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Report

Use of the peroxisome proliferator-activated receptor (PPAR) γ ligand troglitazone as treatment for refractory breast cancer: a phase II study

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Summary

Purpose. To evaluate the therapeutic effects of the peroxisome proliferator-activated receptor (PPAR) γ activating ligand, troglitazone, in patients with refractory metastatic breast cancer.

Experimental design. Patients with advanced breast cancer refractory to at least one chemotherapy regimen (ER negative tumors) or two hormonal regimens (ER positive tumors) were treated with troglitazone at 800 mg PO QD until disease progression, to determine the percentage of patients free of progression at 6 months. Tumor response, toxicity, and changes in serum tumor markers (CEA, CA27.29) that might reflect alteration in tumor differentiation, were also examined.

Results. Twenty-two patients were enrolled before suspension of protocol accrual and treatment when troglitazone was withdrawn from commercial availability following FDA warnings on hepatic toxicity. No objective responses (CR or PR) were observed; only three patients had SD at 8 weeks. Patients came off study for PD (16), DLT (1), FDA withdrawal (2), or other (3) reasons. No patients took troglitazone for more than 20 weeks; all had experienced disease progression or began other systemic therapy within 6 months. All patients with elevated serum tumor markers (CEA and CA27.29) at baseline had rising tumor markers within 8 weeks.

Conclusions. While clinical trials among different patient populations might uncover subtle effects on tumor differentiation, PPARγ activation by troglitazone has little apparent clinical value among patients with treatment-refractory metastatic breast cancer.

Introduction

The peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that regulate transcription, and are members of the nuclear receptor superfamily that includes steroid, retinoid, and thyroid hormone receptors [1, 2]. PPARs have been implicated as important regulators in many physiological functions, including lipid metabolism, atherogenesis, inflammation, and cell differentiation. Three isoforms have been characterized – PPARα, PPARδ, and PPARγ – each of which can form heterodimers with the retinoid X receptors (RXRs). Natural low affinity ligands of PPARs include fatty acids and eicosanoids.

Thiazolidinediones, a commercially available class of antidiabetic drugs [3], are highly selective activating ligands for PPARγ [4]. Laboratory studies have shown that thiazolidinediones stimulate PPARγ in the same concentration range as required for antidiabetic activity [5], and that thiazolidinedione activation initiates a variety of cellular and molecular changes associated with adipocyte differentiation [6]. Troglitazone is one example of a thiazolidinedione drug, and has been shown in clinical trials to induce solid tumor differentiation of liposarcomas both *in vitro* as well as in patients with advanced disease [7, 8]. Treatment with troglitazone led to accumulation of lipid within tumor cells as measured on histology

and NMR spectroscopy, and molecular changes consistent with adipocyte differentiation and consequent decrease in cell proliferation, including decreases in Ki-67 expression, and increased expression of adipsin and aP2. Thiazolidinediones have also been shown to alter *in vitro* growth patterns in models of colorectal cancer [9] and prostate cancer [10, 11].

A variety of laboratory evidence suggests that PPARγ activation may alter the growth characteristics of breast cancer cells. PPARγ is expressed by cell lines derived from primary and metastatic breast tumors. Activators of PPARγ, including troglitazone, cause changes in breast tumor cell growth, including lipid accumulation, suppression of muc-1 (CA27.29) gene expression, and decreased rates of thymidine incorporation [12]. These findings all suggest that activation of PPARγ induces differentiation. Troglitazone can inhibit the growth of MCF-7 breast carcinoma lines by blocking the G1 → S cell cycle transition [13], and can induce apoptosis in the same cell line [14]. *In vivo*, troglitazone has been shown to prevent the preneoplastic mammary lesions arising in DMBA-treated murine mammary glands [15]. Other PPARγ ligands have been shown to inhibit development of breast tumors in a nitrosomethylurea model of carcinogenesis in rats [16]. Thus, a variety of preclinical data suggested that troglitazone might induce cell differentiation and inhibit cell growth among patients with advanced breast cancer. Because troglitazone was a commercially available drug with a well-described and well-tolerated side effect profile, and based on the strength of these preclinical laboratory findings, we initiated a phase II trial of troglitazone as therapy for advanced, refractory breast cancer.

Methods

Patient eligibility: clinical characteristics

Patients with pathological documentation of invasive breast cancer and treatment-refractory (see below) locally advanced (T4; stage IIIB) or metastatic (stage IV) breast cancer with either measurable or evaluable disease (WHO criteria) were eligible. Patients were required to be >18 years of age, with ECOG performance status 0–2, and life expectancy >3 months. Patients requiring diabetes therapy with either insulin or oral antihyperglycemic agents including sulfonylureas, metformin, or thiazolidinediones were ineligible. Because of drug–drug interactions, patients could not use oral contraceptives or cholestyramine while on study. Patients who were pregnant, nursing, or expecting to become pregnant or nurse were ineligible, as were patients with comorbid illnesses that were treatment refractory or that might interfere with the ability to provide informed consent or comply with protocol treatment. Patients were required to have the following laboratory evaluations within 21 days prior to initiation of therapy to be eligible: absolute neutrophil count >1000/mm^3, platelet count >75,000/mm^3, bilirubin <2 mg/dl, SGOT/SGPT <2 × upper limit of normal, glucose <200 mg/dl, creatinine <2 mg/dl.

Patient eligibility: prior therapy

Eligibility was determined by patient stage, tumor hormone receptor status, and by prior therapy. All patients were required to have treatment-refractory breast cancer, as follows:

- *Stage IV, ER and/or PR positive tumors.* Tumor progression/relapse despite at least two prior endocrine therapies in either the adjuvant or metastatic setting.
- *Stage IV, ER and PR negative or unknown tumors.* Tumor progression/relapse despite at least one prior chemotherapy regimen or progression during adjuvant chemotherapy.
- *Stage IIIB tumors.* Tumor progression/relapse despite prior chemotherapy, with tumor burden not treatable with curative intent.

Patients were required to have completed any hormone therapy prior, radiation therapy at least 1 week prior, and chemotherapy or biological therapy (e.g., trastuzumab) at least 2 weeks prior to initiation of protocol treatment. Patients could not have received prior treatment with thiazolidinediones or with RXR ligands. Patients with asymptomatic central nervous system metastases who had received prior cranial radiation and/or surgical resection more than 3 months before enrollment were eligible; patients with other histories of CNS metastases were not.

Treatment plan and dose modification

Patients received troglitazone 800 mg PO in a single daily dose taken with food. This dose was higher than the standard diabetes indication dose of 600 mg QD, but was within the range of doses studied for troglitazone [17] and used in previous oncology trials [7, 8]. Further, this dose could achieve steady-state serum concentrations in excess of 2.82 μg/ml, levels known

to be associated with *in vitro* biochemical effects on tumor cell lines.

Patients were restaged every 8 weeks. Patients with either stable disease, or complete or partial response (WHO criteria) continued on study. Patients with progressive disease were taken off study. Patients were taken off protocol for any grade 3 or greater (NCI common toxicity criteria, version 2.0) toxicity that developed during treatment. Patients developing grade 2 toxicity had treatment held until resolution to grade 0–1, and were then retreated with at 50% dose reduction (i.e., 400 mg PO QD). Patients who redeveloped grade 2 toxicity at the reduced dose were taken off study.

In addition to restaging every 8 weeks, patients were monitored with monthly physical exam, complete blood count, glucose levels, and liver function testing, and with monthly determination of serum tumor markers (CEA and CA27.29 (muc-1)). Tumor markers were followed both as indirect measures of tumor burden and as possible surrogate indicators of tumor differentiation, based on laboratory models [12].

Statistical analysis

The primary study endpoint was determination of the percentage of patients with stable or responding disease after 6 months of treatment with troglitazone. Patients on the study were required to have refractory disease. It was assumed that no more than 10% of the patients entering the study would be without evidence of progression in 6 months if they were untreated, corresponding to a median time to progression of approximately 2 months. The statistical plan noted that troglitazone would be considered useful if 25% of the patients had stable or responding disease 6 months after the start of treatment. The original study plan called for 40 patients to enter the trial. If at least seven of the patients had stable or responding disease at 6 months, the drug would be considered useful for further evaluation. With that study design, there was a 10% chance of deciding to pursue further evaluation if troglitazone had a true stabilization rate of 10%, and a 90% chance of deciding to do further research if troglitazone had a true stabilization rate of 25%.

Protocol accrual and treatment were halted in March 2000 after entry of 22 evaluable patients, when troglitazone was withdrawn from commercial distribution after the United States Food and Drug Administration (FDA) identified unacceptably high rates (1:8000–1:20,000) of acute liver failure associated with troglitazone therapy [18]. The results reflect the experience of those 22 patients.

Protection of human subjects

The study was open for accrual between August 1999 and March 2000 at Dana-Farber Cancer Institute, Brigham & Women's Hospital and Massachusetts General Hospital. The protocol and study were approved by the Scientific Review and Human Protection Committees of Dana-Farber/Partners Cancer Care, which govern clinical trials at these institutions. The study was conducted in accordance with guidelines established by the United States Department of Health and Human Services. All patients provided written informed consent prior to study participation.

Results

Twenty-two women enrolled onto the study before study closure in March 2000. Accrual and treatment were halted prematurely when troglitazone (Rezulin®; Warner-Lambert Company) was withdrawn from the market on March 21, 2000 by the manufacturer following FDA warnings over rare but life-threatening risk of hepatotoxicity. Two patients still receiving protocol-based treatment were taken off study at that time.

The patients had a median age of 53 years (range 36–75). Their sites of disease and number of sites of disease are shown in Table 1. Most patients had three or more sites of disease; 73% had visceral metastases. The patients had tumors highly refractory to treatment. Sixteen of 22 women had received two or more prior chemotherapy regimens, and nearly half had been treated with three or more chemotherapy regimens. Similarly, most had extensive prior hormonal therapy; 64% had received two or more prior hormonal regimens for breast cancer.

The toxicity experience of patients on study is shown in Table 2. Troglitazone was generally well tolerated. Mild upper gastrointestinal distress was the most common treatment related side effect. One patient came off study for nausea/vomiting related to troglitazone. Other side effects were usually caused by symptoms related to the underlying cancer. None of the 22 patients developed serious or life-threatening hepatotoxicity on study. Problems with glucose management were not encountered.

Table 1. Patient population ($n = 22$)

	Number of patients
Characteristic sites of disease	
Breast/chest wall	6
Liver	12
Lung	10
Lymph node	7
Bone	13
Soft tissue	8
Skin	3
Number of disease sites	
1	2
2	7
3	9
4	4
Prior chemotherapy regimens (adjuvant and/or metastatic)	
0	4
1	2
2	6
3	5
4	2
>4	3
Prior hormonal regimens (adjuvant and/or metastatic)	
0	6
1	3
2	7
3	3
4	3

Table 2. Incidence of toxicity ($n = 22$)

	Grade 1	Grade 2	Grade 3	Grade 4
Overall	7	7	3	1
Nausea	6	3	0	0
Anorexia	2	0	0	0
Vomiting	0	2	0	0
Diarrhea	2	0	0	0
GI-other (dyspepsia)	3	1	0	0
SGOT/SGPT	1	1	0	0
Alk phos	2	0	1	0
Change mental status	0	0	0	1
Neurosensory	0	1	0	0
Neuropathy-cranial	0	1	0	0
Constipation	1	0	0	0
Pain	5	2	0	0
Bone pain	2	1	1	0
Fatigue	2	1	0	0
Anxiety	0	2	0	0
Insomnia	0	1	0	0
Wound infection	0	0	1	0
Hemoglobin	2	0	0	0
Hypoglycemia	0	0	0	0
Hyperglycemia	1	0	0	0

The principal endpoint for the study was control of tumor progression (either disease stabilization or response) for 6 months. Sixteen of the total of 22 patients (73%) came off study for progressive disease. Three patients came off study at the discretion of their treating physicians, generally for symptoms or findings suggestive of worsening tumor burden without overt disease progression. One patient came off study with toxicity (nausea/vomiting) related to troglitazone therapy. Two patients were receiving study treatment with troglitazone when it was withdrawn from the market, and came off study at that time. One of those patients had been on study for 90 days, and went on to receive a standard cancer treatment. The other patient had received less than 2 weeks of therapy at the time of study closure. Median time on study was 56 days (range 11–134 days). Of 21 patients evaluable through 8 weeks of treatment, only three (14%) had stable disease. No patient was on study therapy for 6 months; in fact, none exceeded 20 weeks on study. Figure 1 shows the time on study, with 95% confidence intervals. No objective tumor responses (either complete or partial) were observed.

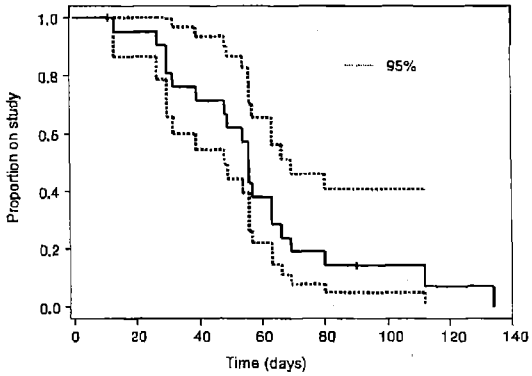
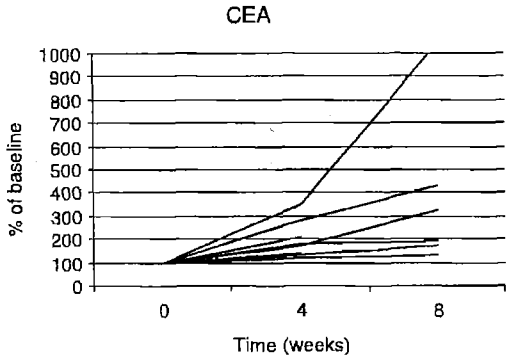
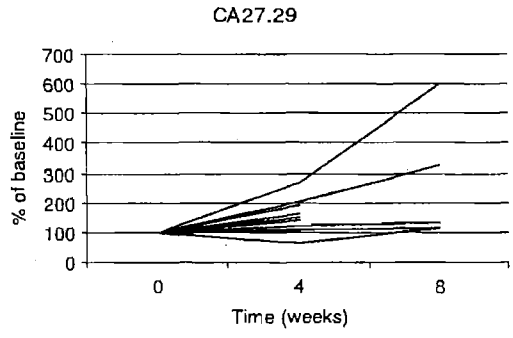


Figure 1. Kaplan–Meier analysis of time on study. Patients on study at the time of protocol closure are censored.

Because preclinical data suggested that PPARγ activation might induce changes in expression of tumor antigens – in particular, decreasing expression of muc-1 (CA27.29) – patients had serum drawn for CEA and CA27.29 determinations at baseline and thereafter on a monthly schedule. Twelve patients had elevated CEA levels (normal 0–3 ng/ml) at some time on study, and had CEA measured at baseline and at least one other timepoint. Fourteen patients had elevated CA27.29 levels (normal 0–38 U/ml) at some time on study, and had CA27.29 measured at baseline and at least one other timepoint. Serial measurement of tumor markers are shown in Figure 2 for CEA (panel A) and CA27.29 (panel B), expressed as a percentage of the baseline level of expression. All patients with elevated tumor markers at baseline and at least one other measured timepoint showed rising markers by 8 weeks of therapy. Serial tumor biopsies were not performed on this trial.



Panel A. Carcinoembryonic antigen (CEA) ($n = 12$)



Panel B. CA27.29 ($n = 14$)

Figure 2. Tumor marker expression following troglitazone therapy.

Discussion

Induction of tumor differentiation with consequent disease control through novel biological mediators is a seminal goal of current clinical research in cancer treatment. The PPARγ nuclear receptor represents a potential target of such therapeutics, because of its widespread expression in a variety of tumor types, and the availability of potent activating ligands. Substantial laboratory experience suggested that PPARγ activation *via* the thiazolidinedione class of antidiabetic drugs might alter growth patterns of breast tumor cells *in vitro* and *in vivo*. For that reason, we undertook a phase II trial of troglitazone, then a commercially available thiazolidinedione, in breast cancer. Among a heavily pretreated patient population with advanced cancer refractory to standard hormonal or chemotherapeutic agents, we did not see objective tumor responses, nor did we find patients with stabilization of disease for at least 6 months. Only 3 of 21 evaluable patients had stable disease at 8 weeks. All patients with elevated serum tumor markers who were followed over time were found to have rising markers within 8 weeks of treatment. We did not encounter unexpected side effects of therapy.

These findings do not suggest overt clinical benefit through administration of thiazolidinediones to patients with refractory breast cancer. Although the study was originally designed to accrue 40 patients; study accrual and treatment were stopped prematurely because troglitazone was withdrawn from the commercial market following reports of rare instances of liver toxicity. Because of the premature closing of the trial, the study cannot exclude the possibility that one quarter of the patients might have experienced disease stabilization for 6 months. However, we do not consider that possibility very likely. The majority of patients (20 of 22; 91%) had stopped study participation on account of worsening clinical status, most within the first 8 weeks. The actuarial curve does not suggest that a large fraction of patients would have continued on study beyond 120 days, much less 6 months. Further, there was no indirect evidence in the way of tumor response or tumor marker response to suggest anticancer activity. Finally, the findings in this trial were qualitatively similar to those recently reported for the use of a selective RXR agonist, bexarotene [19]. That trial, also for refractory breast cancer, demonstrated objective response rates of 3–6% for different strata of patients, and median time to progression of 8 weeks. Collectively, these experiences

suggest that tumor differentiation through PPARγ activation or RXR agonist stimulation is not likely to be of substantial clinical benefit in refractory breast cancer.

The clinical selection criteria may also have affected the apparent clinical value of thiazolidinediones for breast cancer. Treatment utility for all established therapies in breast cancer – hormonal therapy, chemotherapy and biological therapy – declines in more heavily treated patients. Thus, by assessing this agent among such heavily treated patients, some clinical activity may be missed. Similarly, the study was open to patients regardless of the hormone receptor status (ER, PR, HER2) of their tumor, and without knowledge of whether the tumor expressed PPARγ. Evidence suggests that PPARγ may inhibit HER2 and HER3 tyrosine phosphorylation [20], and decrease estrogen production by adipose tissue in the breast [21]. The percentage of breast tumors that express substantial levels of PPARγ is not known. Thus, more tailored patient selection criteria might better identify patients likely to be candidates for PPARγ-targeted therapy.

There is the possibility of combining a PPARγ ligand with another agent may improve the efficacy of such treatment approaches. Because PPARs form heterodimers with RXRs, pairing a thiazolidinedione with a retinoid analogue is one such possible combination. *In vitro* experience suggest that such strategies can heighten the effectiveness of thiazolidinediones at inhibiting preneoplastic mammary lesions in rodent models [14]. It remains to be seen whether combination treatment might offer improved anticancer activity of over monotherapy.

It is additionally possible that PPARγ activation would be more effective in prevention rather than as therapy for malignant tumors. For instance, a recent murine model of colorectal carcinogenesis suggest that the role of PPARγ is most critical before APC-related damage occurs [22]. By inference, PPARγ activation following further steps in tumorigenesis may be ineffective due to multiple other signaling pathway alterations.

In summary, PPARγ activation appears to have little clinical benefit in women with heavily pretreated, refractory breast cancer. Clinical trials among patients with less refractory disease or in combination with other agents may be warranted based on the intriguing preclinical data on this novel nuclear receptor target.

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