A apoptotic killing of B-chronic lymphocytic leukemia tumor cells by allicin generated in situ using a rituximab-alliinase conjugate

Fabian D. Arditti,1 Aharon Rabinkov,1 Talia Miron,1 Yair Reisner,2 Alain Berrebi,3 Meir Wilchek,1 and David Mirelman1

1Departments of Biological Chemistry and 2Immunology, Weizmann Institute of Science; and 3Department of Hematology, Kaplan Medical Center, Rehovot, Israel

Abstract
Allicin, a highly active component from freshly crushed garlic, is produced upon the reaction of the small molecular weight molecule alliin, with the enzyme alliinase (EC 4.4.1.4). Because allicin was shown to be toxic to various mammalian cells in vitro, we devised a novel approach for the therapy of B-cell malignancies based on site-directed generation of allicin. Alliinase was conjugated to the monoclonal antibody rituximab, which recognizes the CD20 antigen, and the resulting conjugate was targeted to CD20+ B chronic lymphocytic leukemia (B-CLL) and other B-cell lymphomas. Upon addition of alliin, allicin was formed in situ, killing the CD20+ tumor B cells via apoptosis. Following a 72-hour treatment, an 85% and 96% reduction was observed in the number of viable B-CLL and EBV-transformed B cells, respectively. Using the human/mouse radiation chimera for the evaluation of allicin, we showed a significant reduction in the number of recovered B-CLL, mantle cell lymphoma, or EBV-transformed B cells. We conclude that our system offers a new powerful and less toxic therapy for B-CLL and other B-cell malignancies. Furthermore, combining alliinase with the appropriate monoclonal antibody may extend the application of this approach to other conditions in which the elimination of a specific cell population is desired. [Mol Cancer Ther 2005;4(2):325–31]

Introduction
Allicin, the biologically active compound from garlic produced upon the interaction of the enzyme alliinase (alliin lyase; EC 4.4.1.4) with its substrate, alliin (S-allyl-L-cysteine sulfoxide; ref. 1), has become in recent years an object of interest in light of its antiproliferative and cytotoxic effect (2–5). Alliinase and alliin are enclosed in different compartments within the garlic clove, so that intact garlic cloves do not contain allicin but rather its precursor, alliin. Following crushing of the garlic clove, alliin is exposed to alliinase, which results in the generation of allicin. Allicin is an unstable, short-lived hydrophobic molecule that penetrates biological membranes with ease and reacts rapidly with free thiol groups (6–8). Therefore, it disappears from the circulation within a few minutes after injection (9). This explains why the versatile and valuable attributes of allicin, including its potent antibiotic and cytotoxic effects, were shown thus far mostly in vitro (3, 4, 6). Nevertheless, we have recently shown that allicin can kill tumor cells if it is generated on the surface of target cells. This was achieved using as a delivery system monoclonal antibodies which have emerged as important therapeutic agents against a number of malignancies (10–14). In our previous study, we conjugated the enzyme alliinase to a monoclonal antibody (mAb) directed against ErbB2, which is expressed on the surface of several tumor cells (15). After the mAb-alliinase conjugate was bound to target tumor cells, the substrate, allicin, was added. In the presence of allicin, the tumor-anchored alliinase produced allicin, which killed tumor cells expressing ErbB2 whereas normal tissues were unharmed. In the present study, we show that the same principle can also be applied to kill malignant B cells expressing either low or high levels of CD20 [human B chronic lymphocytic leukemia (B-CLL) cells or B-cell lymphomas, respectively]. Targeting of alliinase to CD20+ tumor cells was achieved by its conjugation to the humanized anti-CD20 mAb rituximab (Rituxan Mabthera), which is extensively in use in the clinic, mainly for non-Hodgkin lymphomas. Our results show that allicin generated in situ by the targeted rituximab-alliinase conjugate following the addition of allicin was able to kill CD20+ B-CLL cells as well as two other human B-cell lymphoma lines, both in vitro and in vivo.

Materials and Methods

Chemicals
3-(2-Pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP), DTT, and pyridoxal 5′-phosphate were purchased from Sigma (St. Louis, MO). Alliin was synthesized as described (16). Allicin was prepared by applying synthetic allicin onto an immobilized alliinase column (17). Alliin concentration was determined as described (18). 2-Nitro-5-thiobenzoate was synthesized as described (19).

Received 8/24/04; revised 11/23/04; accepted 12/1/04.

Grant support: Grants from Morross Institute for Cancer Research at the Weizmann Institute of Science and from N. Minzly, England.

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.

Requests for reprints: David Mirelman, Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel. Phone: 972-8-9344511; Fax: 972-8-9468256. E-mail: david.mirelman@weizmann.ac.il

Copyright © 2005 American Association for Cancer Research.

Mol Cancer Ther 2005;4(2). February 2005
Biochemical Analysis

Protein concentration was measured at 280 nm, using £280 = 77,000 mol/L•cm⁻¹ (£280 0.1% = 1.54) for alliinase and 210,000 mol/L•cm⁻¹ for purified mAb (£280 0.1% = 1.4). The number of SPDP residues on the modified proteins was determined according to ref. 20. Purification of alliinase was done as described (21). Quantitative determination of alliin and alliin was done by high-performance liquid chromatography as well as by reaction with 2-nitro-5-thiobenzoate (18, 22).

Enzymatic Activity

Alliinase activity was determined by the 2-nitro-5-thiobenzoate method as described (18). The resulting enzymatic activity of the Rituxan-Alliinase conjugate was developed to assay the activity of the enzyme conjugates in a cell-free system. Wells were coated with protein A (5 µg/mL) and quenched with bovine serum albumin (1% in PBS). Binding of PBS-diluted mAballiinase conjugates to the wells was done at 24°C for 2 hours. After removal of unbound conjugates, the activity of adsorbed conjugated alliinase was determined by using 2-nitro-5-thiobenzoate (3 × 10⁻⁴ mol/L) containing alliin (0.6 × 10⁻³ mol/L) in 50 mmol/L phosphate buffer, and 2 mmol/L EDTA (pH 7.2). The decrease in A412 nm was determined after 60 minutes. Wells to which no enzyme was added served as control.

Protein Modifications

SPDP modification of proteins was done as described (15). The modified alliinase was stored in 50% glycerol at -20°C. Modification of rituximab (Hofmann-La Roche, Basel, Switzerland) was done with a 10 molar excess of SPDP over the mAb. The degree of modification was 2.5 to 2.9 SPDP residues/mAb molecule. The modified rituximab was stored in PBS at 4°C. Preparation of rituximab-alliinase conjugate was done as described (15). Conjugates were stored at -20°C in 50% glycerol.

Preparation of Human/Mouse Chimera

Chimeric mice for in vivo experiments were prepared as described (23). Briefly, BALB/c mice were exposed to split lethal total body irradiation. One day later, irradiated mice were injected with bone marrow cells from non-obese diabetic/severe combined immunodeficient mouse donors through the tail vein. All strains of mice were from the Weizmann Institute Animal Breeding Center (Rehovot, Israel). All the animal studies and protocols were approved by the Weizmann Institutional Animal Care and Use Committee.

Tumor Cells and Cultures

B-CLL peripheral blood mononuclear cells (PBMC) were obtained from heparinized whole blood drawn from five patients at Rai stage IV with their informed consent. Blood cells were subjected to Ficoll density gradient centrifugation and the mononuclear cells were diluted to the desired concentration before their injection into mice (100 × 10⁶ cells/mouse, by an i.p. injection) or in vitro (10⁶ cells/mL/well, in 24-well plates) treatment. CD20⁺ mantle cell lymphoma (MCL) line was kindly provided by G. Inghirami (NYU, New York, NY). A CD20⁺ EBV immortalized B-cell line is routinely grown in our lab. Because both of these cell lines induce rapid proliferating tumors, cells were injected i.p. at 4 × 10⁶ cells/mouse or cultured in 24-well plates at 2 × 10⁵ to 3 × 10⁵ cells/mL/well. Cells were recovered by peritoneal lavages or sampled from the culture well and analyzed for the presence of the below mentioned surface markers by fluorescence-activated cell sorting (FACS) analysis.

Fluorescence-Activated Cell Sorting Analysis

The viability of cells isolated from peritoneal washes of engrafted mice was determined 7 to 10 days after the inoculation of human cells by trypan blue dye exclusion or following incubation with a mixture of monoclonal antibodies labeled with different fluorochromes (see below) by FACS analysis. Incubation with the antibodies was for 20 minutes at 4°C. After washing off the antibodies, two or three color analyses were done using a FACScan analyzer (Becton Dickinson, Franklin Lakes, NJ). The following labeled anti-human antibodies were used to recognize specific surface molecules: CD19-FITC (Pan B cells/B-CLL), CD3-PercP (Pan T cell), and CD45-PercP (pan-leukocytes) were purchased from Becton Dickinson; CD20 FITC (B cells, some B-CLL) and CD5-PE (T cells, B-CLL tumor B cells) were purchased from Dako, Glostrup, Denmark.

Detection of Apoptosis by Annexin Staining

Samples from in vitro cultures as well as cell samples from peritoneal washes were incubated with a mixture of selected monoclonal antibodies as described above. After washing off the unbound free antibodies, samples were incubated with 5 µL of Annexin-AFC (Becton MedSystems, Vienna, Austria) or Annexin-APC (PharMingen, San Diego, CA) in annexin buffer for 10 minutes at room temperature. Subsequently, unbound annexin was washed out and samples were analyzed by FACS as described above.

Competitive Inhibition of Conjugate Binding by Rituximab

To examine the CD20 antigen specificity of allicin killing by the rituximab-alliinase conjugate, target cells were preincubated for 30 minutes at 4°C with rituximab alone in excess (20 µg/mL/10⁶ cells). Following preincubation, unbound rituximab was washed off and the cells were incubated with the rituximab-alliinase conjugate at different concentrations under the same conditions as with rituximab alone. After washing off unbound conjugate, the cells were transferred to 6- or 24-well plates and incubated in the presence or absence of the substrate alliin. Cells were assayed for annexin staining and for surface markers by FACS analysis at different time intervals.

Statistical Analysis

Statistical significance was established by the Student’s t test. In vitro experiments were repeated three to five times and in vivo experiments were done thrice. Results are shown as mean ± SD.
Results

Allicin Induces In vitro Apoptosis in a Dose-Dependent Manner

In order to examine whether allicin could override the antiapoptotic barriers in tumor B-CLL cells, we cultured B-CLL PBMC from B-CLL patients at stage IV in the presence of increasing concentrations of pure allicin. As shown in Fig. 1, a dose-dependent apoptotic killing (Annexin-V⁺) of B-CLL tumor cells was induced by allicin following 48 hours in culture. The percentage of apoptotic B-CLL cells increased from background levels (6.6 ± 0.6 %, mean ± SD.) in the absence of allicin to reach a plateau of around 95% at allicin concentrations of 6.5 to 8.1 μg/mL.

Killing of CD20⁺ Tumor B Cells In vitro by Allicin Generated In situ by Rituximab-Alliinase Conjugate

In contrast to most B-cell malignancies, the CD20 antigen is expressed at low levels on B-CLL cell surface. This fact could explain the failure of rituximab alone to induce a considerable response in patients with this malignancy (11, 24–26). Therefore, we used rituximab as a targeting device to direct the enzyme alliinase to the surface of CD20⁺ B-CLL cells, where it can generate cytotoxic allicin upon addition of the substrate, alliin. PBMC (10⁶ cells/well) of all five patients tested. Following removal of unbound conjugate from background levels (6.6 ± 0.6 %, mean ± SD.) in the absence of allicin to reach a plateau of around 95% at allicin concentrations of 6.5 to 8.1 μg/mL.

Additional, we tested the effect of increasing doses of allicin generated by increasing amounts of conjugate on B-cell lymphomas expressing high levels of CD20. As found for B-CLL, allicin generated in situ induced a strong dose-dependent killing of CD20⁺ EBV immortalized cells as well as of MCL cells (Fig. 2A).

In a separate experiment, we evaluated the response of tumor cells to increasing amounts of allicin generated by increasing concentrations of alliin using a constant amount of conjugate (4 μg/well). We found that the number of apoptotic B-CLL cells increased with the concentration of alliin (100 μg/mL). Thus, after the addition of 40, 80, and 160 μg/well of alliin, the number of apoptotic cells increased from a background level of 20% to 61%, 81.7%, and 93.7%, respectively (Fig. 2B). We also examined the progression of the effect mediated by allicin on tumor B-cell surface over time. As seen in Fig. 3, the number of apoptotic (CD19⁺ Annexin-V⁺) tumor B cells, exposed to allicin generated in situ, increased over time, with a highest number of apoptotic cells at the latest time point tested (72 hours). In contrast, when a fixed amount of pure allicin (not generated in situ) was added to the cells, there was no increase in cell death with time. Furthermore, cell death was not observed when conjugate treatment was not followed by the addition of the substrate alliin. In addition, cells that do not contain the CD20 antigen, such as the neuroblastoma cell line LAN-1 or T cells, were not targeted nor damaged by the rituximab-alliinase conjugate (data not shown).

Alliinase Activity Is Specifically Delivered to CD20⁺ Cells by Rituximab

The specificity of the rituximab-alliinase conjugate was investigated by preincubation of the target cells with excess of free rituximab to block the binding sites of the subsequently added mAb-conjugated alliinase. As shown in Fig. 4, preincubation of B-CLL or EBV immortalized cells with free rituximab blocked subsequent binding of the conjugate to tumor B cells and prevented their killing.

Tumoroidal Effect of Targeted Allicin In vivo

In order to assess the in vivo antitumor potential of rituximab-alliinase targeted allicin in a preclinical animal model, we used the human/mouse radiation chimera, which allows engraftment of human tissues, including hematopoietic tumors, in mice (11, 23, 24, 27–29). Chimeric
mice were prepared as described (28, 30). Seven to ten days after i.p. infusion of either 100 × 10⁶ fresh B-CLL PBMC, 4 × 10⁶ MCL cells, or 4 × 10⁶ EBV immortalized B cells, mice were divided into three treatment groups, each consisting of six mice treated as follows: control (only PBS), conjugate only (80 μg/mouse), and conjugate (80 μg/mouse) followed 1 day later by alliin (3 mg/mouse, twice a day, during 3 days). As shown in Fig. 5, a single injection of the conjugate followed by repeated administration of alliin induced a significant reduction in the amount of recovered B-CLL from 63 × 10⁶ ± 12.5 × 10⁶ to 16.5 × 10⁶ ± 3.5 × 10⁶ (n = 3, P < 0.03), MCL from 5.3 × 10⁶ ± 9.6 × 10⁶ to 12 × 10⁶ ± 3.2 × 10⁶ (n = 3, P < 0.03), and EBV immortalized B cells from 75.4 × 10⁶ ± 8.7 × 10⁶ to 15.6 × 10⁶ ± 2.6 × 10⁶ (n = 3, P < 0.01). These effects were observed in peritoneal cell samples taken 24 hours after the last alliin injection.

**Discussion**

We have recently reported the specific and efficient killing of ErbB2+ tumor cells by allicin molecules that were generated in situ from alliin by a conjugate consisting of the enzyme alliinase and a monoclonal antibody specific against ErbB2 (15). In the present study, we show that generation of allicin molecules in situ by a rituximaballiinase conjugate can be used to kill leukemia/lymphoma cells expressing the CD20 cell surface molecule. Generation of allicin at the target tumor cell surface is catalyzed from the substrate Alliin by the enzyme alliinase, which was chemically ligated to the commercially available humanized anti-CD20 mAb, rituximab. Our in vitro and in vivo results show that following the addition of alliin, allicin was generated in situ by the rituximab-alliinase conjugate and this killed B-CLL tumor cells, which express low levels of CD20, as well as two B-cell lines (MCL and EBV transformed B-cell line), which express higher levels of this surface antigen. In contrast, CD20-negative cells like the neuroblastoma LAN-1 or T cells were not affected by the rituximab-alliinase conjugate.

Considering that purified B-CLL cells die shortly in culture if separated from their feeder cells (31–33), we used in our in vitro assays fresh unseparated PBMCs from five B-CLL patients, which contained >90% double positive CD19+CD5+ pathologic cells. These cells survive in culture in the absence of any manipulation for a period of at least 1 to 2 weeks (30). The humanized mAb rituximab is, at present, in extensive clinical use for the treatment of non-Hodgkin lymphoma, which express high levels of CD20 on the cell surface (34–36). In contrast to non-Hodgkin lymphoma and most tumor cells, B-CLL consists of nondividing cells that express low levels of CD20. Nevertheless, several studies have been conducted to analyze the efficacy of standard, escalated, or fractionated doses of rituximab in B-CLL. Overall responses were moderate and complete remission was only occasionally achieved (11, 24–26).

Although a number of studies reported the ability of rituximab to induce apoptosis by itself, the bulk of its cytotoxic activity is known to be mediated by the opsonization of the target cell, leading to antibody-dependent cell cytotoxicity and complement fixation. However, the immune status of the patient is crucial to allow an effective immune response and, very often,

---

**Table 1. Antitumor activity of allicin generated in situ by the rituximab-alliinase conjugate and alliin**

<table>
<thead>
<tr>
<th>B-CLL patients</th>
<th>Untreated</th>
<th>Rtx-all</th>
<th>Alliin</th>
<th>Rtx-all+alliin</th>
<th>% Tumor eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>11,547</td>
<td>11,267</td>
<td>11,547</td>
<td>4,817</td>
<td>58</td>
</tr>
<tr>
<td>#2</td>
<td>11,280</td>
<td>9,131</td>
<td>10,604</td>
<td>3,454</td>
<td>69</td>
</tr>
<tr>
<td>#3</td>
<td>11,079</td>
<td>11,331</td>
<td>N/T</td>
<td>4,727</td>
<td>57</td>
</tr>
<tr>
<td>#4</td>
<td>10,800</td>
<td>12,000</td>
<td>11,625</td>
<td>2,745</td>
<td>75</td>
</tr>
<tr>
<td>#5</td>
<td>12,000</td>
<td>12,000</td>
<td>14,800</td>
<td>1,600</td>
<td>87</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11,341 ± 410</td>
<td>11,145 ± 1,055</td>
<td>12,143 ± 1,585</td>
<td>3,468 ± 1,218</td>
<td>69 ± 11</td>
</tr>
</tbody>
</table>

**SS lymphoma**

SS1: 20,100 21,000 20,400 1,800 91
SS2: 24,300 24,300 20,700 1,440 94
Mean ± SD 21,950 ± 2,144 21,872 ± 2,129 1,988 ± 11,700 2,484 ± 1,509 88 ± 7

**EBV lymphoma**

MCL1: 16,935 17,072 13,683 2,755 84
MCL2: 25,500 25,500 26,500 5,500 78
MCL3: 16,800 15,600 18,800 3,600 79
Mean ± SD 19,744 ± 4,985 19,390 ± 5,342 19,661 ± 6,452 3,951 ± 1,406 80 ± 3

---

* B-CLL obtained from five patients in stage IV with their informed consent.
† This value was calculated as follows: [(untreated – (Rtx-All+alliin)) / untreated] × 100.
‡ SS lymphoma is an EBV immortalized human B cell line grown routinely in our laboratory and MCL is a cell line (Grantas) derived from human Mantle cell lymphoma.
patients are immunocompromised as a result of previous cycles of chemotherapy. Moreover, large amounts of the therapeutic antibody are required to counteract complement inhibitory molecules (i.e., CD55 and CD59), which are present on the surface of malignant B cells (37, 38).

In our study, we enhance the capacity of rituximab to cause tumor cell death by using it as a targeting element. The specificity of rituximab enabled us to anchor the enzyme alliinase to the cell surface of CD20⁺ tumor cells. Because CD20 is not internalized following its binding to rituximab (24, 35), the prolonged presence of the conjugate on the cell surface and repeated administration of high doses of the substrate alliin enabled the continuous in situ enzymatic generation of cytotoxic alliin molecules. Allicin, being a hydrophobic molecule easily, penetrates through membranes and reacts with thiols immediately afterwards (7, 8), thus targeting its effect only in the cells on which it is produced.

The in situ generation of allicin on the targeted cells overcomes the known problem of the short-lived allicin molecules in the circulation. Clinical trials will be needed, however, to verify the lack of toxicity of allicin in humans following its generation in situ. These trials should test also the immunogenicity of the conjugate as a new entity, although the humanized structure of rituximab and the fact that alliinase is a dietary protein that is “known” to the human immune system are expected to minimize unwanted reactivities.

Allicin killed tumor B cells by apoptosis, which could be detected by Annexin-V in in vitro assays. However, owing to annexin being known to occasionally bind nonspecifically to viable B cells in vivo (39), we evaluated our in vivo assays also by determining the amounts of CD19⁺CD45⁺ double positive cells recovered from the peritoneum of engrafted mice as well as by counting trypan-blue-positive versus -negative cells.

In Figure 2, A, conjugate dose dependence of tumor B-cell killing by in situ allicin production. Fresh B-CLL cells (○, ●) obtained from B-CLL patients or two other lymphoma B-cell lines, EBV immortalized B cells (△, ▲), and MCL cell line (○, ●) at 1 × 10⁶ cells/well were incubated with growing amounts (0, 0.25, 1.0, and 4.0 µg/mL) of rituximab-alliinase conjugate for 30 min. After the incubation, unbound conjugate was removed and culture medium with (filled symbols) or without (empty symbols) alliin (100 µg/well) was added. After 48 h in culture, aliquots were sampled and cells were counted and tested for the presence of CD19-FITC and Annexin-Cy5 by FACS analysis as described above. Points, mean (B-CLL, n = 3 for B-CLL samples from three different patients; MCL and EBV immortalized B-cell line, n = 3 for three independent experiments); bars, SD. B, killing of B-CLL cells by allicin production in situ also depends on the concentration of alliin. Fresh B-CLL cells (1 × 10⁶/well) obtained from B-CLL patients were incubated with or without 4 µg conjugate for 30 min. Unbound conjugate was washed out and then alliin was added at increasing amounts as described in the figure. Following 48 h of culture, aliquots were sampled and cells were counted and tested for CD19-FITC and Annexin-Cy5 staining by FACS. Columns, mean (n = 3); bars, SD.

In Figure 3, time dependence of B-CLL cell killing by allicin generated in situ. Fresh B-CLL cells (1 × 10⁶/well) obtained from B-CLL patients were incubated with either 3.25 µg allicin or 4 µg conjugate for 30 min. Only cells incubated with conjugate were washed and subsequently treated with growing amounts of alliin. Following 24 (●), 48 (■), and 72 (□) h of incubation, an aliquot was sampled from each treatment and tested for CD19-FITC and Annexin-Cy5 staining by FACS. Columns, mean (n = 3); bars, SD.
The combination of FACS analysis of peritoneal washes of mice from different treatment groups and trypan blue dye exclusion method allowed the quantitative analysis of the antitumor effect mediated by alliin generated in situ.

The main advantages of the mAb-alliinase conjugate over previously devised antibody-directed enzyme prodrug therapies, immunotoxins, or drug-conjugated antibodies are (a) the very low concentrations of alliinase-mAb conjugate needed for injection because the cytotoxic alliin molecules are continuously generated on the surface of the target cell following the repeated administrations of alliin; (b) the possibility to administer large amounts of the inert prodrug alliin, which is a natural ingredient of garlic; and (c) the selectivity of the alliin killing mechanism.

In order to exert their effect, conjugates of antibodies with toxins or cytotoxic drugs have to penetrate the target cell membrane either by internalization via the antigen receptor or by endocytosis. High concentrations of conjugates are needed because each antibody molecule carries only one or two toxin or drug molecules, and although they are capable of killing the targeted cells, they can also affect normal cells and cause them damage as well. The mode of action of alliin is entirely different: rather than interacting with DNA, alliin chemically modifies free SH (thiol) groups that are usually present in peptides or proteins. Its action with thiol containing proteins. Biochim Biophys Acta 1998;1379:233 –44.

Recently, it was shown that depletion of reduced glutathione due to long-lasting oxidative stress, the main advantages of the mAb-alliinase conjugate over conventional approaches. In addition, by using an appropriate antibody or ligand, the principle of this novel approach shown here for B-cell malignancies could be extended to other tumors as well as to other conditions in which the elimination of a specific population of cells is desired, such as autoimmunity, graft rejection, or graft versus host disease.

In conclusion, the in situ generated alliin technique could offer a more powerful and less toxic therapy than conventional approaches. In addition, by using an appropriate antibody or ligand, the principle of this novel approach shown here for B-cell malignancies could be extended to other tumors as well as to other conditions in which the elimination of a specific population of cells is desired, such as autoimmunity, graft rejection, or graft versus host disease.

**References**


Molecular Cancer Therapeutics

Apoptotic killing of B-chronic lymphocytic leukemia tumor cells by allicin generated *in situ* using a rituximab-alliinase conjugate

Fabian D. Arditti, Aharon Rabinkov, Talia Miron, et al.


Updated version Access the most recent version of this article at:
http://mct.aacrjournals.org/content/4/2/325

Cited articles This article cites 38 articles, 10 of which you can access for free at:
http://mct.aacrjournals.org/content/4/2/325.full#ref-list-1

Citing articles This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/4/2/325.full#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 AARC Publications Department at pubs@aacr.org.

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