Establishment of a Melphalan-resistant Rhabdomyosarcoma Xenograft with Cross-Resistance to Vincristine and Enhanced Sensitivity following Buthionine Sulfoximine-mediated Glutathione Depletion


Departments of Pediatrics [M. C. R, E. I... H. S. F.], Pathology [S. H. R., D. D. B., H. S. F.], and Medicine [G. B. E.], Duke University Medical Center, Durham, North Carolina 27710; The Johns Hopkins Oncology Center, Baltimore, Maryland 21205 [O. M. C.]; Department of Biochemistry, Cornell University Medical College, New York, New York 10021 [O. W. G.]; and Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101 [J. K. H.]

ABSTRACT

A melphalan-resistant human rhabdomyosarcoma xenograft, TE-671 MR, was established in athymic mice by serial melphalan treatment of the parent xenograft, TE-671, at the 10% lethal dosage (LD50); significant resistance was evident after ten passages of the tumor. TE-671 MR demonstrated a doubling time of 3.5 days and a latency period to 1000-mm3 tumors of 27.5 days. The glutathione level of TE-671 MR was 2.36 μmol/g tumor, wet weight, 2-fold higher than the parent line. The glutathione S-transferase activity of TE-671 MR was 117.8 μmol/min/mg protein, essentially unchanged from the parent line. Although TE-671 MR demonstrated cross-resistance to vincristine, dot blot analysis did not reveal an elevated expression of mdrl mRNA in the resistant line. TE-671 MR demonstrated a 9.7-day growth delay following treatment with melphalan at the LD50 (compared to 20.9 days for the parent line). Treatment with L-buthionine-SR-sulfoximine (BSO) resulted in increased sensitivity to melphalan subsequently administered at 50% of the LD50 (melphalan alone, growth delays of 3.7 and 4.6 days in duplicate trials; melphalan plus BSO, growth delays of 7.2 and 9.8 days). Sensitivity to melphalan equal to that of the parent line TE-671 was not achieved, however. Treatment with BSO did not result in significantly enhanced sensitivity to subsequently administered vincristine (50% of the LD50) (vincristine alone, growth delays of 6.8 and 6.9 days in duplicate trials; vincristine plus BSO, growth delays of 10.9 and 7.5 days). These results suggest that generation of melphalan resistance may be associated with development of cross-resistance to vincristine; this resistance may be associated with (although not necessarily mediated by) glutathione elevation; this resistance may be partially overcome by BSO-mediated depletion of glutathione.

INTRODUCTION

Melphalan is a bifunctional alkylating agent with a broad spectrum of activity against human neoplasms, including ovarian carcinoma, neuroblastoma, rhabdomyosarcoma, and medulloblastoma (1–4). However, since resistance to this alkylator frequently develops in patients with initially responsive tumors, evaluation of the mechanisms mediating drug resistance is an important goal offering the possibility of defining modulations useful in reversing and/or bypassing this resistance. Reported mechanisms of resistance to melphalan include elevation of tumor glutathione levels, altered drug transport, and enhanced repair of melphalan-induced DNA interstrand cross-links (5–14). Laboratory generation of drug resistance provides the opportunity to define the mechanisms modulating resistance to a specific antineoplastic agent. However, this approach has been more successful with antimitotobolites and antitumor agents than with alkylating agents (15). Furthermore, those alkylator-resistant human neoplasms available for analysis have generally been generated by in vitro drug exposure with few studies utilizing in vivo therapy (15).

TE-671 is a well-characterized human cell line and xenograft initially reported to be derived from a cerebellar medulloblastoma (16) but now known to be the subline of a rhabdomyosarcoma cell line, RD, established in the same laboratory 8 years earlier (17–20). TE-671 demonstrates marked sensitivity to melphalan in cell culture and in s.c. and i.e. xenografts in athymic nude mice (21, 22). In an effort to identify and modulate resistance of TE-671 to melphalan, we have serially treated and repassed TE-671 growing s.c. in athymic mice with this alkylator. We now report the establishment of a melphalan-resistant (and vincristine-cross-resistant) human rhabdomyosarcoma xenograft, TE-671 MR, and the partial restoration of drug sensitivity when glutathione is depleted by treatment of the whole animal with BSO, an inhibitor of glutathione biosynthesis (23).

MATERIALS AND METHODS

Animals

Male or female athymic BALB/c mice (nu/nu genotype, 6 weeks or older) were used for all in vivo studies and were maintained as described previously (24).

Parent Xenograft (TE-671)

TE-671, a subline of the rhabdomyosarcoma-derived continuous cell line RD (16–20) growing as s.c. xenografts was used in melphalan and vincristine therapy studies and also to initiate the melphalan-resistant xenograft, TE-671 MR. The characterization and transplantation of TE-671 have been described previously (16, 25). Passage numbers for TE-671 relate to the number of passages following the introduction of the cultured cell line into athymic mice.

Establishment of a Melphalan-resistant Human Rhabdomyosarcoma

TE-671 MR was established by serial treatment of animals initially bearing s.c. TE-671 with melphalan (administered as a single i.p. injection in 90 ml/m2 of a 17% dimethyl sulfoxide solution) at a dosage of 100% of the calculated LD50 (71 mg/m2). Following regression and subsequent regrowth of the xenografts, homogenerate prepared from the least responsive tumors was injected into the next generation of animals for similar melphalan treatment. Passage numbers for TE-671 MR begin at the point when TE-671, the parent xenograft, was first exposed to melphalan. Xenograft transplantation was performed as described previously with an inoculation volume of 30 μl (26).

Received 12/1/88; revised 8/1/89; accepted 9/12/89.

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 Supported by NIH Grants CA11898, CA43722, CA44460, CA23099, DK26912, NS20023, and NS00958; ACS Grant CH403; and Bristol-Myers Grant 100-R18.

2 To whom requests for reprints should be addressed, at P. O. Box 2916, Department of Pediatrics, Duke University Medical Center, Durham, NC 27710.

3 The abbreviations used are: i.e., intracranial; BSO, buthionine-SR-sulfoximine; T - C, difference in days between the median time for the tumors of treated (T) and control (C) animals to reach a volume 5 times greater than the volume at the time of treatment; GST, glutathione S-transferase; LD50, 10% lethal dose; GSH, glutathione.

Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 1989 American Association for Cancer Research.
Drugs for Therapy Studies

1-Buthionine-SR-sulfoximine (M, 222.3) (Sigma Chemical Co., St. Louis, MO) was given by i.p. injection (2.5 mmol/kg) at a concentration of 22.5 mg/ml in 0.9% NaCl solution at 12-h intervals for seven doses. Concomitant with these injections, the animals also consumed acidified sterile drinking water (pH 3.0) containing 20 mM BSO. Melphalan, M, = 305, provided by the Burroughs-Wellcome Co. (Research Triangle Park, NC), was administered as a single i.p. dose in a volume of 90 ml/m. The doses were 71 and 36 mg/m2 in 17% dimethyl sulfoximide, which are 100 and 50%, respectively, of the calculated LD10, as determined previously (26). Vincristine (M, 923) (Bristol-Myers Co., New York, NY) was administered as a single i.p. injection in a volume of 90 ml/m2. The doses were 9.8 and 4.9 mg/m2 in 0.9% NaCl solution, which are 100 and 50%, respectively, of the 10% calculated LD10 (27).

Tumor Measurements

The s.c. tumors were measured as described previously (26).

Glutathione Assay

Total glutathione was determined by the method of Tietze (28) and Griffith (29) with minor modifications. Groups of untreated animals bearing s.c. TE-671 and TE-671 MR were used for GSH measurements. Animals bearing TE-671 MR tumors and treated with BSO (1.25 and 2.5 mmol/kg i.p. for seven doses at 12-h intervals concomitant with a 20-mM p.o. regimen as described above) were also assessed for GSH levels. Animal sacrifice, tumor preparation, and GSH analysis were performed as described previously (30).

Glutathione S-Transferase Measurements

GST measurements were performed using the assay of Habig et al. (32) with minor modifications. Groups of untreated animals bearing s.c. TE-671 and TE-671 MR xenografts were used for GST measurements. Xenografts were homogenized in a Brinkman polytron in 0.1 M potassium phosphate buffer/1 mM EDTA, pH 7.4, and handled as described previously (22).

Tumor-doubling Time and Latency

Xenograft-doubling time and latency time to development of 1000-mm3 tumors were determined as described previously (27).

Chromosomal Analysis

Chromosomal analyses of s.c. TE-671 (passage 54) and TE-671 MR (passage 19) xenografts were performed as described previously (25, 33).

Level of Expression of mdr1 mRNA

Extraction of total RNA and selection of polyadenylated RNA was performed as described previously (34). Tumors used were passage 24 TE-671 and passage 20 TE-671 MR xenografts.

PMRD1 was obtained from Dr. I. B. Roninson, Genetics Institute, Chicago, IL. The insert was cleaved from the plasmid using SacI and BamHI. Plasmid pAL containing complementary DNA insert to b-actin was obtained from Dr. D. W. Cleveland, Johns Hopkins University, Baltimore, MD. Both pAl and the mdr1 fragment were radiolabeled by nick translation. Dot blots on nitrocellulose filters were first hybridized to 32P-mdrl (34) and exposed to Cronex film. The blots were dehybridized by boiling in water for 5 min and then rehybridized to 32P-pAI to confirm equal loading of mRNA samples.

Tumor Therapy (TE-671 MR)

Quantitation of Resistance to Melphalan. Groups of ten randomly assigned mice were treated with melphalan (71 or 36 mg/m2) or vehicle when the median tumor volume exceeded 200 mm3. Animals were matched for tumor size, and all animals received vehicle or melphalan on the same day.

Quantitation of Stability of Melphalan Resistance. TE-671 MR, se-
Table 1: Comparison of s.c. xenografts TE-671 and TE-671 MR

<table>
<thead>
<tr>
<th>Response to melphalan</th>
<th>TE-671*</th>
<th>TE-671 MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>71 mg/m²</td>
<td>T-C (days)</td>
<td>20.9</td>
</tr>
<tr>
<td>Regressions</td>
<td>10/10*</td>
<td>1/7</td>
</tr>
<tr>
<td>36 mg/m²</td>
<td>T-C (days)</td>
<td>12.2-16.6</td>
</tr>
<tr>
<td>Regressions</td>
<td>9/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Growth delays of 17.3 and 14.4 days, respectively (Table 2). P > 0.05 for all tumor regressions in comparison to untreated controls.

| Values for TE-671 response to melphalan and GSH were described previously and are shown here for comparative purposes (22, 30).* |

| Number of animals with regressing tumors/number of surviving treated animals. |

<table>
<thead>
<tr>
<th>Delaying time (days)</th>
<th>2.9 ± 0.6*</th>
<th>3.5 ± 0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/g)</td>
<td>1.11 ± 0.15</td>
<td>2.36 ± 0.33</td>
</tr>
<tr>
<td>GST (mmol/min/mg protein)</td>
<td>134.94 ± 40.46</td>
<td>117.81 ± 24.24</td>
</tr>
<tr>
<td>mdr1 expression Low level of mdr1 mRNA No elevation in expression level compared with TE-671</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD.

DISCUSSION

Despite appreciable cytotoxicity of melphalan against a wide spectrum of human neoplasms, including neuroblastoma, rhabdomyosarcoma, ovarian carcinoma, and medulloblastoma (1-4), development of resistance to this (and other alkylating agents) frequently thwart attempts at curative intervention for these malignancies. It is unlikely that a single agent or class of agents will prove curative for human neoplasms, due to the initial presence or emergence of drug-resistant cells (35). Although definition of cross-resistance and collateral sensitivity MR to treatment with melphalan in mice treated or not treated with BSO is summarized in Table 3 and Fig. 1. Previously published data (30) for treatment of TE-671 with melphalan with and without BSO are also shown for comparative purposes.

Melphalan alone (36 mg/m²) produced growth delays of 3.7 and 4.6 days in duplicate trials in TE-671 MR. None of the xenografts (0 of 17) showed any tumor regressions. BSO alone produced no significant growth delay and no tumor regressions (0 of 20). Melphalan (36 mg/m²) plus BSO significantly prolonged the growth delay over melphalan alone with growth delays of 7.2 and 9.8 days, respectively (P < 0.005 and P = 0.002, respectively). Six of 19 xenografts demonstrated tumor regressions.

**Tumor Therapy with Vincristine.** The response of TE-671 MR and TE-671 to treatment with vincristine in mice treated or not treated with BSO is summarized in Table 4. Vincristine alone (4.9 mg/m²) produced growth delays of 6.8 and 6.9 days in duplicate trials in TE-671 MR. None of the xenografts (0 of 18) showed any tumor regressions. BSO alone produced no significant growth delay and no tumor regressions. Vincristine (4.9 mg/m²) plus BSO did not significantly prolong the growth delay over vincristine alone, with growth delays of 10.9 and 7.5 days, respectively (P > 0.01). None of the xenografts (0 of 15) demonstrated tumor regressions.

**Treatment of TE-671-bearing mice with vincristine alone (4.9 mg/m²) produced growth delays of 15.7 and 14.3 days. Treatment of mice bearing TE-671 tumors with vincristine plus BSO resulted in growth delays no different from those seen with vincristine alone (11.2 and 15.7 days, respectively) (P > 0.01).**

<p>| Table 2: Response of s.c. xenografts TE-671 and TE-671 MR to vincristine |
|-------------------|-------|--------|</p>
<table>
<thead>
<tr>
<th>Vincristine dose (mg/m²)</th>
<th>TE-671</th>
<th>TE-671 MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.8</td>
<td>17.3</td>
<td>3/8</td>
</tr>
<tr>
<td>4.9</td>
<td>14.3</td>
<td>1/10</td>
</tr>
</tbody>
</table>

P < 0.002 for all growth delays (T - C) in comparison to untreated controls. P > 0.05 for all tumor regressions in comparison to untreated controls.

Number of animals with regressing tumors/number of surviving treated animals.

Table 3: Response of s.c. TE-671 and TE-671 MR xenografts to melphalan ± i-buthionine-SR-sulfoximine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TE-671*</th>
<th>TE-671 MR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan (36 mg/m²)</td>
<td>12.6</td>
<td>9/9</td>
</tr>
<tr>
<td>BSO</td>
<td>0/9 (NS)</td>
<td></td>
</tr>
<tr>
<td>Melphalan + BSO</td>
<td>17.2</td>
<td>9/9</td>
</tr>
<tr>
<td>BSO</td>
<td>7.2</td>
<td>0/10 (NS)</td>
</tr>
</tbody>
</table>

Experiment 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TE-671*</th>
<th>TE-671 MR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan (36 mg/m²)</td>
<td>16.6</td>
<td>8/9</td>
</tr>
<tr>
<td>BSO</td>
<td>4.6</td>
<td>0/8 (NS)</td>
</tr>
<tr>
<td>Melphalan + BSO</td>
<td>22.9</td>
<td>8/9</td>
</tr>
<tr>
<td>BSO</td>
<td>9.8</td>
<td>4/9 (NS)</td>
</tr>
</tbody>
</table>

Experiment 2

| Data for TE-671 were presented previously and are shown here for comparative purposes (30).* |

| Experiment regimens: BSO (2.5 mmol/kg) i.p. every 12 h for seven doses concomitant with a 20 mm solution in acidified sterile water for 72 h, followed by melphalan (0.50 LD₅₀) in a single i.p. dose. BSO was started approximately 3 days before the tumors reached 200 mm³. |

**Number of animals with regressing tumors/number of surviving treated animals.**

P < 0.001 for all experiments unless otherwise designated. NS, not significant (P > 0.01).

**Growth delay for melphalan + BSO is significantly longer than melphalan alone:** P = 0.01 and 0.001 for Experiments 1 and 2, respectively.

**Growth delay for melphalan + BSO is significantly longer than melphalan alone:** P ≤ 0.005 and 0.002 for Experiments 1 and 2, respectively.
Fig. 1. Groups of nine to ten randomly assigned mice with s.c. TE-671 MR tumors were treated with BSO alone (2.5 mmol/kg every 12 h by i.p. injection for seven doses plus concomitant administration of an additional solution in acidified drinking water), melphalan alone (50% of the 10% lethal dose administered by i.p. injection), BSO plus melphalan, or an equivalent volume of drug vehicle.

Table 4 Response of s.c. TE-671 and TE-671 MR xenografts to vincristine ± i-buthionine-SR-sulfoximine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TE-671*</th>
<th>TE-671 MR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine (4.9 mg/m²)</td>
<td>15.7</td>
<td>6.8 (P = 0.002)</td>
</tr>
<tr>
<td>BSO</td>
<td>0.3 (NS)</td>
<td>0.4 (NS)</td>
</tr>
<tr>
<td>Vincristine + BSO</td>
<td>11.2</td>
<td>0/10 (NS)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine (4.9 mg/m²)</td>
<td>14.3</td>
<td>6.9</td>
</tr>
<tr>
<td>BSO</td>
<td>−0.3 (NS)</td>
<td>0.6 (NS)</td>
</tr>
<tr>
<td>Vincristine + BSO</td>
<td>15.7*</td>
<td>7.5*</td>
</tr>
</tbody>
</table>

* Experiment regimen: BSO (2.5 mmol/kg) i.p. every 12 h for seven doses concomitant with an additional solution in acidified drinking water for 72 h, followed by vincristine (0.50 LD₅₀) in a single i.p. dose. BSO was started approximately 3 days before the tumors reached 200 mm³.

In children treated shortly following diagnosis (3). However, the striking failure of melphalan to produce responses (1 of 13) in patients with recurrent rhabdomyosarcoma highlights the profound clinical impact produced by the development of resistance to this alkylating agent. Generation of a melphalan-resistant xenograft derived from TE-671 was accordingly attempted in order to facilitate identification (and modulation) of the mechanism(s) mediating resistance.

TE-671 MR, generated by sequential treatment and repassage of the melphalan-sensitive parent xenograft TE-671, demonstrates relative melphalan resistance, as well as cross-resistance to vincristine. Previous investigations have demonstrated resistance to melphalan to be multifactorial; identified mechanisms include decreased melphalan uptake (5, 6), increased intracellular glutathione levels protective of critical cellular targets (7-10), increased cellular detoxification (11), and enhanced capacity to repair damaged DNA (12-14). These studies, performed in murine and human ovarian human neoplasms, provided a foundation for our studies designed to identify and modulate resistance in TE-671 MR.

The current studies with TE-671 MR demonstrated a 2-fold elevation of glutathione levels compared to the parent xenograft TE-671. BSO pretreatment significantly increased sensitivity to melphalan with growth delays increasing from 3.7 and 4.6 days (in duplicate trials) to 7.2 and 9.8 days (duplicate trials). Growth delays typical of the parent xenograft (12.6-16.6 days) were not obtained, however. Although it is tempting to attribute the melphalan resistance of TE-671 MR to the elevation of glutathione, all studies (including ours) documenting partial restoration of sensitivity to melphalan-resistant cells by the use of BSO-mediated glutathione depletion must recognize that similar glutathione depletion can further sensitize cells with normal melphalan sensitivity (36), raising the possibility that the mechanisms accounting for increased melphalan resistance may be independent of the elevated levels of glutathione (which may then represent an epiphenomenon). Clarification of these possibilities will await measurement of glutathione-melphalan adducts in resistant and sensitive cell lines (37). Nevertheless, modulation of glutathione metabolism resulting in intratumoral patterns may help to bypass drug resistance by incorporation into therapeutic regimens of additional active antineoplastic agents. Identification of mechanisms mediating drug resistance would facilitate definition of modulations effective in restoring sensitivity, with consequent improvements in clinical outcome. Although the use of cell lines and tumors with laboratory-generated drug resistance may provide the means to further an understanding of resistance to antineoplastic agents, this approach has been successful mainly with nonalkylating agents (such as the antimetabolites) (15). Although human cell lines resistant to alkylating agents have been generated in vitro, few reports of in vivo drug exposure regimens resulting in resistance have been described (15).

TE-671, initially reported to be derived from a human medulloblastoma but now known to be a subline of the human rhabdomyosarcoma cell line RD (16-20), demonstrates considerable sensitivity to melphalan, mirroring clinical experience in the treatment of rhabdomyosarcoma (with 12 of 13 responses
depletion of this thiol may represent an effective, albeit non-specific, approach to enhancing the activity of melphalan and possibly other alkylating agents in resistant neoplasms.

Although several laboratory reports have demonstrated that GST activity is elevated in tumor cells resistant to antineoplastic agents [cyclophosphamide (38), chlorambucil (39, 40), and mitomycin (41)], the role of GST in clinical drug resistance is not yet defined. Since there was no difference in total GST activity (measured with 1-chloro-2,4-nitrobenzene as substrate) between TE-671 and TE-671 MR, it is not likely that changes in GST activity account for melphalan resistance. The results do not, however, preclude the possibility that elevation of a specific isomer of GST occurs.

Karyotypic analysis showed no cytological evidence of gene amplification. No new structural abnormalities, double minute chromosomes, abnormally banded regions, or homogeneously staining regions were seen in TE-671 MR. This result was anticipated since alkylator resistance, unlike resistance to antimetabolites, is rarely associated with gene amplification (42).

The finding of cross-resistance to vincristine in TE-671 MR is preceded by the report of Horton et al. (34), which showed similar cross-resistance to vincristine developing in a human rhabdomyosarcoma xenograft with primary (laboratory-generated) resistance to melphalan. Since melphalan resistance in TE-671 MR is associated with increased glutathione levels and reversed by glutathione depletion, the effect of glutathione depletion on vincristine resistance was also investigated. These studies failed to demonstrate significantly increased vincristine activity in glutathione-depleted TE-671 MR tumors. Enhancement of vincristine activity was also not seen following glutathione depletion in the parent xenograft, TE-671. As noted previously glutathione depletion does enhance melphalan activity in both TE-671 (30) and TE-671 MR (present results). The studies with vincristine suggest that cellular glutathione levels are not an important modulator of vincristine cytotoxicity in these tumors and suggest further that increased glutathione content cannot be the only difference between TE-671 MR and the parent strain. In an effort to identify additional differences which might account for vincristine cross-resistance, we examined the expression of the mdrl gene in TE-671 and TE-671 MR; no differences were found. This result is consistent with a previous report indicating that primary resistance to alkylating agents was not mediated by a P-glycoprotein multiple drug resistance (mdr) phenotype in human cervical carcinoma cell lines (43). At present the molecular mechanism of vincristine cross-resistance in TE-671 MR remains unknown.

The role of TE-671 MR as a model for studying melphalan resistance (and the cross-resistance/collateral sensitivity to other agents) in human neoplasia is presently undefined. Mechanisms of resistance demonstrated in vitro may not be representative of mechanisms of resistance occurring clinically. For example, despite clear evidence of a marked increase in dihydrofolate reductase in tumor cell lines with in vitro-generated methotrexate resistance (44), to our knowledge even minimal elevation of tumor cell dihydrofolate reductase has not been shown in any patient failing to respond to methotrexate (45). Analysis of human neoplasms with in vivo-generated resistance to alkylating agents such as in TE-671 MR has only rarely been described (15), and it is possible that the mechanisms of resistance to melphalan of TE-671 MR may be the same as those mediating clinical resistance. Studies are in progress in our laboratory attempting to establish cell lines and xenografts derived from patient medulloblastoma specimens with de novo or clinically acquired alkylator resistance. Such studies may allow comparison of clinical and in vivo-generated drug resistance.

ACKNOWLEDGMENTS

Editorial assistance on this manuscript was rendered by Ann Tammariz.

REFERENCES

23. Griffith, O. W. Mechanism of action, metabolism and toxicity of buthionine

Downloaded from cancerres.aacjournals.org on April 15, 2017. © 1989 American Association for Cancer Research.


Establishment of a Melphalan-resistant Rhabdomyosarcoma Xenograft with Cross-Resistance to Vincristine and Enhanced Sensitivity following Buthionine Sulfoximine-mediated Glutathione Depletion


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/49/24_Part_1/6917

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.