Lonidamine as a Modulator of Alkylating Agent Activity in Vitro and in Vivo

Beverly A. Teicher, Terence S. Herman, Sylvia A. Holden, Ron Epelbaum, Shi-de Liu, and Emil Frei III

Dana-Farber Cancer Institute [B. A. T., T. S. H., S. A. H., R. E., S-d. L., E. F.] and Joint Center for Radiation Therapy [B. A. T., T. S. H.], Boston, Massachusetts 02115

ABSTRACT

We are searching for relatively nontoxic compounds that can positively modulate the efficacy of antitumor alkylating agents. Lonidamine inhibits cellular energy metabolism and could potentially increase damage by alkylating agents if cellular defenses are energy requiring. Exposure of cells to lonidamine (500 μM) for 2 h under hypoxic conditions followed by 1-h exposures to lonidamine plus alkylating agents under normally oxygenated conditions in vitro significantly increased the cell kill achieved by cis-diaminedichloroplatinum(II) (CDDP) approximately 5-fold and by d-tetraplatin approximately 10-fold at 90% inhibitory concentration in MCF-7/CDDP (CDDP-resistant) cells. Carboplatin cytotoxicity, however, was little changed. In the MCF-7 parent cell line, treatment with lonidamine increased CDDP cytotoxicity by approximately 10-fold, d-tetraplatin by approximately 10-fold, and carboplatin by approximately 6-fold at the 90% inhibitory concentration. For l-phenylalanine mustard (melphanal), N,N',N''-trithelylenethiophosphoramide (thiotepa), and N,N'-bis(2-chloroethyl)-N-nitrosourea, little resistance was evident in the MCF-7/CDDP lines compared with the parent line. Treatment with lonidamine increased the cytotoxicity of each drug by 1.5- to 3-fold in both cell lines. When exposure to lonidamine was extended to 24 h before and after 12 h after drug exposure in MCF-7 normally oxygenated cultures, CDDP (250 μM) cytotoxicity was increased by approximately 100-fold, but melphanal cytotoxicity was increased only 2- to 3-fold over the concentration range tested. In the FSallC murine fibrosarcoma tumor system, five i.p. injections of 50 mg/kg of lonidamine over 36 h increased the tumor cell kill by CDDP and carboplatin approximately 2- to 3-fold over the dose range tested when the platinum complexes were given i.p. immediately after the third lonidamine injection. When cyclophosphamide and thiotepa were given in the same schedule, 10-fold increases in tumor cell killing were evident on tumor excision assay over the dosage ranges. The increase in bone marrow toxicity caused by lonidamine in addition to the alkylating agents was less than for tumor cells. Finally, in the EMT6 murine mammary carcinoma, use of lonidamine at 500 mg/kg twice daily along with CDDP, carboplatin, thiotepa, and cyclophosphamide significantly increased tumor growth delays by approximately 1.6- to 3.0-fold. The results suggest that lonidamine can positively modulate antitumor alkylating agent cytotoxicity and may be a clinically useful adjunctive therapy with these drugs.

INTRODUCTION

Lonidamine, 1-[(2,4-dichlorophenyl)methyl]-2H-indazole-3-carboxylic acid, affects the energy metabolism of cells (1-6). In both normal and neoplastic cells, oxygen consumption is strongly inhibited by this drug. Furthermore, in tumor cells, aerobic and anaerobic glycolysis are additionally affected (1, 3-6). Based on these data, mitochondria have been considered the primary intracellular targets of the drug. As data have been accrued, it has become evident that lonidamine is not equally effective in all cell types and that the mitochondrial effects of lonidamine can be reversible if the drug is removed after short time exposure (7). Ultrastructural studies by DeMartino et al. (2) indicate that the inhibition of energy metabolism in cells by lonidamine is a consequence of damage to the inner and outer mitochondrial membranes which leads to inhibition of respiration and glycolysis and finally loss of cell viability (2).

Lonidamine could be an important component of a combined modality regimen if repair of damage by a cytotoxic treatment is an energy-dependent process. Working with Chinese hamster HA-1 cells in culture, Hahn et al. (8) showed that, at concentrations achievable in vivo, lonidamine inhibited the repair of potentially lethal damage caused by X-rays, methyl methane sulfonate, bleomycin, and hyperthermia. Kim et al. (9-11) showed that lonidamine potentiated the effects of radiation and the effects of hyperthermia (10) in murine tumor models. Lonidamine has also been shown to enhance the cytotoxicity of several alkylating agents (12) as well as Adriamycin in culture (13).

We are searching for drugs that are relatively nontoxic themselves but which may positively and selectively modulate the cytotoxicity of alkylating agents in tumor cells. The present studies were undertaken to examine the potential of lonidamine to enhance the activity of alkylating agents in vitro against MCF-7 and MCF-7/CDDP (CDDP resistant) human breast carcinoma cells and against the FSallC murine fibrosarcoma and EMT6 murine mammary carcinoma in vivo.

MATERIALS AND METHODS

Drugs. Lonidamine was obtained as a gift from DeSanctis Consultants (Montreal, Canada), prepared in PBS, and stored at −20°C. CDDP (cisplatin) and carboplatin were gifts from Dr. Donald H. Picker and Dr. Michael J. Abrams, Johnson Matthey, Inc. (West Chester, PA), and were prepared in PBS and stored at −20°C. d-Tetraplatin was a gift from the Upjohn Co. (Kalamazoo, MI). Melphalan (l-phenylalanine mustard) and CTX were purchased as pure powders from Sigma Chemical Co. (St. Louis, MO). L-PAM was dissolved in HCl-acidified ethanol and diluted with PBS just prior to use. BCNU and thiotepa were purchased from the Dana-Farber Cancer Institute pharmacy.

Cell Lines. The MCF-7 cell line is a human adenocarcinoma of the breast, developed by Dr. M. Rich of the Michigan Cancer Foundation. This line is estrogen receptor positive and retains certain characteristics of breast adenocarcinoma. MCF-7 has been used as a model for in vivo studies of breast carcinoma (14, 15). MCF-7 human breast carcinoma cells grow as monolayers in Dulbecco's minimal essential medium supplemented with antibiotics, t-glutamine, and 10% fetal bovine serum. This cell line has a plating efficiency of 25 to 40%.

The MCF-7/CDDP cell line was developed by a dose escalation protocol, previously (16, 17). The resistant subline was screened for degree of resistance, generation times similar to those of the parent line, and relative stability of resistance (up to 2 mo). Every 6 months, the MCF-7/CDDP cell line was screened for degree of resistance, generation times similar to those of the parent line, and relative stability of resistance (up to 2 mo). Every 2 mo, a vial of early passage cloned cells was used to ensure that all experiments were carried out with the same subline. The MCF-7/CDDP line was used for in vivo studies as described previously (16, 17).

Received 8/14/90; accepted 11/9/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1 This work was supported by a grant from DeSanctis Consultants, Montreal, Canada; National Cancer Institute Grant 1P01-CA38493; and a grant from the Mathers Foundation.

2To whom requests for reprints should be addressed.

The abbreviations used are: CDDP, cis-diaminedichloroplatinum(II); L-PAM, l-phenylalanine mustard (melphanal); thiotepa, N,N',N''-trithelylenethiophosphoramide; CTX, cyclophosphamide; BCNU, N,N'-bis(2-chloroethyl)-N-nitrosourea; CFU-GM, granulocyte-macrophage colony-forming unit; PBS, phosphate-buffered saline.
CDDP cells are grown under the same medium and serum conditions as are the MCF-7 parent cell line and have a plating efficiency of 25 to 30%.

Survival Studies. MCF-7 cells or MCF-7/CDDP cells in exponential growth were treated with lonidamine (500 µM) for 2 h under hypoxic conditions (18) and then exposed to various concentrations of the alkylating agents along with the lonidamine for 1 h under normally oxygenated conditions. In other experiments, MCF-7 cells in exponential growth were exposed to lonidamine (500 µM) under normally oxygenated conditions for 24 h prior to exposure to the combination of lonidamine (500 µM) and various concentrations of CDDP or L-PAM for 1 h and followed by an additional 18 h of exposure to lonidamine (500 µM). Following treatment, cells were washed 3 times with 0.9% PBS solution and suspended by treatment with 0.25% trypsin. The cells were plated in duplicate at 3 dilutions for colony formation. After 2 wk the colonies were visualized by staining with crystal violet, and colonies of 50 cells or greater were counted. The results were expressed as the surviving fraction of treated cells compared with vehicle-treated control cells.

Tumor Lines. The FSall fibrosarcoma (19, 20) adapted for growth in culture (FSallC) (20) was carried in male C3H/FeJ mice (The Jackson Laboratory, Bar Harbor, ME). For the experiments, 2 x 10⁶ tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of 8- to 10-wk-old male C3H/FeJ mice.

The EMT6 murine mammary carcinoma is an in vivo-in vitro tumor system (21-24). The EMT6 tumor was carried in BALB/c mice (Taconic Farms, Germantown, NY). For the experiments, 2 x 10⁶ tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of BALB/c mice 8 to 10 wk old.

Tumor Excision Assay. For each experiment, two tumors were implanted per mouse, and there were two animals at each dosage level; therefore, four tumors were pooled at each point. When the tumors were approximately 100 mm³ in volume (about 1 wk after tumor cell implantation), five lonidamine doses (50 mg/kg) were administered every 7 to 10 h over 36 h. The alkylating agents were administered as single doses by i.p. injection (0.2 ml) along with the third lonidamine (50 mg/kg) injection. Mice were sacrificed 24 h after alkylating agent treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage and then soaked in 95% ethanol. The tumors were excised, and a single cell suspension was prepared as described previously (25). The untreated tumor cell suspensions had a plating efficiency of 8 to 12%. The results are expressed as the surviving fraction ± SE of cells from treated groups compared with untreated controls.

Bone Marrow Toxicity. Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle, and CFU-GM assay was carried out as described previously (25). Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher Scientific, Springfield, NJ). The results from three experiments, in which each group was measured at three cell concentrations in duplicate, were averaged. The results are expressed as the surviving fraction of treated groups compared with untreated controls from three independent experiments.

Bone Marrow Toxicity. Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle, and CFU-GM assay was carried out as described previously (25). Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher Scientific, Springfield, NJ). The results from three experiments, in which each group was measured at three cell concentrations in duplicate, were averaged. The results are expressed as the surviving fraction of treated groups compared with untreated controls from three independent experiments.

Tumor Growth Delay. For the experiments, 2 x 10⁶ EMT6 tumors were prepared from a brei of several stock tumors prepared from a brei of several stock tumors were implanted i.m. into the legs of BALB/c mice 8 to 10 wk old. When the tumors were approximately 100 mm³ in volume (Day 7 after tumor cell implantation), treatment was initiated. LONIDAMINE (50 mg/kg) was administered i.p. daily on Days 7 to 12 just prior to the alkylating agent or twice daily on Days 7 to 12 just prior to and during exposure to lonidamine. Each alkylating agent was administered i.p. alone or in combination with lonidamine or D-tetraplatin on the daily or twice daily schedule. The alkylating agent treatment schedules were the same as the CDDP treatment schedules (3.3 mg/kg) on Days 7, 9, and 11; carboplatin (25 mg/kg) on Days 7 to 12; CTX (100 mg/kg) on Days 7, 9, and 11; and thiotepa (5 mg/kg) on Days 7 to 12. These are standard doses and schedules for these drugs.

The results were expressed as the days taken by each individual tumor to reach 500 mm³ compared with the untreated controls. Tumor volume was calculated as a hemiellipsoid. Untreated EMT6 tumors reached 500 mm³ in 12.2 ± 0.4 days. Each treatment group had seven animals, and the experiment was repeated 3 times. Days of tumor growth delay are the means ± SE for the treatment group compared with the controls.

RESULTS

Exposure to lonidamine (500 µM) for 2 h under hypoxic conditions followed by an additional 1-h exposure under normally oxygenated conditions did not alter the survival of exponentially growing MCF-7 or MCF-7/CDDP cells compared with untreated controls; however, this exposure to lonidamine increased the sensitivity of both cell lines to several alkylating agents (Fig. 1). The MCF-7/CDDP cell line is about 5-fold resistant to CDDP at the 90% inhibitory concentration compared with the MCF-7 parent cell line. Exposure of the MCF-7/CDDP cells to lonidamine prior to and during CDDP exposure increased the sensitivity of that cell line to CDDP by about 5- to 7-fold, so that the MCF-7/CDDP cells became as sensitive to CDDP as were the MCF-7 parent cells. A marked increase in CDDP cytotoxicity was observed in the MCF-7 parent cells upon prior and concomitant exposure to lonidamine. The increase in cytotoxicity was more than 10-fold with 100 µmol of CDDP and more than 100-fold with 500 µmol of CDDP. The cytotoxicity of carboplatin was increased only slightly in the MCF-7/CDDP cells by the addition of lonidamine prior to and during carboplatin exposure. In the MCF-7 parent cells, on the other hand, there was a larger increase in carboplatin cytotoxicity by prior and concomitant exposure to lonidamine which reached a maximum of about 10-fold between 250 and 500 µmol of carboplatin. As occurred with CDDP, exposure to lonidamine prior to and during exposure to D-tetraplatin increased the sensitivity of the MCF-7/CDDP cells to the drug so that they were as sensitive to D-tetraplatin as were the MCF-7 parent cells. In the MCF-7 parent cell line, the increase in the cytotoxicity of D-tetraplatin by prior and concomitant exposure to lonidamine was greater than 10-fold at 10 to 50 µM D-tetraplatin and less at higher concentrations of the platinum drug.

Fig. 1. Survival of the MCF-7 human breast carcinoma parent cell line (•, O) and CDDP-resistant MCF-7/CDDP subline (□, □) to various alkylating agents in the presence (□, □) or absence (•, •) of lonidamine (500 µM). Points, mean of three independent experiments; bars, SE.
In these cell culture experiments, small increases in the cytotoxicity of L-PAM, thiotepa, and BCNU were observed when MCF-7 or MCF-7/CDDP cells were exposed to lonidamine prior to and during exposure to each of the drugs (Fig. 1). With L-PAM, addition of lonidamine to treatment with the alkylating agent increased the cytotoxicity of the drug 2- to 3-fold in both cell lines. With thiotepa, the increase in cytotoxicity with the addition of lonidamine prior to and during thiotepa treatment was 4- to 5-fold. Finally, with BCNU, there was dose modification of the drug toxicity in the presence of lonidamine prior to and during drug treatment, so that the increase in BCNU cytotoxicity at low drug concentrations was about 2-fold, and at high drug concentrations, the increase was about 5-fold.

Even under prolonged exposure times, lonidamine showed only limited direct cytotoxicity toward MCF-7 parent cells producing a surviving fraction of 0.75 after 42-h exposure to 500 μmol of the drug (Fig. 2). When normally oxygenated MCF-7 cells were exposed simultaneously to lonidamine (500 μM) and various concentrations of CDDP for 1 h followed by continued exposure to lonidamine (500 μM) for 12 h, a dose modification of the CDDP toxicity resulted, increasing from about 2.5-fold at 50 μM CDDP to about 50-fold at 250 μM CDDP. When exposure to lonidamine (500 μM) was maintained for 24 h prior to, during CDDP exposure, and 12 h post-CDDP treatment, an additional increase in CDDP cytotoxicity was observed so that 50- to 100-fold more cells were killed by the combination treatment at 100 to 250 μM CDDP than by CDDP exposure alone. A similar study was conducted with L-PAM, and much lesser effects were seen with the addition of lonidamine to L-PAM treatment. With lonidamine (500 μM) exposure for 24 h prior to, during, and for 12 h after L-PAM treatment, there were increases of 2- to 3-fold in cell killing compared with L-PAM alone.

Using the FSaIIIC fibrosarcoma in vivo-in vitro tumor system, the responses of tumors in vivo was examined to increasing single doses of alkylating agents in the presence or absence of lonidamine (Fig. 3). Tumor cell kill was quantified by colony formation, and the survival of bone marrow from the same animals was measured by the CFU-GM assay. The treatment schedule for lonidamine was five i.p. injections of 50 mg/kg given at 7- to 10 h intervals over 36 h with the alkylating agent being administered as a single dose i.p. immediately following the third lonidamine injection. Lonidamine, on this treatment schedule, resulted in about a 50% killing of the tumor cells and no toxicity to the bone marrow CFU-GM. Each of the four alkylating agents produced log-linear increasing tumor cell killing with increasing dose of the drug. The addition of lonidamine prior to, during, and following treatment with CDDP resulted in a 2- to 3-fold increase in tumor cell killing and a smaller increase in bone marrow CFU-GM killing. A very similar effect was observed with carboplatin in vivo where, in the presence of lonidamine treatment, there was a 2- to 2.5-fold increase in the killing of FSaIIIC tumor cells and a smaller increase in the killing of bone marrow CFU-GM.

The addition of lonidamine to treatment with cyclophosphamide in vivo resulted in 10-fold additional tumor cell killing in the normal therapeutic range of CTX doses (100 to 300 mg/kg) and a much smaller increase in the killing of bone marrow CFU-GM of 0- to 3-fold in that same dosage range (Fig. 3). There was a similar 10-fold increase in the killing of tumor cells by thiotepa with the addition of lonidamine to treatment with that drug. The 10-fold increase in tumor cell killing by thiotepa persisted over the entire dosage range (10 to 30 mg/kg) examined. The killing of bone marrow CFU-GM by thiotepa with the addition of lonidamine increased to a lesser extent than did the tumor cell killing, so that there was equal or greater tumor cell than bone marrow CFU-GM killing by thiotepa over the dosage range examined.

The EMT6 mouse mammary carcinoma was used for tumor growth delay studies (Table 1). Lonidamine was administered either daily or twice daily for 5 days at a dose of 50 mg/kg. On the daily schedule, lonidamine was administered i.p. immediately prior to the alkylating agent, which was also administered i.p. On the twice daily schedule, the first dose of lonidamine was administered i.p. immediately prior to the alkylating agent, and the second dose was administered i.p. 4 h later. Lonidamine on either treatment schedule had only a small effect on tumor growth. Each of the alkylating agents was administered on a multiple dose schedule over the 5-day period of lonidamine.
LONIDAMINE AS A MODULATOR OF ALKYLATING AGENTS

The EMT6 tumor was grown subcutaneously in BALB/c mice. Treatment was administered on Day 7 post-tumor cell implantation when the tumors were 50 to 100 mm³ in volume. Tumor growth delay is the difference in days for treated tumors to reach 500 mm³ compared with untreated controls.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Lonidamine + alkylating agent (5 x 50 mg/kg)</th>
<th>Lonidamine (5 x 50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lonidamine</td>
<td>1.0 ± 0.4</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>CDDP (3 x 3.3 mg/kg)</td>
<td>6.5 ± 1.5</td>
<td>11.0 ± 1.3</td>
</tr>
<tr>
<td>Carboplatin (5 x 25 mg/kg)</td>
<td>4.9 ± 0.7</td>
<td>11.2 ± 1.3</td>
</tr>
<tr>
<td>CTX (3 x 100 mg/kg)</td>
<td>9.7 ± 1.3</td>
<td>21.3 ± 2.0</td>
</tr>
<tr>
<td>Thiotepa (5 x 5 mg/kg)</td>
<td>5.7 ± 1.1</td>
<td>16.5 ± 1.8</td>
</tr>
</tbody>
</table>

Mean ± SE.

administration. There was no change in the tumor growth delay produced by CDDP with once daily lonidamine but, with twice daily lonidamine, there was a 1.7-fold increase in tumor growth delay from about 6.5 days to about 11.0 days. The tumor growth delay produced by carboplatin was only slightly increased by daily lonidamine treatment. However, twice daily lonidamine along with carboplatin resulted in a 1.7-fold increase in tumor growth delay from about 4.9 days to about 8.2 days. There was no significant increase in the tumor growth delay produced by CTX with the addition of daily lonidamine, but, again, twice daily lonidamine resulted in a 2.2-fold increase in tumor growth delay compared with CTX alone. The tumor growth delay produced by cyclophosphamide was about 9.7 days, and that of the combination treatment was about 21.3 days. A similar pattern persisted with thiotepa treatment in that there was a small increase in tumor growth delay with the addition of daily lonidamine but a larger increase of about 3-fold with daily thiotepa and twice daily lonidamine.

DISCUSSION

Treatments or agents that are relatively nontoxic but can modulate the cytotoxicity of bifunctional alkylating drugs in tumors could be very useful in combination chemotherapeutic regimens for the treatment of clinical neoplastic diseases. Potential modulators of alkylating agent cytotoxicity include the following: (a) the topoisomerase II inhibitors such as etoposide, VM-26, and novobiocin; (b) the nitroimidazole radiosensitizers such as misonidazole and etanidazole; (c) agents or treatments that inhibit or prevent DNA repair, such as 3-aminobenzamide, pentoxifylline, or hyperthermia; (d) agents that increase tumor oxygenation, such as Fluosol-DA/carbogen and nicotinamide; and (e) agents that interfere with cellular energy production, such as rhodamine-123 and lonidamine. Most of these treatments or agents, even those that are highly cytotoxic in their own right, have been shown to be effective additions to other single treatments and combinations. Lonidamine has undergone Phase II testing in clinical trials (26–29) and has shown promising results in preliminary reports of clinical trials in combination with radiation therapy (30) and hyperthermia (31).

In cell culture, prior exposure of the cells to lonidamine under hypoxic conditions and then simultaneous exposure to lonidamine and the alkylating agent under normally oxygenated conditions resulted in significant increases in the cytotoxicity of CDDP, carboplatin, and cisplatin in MCF-7 human breast carcinoma cells. The presence of lonidamine in these experiments essentially overcame the CDDP resistance of the MCF-7/CDDP cells, but the degree of enhancement of cytotoxicity of CDDP, carboplatin, and cisplatin by lonidamine in the MCF-7/CDDP cells was much less than in the MCF-7 parent cell line. In cell culture, much lesser effects were seen with L-PAM, thiotepa, and BCNU. In vivo, however, a somewhat different pattern of enhancement in alkylating agent activity was observed. Lonidamine, administered prior to, during, and after alkylating agent treatment in the tumor cell survival assay using the FSaIIC fibrosarcoma, produced essentially additive tumor cell killing with both CDDP and carboplatin. However, greater than additive enhancement in (calculated by product of the surviving fractions) tumor cell killing was probably obtained with CTX and thiotepa. With each of these four drugs, in general, the addition of lonidamine to the alkylating treatment resulted in a greater increase in the killing of tumor cells than in the killing of bone marrow CFU-GM, indicating that there would be an increase in therapeutic index if bone marrow toxicity was dose limiting.

In the EMT6 mouse mammary carcinoma growth delay studies, lonidamine on two different schedules was added to standard doses of each of the four alkylating agents without any evidence of an increase in the toxicity of the combination treatment compared with the alkylating agent alone. For CDDP, carboplatin, CTX, and thiotepa, significant increases in tumor growth delay were observed only with the twice daily schedule of lonidamine. As in the tumor cell survival assay, lesser increases were seen with the platinum drugs, CDDP and carboplatin, than with the mustard-type alkylating drugs, CTX and thiotepa.

These results indicate that lonidamine has the potential to increase the efficacy of antineoplastic alkylating agents without a reduction in the dosage of the alkylating agents and that a greater potentiation of the effect of the alkylating agents may occur in the tumor compared with the bone marrow. Although in cell culture lonidamine potentiated the cytotoxicity of platinum drugs to a greater degree than it potentiated the cytotoxicity of mustard-type drugs, in vivo, larger enhancement in both the FSaIIC fibrosarcoma and the EMT6 mammary carcinoma was observed with the mustard-type drugs than with the platinum drugs. Clinical protocols utilizing lonidamine with CTX are being considered.

REFERENCES

Lonidamine as a Modulator of Alkylating Agent Activity \textit{in Vitro} and \textit{in Vivo}


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/3/780

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.