statistically significant associations between high ER expression and both disease stabilization as measured by CT scan and CA125 response/stabilization. Furthermore, analysis of the CA125 end point indicated a highly significant trend between increasing expression of ER and likelihood of CA125 stabilization or response. These results are consistent with the view that the higher the level of ER expression, the more likely that a tumor is to be growth regulated by estrogen. In experimental models of ovarian cancer, we have observed that ovarian cancer cells with a moderate to high expression of ER demonstrate a growth response to estrogen and antiestrogens (3–5). For breast cancer, the likelihood of response to tamoxifen increases in parallel with increasing ER expression (2, 20). Further support for estrogen regulation was obtained from observations of the PR.

We have demonstrated previously that the PR is under estrogen control in ER-positive ovarian cancer models, and
inhibition in ovarian cancer, but the precedent for endocrine disease stabilization or CA125 responses on letrozole treatment. Cells by 17\(^{-}\)estradiol (9), no associations were observed with /H9252/lated (albeit to a small degree) in ER-positive ovarian cancer positive breast disease (26). Although the HSP27 was modu-
more effective than tamoxifen in ER-positive/EGF receptor-breast cancer, and in a recent report, letrozole was significantly increased expression has been associated, similar to erbB2, because estrogen tends to down-regulate EGF receptor (8), and increased signaling of the Ras/ERK pathway as a result of erbB2 overexpression may result in estrogen-independent activation of the ER (24). It is feasible that disruption of this pathway, by herceptin for example, might allow antiestrogen strategies to have greater effect, and we are exploring this in experimental models.

Several other markers were also evaluated. High erbB-2 expression was associated with CA125 progression on letrozole treatment, and this observation is consistent with numerous studies of tamoxifen in breast cancer that indicate an association between high erbB2 and tamoxifen resistance (22, 23). Increased signaling of the Ras/ERK pathway as a result of erbB2 overexpression may result in estrogen-independent activation of the ER (24). It is feasible that disruption of this pathway, by herceptin for example, might allow antiestrogen strategies to have greater effect, and we are exploring this in experimental models.

High levels of EGF receptor expression were associated with CA125 stabilization or response. This was unexpected because estrogen tends to down-regulate EGF receptor (8), and increased expression has been associated, similar to erbB2, with tamoxifen resistance in breast cancer (25). However, differences are beginning to emerge between letrozole and tamoxifen in breast cancer, and in a recent report, letrozole was significantly more effective than tamoxifen in ER-positive/EGF receptor-positive breast disease (26). Although the HSP27 was modulated (albeit to a small degree) in ER-positive ovarian cancer cells by 17\(^{-}\)estradiol (9), no associations were observed with disease stabilization or CA125 responses on letrozole treatment.

There have been no other trials of selective aromatase inhibition in ovarian cancer, but the precedent for endocrine therapy was set by a number of small trials of tamoxifen therapy giving an average response rate of 9% (range, 0–23%) and some durable periods of stable disease (12). Progestogens were less active, and no responses were seen in a small study of aminoglutethimide (12). No clinical responses to letrozole were observed in this study, although the marker response rate, thought by some to be a surrogate for clinical response, was 8%. Possible explanations for this low level of efficacy include inadequate dosing, poor patient selection, or a preponderance of tumors with few steroid hormone receptors. The low response to letrozole was unlikely to be attributable to inadequate dose because the 2.5-mg dose of letrozole used in this study has proven efficacy in breast cancer and has been shown to suppress estradiol levels by 95% within the first 2 weeks of treatment in postmenopausal women (27). Studies in breast cancer have shown that certain patient and tumor characteristics are more likely to be associated with a response to endocrine therapy, including long disease-free interval, absence of visceral sites, and lower tumor burden. In ovarian cancer, there is an inverse relationship between the size of metastatic deposits and the likelihood of response to chemotherapy. Thirty % of our patients had visceral involvement or metastatic deposits >5 cm and were perhaps unlikely to respond to endocrine therapy (Table 1). Fifty % of patients were <2 years from diagnosis, although there were 7 patients who had survived for >5 years. In the stable disease group, no one had received more than two lines of chemotherapy, and there were no responses in patients who had visceral disease. The endometrioid tumors were relatively over-represented because 4 of 11 patients with endometrioid histology had stable disease, compared with 4 of 43 serous tumors. There was no clear relationship between time from diagnosis and stable disease, because the median time from diagnosis was the same (23 months) in this subgroup compared with the whole group. Because this was a nonrandomized study, it could be that some of those tumors with the highest ER and PR levels may have an intrinsically more favorable course to their disease because of biological differences in the tumor, rather than any stabilizing effect attributable to letrozole. Therefore, the identification of a potentially endocrine-sensitive subgroup forms the basis of a future randomized study of endocrine therapy versus...
placebo, where approximately 1 of 3 patients screened would be eligible for entry on the basis of ER and PR criteria. In this group of patients, 25% demonstrated a CA125 response.

It is evident from this and from other studies that only a small minority of unselected patients with advanced ovarian cancer will benefit from endocrine therapies. Those that do, however, may have sustained responses or prolonged periods of stable disease. The potential for intervention with endocrine therapies when the tumor burden is low after primary chemotherapy has not yet been explored. The ability to characterize those patients likely to respond will be invaluable to these approaches. The results of this study suggest that ER and PR expression measured by immunohistochemistry in primary tumor blocks is sufficient to define the group of patients with the highest probability of benefit from endocrine therapy. These are the patients who should be selected for future trials of antineoplastic therapy.

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REFERENCES


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