Regional Treatment of Epidermal Growth Factor Receptor vIII-expressing Neoplastic Meningitis with a Single-Chain Immunotoxin, MR-1

Gary E. Archer, John H. Sampson,
Ian A. J. Lorimer, Roger E. McLendon,
Chien-Tsun Kuan, Allan H. Friedman,
Henry S. Friedman, Ira H. Pastan, and
Darell D. Bigner

Departments of Pathology [G. E. A., R. E. M., H. S. F., D. D. B.], Surgery (Neurosurgery) [G. E. A., J. H. S., A. H. F., H. S. F., D. D. B.], and Pediatrics [H. S. F.], Duke University Medical Center, Durham, North Carolina 27710; Ottawa Regional Cancer Centre, Ottawa, Ontario, K1H 8L6 Canada [I. A. J. L.]; and Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892-4225 [I. H. P.]

ABSTRACT

The incidence of neoplastic meningitis is on the rise. Neoplastic meningitis can result from a direct seeding of the neuraxis by primary brain tumors or by hematogeneous spread of systemic solid tumors. A frequent genetic alteration in primary brain tumors such as gliomas is an in-frame deletion in the epidermal growth factor receptor (EGFR) gene EGFRvIII, which brings together what were normally distant polypeptide sequences in the intact receptor. A novel glycinine is formed at the fusion junction, resulting in a unique and tumor-specific target. By using phage display, we have isolated a single-chain antibody specific for the EGFRvIII mutation and expressed it with a modified form of the Pseudomonas exotoxin to form the immunotoxin MR1scFvPE38KDEL (MR-1). The multiple dose toxicity and therapeutic efficacy of MR-1 immunotoxin were tested in an athymic rat model of neoplastic meningitis. The maximally tolerated doses in non-tumor-bearing rats were three doses of 3 μg each. For therapeutic studies, the target was a neoplastic meningitis induced by intrathecal inoculation of the EGFRvIII-expressing human glioma U87MG.ΔEGFR. A dose escalation study compared the survival of three equal doses of 1, 2, and 3 μg of MR-1 immunotoxin with saline or 3 μg of the control immunotoxin specific for the interleukin 2 receptor, anti-Tac. All animals treated with three doses of saline or 3 μg of anti-Tac died, with median survival of 7 and 10 days, respectively. There were 75% (six of eight) long-term survivors in the group treated with three doses of 1 μg and 57% (four of seven) long-term survivors in the groups treated with three doses of either 2 or 3 μg of MR-1 immunotoxin. None of the MR-1 immunotoxin-treated groups reached median survival by the termination of the study at 53 days. Therefore, median survival was estimated to be >53 days, resulting in an estimated increase in median survival of >657% compared with saline and 430% versus anti-Tac. Compartmental therapy with three doses of 2 μg of MR-1 immunotoxin is effective in the treatment of EGFRvIII-expressing neoplastic meningitis. This dose was found to have no clinical or histopathological effects on non-tumor-bearing animals. MR-1 immunotoxin is, therefore, considered specific and safe within its therapeutic window. Phase I clinical trials for tumors invading the intrathecal space that express the EGFRvIII target should be initiated.

INTRODUCTION

The intrathecal compartment provides a reservoir for tumor growth, resulting in the development of neoplastic meningitis, most likely due to a failure of systemically administered chemotherapy agents to reach a therapeutic level in the cerebrospinal fluid (1). Neoplastic meningitis can result from a direct seeding of the neuraxis by primary brain tumors such as medulloblastomas, or it may result from hematogeneous dissemination of systemic solid tumors such as breast and lung adenocarcinomas and melanomas. To overcome the limitations of systemic delivery of chemotherapeutic agents, direct delivery into the intrathecal space has been attempted successfully. However, because of the limited number of therapeutic agents approved for direct intrathecal injection, drug resistance, and tumor cell heterogeneity, the effective treatment of neoplastic meningitis is limited.

Immunotoxins are specific cytotoxic reagents constructed by linking a specific ligand, such as an antibody or growth factor ligand, with naturally occurring protein toxins. The toxins most commonly used are plant or bacterial toxins, which inhibit protein synthesis. Immunotoxins have the advantage of not being affected by tumor cell hypoxia, as are some radiolabeled MAb, and they are more efficient than MAb-drug conjugates (2). Improved methods of genetic engineering have allowed construction of smaller antigen-binding fragments. These single-chain antibody variable domains (scFv) can be fused to a protein toxin, resulting in a homogeneous product. Single-chain...
immunotoxins have an advantage over intact IgG antibodies chemically coupled to toxins. The smaller size of the scFv immunotoxins allows greater tumor penetration, which can lead to increased therapeutic efficacy (3).

Many immunotoxins have been made that recognize a wide variety of tumor-associated antigens. We have made a number of MAb s that recognize a deletion mutant of the EGFR, EGFRvIII. The in-frame deletion in the EGFR gene brings together what were normally distant polypeptide sequences in the intact receptor. At the fusion junction, a novel glycine is formed, resulting in a unique and tumor-specific target (4). EGFRvIII is expressed on the cell surface of 52% of all glioblastoma multiformes, 16% of non-small lung carcinomas, and 27% of breast carcinomas (5). In this study, we investigated an immunotoxin, MR-1, in which the anti-EGFRvIII scFv antibody was isolated by phage display and fused to a Pseudomonas exotoxin that was modified to remove the natural cell binding domain (PE38KDEL). MR-1 was tested for toxicity and therapeutic efficacy against a human EGFRvIII-expressing cell line in a neoplastic meningitis model in athymic rats using compartmental administration and was shown to have significant efficacy and no toxicity at therapeutically effective doses.

MATERIALS AND METHODS

Immunotoxins. The scFv domain of the MR1scFvPE38KDEL immunotoxin was isolated by phage display from an immunized mouse and fused to domains II and III of Pseudomonas exotoxin (6). The scFv of the MR-1 immunotoxin was shown by competitive binding analysis to be specific for cells expressing the EGFRvIII mutation (7).

Anti-TacFvPE38KDEL (anti-Tac) binds to the interleukin 2 receptor, which is not found on the U87MG.ΔEGFR cell line, and was used in these experiments as an immunotoxin control (8). The cytotoxic activity of these immunotoxins on the U87MG.ΔEGFR cells was determined by inhibition of protein synthesis. Target cells, 2 × 10^6 cells in 200 μl of complete medium, were incubated with various concentrations of immunotoxins, for 20 h in 96-well plates. Protein synthesis inhibition was measured after a 4-h [3H]leucine pulse (9).

Cell Line. The human glioblastoma cell line U87MG.ΔEGFR was obtained from W. Cavenee Ludwig Institute for Cancer Research, University of California at San Diego, La Jolla, CA; Ref. 10. The number of EGFRvIII receptors per cell was determined by quantitative flow cytometry to be 4.1 × 10^5 (11). Athymic mouse xenografts of U87MG.ΔEGFR were used as a source of material for initiation of neoplastic meningitis in athymic rats. Tumors were initiated s.c. in athymic mice by the injection of 10^7 cells.

Animal Model. Female athymic rats, Big:NIMRmu/mu, 5–6 months of age, were maintained in the Duke University Cancer Center Isolation Facility. Subarachnoid catheters were placed as described by Fuchs et al. (12), a modification of the method of Kooistra et al. (13). Briefly, rats were placed in a Kopf stereotactic frame (David Kopf Instruments, Tujunga, CA) with the neck flexed at 90°. A midline sagittal incision was made from the inion to the laminar arch of C1. The atlantooccipital membrane was exposed, and the underlying cisterna magna dura were opened. A PE-10 catheter (Intramedic; Clay Adams, Franklin Lakes, NJ) with a 5-0 stainless steel wire stylet was inserted into the subarachnoid space and passed along the posterior aspect of the spinal cord so that the tip rested in the lumbar region (8.5 cm). A loose knot was tied in the catheter and fixed with dental epoxy (Lang Dental Manufacturing Co., Chicago, IL). The catheter was passed through the skin lateral to the incision, and the wound was closed with surgical clips. Bolus injections of tumor cells or MR-1 immunotoxin into the intrathecal space were made through the catheter using a Hamilton syringe and injector equipped with a 30-gauge needle. All injections were followed with a 20-μl saline flush. U87MG.ΔEGFR neoplastic meningitis was initiated from xenografts growing s.c. in athymic mice. The tumors were harvested as described previously (14), cut into fine pieces, and dissociated into a single-cell suspension with 0.5% collagenase in a trypsinization flask for 2 h, stirring at room temperature. The cells were washed, and viable cells were isolated on a Ficoll density gradient. The cells were washed twice in PBS and resuspended at a concentration of 1.25 × 10^8 viable cells/ml. Forty μl (5 × 10^5) cells were injected via the indwelling subarachnoid catheter. The natural progression of untreated rats was a progressive loss of hindlimb motor function, followed by death. The median survival was 9 days.

RESULTS

Immunotoxins. In vitro cytotoxicity of the MR-1 immunotoxin showed a 50% inhibition of protein synthesis of 19.25 ng/ml against the human glioma EGFRvIII-expressing cells U87MG.ΔEGFR and >10,000 ng/ml for the non-expressing parent cell line U87MG. In vitro cytotoxicity of anti-Tac immunotoxin against both the EGFRvIII-expressing cell line U87MG.ΔEGFR and the non-expressing U87MG showed a 50% protein synthesis inhibition of >10,000 ng/ml.
Immunotoxin Treatment of EGFRvIII Neoplastic Meningitis

Three doses of 20 μg of MR-1 immunotoxin with saline-treated animals. All animals that died, hemorrhage was seen in one of six and one of two animals that died, as well as one of two animals given saline. Edema was found in one of six animals treated with 5 μg of MR-1 immunotoxin, respectively, as well as one of two animals treated with 2 and 3 μg, respectively. No other histological changes were seen in animals that survived the observation period of 45 days. In surviving animals treated with saline, hemorrhage and demyelination were found in one of eight and three of eight animals, respectively. A ventriculitis/meningitis was found in the one 1-μg animal and both of the 3-μg animals that died, as well as one of two animals that died in the saline control group, and is believed to be the cause of death in these animals. In MR-1 immunotoxin-treated animals that died, hemorrhage was seen in one of six and one of four animals treated with 5 and 15 μg, respectively. Necrosis was found in one of six animals treated with 5 μg. Demyelination was seen in two of two and one of three animals treated with 3 and 4 μg of MR-1 immunotoxin, respectively, as well as one of two animals given saline. Edema was found in one of one, two of three, and two of six animals receiving 1, 4, and 5 μg of MR-1 immunotoxin, respectively. On the basis of the survival data in non-tumor-bearing rats, we concluded that doses of MR-1 immunotoxin up to 3 μg were safe. MR-1 toxicity data are summarized in Table 1.

Intrathecal Treatment of EGFRvIII-positive Neoplastic Meningitis. The therapeutic efficacy of MR-1 immunotoxin against the EGFRvIII-expressing human glioblastoma U87MG.AEGFR was investigated in three separate experiments. In experiment 1, groups of tumor-bearing athymic rats were treated with three doses of 1, 2, or 3 μg of MR-1 immunotoxin, and survival was compared with saline-treated rats. The experiment was terminated after 180 days, and all surviving rats were termed LTSs. All of the saline-treated controls died, with a median survival of 12.5 days. Rats treated with three doses of 1 or 2 μg of MR-1 did not reach median survival before the termination of the experiment; therefore, the median survival was >180 days, resulting in an IMS of >1340% (P < 0.001). There were 50% (4 of 8) LTSs in the group treated with 1 μg and 60% (6 of 10) LTSs in the group of rats treated with 2 μg. Animals treated with three 3-μg doses had a median survival of 162.5 days, IMS of 1200% (P < 0.001) and 40% (4 of 10) LTSs (Fig. 1). Microscopic examination of the neuraxis of MR-1 immunotoxin-treated animals showed no tumor in the LTSs in the groups treated with three doses of either 1 or 3 μg. However, in one of the six LTSs treated with three doses of 2 μg, a small nest of tumor was found around the cauda equina.

In the second experiment, the 1-, 2-, and 3-μg doses of MR-1 immunotoxin were repeated, and survival was compared with a group of animals that received three doses of 3 μg of the control immunotoxin anti-Tac. The experiment was terminated after 157 days, and all surviving rats were termed LTSs. All of the animals treated with three doses of 3 μg of anti-Tac died with a median survival of 9 days. Rats treated with three 1-μg doses of MR-1 immunotoxin did not reach median survival by the end of the experiment, resulting in a median survival of >157 days and a corresponding IMS of >1644% (P = 0.008), with 55.5% (5 of 9) LTSs. Animals treated with three 2-μg doses of MR-1 immunotoxin did not reach median survival by the end of the experiment, resulting in a median survival of >157 days and an IMS of 1494%, (P = 0.008), with 50% (4 of 8) LTSs. Animals treated with three 3-μg doses of MR-1 immunotoxin had a median survival of 47 days, resulting in an IMS of 422% (P = 0.008), with 37.5% (3 of 8) LTSs (Fig. 2).

Histological examination of the neuraxis showed that one of four LTSs in the group treated with three 1-μg doses of MR-1 immunotoxin and one of five LTSs in the group treated with three 2-μg doses of MR-1 immunotoxin had microscopic tumors. None of the other LTSs in any of the MR-1 immunotoxin-treated groups had any evidence of tumor.

In the third experiment, the therapeutic efficacy of 1, 2, and 3 μg of MR-1 immunotoxin was compared with saline and three 3-μg doses of the control immunotoxin anti-Tac. The experi-

### Table 1 Histological findings in non-tumor-bearing rats

<table>
<thead>
<tr>
<th>MR-1 immunotoxin dose</th>
<th>Survival</th>
<th>Hemorrhage</th>
<th>Necrosis</th>
<th>Demyelination</th>
<th>Edema</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 μg</td>
<td>4/5</td>
<td>0/1</td>
<td>0/4</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>2 μg</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>3 μg</td>
<td>2/5</td>
<td>0/2</td>
<td>0/3</td>
<td>0/2</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>4 μg</td>
<td>1/4</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>5 μg</td>
<td>2/8</td>
<td>1/6</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>10 μg</td>
<td>2/4</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>15 μg</td>
<td>1/5</td>
<td>0/4</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>20 μg</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Saline</td>
<td>8/10</td>
<td>0/2</td>
<td>1/8</td>
<td>0/2</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

a Dead, died before day 42. Lived, killed on day 42.

b Cause of death, ventriculitis/meningitis.
ment was terminated after 53 days, and all surviving rats were termed LTSs. All animals treated with three doses of 1 and 2 μg of anti-Tac died, with median survival of 7 and 10 days, respectively. None of the rats treated with MR-1 immunotoxin had reached median survival at the termination of the experiment. Therefore, the median survival of all MR-1 immunotoxin treatment groups was >53 days, with IMS >657% versus saline and 430% versus anti-Tac. There were 75% (6 of 8) LTSs in the group treated with three doses of 1 μg of MR-1 immunotoxin had a median survival of 162.5 days (P < 0.001). MR-1 immunotoxin was given on days 3, 5, and 7 after tumor inoculation.

DISCUSSION

In this study, we investigated the compartmental delivery of an immunotoxin that reacts with the tumor-specific epitope of EGFRvIII for the treatment of neoplastic meningitis. Because of improved treatment of systemic solid tumors, the incidence of neoplastic meningitis is an increasing complication of breast and lung adenocarcinomas and melanomas, as well as a direct result of primary central nervous system tumors. The most likely cause of central nervous system relapse of solid tumors resulting in the development of neoplastic meningitis is a failure of tumoricidal agents, administered systemically, to achieve a therapeutic level in the intrathecal space (1). Once established, patients with neoplastic meningitis have a median survival of only 2–3 months (15, 16). In an effort to overcome the limitations of systemic delivery of chemotherapeutic agents, direct delivery into the intrathecal compartment, either through the lumbar cistern or an indwelling ventricular reservoir, is actively being investigated. Because of drug resistance, tumor cell heterogeneity, and a limited number of drugs that have been approved for intrathecal use, only limited success has been achieved. A continued search is on for reagents with greater tumor specificity.

The EGFR gene is often overexpressed in malignant gliomas and frequently rearranged to produce deletion mutations. Seven types of deletion mutations have been described. The most common type of deletion is the type III, in which an in-frame deletion of 801 bp in the extracellular domain brings together distant regions of the polypeptide chain (4). At the fusion junction, a novel glycine residue is formed, generating the tumor-specific epitope. There is a constituent activation of the tyrosine kinase of the EGFRvIII, which confers a ligand-independent transforming activity in vivo (10, 17, 18). The EGFRvIII protein is expressed on the cell surface of 52% of all gliomas, 16% of non-small cell lung carcinomas, and 27% of breast carcinomas, tumors that can seed the leptomeninges and cause neoplastic meningitis (5, 19). A panel of MAbs, which are specific for the EGFRvIII and do not cross-react with the wild-type receptor, have been developed in our laboratory (5).

Immunotoxins are a class of cytotoxic reagents, constructed by altering the receptor specificity of naturally occurring protein toxins by genetically deleting the general cell binding domain and replacing it with a ligand-specific moiety. The ligand specificity of the immunotoxin is most often directed by a MAb recognizing a tumor-associated antigen. Because immunotoxins tend to be larger molecules than other chemotherapeutics, compartmental administration is an ideal method of delivery for achieving high local concentrations. In addition, immunotoxins should not be effected by hypoxic conditions that reduce the effectiveness of radiation. Immunotoxins are also more efficient in cell killing with estimations of as few as one toxin molecule being required to kill a single tumor cell (2). Most chemotherapeutic drugs often require >10^5–10^6 moles...
cules/cell to cause cell death (2). Zovickian and Youle (20) were the first to use immunotoxins for the compartmental treatment of neoplastic meningitis.

For the construction of the MR-1 immunotoxin used in this study, the single-chain antibody variable domain was isolated by phage display and fused with domains II and III of *Pseudomonas* exotoxin A. The MR-1 immunotoxin had better cytotoxic activity than previously isolated intact IgG anti-EGFRvIII MAbs constructed by chemical conjugation to the modified *Pseudomonas* toxin (21). The choice of the scFv MR-1 immunotoxin for compartmental therapy was not based solely on its increased cytotoxicity. The smaller size of the MR-1 immunotoxin compared with the intact IgG anti-EGFRvIII chemically conjugated immunotoxins should achieve greater tumor penetration, resulting in an increased therapeutic effect. A comparison of the treatment benefits of an immunotoxin, LMB-1, constructed with an intact MAb, B3, which recognizes Le$^a$ and several closely related carbohydrate antigens and a scFv immunotoxin, LMB-7 (constructed from the murine MAb B3), showed that the scFv immunotoxin LMB-7 had a greater effect on overall survival and specifically, LTSs compared with the chemically conjugated LMB-1 immunotoxin (3, 22).

The MR-1 immunotoxin was effective in the treatment of EGFRvIII positive neoplastic meningitis. Three equal doses of 2 mg were effective in increasing median survival. Treatment with three doses of 3 μg of MR-1 immunotoxin was statistically significant compared with saline and control immunotoxin anti-Tac. Animals treated with three doses of saline or 3 μg of anti-Tac died with median survivals of 7 and 10 days, respectively. Median survival for all MR-1 immunotoxin treatment groups was >53 days. MR-1 immunotoxin was given on days 3, 5, and 7 after tumor inoculation.

**Fig. 2** Comparison of MR-1 immunotoxin treatment of EGFRvIII-positive neoplastic meningitis with the control immunotoxin anti-Tac. Median survival of anti-Tac-treated animals was 9 days. Animals treated with three doses of 1, 2, and 3 μg of MR-1 immunotoxin had median survivals of 143.5 days ($P = 0.008$), >157 days ($P = 0.003$), and 47 days ($P = 0.008$), respectively. MR-1 immunotoxin was given on days 3, 5, and 7 after tumor inoculation.

**Fig. 3** Comparison of MR-1 immunotoxin treatment with saline and control immunotoxin anti-Tac. Animals treated with three doses of saline or 3 μg of anti-Tac died with median survivals of 7 and 10 days, respectively. Median survival for all MR-1 immunotoxin treatment groups was >53 days. MR-1 immunotoxin was given on days 3, 5, and 7 after tumor inoculation.
significant in increasing median survival; however, the increases and percentage of LTSs were not as great as those seen with 1 or 2 µg. The 3-µg dose of MR-1 immunotoxin did not cause any clinical symptoms in non-tumor-bearing rats; however, there was some evidence of demyelination on histological examination of these animals. It may be that a total dose of 9 µg of MR-1 immunotoxin (3 × 3 µg) is reaching the upper limit of the therapeutic window, and although no clinical symptoms were seen in non-tumor-bearing rats, the addition of tumor burden may influence the clinical outcome. The therapeutic window for the MR-1 immunotoxin in rats may be somewhat narrow. However, because of an inability to accurately determine the pharmacokinetics in the rat model, it is impossible to determine the therapeutic window when treatment is designed for human trials. The design of human trials must address the issue of pharmacokinetics and the frequency of dose. Most likely, the human therapy would benefit from a constant infusion of MR-1 immunotoxin rather than the bolus dosing done in the rat model. Our use of this model was to prove that in vivo MR-1 immunotoxin was therapeutically effective at a nontoxic dosing. This represents the first therapeutic study with the tumor-specific immunotoxin MR-1. The tumor that was used to initiate the neoplastic meningitis was a human glioma, U87MG, that was transfected to express the EGFRvIII. Quantitative flow cytometry showed that the U87MG.AEGR cells expressed 4.1 × 10^5 EGFRvIII receptors/cell (12). Quantitative analysis of five human glioma biopsies showed a range of 2.7 × 10^5 to 6.8 × 10^5 in four of the five, and the fifth biopsy had 2.7 × 10^4 EGFRvIII receptors/cell (12). These data suggest that although the U87MG.AEGR has been artificially made to express the EGFRvIII, it is an accurate representation of a de novo EGFR-vIII-expressing human glioma. The U87MG.AEGR cells do express wild-type EGFR; however, it is unlikely that the MR-1 immunotoxin is exerting its therapeutic effect through the wild-type receptor because of the specificity of the MR-1scFv (8): (a) the scFv portion of the MR-1 immunotoxin has been shown to be specific for the EGFRvIII and will not cross-react with wild-type EGFR; and (b) the cytotoxicity of the MR-1 immunotoxin for the untransfected U87MG was >10,000 ng/ml (7). To test the specificity of the treatment effect, MR-1 treatment was compared with a control immunotoxin. The control immunotoxin chosen was the single-chain Fv immunotoxin anti-Tac. Anti-Tac immunotoxin is constructed with the same form of Pseudomonas exotoxin A (PE38KDEL) as the MR-1 immunotoxin. The anti-Tac immunotoxin is specific for the interleukin 2 receptor and required >10,000 ng/ml for cytotoxicity of the U87MG.AEGR. A comparison of the median survival of animals treated with anti-Tac immunotoxin and saline showed no statistical difference (P > 0.5). This indicates that the treatment effect seen with the MR-1 immunotoxin is specific and not due to a non-specific interaction with the toxin portion of the molecule.

There have been other reports of the intrathecal treatment of animal models of neoplastic meningitis. Zovickian and Youle (20) treated the guinea pig B-cell lymphoma L2C with the anti-idiotypic Mab M6 conjugated to intact ricin. The authors reported a 5-day increase in median survival after M6 immunotoxin treatment. A modification of the L2C lymphoma neoplastic meningitis animal model in which an indwelling catheter was used for treatment with the M6 immunotoxin showed that the median survival of the treated animals could be significantly extended by 28 to 32.5 days, when the animals were treated with multiple doses (23). Multiple dosing with LMB-1 immunotoxin was also shown to cause increased median survival compared with single-dose administration. Most importantly, the multiple dose regime produced 20% LTSs (22). Our dosage regime was based on the fact that the immunotoxin would be removed from the cerebrospinal fluid by bulk flow, resulting in a cerebrospinal fluid half-life <12 h and requiring repeated administration to maintain adequate tumoricidal levels. Future clinical trials must be designed to assess the pharmacokinetics of intrathecal immunotoxins to maximize treatment efficacy and to minimize the production of antibodies to the Pseudomonas exotoxin.

The EGFRvIII is a tumor-specific target that occurs on clinically relevant tumors that can invade the intrathecal space and cause neoplastic meningitis. MR-1 immunotoxin was shown to be effective in increasing median survival and producing LTSs at a level that was nontoxic in non-tumor-bearing rats. Clinical trials for the compartmental treatment of EGFR-vIII-positive neoplastic meningitis are being designed.

**ACKNOWLEDGMENTS**

We thank Catherine McLaughlin for excellent technical assistance in the performance of these studies.

**REFERENCES**


Regional Treatment of Epidermal Growth Factor Receptor vlll-expressing Neoplastic Meningitis with a Single-Chain Immunotoxin, MR-1


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/5/9/2646

Cited articles
This article cites 23 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/5/9/2646.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/5/9/2646.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.