Synergistic Potentiation of in Vivo Antitumor Activity of Anti-Human T-Leukemia Immunotoxins by Recombinant α-Interferon and Daunorubicin

Solichiro Yokota, Hideki Hara, Yi Luo, and Ben K. Seon

Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

In the present study, immunotoxins (ITs) containing ricin A chain (RA) and anti-human T leukemia monoclonal antibodies SN1 and SN2 were used with or without α-interferon (IFN) and/or daunorubicin (DNR) for in vivo tumor suppression. SN1 and SN2 are directed toward two unique human T-leukemia-associated cell surface antigens, TALLA and GP37, respectively. As the tumor model, we used nude mice bearing ascitic tumors of Ichikawa, a human T acute lymphoblastic leukemia cell line. In initial studies, we investigated the effect of the IT injection schedule on the efficacy of ITs in the in vivo suppression of the ascitic tumors. Four doses of 20 µg each of SN1-RA and SN2-RA completely suppress the tumor growth in 100% of the treated mice when the IT treatment is initiated either 1 or 2 days after tumor inoculation of 1.6 × 10⁷ Ichikawa cells into the mice. Subsequently, we investigated the potentiating effects of IFN and DNR on the in vivo antitumor activity of ITs. To this end, we chose to initiate the treatment 4 days after the tumor inoculation when IT treatment alone is only partially effective. ITs (10 µg each of SN1-RA and SN2-RA) plus IFN (2 × 10⁶ IU) or ITs plus IFN plus DNR (5 µg) completely suppress tumor growth in 100% of the treated mice while similar treatment with any one of the three agents is only partially effective. Similar treatment with ITs plus DNR or IFN plus DNR results in complete suppression of tumor growth in 80% of the treated mice. These results were reproducible in a repeated experiment. To gain information about the mechanisms involving the IFN potentiation of IT activity, we carried out several experiments. The cell surface expression of TALLA and GP37 was slightly augmented by the in vitro incubation of Ichikawa cells with IFN as measured by fluorescence-activated cell sorter analysis. The degree of the increase in either TALLA or GP37 was significantly smaller than that of HLA class I antigens in the same experiment. In in vitro experiments, IFN did not show any significant cytotoxic activity against Ichikawa cells or augment the cytotoxic activity of ITs against Ichikawa cells. On the other hand, injections of IFN into nude mice augmented activity of macrophages and NK cells; however, Ichikawa leukemia cells were rather resistant to the NK cell lysis. These results collectively indicate that IFN potentiates the in vivo antitumor activity of our anti-human T leukemia ITs primarily by host-mediated effector mechanisms but not by direct action of IFN on leukemia cells. Particularly, the activation of macrophages by IFN in the tumor-bearing nude mice appears to be the major factor in the potentiation of the in vivo antitumor activity of the ITs in the present study. Furthermore, the present results clearly demonstrate the superior therapeutic efficacy of combined therapy, especially with ITs plus IFN, to therapy with ITs, IFN, or DNR alone.

INTRODUCTION

The concept of antibody-guided specific delivery of cytotoxic agents to tumor targets has gained widespread attention recently. In this regard, a chimeric molecule, IT, consisting of antibody and toxin or its subunit is of particular interest (reviewed in Refs. 1-3). Among the various known toxins and their subunits, the A chain subunit of ricin (a plant toxin) is the substance most widely used for preparing ITs. Ricin is composed of two disulfide-linked subunits, i.e., A and B chains. The B chain is a lectin which is specific for galactose present on the surface of a wide variety of cells. The A chain is an enzyme which catalytically and irreversibly inhibits protein synthesis in the cytoplasm of the cells by acting on tRNA (4). Thus, the ricin A chain is an attractive cytotoxic agent to be delivered by antitumor antibodies to the tumor targets.

Previously, a number of investigators including ourselves reported that ITs containing RA and various mAbs can effectively kill tumor cells in vitro (reviewed in Refs. 1-3 and 5). More recently, several investigators reported various degrees of efficacy of such ITs in the in vivo suppression of tumor growth (see "Discussion"). We showed that our human T leukemia-specific ITs containing RA and mAbs SN1 and SN2 were capable of completely suppressing the in vivo growth of human T leukemia cells without any overt undesirable toxicity (6). Neither control RA conjugate nor naked (unconjugated) mAbs (SN1 and SN2) were effective in suppressing the same tumors in nude mice (6). Furthermore, SN1-RA and SN2-RA showed specific killing of human T leukemia cells in vitro (7). SN1 and SN2 define two unique human T leukemia-associated cell surface antigens, termed TALLA and GP37, respectively, and show highly selective reactivity with human T malignant cells (8-12). We have been using a mixture of two ITs targeted to different antigens on tumor cells to minimize the problem of tumor heterogeneity. In the above in vivo study, however, treatment with ITs was initiated 2 h after each nude mouse was inoculated with 1.6 × 10⁷ cells of Ichikawa, a human T acute lymphoblastic leukemia cell line. In the present study, we first investigated the effect of IT injection schedule on the efficacy of ITs in in vivo tumor suppression. In order to further improve the efficacy of ITs, we studied if recombinant human IFN-α and DNR, a widely used anti-human leukemia chemotherapeutic agent, can potentiate the in vivo antitumor activity of ITs. It is important to note that IT, IFN, and DNR exert their antitumor action by individually different mechanisms (see "Discussion").

MATERIALS AND METHODS

Mice. Female BALB/c AnNCr-nu (nu/nu) and athymic NCr-nu (nu/nu) mice, 5 to 6 weeks old, were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) through the Frederick Cancer Research Facility of the National Cancer Institute. The mice were housed under sterile conditions in cages with filter bonnets in a laminar flow unit (LabView). The mice were fed standard mouse chow and water ad libitum. Mice were between 8 and 10 weeks of age when used for therapy experiments.

Human Tumor Established in Nude Mice. An ascitic tumor was established by inoculating nonpreconditioned nude mice with 1.6 × 10⁷ Ichikawa (a human T acute lymphoblastic leukemia cell line) cells as described previously (6). Transplantability of the tumor was 100%. This animal model was described previously (6, 13).
Reagents. N-Succinimidyl-3-(2-pyridyl disulfide)propionate was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Purified RA was obtained from E-Y Laboratories, Inc. (San Mateo, CA) and Inland Laboratories (Austin, TX). DNR, poly(I)-poly(C), and carrageenan (Sigma type IV) were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human IFN-α, from Schering-Plough Corp. (Kenilworth, NJ), was dissolved in PBS immediately prior to use.

Preparation of Immunoconjugates. The IgG was isolated from BALB/c murine ascites containing mAb SN1 (IgG1c), mAb SN2 (IgG1c), or MOPC 195 variant plasmacytoma IgG (IgG1c) by DE52 chromatography followed by gel filtration on a Sephadex G-150 column or affinity chromatography on a protein A-Sepharose column. The purified IgGs were individually conjugated with RA and the conjugation products were characterized as described previously (7, 14).

In Vitro Cytotoxicity Test. The cytotoxic activity of ITs and IFN was determined as described previously (7, 14). Briefly, Ichikawa cells were suspended in RPMI 1640 supplemented with 8% (v/v) heat-inactivated FBS, penicillin (100 units/ml), and streptomycin (50 μg/ml) at a concentration of 7.5 × 10^5 cells/ml. One ml of the cell suspension was placed, in triplicate, into individual wells of 24-well flat bottomed plates (Flow Laboratories, McLean, VA). ITs (SN1-RA plus SN2-RA) and/or IFN was added to the individual wells and the plates were incubated for 3 days. On day 2, a portion of each cell culture supernatant was replaced with fresh cell culture medium. The cells were counted daily and the viability of the cells was determined using trypan blue.

In Vitro Effect of IFN on the Expression of TALLA and GP37 on Human T Leukemia Cells. Ichikawa cells were suspended in RPMI 1640 containing 10% FBS at 1 × 10^6 cells/ml and incubated with or without IFN (10^2 to 10^6 IU/ml) for 48 h at 37°C in a humified atmosphere of 5% CO2 in air. The cells were washed three times and portions of the cells were allowed to react with mAbs SN1, SN2, E2-1B1, or an isotype-matching control mouse IgG (MOPC 195 variant; IgG1c); E2-1B1 is a mAb directed to a public determinant of the heavy chain of HLA Class I antigens. The treated cells were reacted with fluorescein isothiocyanate-labeled F(ab’)2 of sheep anti-mouse IgG for FACS analysis (15).

In Vivo Treatment of Ascitic Tumors with ITs. Fifteen nude mice which were inoculated i.p. with 1.6 × 10^7 Ichikawa cells were divided into 3 groups. One group not treated with IT served as a control. Treatment of each of the two remaining groups of mice was initiated by i.p. injection of ITs (a) 1 day or (b) 2 days after the tumor inoculation.

The injection of ITs was repeated for each group 1, 6, and 29 days after the initial injection.

In Vivo Treatment of Tumors with ITs, IFN, and/or DNR. In the present study, efficacy of combined use of ITs with IFN and/or DNR was investigated. In a separate experiment, treatment with ITs alone was shown to be only partially effective when the treatment of the tumor-bearing mice was initiated 4 days after tumor inoculation. In the present experiment, 40 mice which had been inoculated with 1.6 × 10^7 Ichikawa cells were divided into 8 groups. On day 4 after the tumor inoculation, each group of mice was treated with (a) PBS (control), (b) ITs (10 μg each of SN1-RA and SN2-RA), (c) IFN (2 × 10^6 IU), (d) DNR (5 μg), (e) ITs plus IFN, (f) ITs plus DNR, (g) IFN plus DNR, and (h) ITs plus IFN plus DNR. The treatment was repeated 4 times, i.e., 1, 2, 3, and 23 days after the initial treatment. A separate experiment indicated that injections of ITs and IFN at different times were less effective than the simultaneous injections of ITs and IFN.

Follow-up of the Treatment Efficiency. During the treatment, mouse weight was measured three times a week using an electronic balance (OHAUS Model GT210) and the mice were monitored daily for mortality.

Effect of IFN on the Activity of NK Cells and Macrophages in Nude Mice. Individual nude mice were given i.p. injections of 2 × 10^3 IU of IFN daily 4 times. Twenty-four h after the last injection, the peritoneal cells were harvested by peritoneal lavage with cold PBS using a syringe with an 18-gauge needle. In two separate experiments, 3.8 and 4.6 × 10^6 cells/mouse were obtained. The NAPC were prepared by incubating peritoneal cells in a 100-mm-diameter plastic dish (1015-01; Encore Plastic, Toronto, Ontario, Canada) in a humidified 5% CO2 atmosphere at 37°C for 2 h. To prepare APC, the peritoneal cells were suspended in RPMI 1640 containing 10% FBS at a concentration of 5 × 10^5, 1 × 10^6, or 2 × 10^6 cells/ml. Then, 200 μl of the cell suspensions were added into individual wells of flat-bottomed microtiter plates (Falcon No. 3040) and incubated for 2 h. The plates were washed to remove NAPC, and the remaining adherent cell monolayers were used as macrophage monolayers. The yields of NAPC and APC from peritoneal cells were approximately 70 and 30%, respectively. The NAPC fraction and APC monolayer were used as the effector cells of NK and macrophage activity, respectively. The NK and macrophage activity was determined using 4- and 18-h 51Cr release assays, respectively, using 51Cr-labeled target tumor cells (16). Control activated NK cells were obtained from spleens of BALB/c mice which had been given i.p. injections of 100 μg of poly(I)-poly(C) (17).

In the macrophage activity assay, the cytolytic activity of the APC was measured against 51Cr-labeled Ichikawa leukemia cells in the absence and in the presence of carrageenan (25 μg/ml). Carrageenan is known to abolish the activity of macrophages (18).

RESULTS

Effect of IT Injection Timing on the Antitumor Efficacy. Complete suppression of tumor growth was observed for all treated mice when treatment of tumor-bearing nude mice with two anti-human T leukemia ITs, SN1-RA and SN2-RA, was initiated 1 (Fig. 1, group a) or 2 days (Fig. 1, group b) after tumor inoculation with 1.6 × 10^7 Ichikawa leukemia cells. The injection was repeated three times for each group on 1, 6, and 29 days after the initial injection. All untreated control mice died within 10 weeks after the tumor inoculation (Fig. 1, group c). These results were reproducible in another experiment. Two repeating injections of ITs (on 4 and 28 days after the initial injection) appear to be less effective than the three repeating injections described above. For instance, the following results were obtained from a set of experiments using two repeating injections of 20 μg each of SN1-RA and SN2-RA: (a) complete suppression of tumor growth for 5 of 5 tumor-bearing mice when the treatment was initiated 2 h after the tumor inoculation; (b) complete suppression of the tumor growth for 4 of 5 tumor-bearing mice by the treatment initiated 1 day after the tumor inoculation (the remaining mouse died on day 53); (c) all 5 tumor-bearing mice died within 9 weeks when the treatment was initiated 4 days after the tumor inoculation (median survival time, 53.2 days); and (d) all 5 control (untreated)
tumor-bearing mice died within 8 weeks after the tumor inoculation (median survival time, 46.8 days).

Combination Therapy Using IT, IFN, and DNR. We investigated whether IFN and DNR potentiate the in vivo antitumor activity of IT. In this experiment, treatment of the tumor-bearing mice with therapeutic agents was initiated 4 days after tumor inoculation when treatment with ITs alone was only partially effective. Forty nude mice which had been inoculated with $1.6 \times 10^7$ T leukemia cells were divided into 8 groups. On day 4 after the tumor inoculation, each group of mice was treated as described in "Materials and Methods," and the results are summarized in Fig. 2. Treatment of the tumor-bearing mice with IFN (2 $\times$ 10^4 IU) or DNR (5 $\mu$g) extended the survival times of the treated mice only slightly; median survival times for the control, IFN-treated, and DNR-treated groups were 60.8, 68.6, and 89.8 days, respectively (Fig. 2A). Treatment with ITs (10 $\mu$g each of SN1-RA and SN2-RA) extended the survival time only slightly except for one mouse for which complete suppression of tumor growth was observed (Fig. 2B). In contrast, ITs plus IFN or ITs plus IFN plus DNR completely suppressed tumor growth in all of the treated mice. Thus, therapy with any of the four combinations used was more effective than therapy with ITs, IFN, or DNR alone. Among the four combinations, ITs plus IFN and ITs plus IFN plus DNR were the most effective. The above results were reproducible in a repeated experiment; again, ITs plus IFN or ITs plus IFN plus DNR completely suppressed tumor growth in all of the treated mice (5 mice/group). A minor difference observed between the two experiments was that treatment with DNR plus IFN resulted in complete suppression of tumor growth in 60%, instead of 80%, of the treated mice.

The antitumor efficacy of ITs, IFN, and DNR was also followed by measuring the body weight of individual mice three times a week. The increase in body weight correlated with the ascitic tumor increase and with the corresponding increase in death rate; an example of the body weight results is shown in Fig. 3. Treatment with ITs plus DNR or IFN plus DNR resulted in complete suppression of tumor growth in 80% of the treated mice. Among the four combinations, ITs plus IFN and ITs plus IFN plus DNR were the most effective. The above results were reproducible in a repeated experiment; again, ITs plus IFN or ITs plus IFN plus DNR completely suppressed tumor growth in all of the treated mice (5 mice/group). A minor difference observed between the two experiments was that treatment with DNR plus IFN resulted in complete suppression of tumor growth in 60%, instead of 80%, of the treated mice.

The antitumor efficacy of ITs, IFN, and DNR was also followed by measuring the body weight of individual mice three times a week. The increase in body weight correlated with the ascitic tumor increase and with the corresponding increase in death rate; an example of the body weight results is shown in Fig. 3.

Effect of IFN on the Expression of TALLA and GP37 on Leukemia Cells. To gain information about the mechanisms involving IFN potentiation of IT activity, we investigated whether IFN augments the expression of two leukemia-associated antigens, TALLA and GP37, defined by mAb E2-1B1. Ichikawa leukemia cells were incubated in vitro with 10-fold serial dilutions (10^2 to 10^6 IU/ml) of IFN and control medium. The cell surface expression of TALLA, GP37, and HLA class I antigens was determined by FACS analysis (see "Materials and Methods"). A typical example of FACS analysis data is shown in Fig. 4. Treatment of Ichikawa cells with IFN resulted in a substantial increase in the expression of HLA class I antigens (defined by mAb E2-1B1) but resulted in smaller increases in the expression of TALLA and GP37.
It should be noted that IFN is known to induce an increase in cell surface expression of HLA class I antigens (reviewed in Refs. 19 and 20).

**In Vitro Cytotoxicity Activity of IFN, IT, and IFN plus IT.** To investigate if IFN exerts a direct cytotoxic action against Ichikawa cells, the leukemia cells were cultured in vitro in the absence (control) and presence (10² to 10⁴ IU/ml) of IFN for 3 days and viability of the cells was determined daily. No effect of IFN on the viability of the leukemia cells was observed (data not shown). Subsequently, we investigated if IFN potentiates in vitro the cytotoxic activity of IT against the leukemia cells. The results are shown in Fig. 5. IFN did not potentiate the in vitro cytotoxic activity of ITs at all. Furthermore, IFN did not show any direct cytotoxicity against the leukemia cells as found in an earlier experiment (see above).

**Augmentation of Cytotoxic Activity of NK Cells and Macrophages in Nude Mice by IFN.** We determined the degree of augmentation of NK and macrophage antitumor activity in nude mice by IFN. Some of the results obtained for NK augmentation are shown in Table 1. In this study, we compared the cytotoxic activity of NK cell fractions from IFN-injected and IFN-nontreated nude mice. Poly(I)-poly(C)-induced NK cells from normal BALB/c mice was also included in this study as a positive control. Ichikawa target cells were tested in parallel with two well known NK target cells, YAC-1 (a mouse lymphoma cell line) and K-562 (a human leukemia cell line). Injection of IFN into nude mice substantially augmented NK activity, particularly at the higher (80 and 40) effector/target ratios. The relative degree of augmentation against the three target cells was YAC-1 > K-562 > Ichikawa. Note that the relative susceptibility of the three target cells against the control poly(I)-poly(C)-augmented NK cells was YAC-1 > K-562 > Ichikawa (Table 1). These results indicate that Ichikawa cells are rather resistant to NK cytolysis by either IFN-induced or poly(I)-poly(C)-induced NK cells. Addition of carrageenan (25 µg/ml) to the assay solution did not affect the results (data not shown); therefore, we conclude that contaminating macrophages, if any, did not contribute significantly to the above cytolyis (see below and Ref. 18). The results of macrophage activation by IFN are summarized in Table 2. To ascertain that the antitumor cytotoxicity of the isolated macrophage preparation is indeed due to macrophages, the assay was carried out in the presence as well as in the absence of carrageenan. As shown in Table 2, IFN substantially augmented macrophage cytotoxicity. The activity was completely abolished by carrageenan which supports our view that the cytotoxicity is due to macrophages.

### DISCUSSION

An important therapeutic application of antitumor IT will be in serotherapy. In this regard, a number of investigators including ourselves reported in vivo studies of animal and human tumors using various ITs (reviewed in Refs. 1, 3, and 21). Most human tumor studies were carried out using nude mice carrying human tumors (6, 14, 22–28), except for a few studies where clinical trials of cancer patients were performed (29–32). Although T-cell functions are deficient in nude mice, nude mice possess many other important biological and immunological functions such as NK cell, B-cell, and macrophage activity (33–35) and can generate a variety of biologically important products such as interleukin 2, IFNs, and tumor necrosis factors (36–38). Thus, nude mice, particularly nonpreconditioned (not X-irradiated or splenectomized) nude mice, carrying human tumors provides us with a valuable and useful model for studying the in vivo efficacy of antitumor ITs.

Previously, Kitahara et al. (13) and our group (6) established an ascitic tumor of human T leukemia in nonpreconditioned nude mice. More recently, we succeeded in establishing an ascitic tumor of human non-T leukemia in nonpreconditioned nude mice (15). In the present study, we used the ascitic tumor of human T leukemia.

Among various toxins and toxin subunits available, the A chain subunit of ricin, a plant toxin from castor beans, has been most widely used for preparing an IT. Among various toxins and toxin subunits available, the A chain subunit of ricin, a plant toxin from castor beans, has been most widely used for preparing an IT. We have been using RA in preparing our anti-human leukemia ITs (5–7, 14, 39). RA per se is not an effective cytotoxic agent against intact target cells because of its inability to bind efficiently to cell surfaces.
and to traverse the cell membranes. However, RA becomes highly cytotoxic when delivered to the cytoplasm of the target cells by an appropriate delivery vehicle such as the ricin B-chain and an effective antibody. In cells, RA catalytically removes adenine from 28S rRNA (4) causing irreversible inhibition of protein synthesis and ultimately cell death. Thus, conjugates of RA with an appropriate antitumor mAb may provide an IT specifically targeted on the tumor cells that react with the mAb.

Although antitumor RA conjugates appear to have good potential for in vivo therapy of cancer patients, some problems involving RA ITs remain to be studied. One of these is the need for potentiating the in vivo cytotoxic activity of antitumor ITs. In the present study, we investigated such potentiation by using IFN and DNR. Indeed, both IFN and DNR showed strong synergistic in vivo potentiating of IT activity (Fig. 2).

DNR is an antibiotic of the rhodomycin group and is being widely used for treating human leukemias; DNR inhibits DNA and RNA synthesis in the cells (40). Thus, the mechanism by which DNR kills target cells is different from the cytotoxic mechanism of RA ITs (see above). The inhibition of the polynucleotide synthesis by DNR will result not only in the inhibition of proliferation of target cells but also in the eventual inhibition of the protein synthesis in the cells because polynucleotides are necessary for protein synthesis. Thus, the combined action of DNR and ITs will exert cytotoxic activity against leukemia cells through multiple mechanisms which probably accounts for the observed synergistic antileukemia activity by ITs and DNR (see Fig. 2).

Recently, Griffin et al. (41) reported that doxorubicin, another chemotherapeutic agent, potentiated in vivo antitumor activity of an anti-human transferrin receptor IT.

IFN exerts a variety of effects on tumor cells at the cellular level (20, 42). These effects may be divided into two groups, i.e., effects by a direct action of IFN on the tumor cells and the host-mediated effects. To gain information about the mechanisms by which IFN and our anti-human leukemia ITs exert synergistic antitumor effect in vivo, we carried out several experiments. These experiments include studies of (a) effect of IFN on the cell surface expression of leukemia-associated antigens TALLA and GP37, (b) direct cytotoxic activity of IFN against human leukemia (Ichikawa) cells, (c) IFN-mediated potentiation of IT activity against human leukemia cells in vitro, and (d) in vivo effect of IFN on activation of NK cells and macrophages in nude mice. The results of these tests are presented in Figs. 4 and 5, in Tables 1 and 2, and in “Results.” The test results collectively indicate that IFN potentiates the in vivo antitumor activity of our anti-human leukemia ITs primarily by host-mediated effector mechanisms but not by direct action of IFN on the leukemia cells. IFN potentiated both NK cell and macrophage activity in the tumor-bearing nude mice (Tables 1 and 2). However, Ichikawa leukemia cells were rather resistant to the NK cell lysis (Table 1). Therefore, the macrophage activation by IFN appears to be the major factor in the synergistic potentiation of antileukemia activity of ITs by IFN in this study.

Recently, Basham et al. (43) and Cameron et al. (44) studied murine tumor models and reported that host effector mechanisms were important in potentiating antitumor activity of an antiidiotype antibody or interleukin 2 by human IFN-α. These results are consistent with those presented here recently. Some investigators (29–32) conducted clinical trials of their RA-containing ITs in patients with leukemias and solid tumors (melanoma and colorectal carcinoma). Although these therapeutic attempts were, in general, not successful in inducing prolonged remission of the treated patients, most patients (particularly leukemia patients) tolerated the IT therapy well. However, several changes may be needed to achieve better therapeutic efficacy. One necessary change will be to use a cocktail of effective antitumor ITs and another will be to use appropriate in vivo potentiators of IT. The present results suggest that IFN and certain chemotherapeutic agents (e.g., DNR) may be used as such potentiators.

ACKNOWLEDGMENTS

We wish to thank H. Tsai and J. Prendergast for their skillful technical assistance and Dr. J. Krasner, D. Ovak, and C. Zuber for their help in the preparation of the manuscript.

REFERENCES


Synergistic Potentiation of In vivo Antitumor Activity of Anti-Human T-Leukemia Immunotoxins by Recombinant α-Interferon and Daunorubicin

Soichiro Yokota, Hideki Hara, Yi Luo, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/1/32

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.