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# Trifluoperazine as a modulator of multidrug resistance in refractory breast cancer

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**Abstract** Overexpression of P-glycoprotein (P-gp) has been implicated as the mechanism of multidrug resistance (MDR) in a number of human cancers, including carcinoma of the breast. We conducted a clinical trial to determine whether the P-gp inhibitor, trifluoperazine, could sensitize patients with refractory breast cancer to vinblastine chemotherapy. Adult patients with histologically confirmed, refractory, advanced breast cancer were treated with vinblastine at a dose of 1.7 mg/m$^2$ per day by continuous infusion for five consecutive days. Patients who did not respond after two cycles were subsequently treated with vinblastine plus trifluoperazine at a dose of 8 mg twice daily during the five days of chemotherapy. In patients from whom tumor samples were available, the expression of P-gp was determined by immunocytochemistry. Of 35 patients enrolled, 30 were evaluable, 2 of whom (7%) achieved a partial response to vinblastine alone. Among the 16 patients treated with vinblastine plus trifluoperazine there was one response (6%) which lasted 16 weeks. Tumor samples were available from 16 patients, and 14 (87%) were immunoreactive for P-gp. P-gp expression was detected both in the patient who responded to vinblastine plus trifloperazine and in one of the two patients who responded to vinblastine alone. Continuous-infusion vinblastine demonstrated limited activity in this study. Furthermore, trifluoperazine did not effectively reverse established resistance to vinblastine. This failure may be related the presence of multiple mechanisms of drug resistance in this heavily pretreated population, or because ineffective concentrations of the modulator were achieved in vivo. Future studies should evaluate more effective modulators, and attempt to reverse MDR earlier in the course of treatment, before other forms of resistance can develop.

**Key words** Breast cancer · Multidrug resistance · Trifluoperazine · Vinblastine

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## Introduction

Multidrug resistance (MDR) occurs when cancer cells display cross-resistance to a broad spectrum of structurally unrelated chemotherapeutic drugs. The most common mechanism of MDR is the overexpression of P-glycoprotein (P-gp), a membrane-associated drug efflux pump. A broad range of bulky, hydrophobic compounds competitively inhibit P-gp and reverse MDR both in cell culture and in animal models [8]. For example, nontoxic doses of trifluoperazine can reduce in vitro resistance to vinblastine five- to tenfold and improve survival in mice bearing MDR tumors [9, 41].

The clinical evaluation of inhibitors of P-gp is being actively pursued [1–5, 19, 24, 25, 29, 35–37, 40, 43–46]. For example, Miller et al. treated 36 patients with resistant tumors with the combination of doxorubicin

and trifluoperazine [24]. Seven responses (19%) were observed. Notably, all the responders had tumors previously sensitive to doxorubicin alone. Among the 15 patients who had never responded to doxorubicin, there were no responses to the combination containing trifluoperazine. One possible explanation of this finding is that in selected tumors which are initially sensitive to chemotherapy, resistance develops due to the overexpression of P-gp, which can be overcome by adding competitive P-gp inhibitors to the treatment regimen.

Therefore, successful clinical modulation of drug resistance by P-gp inhibitors may require the selection of a patient population with tumors that are usually initially sensitive to chemotherapy but frequently develop resistance following treatment. One such population are patients with advanced breast cancer, a disease in which the majority of patients initially respond to chemotherapy but very few are cured. Accordingly, we conducted a study of continuous infusion vinblastine in patients with refractory breast cancer to determine if resistance could be overcome by the addition of the P-gp inhibitor trifluoperazine.

## Methods

Patients with histologically confirmed, measurable breast cancer resistant to anthracyclines were eligible for this study. Anthracycline resistance was defined as progression during treatment for advanced disease, or relapse within 12 months of adjuvant therapy with a chemotherapy regimen containing an anthracycline or an anthracene. Patients were required to be at least 18 years of age, have an ECOG performance status $\leq 2$, and adequate marrow, hepatic, and renal function (WBC $\geq 3500/\mu l$, platelets $\geq 100\,000/\mu l$, bilirubin $\leq 2.5$ mg/dl, albumin $> 3$ mg/dl and creatinine $< 2.5$ mg/dl). Patients with brain metastases were eligible provided the central nervous system disease was stable and they had other metastases which could be measured. Patients who had previously received vinblastine by continuous infusion, had a serious intercurrent medical or psychiatric illness, who were pregnant, or who required concurrent treatment with an antipsychotic drug were excluded.

Prior to beginning treatment, a complete history was obtained from each patient and each received a physical examination which included an assessment of mental status. Laboratory studies included a complete blood count with differential, serum chemistries (albumin, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase), a chest X-radiograph, CT scans of the chest and abdomen, and a bone scan. Patients with skin or subcutaneous metastases readily accessible to biopsy were asked to provide tumor tissue for analysis of MDR expression, but consent for this biopsy was not required for participation in the study.

A permanent central venous access device was placed before treatment. Therapy was generally delivered in the outpatient clinic by an infusion pump. During the first cycle of chemotherapy, patients received vinblastine alone at a dose of 1.7 mg/m$^2$per day by continuous infusion for five consecutive days. Chemotherapy was repeated every 21 days, provided the blood counts were adequate and there were no serious nonhematologic toxicities. If these conditions were not met, treatment was delayed for at least 1 week to allow for recovery.

The dose of vinblastine was modified on subsequent chemotherapy cycles according to nadir blood counts. In patients experiencing serious hematologic toxicity (WBC $< 1000/\mu l$, platelets $< 50\,000/\mu l$), the dose of vinblastine was reduced by 20%. Dose escalations by 20% were permitted in patients who had no serious hematologic toxicity during the previous chemotherapy cycle.

The response to vinblastine alone was evaluated after two cycles of therapy. For patients who were not responding to treatment, trifluoperazine was added at a dose of 8 mg twice daily for 5 days during subsequent cycles of vinblastine chemotherapy. If neurocortical or neurocerebellar side effects developed which were deemed by the patient to be intolerable, treatment was held until the toxicity resolved and the dose of trifluperazine on subsequent cycles was reduced by 25%.

Response was defined according to ECOG criteria and measured from the time of response to the time of disease progression [28]. Toxicity was defined according to NCI Common Toxicity criteria. The expression of P-gp was determined on representative paraffin-embedded tumor samples by immunocytochemistry performed according to the avidin-biotin-peroxidase method of Hsu et al. [17], with modifications to optimize the assay for the antibodies used [7, 12, 33, 38]. Four antibodies directed against different epitopes of P-gp were used, including MRK-16 (generous gift from Dr. T. Tsuruo, University of Tokyo), JSB-1 (generous gift from Dr. J. Scheper, Free University, Amsterdam), UIC-2 (generous gift of Dr. I. Roninson, University of Illinois), and MDR (Oncogene Science, Manhassett, N.Y.). Overexpression of P-gp was defined as membrane staining above background with at least two different antibodies. Paraffin-embedded sections of normal kidney were stained along with the patient samples. Renal tubules express P-gp and were used as a positive control while the glomeruli, which do not express P-gp, served as a negative control to assess background staining. No attempt was made to quantify the intensity of staining.

This protocol and its subsequent revisions were approved by the Yale University Human Investigation Committee. Informed written consent was obtained from all patients. Patients were enrolled and treated at the Yale-New Haven Hospital and its affiliated hospitals. All data were collected and tabulated by the Clinical Research Office of the Yale Comprehensive Cancer Center.

## Results

Of 35 patients enrolled into this study, 4 were ineligible, 1 because of a prior diagnosis of a second malignancy within the previous 3 years, 1 because she had not received prior anthracycline chemotherapy, and 2 because of either inadequate documentation of pretreatment albumin or an inappropriately low albumin.

The patient characteristics are shown in Table 1. All of the patients were women, the median age was 56 years, and the majority had an excellent performance status. The most common sites of metastatic disease were the soft tissue and bone, although 15 patients had liver involvement and 7 had brain metastases. All patients had received extensive prior treatment: 29 (94%) had received $\geq 6$ prior cycles of chemotherapy, 18 (58%) had received prior radiation therapy, and 16 (52%) had been treated with hormones. The median number of prior chemotherapy regimens was 2 (range 1–6), including two patients who had undergone an autologous bone marrow transplantation. Resistance to anthracycline-containing chemotherapy was documented in all of the patients, and 9 (29%) had received other naturally derived chemotherapy drugs.

**Table 1** Patient characteristics

| | *n* (%) |
|---|---|
| Eligible | 31 |
| Evaluable | 30 |
| Age (years) | 56 (29–72) |
| Performance status | |
| 0 | 16 (52) |
| 1 | 11 (35) |
| 2 | 4 (13) |
| Sites of disease | |
| Bone | 19 (61) |
| Lung | 10 (32) |
| Liver | 15 (48) |
| Soft tissue | 21 (68) |
| Brain | 7 (23) |
| Prior therapy | |
| Autotransplant | 2 (6) |
| Radiotherapy | 18 (58) |
| Hormonal | 16 (52) |

**Table 2** Responses to treatment to treatment with continuous infusion vinblastine alone, or vinblastine plus trifluoperazine. (*CI* confidence interval, *Eval pts* evaluable patients; *CR* complete response, *PR* partial response)

| | Vinblastine | | | Vinblastine plus trifluoperazine | | |
|---|---|---|---|---|---|---|
| | *n* | % | 95%CI | *n* | % | 95%CI |
| Eval pts | 30 | | | 16 | | |
| CR | 0 | 0 | 0 | 0 | 0 | 0 |
| PR | 2 | 7 | 0–16 | 1 | 6 | 0–18 |

One of the 31 eligible patients discontinued treatment during the first cycle of vinblastine because of the rapid development of disease-related hepatic insufficiency. Therefore, there were 30 patients who received at least one complete cycle of chemotherapy and were included in the analysis for response and toxicity (Tables 2 and 3). There were no significant differences in response rate if the three ineligible patients who were treated were included.

There were two patients (7%) who achieved a partial response (PR) to continuous infusion vinblastine alone (95% confidence interval 0–16%). The duration of these responses were 15 and 44 weeks. Neither of these patients had liver or CNS involvement, and neither was retreated with the combination of vinblastine plus trifluoperazine at the time of disease progression.

The vinblastine plus trifluoperazine combination was used to treat 16 patients (Table 2). The most common reasons for patients not crossing over to the combination were patient refusal or inadequate performance status secondary to disease progression. One patient among these 16 patients (6%), who had stable disease following two cycles of vinblastine alone, achieved a PR in lung and liver lesions lasting 4 months when treated with the combination (95% confidence interval 0–18%). A second patient had complete clearing of a $3 \times 2.5$-cm subcutaneous metastasis which lasted $> 4$ weeks, but this patient was lost to follow-up before a reevaluation of her liver disease was obtained to confirm systemic response. The median survival for all patients on the study was 6.5 months, with a 1- and 2-year survival of 23% and 3%, respectively.

**Table 3** Maximum toxicity experienced during any chemotherapy cycle

| Toxicity | ECOG grade | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| WBC | 2 | 2 | 5 | 10 | 11 |
| PMN | 4 | 2 | 1 | 4 | 19 |
| Platelet | 23 | 4 | 1 | 1 | 1 |
| Anemia | 5 | 7 | 11 | 7 | 0 |
| Nausea | 21 | 5 | 1 | 3 | 0 |
| Stomatitis | 22 | 4 | 2 | 1 | 1 |
| Diarrhea | 27 | 2 | 0 | 1 | 0 |
| Constipation | 23 | 2 | 1 | 4 | 0 |
| CNS | 19 | 2 | 7 | 2 | 0 |
| Flu-like symptoms | 17 | 6 | 5 | 2 | 0 |

## Toxicity

Hematologic side effects associated with continuous infusion vinblastine were significant in this heavily pretreated population (Table 3). Myelotoxicity consisted of serious neutropenia (23), anemia (7), and thrombocytopenia (2). One patient died of sepsis within 4 weeks of her fourth cycle of chemotherapy, but was not neutropenic when this complication developed. In the group of 16 patients who crossed over to vinblastine plus trifluoperazine, there were two hospitalizations for neutropenic fever when vinblastine was administered alone, and two hospitalizations when trifluoperazine was added to this chemotherapy. Furthermore, comparison of the first cycle of treatment following the addition of trifluoperazine to the previous cycle in which only vinblastine was given demonstrated no significant difference in nadir white cell, neutrophil, or platelet counts ($P \geq 0.2$, Student's *t*-test). Therefore, it did not appear that this modulator significantly increased the hematologic toxicity of vinblastine.

Other significant side effects included nausea, which limited oral intake and was associated with multiple episodes of vomiting in three patients, constipation (four patients), and muscle and joint pains (two patients). Severe stomatitis developed in two patients and one required enteral nutritional support. Both episodes of severe stomatitis occurred in patients receiving the combination of vinblastine and trifluoperazine. The side effects directly related to trifluoperazine were

primarily limited to CNS toxicity, consisting of agitation or anxiety (eight patients) and somnolence or depression in four patients.

### MDR1 gene expression

Tumor samples appropriate for analysis were obtained from 16 patients. Immunoreactivity for P-gp was detectable in 14 of these patients (87%). In one patient samples were available from both the breast primary and a lung metastasis. The primary tumor was immunoreactive, while no P-gp was detected in the metastasis. Tissue was available from one of the two responders to vinblastine alone, and from the patient who developed skin clearing after trifluoperazine was added to the chemotherapy regimen. In both of these samples P-gp was detectable.

## Discussion

The significance of P-gp as a mechanism of clinical drug resistance remains uncertain. Both retrospective and prospective studies, however, have found that in certain tumor histologies P-gp expression correlates with sensitivity to chemotherapy, response duration, and overall survival. Tumor histologies in which P-gp is an important prognostic feature include acute leukemias, cancers of the breast and ovary, pediatric tumors, and small-cell lung carcinoma [6, 16, 23, 27, 30, 42]. These tumors all tend to be sensitive to chemotherapy initially, but usually recur with resistant disease. The prevalence of P-gp overexpression in previously untreated breast cancer ranges from $< 10\%$ to 85% depending on the method of detection used and the definition of a positive result. Using slot blot analysis, one of the largest series identified increased *mdr1* message in 7/55 (13%) previously untreated patients [11]. By various methods and using an immunohistochemical definition of requiring staining in at least 5% of cells, studies which have examined mixed groups of breast cancer patients (previously untreated and treated) have shown a prevalence of MDR of 20% (28/141) in untreated patients and 54% (48/89) in treated patients [11, 20, 31, 32, 34]. Overexpression of P-gp, therefore, may be the dominant mechanism in acquired drug resistance in breast cancer, and this may be an optimal setting to evaluate inhibitors of P-gp.

The prognosis for patients with doxorubicin-resistant breast cancer is poor. Although the reported activity of "salvage" chemotherapy is variable (response rate 0–57%), response duration tends to be brief, and few patients survive beyond 1 year [15]. In this setting, vinblastine, given as a continuous infusion, has been evaluated by a number of investigators with conflicting results. Although two series found no activity in a total of 32 patients treated [18, 39], a much larger study reported by Fraschini et al. showed an overall response rate of 37%, including 5 complete responders, in 106 women with breast cancer refractory to doxorubicin [10]. Based on these promising results, preclinical data showing that trifluoperazine is more efficient at modulating resistance to vinca alkaloids than it is to other cytotoxins, and evidence that prolonged exposure to chemotherapy may be more effective against MDR tumors [21], we selected a 5-day infusion of vinblastine to treat patients on this study. Despite significant marrow toxicity, we were not able to confirm significant antitumor activity for this schedule of single-agent vinblastine.

The dose of trifluoperazine chosen in this trial was based on our prior experience using this drug as a modulator of chemotherapy [13, 14]. In an earlier phase I study, we determined that dose-limiting CNS and neuromuscular toxicity occurred when $\geq 9$ mg trifluoperazine twice daily was administered in the outpatient setting [13]. In contrast, Miller et al. reported that higher doses of trifluoperazine could safely be given to hospitalized patients when anticholinergics were used to treat extrapyramidal side effects [24]. However, in the study by Miller et al., clinical responses were observed at all tested dose levels, and even at the highest doses serum concentrations of trifluoperazine were much lower than the concentrations required to modulate MDR in vitro. Since trifluoperazine and most other inhibitors have a large apparent volume of distribution, serum concentrations may not adequately reflect the drug concentration in tumor cells. For example, Trump et al. were able to obtain simultaneous plasma and tumor samples from two patients receiving treatment with tamoxifen as a P-gp inhibitor in combination with vinblastine. In these two tumor samples, the concentrations of tamoxifen and *N*-desmethyltamoxifen were three- and eight fold greater than in the corresponding plasma [40]. A higher concentration of the modulator in tissue may explain the lack of correlation between the achievable plasma concentrations of P-gp inhibitors and the likelihood of response. Accordingly, we elected to use a tolerable, fixed dose of trifluoperazine and did not monitor plasma concentrations.

In the clinical studies of drug resistance modulation reported to date, the response rates have ranged from 7% to as high as 71% [1–5 19, 24, 25, 29, 35–37, 40, 43–46]. In some of these studies, however, cytotoxic drugs were used in continuous infusion schedules in patients who had previously received chemotherapy only by bolus administration. This alteration in drug schedule may itself account for the activity observed [22, 44]. In order to avoid this potential confounding variable, patients in our study were treated with vinblastine alone prior to receiving the same schedule of vinblastine plus trifluoperazine. In retrospect, this design may also be flawed. Almost half of the patients in our study did not receive treatment with the P-gp inhibitor, generally because of disease progression or

patient refusal. This problem has been observed in other studies and suggests that this is an inefficient design for initial trials of new modulating agents [26, 43, 46]. In addition, the inclusion of a modulator can alter the pharmacokinetics of the chemotherapy (1, 3, 22). Thus, responses attained when the modulator is added could be solely due to an increase in the dose intensity of the chemotherapy. Furthermore, exposure to additional chemotherapy prior to testing a P-gp inhibitor may induce additional non-P-gp mechanisms of resistance. It is therefore not surprising that studies using this design have been inconclusive.

A randomized trial, therefore, may be the best approach for evaluating the activity of a modulator. In breast cancer, two randomized studies have shown conflicting results. Wishart et al. found no advantage when orally administered quinidine was added to epirubicin in patients receiving their first chemotherapy regimen for advanced disease [45]. In contrast, preliminary results of another study showed a benefit when verapamil was added to the chemotherapy regimen [2]. Belpomme et al. treated 99 patients with anthracycline-resistant disease with a chemotherapy regimen consisting of bolus vinblastine 2 mg/m$^2$ on days 1 and 10, and 5-fluorouracil, 400 mg/m$^2$ per day given as a continuous infusion on days 1–10. Patients randomized to receive verapamil took 120 mg orally twice daily throughout the 28 day treatment cycle. The response rate (27% vs 11%) and survival (11.2 vs 6.6 months) was significantly better for the group receiving verapamil ($P = 0.04$). These discrepant results may be related to the patients included in these studies or the combination of the modulator-cytotoxin chosen. A more precise evaluation of the clinical significance of MDR modulation awaits the development of better tools for inhibiting P-gp, and assays for assessing its function.

As new, more potent inhibitors of P-gp are identified, future clinical trials should first focus on determining the maximum tolerated dose and the effect of the inhibitor on the pharmacokinetics of the cytotoxic drugs. Randomized studies could then be conducted in previously untreated patients, administering chemotherapy alone or with the best inhibitors of P-gp. These studies should be confined to diseases in which P-gp has been shown to be of prognostic importance, and therefore possibly a primary mechanism of acquired drug resistance. Despite the minimal activity demonstrated in this trial, breast cancer, a common tumor which displays acquired drug resistance, re-:mains a good model to explore the clinical significance of P-gp-mediated MDR. As more effective modulators are identified, they should be tested in randomized trials of previously untreated patients with this disease.

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