Buthionine Sulfoximine Enhancement of Arsenic Trioxide-Induced Apoptosis in Leukemia and Lymphoma Cells Is Mediated via Activation of c-Jun NH₂-Terminal Kinase and Up-regulation of Death Receptors

Duo Chen, Rosemarie Chan, Samuel Waxman, and Yongkui Jing

Division of Hematology/Oncology, Department of Medicine, Mount Sinai School of Medicine, New York, New York

Abstract

The mechanism of apoptosis induced by treatment with As₂O₃ alone or in combination with buthionine sulfoximine (BSO) was studied in NB4, U937, Namalwa, and Jurkat cells. As₂O₃ at concentrations <2 μmol/L induced apoptosis in NB4 cells and Namalwa cells but not in U937 and Jurkat cells. As₂O₃-induced apoptosis in NB4 cells and Namalwa cells correlated with increase of H₂O₂ and caspase activation without activation of c-Jun NH₂-terminal kinase (JNK). BSO (10 μmol/L) depleted the reduced form of intracellular glutathione without inducing apoptosis but synergized with 1 μmol/L As₂O₃ to induce apoptosis in all four cell lines. This synergy correlated with JNK activation. Treatment with As₂O₃ plus BSO, but not with As₂O₃ alone, increased the levels of death receptor (DR) 5 protein and caspase-8 cleavage. The JNK inhibitor SP600125 inhibited the increase in DR5 protein and attenuated apoptosis induced by treatment with As₂O₃ plus BSO. These observations suggest that a DR-mediated pathway activated by JNK is involved in apoptosis induced by treatment with As₂O₃ plus BSO. (Cancer Res 2006; 66(23): 11416-23)

Introduction

As₂O₃ treatment induces clinical remission in acute promyelocytic leukemia (APL) patients (1–3). Mechanism studies suggest that both apoptosis and partial differentiation induction account for the therapeutic effect of As₂O₃ in APL patients (4, 5). Although clinical trials using As₂O₃ in other forms of leukemia, lymphoma, and solid tumors have been initiated, therapeutic effects as in APL have not been observed (6–8). Combination of other agents with As₂O₃ has shown improvement in therapeutic effectiveness in vitro and in vivo (9–12). As₂O₃ at low concentrations (1–2 μmol/L) selectively induces apoptosis in APL-derived NB4 cells and in some lymphoma cells, whereas other leukemia cells and solid tumor cells are insensitive to As₂O₃-induced apoptosis (13–15). As₂O₃ induces apoptosis in APL cells through a mitochondria-mediated pathway resulting from the accumulation of H₂O₂ (10, 16, 17). Leukemia and lymphoma cells with lower intracellular levels of reduced glutathione (GSH) are much more sensitive to As₂O₃-induced apoptosis than cells with higher levels (10, 18). Agents with the ability to decrease intracellular GSH levels enhance As₂O₃-induced apoptosis (10, 19, 20). Among the agents that enhance As₂O₃-induced apoptosis, buthionine sulfoximine (BSO) is the most effective (17, 21, 22).

In the present study, the apoptotic induction ability and mechanism of As₂O₃ alone and in combination with BSO were compared in two leukemia and two lymphoma cell lines in vitro. The data indicate that As₂O₃ induces apoptosis at low concentrations in NB4 and Namalwa cells, but not in U937 and Jurkat cells, through a H₂O₂-mediated pathway. This pathway can be inhibited by the antioxidant N-acetylcysteine (NAC). However, As₂O₃ in combination with BSO synergistically induced apoptosis in all the cell lines tested primarily through activation of c-Jun NH₂-terminal kinase (JNK), which up-regulated death receptor (DR) 5 and the caspase-8-mediated pathway.

Materials and Methods

Reagents. As₂O₃ (0.1%) solution was kindly supplied by Cell Therapeutic, Inc. (Seattle, WA) BSO, ethidium bromide (EB), acridine orange (AO), NAC, catalase, and SP600125 were purchased from Sigma Chemical Co. (St. Louis, MO). Z-VAD-FMK and Z-IETD-FMK were obtained from Calbiochem (La Jolla, CA). Antibodies to Bcl-2 and poly(ADP-ribose) polymerase (PARP) were obtained from Roche Diagnostics Corp. (Indianapolis, IN). Caspase-3 and caspase-8 are from BD Biosciences (San Diego, CA). DR5 and DR4 were from Axxora (San Diego, CA). Bid, JNK, p38, extracellular signal-regulated kinase (ERK), and phosphorylated ERK (p-ERK) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phosphorylated JNK (p-JNK), phosphorylated p38 (p-p38), and IκBα are from Cell Signaling Technology (Beverly, MA).

Cell lines. U937 and NB4 leukemia cells were cultured in RPMI 1640 supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, 1 mM/L t-glutamine, and 10% heat-inactivated fetal bovine serum (FBS). Namalwa and Jurkat human lymphoma cells were cultured in RPMI 1640 adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM/L HEPES, 1.0 mM/L sodium pyruvate, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% FBS. Cells in logarithmic growth were seeded at 1 × 10⁶ per mL for studies.

Quantitation of apoptotic cells. Apoptotic cells were examined morphologically after staining with AO and EB (10) and by using fluorescence-activated cell sorting (FACS) after staining with Annexin V-FITC (23). For morphologic analysis, briefly, 1 μL of stock solution containing 100 μg/mL AO and 100 μg/mL EB was added to 25 μL cell suspension. The apoptotic cells (i.e., those showed nuclear shrinkage and blebbing) and apoptotic bodies were analyzed with the aid of a fluorescence microscopy. The percentage of apoptotic cells was calculated after counting total 300 cells. For FACS analysis, about 5 × 10⁵ to 10 × 10⁵ cells were washed twice with PBS and then labeled with Annexin V-FITC and propidium iodide (PI) in medium-binding reagent according to the Annexin V-FITC apoptosis detection kit instruction provided by the manufacturer (Oncogene, Cambridge, MA). Fluorescent signals of FITC and PI were detected respectively at 518 nm and at 620 nm on FACS (Becton Dickinson, San Jose, CA). The log of Annexin V-FITC fluorescence was

Requests for reprints: Yongkui Jing, Division of Hematology/Oncology, Department of Medicine, Mount Sinai School of Medicine, Box 1178, One Gustave L. Levy Place, New York, NY 10029-6547. Phone: 212-241-6775; Fax: 212-996-5787; E-mail: yongkui.jing@mssm.edu.

© 2006 American Association for Cancer Research. Cancer Res 2006; 66: (23). December 1, 2006 11416 www.aacrjournals.org Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 2006 American Association for Cancer Research.
displayed on the X axis and the log of PI fluorescence was displayed on the Y axis. The data were analyzed by the CellQuest program (Becton Dickinson). For each analysis, 10,000 events were recorded.

**Intracellular H2O2 production.** Intracellular H2O2 levels were determined, as reported previously, using 5,6-carboxy-2′,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR ref. 14). Briefly, 2 hours before ending the indicated treatment, 0.5 μmol/L DCFH-DA was added to the medium. The fluorescence intensity was measured by FACScan.

**Measurement of intracellular GSH.** Intracellular GSH contents were measured using a Glutathione Assay kit (Calbiochem, San Diego, CA). In brief, 5 × 10⁶ cells were homogenized in 5% metaphosphoric acid using a Teflon pestle (Racine, WI). Particulate matter was separated by centrifugation at 4,000 × g. The supernatant solution was used for GSH measurement according to the manufacturer’s instructions. The GSH content was expressed as nmol/10⁶ cells.

**Western blot analysis.** Cells were centrifuged, washed with cold PBS, and lysed on ice for 30 minutes in radioimmunoprecipitation assay buffer (1% PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) containing protease and phosphatase inhibitors [1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na3VO4, 5 mmol/L NaF, and 1× protease inhibitor (Boehringer Mannheim GmbH, Mannheim, Germany)]. Protein concentrations were determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Total protein (50 μg) was electrophoresed on 8% to 12% SDS polyacrylamide gels and transferred to nitrocellulose membranes (Amersham, Piscataway, NJ). After incubating with 5% nonfat milk for 1 hour, the membranes were incubated with the primary antibody indicated overnight at 4°C, washed with TBS (pH 6.8) and Tween 20 (TBS-T) thrice, incubated with secondary antibody for an hour at room temperature, and washed with TBS-T thrice. The immunocomplex was visualized by enhanced chemiluminescence Western blotting detection reagents (Amersham Biosciences, Buckinghamshire, United Kingdom).

**Statistics.** Data were analyzed for statistical significance using the Student’s t test (Microsoft Excel, Microsoft Corp., Seattle, WA). Differences were considered significant at *P* < 0.05.

**Results**

**As2O3 at concentrations below 2 μmol/L induces apoptosis in NB4 and Namalwa cells but not in Jurkat and U937 cells.** The apoptotic induction ability of As2O3 in NB4, U937, Namalwa, and Jurkat cells after treatment at concentrations of 1 to 2 μmol/L for 1 to 3 days was compared. As2O3 induced apoptosis at 1 μmol/L in NB4 cells but not in Namalwa, Jurkat, and U937 cells (Fig. 1A). When As2O3 concentration was increased to 2 μmol/L, apoptotic cells were observed in both NB4 and Namalwa cells but not in Jurkat and U937 cells (Fig. 1B). These results as well as those in previous reports indicate that As2O3 selectively induces apoptosis in some but not all leukemia and lymphoma cells (14, 15, 17).

**As2O3-induced apoptosis correlates with increase of H2O2 accumulation and caspase-3 activation among the four cell lines.** Several pathways have been reported to mediate As2O3-induced apoptosis (14, 21, 24, 25) and some factors involved in these pathways have been examined here. As shown in Fig. 2A, As2O3 at 2 μmol/L induced PARP cleavage and decreased procaspase-3 levels in NB4 and Namalwa cells. These findings correlate with apoptotic induction (Fig. 1A). The protein levels of Bel-2, ERK, JNK, and p38 were not changed by As2O3 treatment in any of these cell lines even to those where it induced apoptosis. ERK, p38, and JNK activation was investigated by comparing the levels of their phosphorylated forms to their nonphosphorylated ones. p-ERK was detected in all of the cell lines, but the levels of its phosphorylated form were not changed by As2O3 treatment.

**Figure 1.** As2O3 induces apoptosis at lower concentrations in NB4 and Namalwa cells than in U937 and Jurkat cells. The percentage of apoptotic cells was determined with the aid of a fluorescence microscope after staining with AO and EB. A, cells were treated with 1 μmol/L As2O3 for 1 to 3 days. B, cells were treated with 2 μmol/L As2O3 for 1 day. **P < 0.001 compared with control cells**.
BSO synergizes with As$_2$O$_3$ to induce apoptosis by activating both JNK and caspases. NB4, U937, Namalwa, and Jurkat cells were treated with either 1 μmol/L As$_2$O$_3$ or 10 μmol/L BSO alone or in combination for 24 or 48 hours (Fig. 4A). Neither BSO nor As$_2$O$_3$ alone significantly induced apoptosis in these cell lines after treatment at this short time period. However, BSO in combination with As$_2$O$_3$ synergistically induced apoptosis in all of these lines. BSO depleted intracellular GSH levels in all of these

Figure 2. As$_2$O$_3$ induces PARP cleavage and H$_2$O$_2$ accumulation, but not JNK phosphorylation, in NB4 and Namalwa cells. A, Western blot analysis of PARP, procaspase-3, Bcl-2, ERK, JNK, and p38. The cells were treated with 2 μmol/L As$_2$O$_3$ for 24 hours. B, H$_2$O$_2$ levels. The cells were treated with 2μmol/L As$_2$O$_3$ for 24 hours and H$_2$O$_2$ levels were measured by FACS after staining with DCFH-DA as described in Materials and Methods. Dark line, treated with As$_2$O$_3$; light line, without treatment. The peak shift to right means an increase of H$_2$O$_2$ amount. C, NAC and Z-VAD-FMK inhibit As$_2$O$_3$-induced apoptosis in NB4 and Namalwa cells. NB4 and Namalwa cells were treated for 24 hours with 2 μmol/L As$_2$O$_3$ alone or after pretreatment with 10 mmol/L NAC or 50 μmol/L Z-VAD-FMK for 4 hours. Apoptotic cells were detected using FACS after staining with Annexin V-FITC.

Figure 3. As$_2$O$_3$ induces apoptosis and PARP cleavage, but not JNK phosphorylation, in a dose-dependent pattern in NB4 cells but not in U937 cells. A, cell growth inhibition. Both NB4 and U937 cells were treated at the indicated concentrations for 24 hours. Cell number was determined with the aid of a hemocytometer; B, apoptotic cells. Both NB4 and U937 cells were treated with As$_2$O$_3$ at the indicated concentrations for 24 hours. The percentage of apoptotic cells was determined with the aid of a fluorescence microscope after staining with AO and EB. C, Western blot analysis of PARP, caspase-3, Bcl-2, ERK, JNK, and p38. Both cell types were treated with As$_2$O$_3$ at the indicated concentrations for 24 hours.
cell lines (Fig. 4B). BSO in combination with \( \text{As}_2\text{O}_3 \) induced PARP cleavage and decreased procaspase-3 levels in all of these cell lines (Fig. 4C). None of the treatments altered the levels of Bcl-2 protein (Fig. 4C). \( \text{As}_2\text{O}_3 \) and BSO treatment strongly induced level of p-JNK and weakly induced level of p-p38 in all cell lines (Fig. 4D).

U937 and Jurkat cells were treated with 1 \( \mu \text{mol/L} \) \( \text{As}_2\text{O}_3 \) in combination with 10 \( \mu \text{mol/L} \) BSO for 6 to 72 hours to further investigate the induced apoptotic pathways. \( \text{As}_2\text{O}_3 \) plus BSO significantly induced apoptosis in both cell lines after 48 hours of treatment (Figs. 4A and 5A). Time course of the protein levels of PARP, procaspase-3, p-JNK, and p-p38 were determined in both cell lines after treatment with \( \text{As}_2\text{O}_3 \) in combination with BSO (Fig. 5B). PARP and procaspase-3 cleavages were correlated with increased levels of p-JNK in both cell lines. p-p38 was significantly increased in U937 cells but not in Jurkat cells. These data suggest that both caspase- and JNK-activated pathways may contribute to \( \text{As}_2\text{O}_3 \) plus BSO–induced apoptosis.

\( \text{H}_2\text{O}_2 \) levels were measured in U937 cells after treatment with \( \text{As}_2\text{O}_3 \) plus BSO. As shown in Fig. 5C, \( \text{H}_2\text{O}_2 \) increased in U937 cells.

**Figure 4.** Effects of \( \text{As}_2\text{O}_3 \) combined with BSO on apoptotic induction and specific protein levels. A, apoptotic cells. Cells were treated with and without 1 \( \mu \text{mol/L} \) \( \text{As}_2\text{O}_3 \), 10 \( \mu \text{mol/L} \) BSO, and their combination for 24 hours for NB4 and Namalwa cells and for 48 hours for U937 and Jurkat cells. The percentage of apoptotic cells was determined with the aid of a fluorescence microscope after staining with AO and EB. B, GSH levels. The cells were incubated in the absence and presence of 10 \( \mu \text{mol/L} \) BSO for 48 hours. GSH levels were measured using GSH detection kits as described in Materials and Methods. C, PARP, caspase-3, and Bcl-2 levels. D, ERK, p38, and JNK as well as their phosphorylated forms. Cells were incubated with and without 1 \( \mu \text{mol/L} \) \( \text{As}_2\text{O}_3 \), 10 \( \mu \text{mol/L} \) BSO, and their combination for 24 hours for NB4 and Namalwa cells and 48 hours for U937 and Jurkat cells. The level of each protein was detected using specific antibodies as described in Materials and Methods.
after treatment with As$_2$O$_3$ plus BSO. NAC or catalase blocked H$_2$O$_2$ accumulation (Fig. 5C) but not apoptotic induction in U937 cells (Fig. 5D). Moreover, NAC and catalase did not block JNK activation in U937 cells after treatment with As$_2$O$_3$ plus BSO (data not shown). These results suggest that increased H$_2$O$_2$ production is not necessary in As$_2$O$_3$ plus BSO–induced apoptosis.

DR5 is increased in apoptotic cells after treatment with As$_2$O$_3$ in combination with BSO. DR4 and DR5 protein levels were measured in U937 cells after treatment with As$_2$O$_3$ or BSO alone and in combination. DR4 was highly expressed in U937 cells and its level was not increased after treatment by either agent alone or in combination (Fig. 6A). DR5 was weakly expressed in U937 cells and its level was induced after treatment with As$_2$O$_3$ plus BSO but not after treatment with either agent alone. Correlated with the DR5 induction, procaspase-8 and Bid protein levels were cleaved in U937 cells after treatment with As$_2$O$_3$ plus BSO but not after treatment with either agent alone. These data suggest that DR5, but not DR4, participate in As$_2$O$_3$ plus BSO–induced apoptosis. Recently, it has been found that DR5 levels can be increased by JNK activation (26). To investigate a possible connection between JNK activation and DR5 induction in As$_2$O$_3$ plus BSO–treated cells, JNK inhibitor SP600125 was used. PARP and procaspase-8 cleavages, increased p-JNK, and DR5 protein levels after treatment with As$_2$O$_3$ plus BSO were inhibited after the addition of SP600125 (Fig. 6B). These data suggest that activated JNK increases DR5 protein levels, which in turn mediates As$_2$O$_3$ plus BSO–induced apoptosis.

Caspase inhibitor, Z-VAD-FMK, and JNK inhibitor, SP600125, inhibited apoptosis induced by As$_2$O$_3$ in combination with BSO. The requirement of caspase activation or JNK activation in combination treatment–induced apoptosis was evaluated by using a JNK inhibitor, SP600125, and a caspase inhibitor, Z-VAD-FMK. Both agents significantly inhibited As$_2$O$_3$ plus BSO–induced apoptosis in U937 cells (Fig. 6C). These results suggest that both
caspase- and JNK-mediated pathways participate in As$_2$O$_3$ plus BSO–induced apoptosis.

Discussion

As$_2$O$_3$ is an apoptosis inducer in many kinds of cancer cell lines \textit{in vitro} and acts in some leukemia and lymphoma cells at very low concentrations (10, 14, 15, 17, 24, 27, 28). Among the leukemia and lymphoma cells, APL and B-cell lymphoma cells are more sensitive to As$_2$O$_3$-induced apoptosis than other types of leukemia and lymphoma cells (14, 15, 17). One of the reasons to account for this selectivity has been thought to be, at least partially, due to lower levels of cellular GSH (10, 29). However, Jurkat cells containing lower levels of GSH than NB4 cells (Fig. 4B) are not sensitive to As$_2$O$_3$-induced apoptosis at the low concentration (1 μmol/L; Fig. 1B), suggesting that other factors may compensate for the lower levels of GSH and account for their insensitivity to As$_2$O$_3$ treatment. Because As$_2$O$_3$ induces apoptosis at low concentrations through a H$_2$O$_2$-mediated pathway (Fig. 2B and C), one possibility might be higher cellular levels of glutathione peroxidase and glutathione-S-transferase (14, 23), which scavenge H$_2$O$_2$ catabolism. Because these enzymes use GSH as a substrate to catalyze H$_2$O$_2$, depletion of GSH will decrease the activities of these enzymes and in turn sensitize the cells to As$_2$O$_3$-induced apoptosis.

![Figure 6. DR5 up-regulation, caspase-8 activation, and apoptotic induction in U937 cells after treatment with As$_2$O$_3$ plus BSO are inhibited by JNK inhibitor SP600125.](www.aacrjournals.org)
Many agents, such as green tea, ascorbic acid, phosphatidylinositol 3-kinase (PI3K)/Akt inhibitor, and BSO enhance As2O3-induced apoptosis (10, 19, 30–32). One common mechanism of these agents that enable them to synergize with As2O3 seems to be due to their ability to decrease intracellular GSH levels. It has been found that the enhanced effect of ascorbic acid on As2O3-induced apoptosis correlated with the depletion of GSH in myeloma cells and As2O3 in combination with ascorbic acid has been put into clinical trials (19, 33–35). Among these agents that depleted intracellular GSH, BSO is the most effective. BSO has been found to enhance As2O3-induced apoptosis in many types of hemopoietic and solid tumors (10, 17, 22, 36). Consistent with previous reports, BSO significantly depleted intracellular GSH levels (Fig. 4B) and enhanced As2O3-induced apoptosis in both As2O3-sensitive and As2O3-insensitive leukemia and lymphoma cells (Fig. 4A). These data suggest that As2O3 plus BSO might be an effective treatment for leukemias and lymphomas.

Many mechanisms have been proposed to be involved in As2O3-induced apoptosis [e.g., production of reactive oxygen species (ROS) and activation of caspase through both mitochondrial-dependent and mitochondrial-independent pathways; refs. 14, 16, 37, 38]. Recently, it has been found that JNK and mitogen-activated protein kinase are involved in As2O3-induced apoptosis (21, 39–41). By using NB4 and Namalwa cells which are particularly sensitive to As2O3-induced apoptosis at low concentrations (Fig. 1), we have found that H2O2 production (Fig. 2B), but not JNK and p38 activation (Figs. 2A and 3C), is correlated with As2O3-induced apoptosis. As2O3 induced H2O2 accumulation and caspase-3 activation in NB4 and Namalwa cells (Fig. 2A and B). This apoptotic effect was blocked by the antioxidant, NAC, and by the caspase inhibitor, Z-VAD-FMK (Fig. 2C). As2O3 at this concentration did not activate JNK and p38 (Fig. 2A). These results suggest that ROS-mediated signaling is a main pathway of As2O3-induced apoptosis at low concentrations.

Unlike the apoptotic mechanism described above, PARP and caspase-3 cleavages in these cell lines after treatment with As2O3 plus BSO were correlated with an increase of p-JNK (Figs. 4D and 5B). Because the JNK inhibitor SP600125, but not antioxidants NAC and catalase, inhibited the apoptosis induced by combination of As2O3 and BSO (Figs. 5A and 6C), it seems that JNK activation contributes to As2O3 plus BSO-induced apoptosis through a H2O2-independent pathway. Recently, it has been found that activated JNK increases the cellular levels of DRs (26). By comparing the DR4 and DR5, it was found that DR5, but not DR4, was induced after treatment of As2O3 plus BSO but not with each agent alone (Fig. 6A). Up-regulation of DR5 correlated with caspase-8 activation, JNK activation, and apoptotic induction in U937 cells (Fig. 6B). Inhibition of JNK by SP600125 decreased DR5 up-regulation and apoptotic induction in U937 cells treated with As2O3 plus BSO (Fig. 6B and C). Nuclear factor-κB (NF-κB) has been found to contribute to the activation of JNK and up-regulation of DR5 (26). NF-κB activity is inhibited by IκBα and it has been found that the IκBα protein was regulated by As2O3 treatment (42, 43). We observed that IκBα was degraded by the combination treatment but not either agent alone (Fig. 6A). The IκBα degradation is correlated with JNK activation. These results suggest that As2O3 plus BSO may induce apoptosis through a JNK-regulated DR-mediated pathway due to activation of NF-κB.

Acknowledgments

Received 1/31/2006; revised 8/15/2006; accepted 9/22/2006.

Grant support: NIH grant R01CA93533.

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.

We thank Dr. William Scher for the critical reading of the article.

References


Cancer Res 2006; 66: (23), December 1, 2006 14122 www.aacrjournals.org Cancer Research


Buthionine Sulfoximine Enhancement of Arsenic Trioxide-Induced Apoptosis in Leukemia and Lymphoma Cells Is Mediated via Activation of c-Jun NH2-Terminal Kinase and Up-regulation of Death Receptors

Duo Chen, Rosemarie Chan, Samuel Waxman, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/23/11416

Cited articles
This article cites 43 articles, 22 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/23/11416.full.html#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
/content/66/23/11416.full.html#related-urls

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.