Regressions and Cures of Melanoma Xenografts following Treatment with Monoclonal Antibody β-Lactamase Conjugates in Combination with Anticancer Prodrugs

David E. Kerr,1 George J. Schreiber, Vivekananda M. Vrudhula, Hákan P. Svensson, Ingegerd Hellström, Karl Erik Hellström, and Peter D. Senter

Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

ABSTRACT

Cephalosporin doxorubicin (C-Dox) and 7-(4-carboxybutanamido)-cephalosporin mustard (CCM) are prodrugs that are catalytically converted by Enterobacter cloacae β-lactamase (bL) to the active anticancer agents doxorubicin and phenylenediamine mustard, respectively. Both prodrugs were less cytotoxic to the 3677 human melanoma line than their respective drugs and were activated in an immunologically specific manner by 96.5-bL, a mAb-bL conjugate that binds to 3677 cell surface antigen. Similar results were obtained using the CCM prodrug on SK-MEL 28 human melanoma cells. Experiments in mice with established s.c. 3677 tumors demonstrated that although no tumors were cured in mice receiving the 96.5-bL/C-Dox combination, the activities were greater than those obtained from systemic doxorubicin treatment or from administration of the nonbinding conjugate P1.17-bL in combination with C-Dox. In contrast, when CCM was used as a prodrug, cures of established 3677 tumors were obtained in 80% of the 96.5-bL treated animals. This combination was also able to induce regressions of large 3677 tumor masses (800 mm³) without any apparent toxic side effects. We conclude that 96.5-bL, in combination with C-Dox or CCM has greater antitumor activity than systemic treatment with the corresponding drugs and that CCM is a more effective prodrug than C-Dox for treating human 3677 melanoma xenografts.

INTRODUCTION

A great deal of research is being done to improve the efficacy of chemotherapeutic agents for cancer therapy. An approach that has received attention recently concerns the use of mAb-enzyme conjugates for the activation of anticancer prodrugs (reviewed in Refs. 1–3). In this two-step targeting strategy, a mAb-enzyme conjugate is allowed to localize to tumors prior to the administration of a relatively nontoxic prodrug. The enzyme then converts the prodrug into an active anticancer agent. Provided that the conjugate localizes primarily to the tumor mass, selective prodrug activation should occur. Several studies suggest that mAb-enzyme conjugates can generate drugs intratumorally (4–7) and that mAb-enzyme/prodrug combinations can lead to better therapeutic effects than systemic drug therapy (1–3, 6–9).

β-Lactamases are of particular interest for this targeting strategy because of their high turnover rates, broad substrate specificities, and their abilities to effect the release of a wide range of mechanistically distinct chemotherapeutic agents (7, 9–18). Several previous studies have demonstrated that anticancer agents such as phenylenediamine mustard (14–16), doxorubicin (7, 9, 10, 13), platinum complexes (17), a Vinca alkaloid (9), and taxol (18) can be appended to cephalosporins, forming prodrugs that are substrates for β-lactamases. In vivo studies using human lung adenocarcinoma (7, 14) and colon carcinoma (9) tumor xenograft models in nude mice indicate that mAb-β-lactamase conjugates in combination with cephalosporin-containing prodrugs can lead to pronounced antitumor activities. Mechanistic insight into this activity was provided in one of the studies, in which it was shown that treatment with a mAb-β-lactamase conjugate and a doxorubicin prodrug resulted in intratumoral generation of doxorubicin in concentrations that were greater than those achieved with systemic doxorubicin administration (7). These results prompted us to investigate other tumor models and to compare different prodrugs for therapeutic efficacy.

Here we describe the use of an antimelanoma mAb-β-lactamase conjugate for prodrug activation. The mAb used, 96.5, is known to bind with high affinity to the melanotransferrin antigen (p97), which is present on the cell surface of most human melanomas and some carcinomas (19). In vitro studies indicate that the drugs, doxorubicin and PDM,2 can be generated in an immunologically specific manner from the respective cephalosporin-based prodrugs, C-Dox and CCM. Therapeutic responses, including complete regressions and cures of established melanoma xenografts, can be obtained using 96.5-β-lactamase in combination with C-Dox or CCM.

MATERIALS AND METHODS

Proteins and Cell Lines. bL was obtained and purified as described previously (14). The 3677 cell line was established at Bristol-Myers Squibb (Seattle, WA) from a tumor biopsy obtained from a patient with metastatic melanoma. SK-MEL 28 cells were obtained and propagated as described previously (20). 96.5, a murine mAb of the IgG2a isotype, binds to the p97 antigen, which is expressed on most melanoma cells (19), including 3677 and some carcinomas (14). In vitro studies indicate that the drugs, doxorubicin and PDM,2 can be generated in an immunologically specific manner from the respective cephalosporin-based prodrugs, C-Dox and CCM. Therapeutic responses, including complete regressions and cures of established melanoma xenografts, can be obtained using 96.5-β-lactamase in combination with C-Dox or CCM.

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1 To whom requests for reprints should be addressed, at Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, WA 98121.

2 The abbreviations used are: PDM, phenylenediamine mustard; C-Dox, cephalosporin doxorubicin; bL, Enterobacter cloacae β-lactamase; CCM, 7-(4-carboxybutanamido)-cephalosporin mustard.
96.5 Binding to 3677 and SK-MEL 28 Cells. 96.5 was radiolabeled with \(^{125}\text{I}\) to a specific activity of 3 mCi/mg using Iodogen (Pierce, Rockville, IL) as described previously (24). 3677 and SK-MEL 28 cells were aliquoted into 96-well plates (1.5 \times 10^5 cells/well) containing 0.3–5 nM \(^{125}\text{I}\)-labeled 96.5. The cells were incubated with the mAb for 60 min on ice and then separated from unbound material by centrifugation through silicone oil. After freezing, the centrifuge tubes at \(-78^\circ\text{C}\), the cell pellets were cut away from the supernatants. The radioactivity of both the pellets and the supernatants were counted with a gamma counter. Using these conditions, less than 10% of the radioactivity was associated with the pellet in the presence of a 100-fold excess of unlabeled 96.5. The affinity constant and number of \(^{125}\text{I}\)-labeled 96.5 molecules bound/cell at saturation were determined by Scatchard analysis (25).

In Vitro Cytotoxicity. 3677 and SK-MEL 28 cells in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% fetal bovine serum containing penicillin (60 \mu\text{g/ml}) and streptomycin (0.1 mg/ml) were plated into 96-well microtiter plates at 1.5 \times 10^5 cells/well and allowed to adhere at 37°C. Two days later, the cells were treated with either of the mAb-bL conjugates at 0.25 \mu\text{g} mAb component/ml or with media alone. In experiments with CCM, some cells were treated with a saturating concentration (0.5 mg/ml) of unconjugated 96.5 prior to treatment with 96.5-bL. After 30 min at 4°C, the plates were washed three times with antibiotic-free RPMI 1640 containing 10% FBS. Various concentrations of C-Dox or CCM were added to cells that were treated with either mAb-bL or with media alone. The cells were incubated with drug or prodrug for 1 h at 37°C and then washed three times with IMDM. Incubation was continued for 24 h at 37°C. The cells were pulsed for 18 h with \(^{125}\text{I}\)-labeled 96.5. The cells were incubated with the mAb for 60 min on ice and then separated from unbound material by centrifugation through silicone oil. After freezing, the centrifuge tubes at \(-78^\circ\text{C}\), the cell pellets were cut away from the supernatants. The radioactivity of both the pellets and the supernatants were counted with an LKB Wallac \(\beta\)-plate scintillation counter.

Animal Studies. All experiments were performed using athymic female nu/nu mice (Harlan Sprague-Dawley, Indianapolis, IN) and were initiated when the animals reached an age of 6–10 weeks. 3677 tumors were initially established by injecting (i.s.c.) 2 \times 10^6 cells in PBS in the flank. The resulting tumors were excised, cut into small pieces, and then reinjected s.c. (this constitutes passage 1). Experiments were performed using tumors from \(\text{in vivo}\) passages 1–3. All animals were monitored daily for general health, and every 3–7 days for tumor growth and body weight. Tumor volumes were estimated using the formula: longest dimension \times perpendicular dimension^2/2. Statistical analyses were performed using either a Student’s \(t\) test or a Mann-Whitney rank-sum test (Sigmastat software; Jandel Scientific, San Rafael, CA).

Conjugate Localization Experiments. Localization experiments were performed as described previously (26). Briefly, the bL-containing conjugates were labeled with \(^{125}\text{I}\) using Iodogen and diluted with unlabeled conjugate, such that each mouse received (i.v.) 0.75 mg mAb component/kg containing approximately 10 \mu\text{Ci} (370 kBq) of radioactivity. After 72 or 96 h, the mice (three/group) were anesthetized, bled through the orbital plexus, and sacrificed. Selected tissues were removed, weighed, and counted with a gamma counter.

Therapy Experiments. 3677 tumor-bearing mice (four to six/group) were treated with 96.5-bL or P1.17-bL (i.v.; 1 mg mAb component/kg), followed 72 h later by C-Dox or CCM (i.v.; doses indicated in figure legends). This was repeated on a weekly basis for a total of three rounds of treatment. Other groups were treated with C-Dox, Dox, CCM, or PDM, according to the same schedule, but without prior conjugate treatment. Maximum tolerated doses resulted in no deaths and weight losses of <20% compared to the weight at the beginning of the experiment. These doses were within 50% or less of doses where drug-related deaths occurred. The tumor mass did not have a noticeable effect on weight throughout the duration of the experiment. Partial tumor regression reflects a decrease in tumor volume to \(\leq\)50% of the tumor volume at the initiation of therapy. Complete regression refers to a tumor that for at least 2 weeks is not palpable. A cure is defined as an established tumor that is not palpable for \(\geq10\) tumor volume doubling delays (the time in days that it takes for control tumors to double in size).

RESULTS

Melanoma Models. The 3677 human melanoma line was developed from a tumor biopsy taken from a patient with metastatic melanoma. Scatchard analysis of the binding of radiolabeled 96.5 to 3677 cells indicated that the mAb bound with a dissociation constant of 0.7 nM (Fig. 1A). This mAb has been shown previously to bind the p97 antigen (19). At saturation, there were approximately \(2.1 \times 10^4\) molecules of 96.5 bound/cell. As previously described (20), the 96.5 mAb also bound to SK-MEL 28 cells. Scatchard analysis (Fig. 1B) indicated that this cell line bound significantly more molecules of 96.5 (3.0 \times 10^5/cell) than did 3677, albeit with slightly lower affinity (3.0 nM). Fluorescence-activated cell sorter analysis indicated that both cell lines bound 96.5 in a uniform manner (data not shown). It was possible to establish these cell lines \(\text{in vivo}\) by injecting the cells s.c. into nude mice and then passaging the tumors that grew out. The 3677 line grew in a much more reproducible manner than SK-MEL 28 and was thus the subject of most of the studies described here.

Conjugate Preparation and Cytotoxicity Experiments. Conjugates of bL were prepared by combining maleimide-substituted enzynme with 96.5-F(ab’)-SH or P1.17-F(ab’)-SH. A competition binding assay (15) indicated that the purified mAb-bL conjugates (molecular weight, approximately 90,000) had the same antigen-binding characteristics as their respective F(ab’) fragments. In addition, enzymatic activity assays using nitrocefin as substrate (15) indicated that the bL activity was preserved (data not shown). These results were expected, based on previous studies using the same conjugation methodology (21).

The known cephalosporin-containing prodrugs C-Dox and CCM (Fig. 2) were prepared from the respective drugs, doxorubicin and PDM (14, 23). It has been shown previously that C-Dox and CCM were less cytotoxic than doxorubicin and PDM, respectively, and that the active drugs were released upon bL-catalyzed hydrolysis (14, 23). Therefore, it was expected that the prodrugs would be relatively noncytotoxic to 3677 and SK-MEL 28 melanoma cells \(\text{in vitro}\) and that prodrug activation could be mediated by mAb-bL conjugates that bound to cell surface antigens. To test this, the cells were treated with the mAb-bL conjugates, washed, and then exposed to either C-Dox or CCM. The cytotoxic effects of these combinations were compared to
no preferential intratumoral accumulation ($P = 0.686$ and $1.000$ for P1.17-bL uptake in tumor versus blood at 72 and 96 h, respectively). Based on these results, the time interval between conjugate and prodrug administration for subsequent in vivo therapy experiments was 72 h.

Therapy with C-Dox. In vivo therapy experiments were performed using the mAb-bL conjugates in combination with C-Dox in

those of the drugs or the prodrugs without prior conjugate treatment. Unconjugated 96.5 has already been shown not to affect in vitro cell growth (20).

C-Dox (IC$_{50}$, 4.4 µM) was approximately 9-fold less toxic to 3677 cells than doxorubicin (IC$_{50}$, 0.5 µM; Fig. 3A). This modest difference in cytotoxic activity is consistent with a previous report using three other cell lines (7). An increase in cytotoxic activity was obtained on cells that were treated with 96.5-bL prior to C-Dox. The activity of this combination (IC$_{50}$, 1 µM) was approximately 3 times greater than that obtained using the nonbinding control conjugate in combination with C-Dox. A greater differential between drug and prodrug cytotoxicity was obtained with the nitrogen mustards. CCM (IC$_{50}$, 6 µM) was approximately 26 times less toxic to 3677 cells than PDM and was fully activated on cells that were pretreated with 96.5-bL (Fig. 3B). Activation was immunologically specific, since the cytotoxic effect of the 96.5-bL + CCM combination was reduced on cells that were exposed to saturating concentrations of unconjugated 96.5 prior to conjugate exposure. Furthermore, the extent of C-Dox activation was lower on P1.17-bL- than on 96.5-bL-treated cells. Similar results were obtained on SK-MEL 28 cells (Fig. 3C). Incomplete removal of unbound conjugate might account for the small amount of prodrug activation observed in the P1.17-bL-treated cells (Fig. 3B). These experiments establish that C-Dox and CCM are less toxic than their corresponding drugs and that the 96.5-bL conjugate is able to effect prodrug activation. This is significant in light of the relatively low level of p97 antigen expression on 3677 cells compared to SK-MEL 28 and other melanoma cell lines (19).

Conjugate Localization. Studies were undertaken in nude mice to establish the extent of mAb-bL conjugate localization in s.c. 3677 tumor xenografts and to determine an appropriate time interval between the administration of conjugate and prodrug. Radiolabeled 96.5-bL and P1.17-bL conjugates were injected i.v. (0.75 mg/kg) into mice that had s.c. 3677 tumors of approximately 200 mm$^3$ in volume. The amount of radioactivity in the tumors, blood, and several other tissues was determined 72 and 96 h later. It was found that the concentration of 96.5-bL in tumors was much higher (>10-fold) than in any of the other tissues measured (Fig. 4). This was most likely due to binding to the p97 antigen on tumor cells, since P1.17-bL showed
mice that had established s.c. 3677 tumors. Treatment was initiated 10 days after tumor implant, at which time the tumors were approximately 150 mm³ in volume. The mAb-bL conjugates were injected i.v. at 1 mg mAb component/kg body weight. After 72 h, C-Dox (40 or 60 mg/kg) was administered i.v. This dosing schedule was carried out a total of three times on a weekly basis. The therapeutic effects were compared to those of doxorubicin at the maximum tolerated dose under this dosing regimen (6 mg/kg). Unconjugated 96.5 was not used in the experiments, since previous studies have shown it to be devoid of in vivo antitumor activity (27). For all of the in vivo experiments, the maximum tolerated dose is defined as a dose level that gave less than 20% weight loss, no treatment-related deaths, and was within 50% of the dose where such events took place.

Doxorubicin had little to no effect on the growth of 3677 tumors in mice (Fig. 5). In contrast, a significant delay in tumor growth was obtained in mice that received 96.5-bL 72 h prior to treatment with C-Dox at 60 mg/kg/injection. The antitumor effect of this combination was not apparent until after the second round of therapy. At the completion of prodrug treatment (day 27), the tumors underwent regression, reached their nadir at day 47, and then began to grow. The antitumor effect of the mAb-bl + C-Dox therapy was immunologically specific, since P1.17-bl in combination with 60 mg/kg/injection C-Dox was less effective than the corresponding 96.5-bl group. With both conjugates, a steep prodrug dose-response was observed, since 40 mg/kg/injection C-Dox was less effective than the higher dose.

Although the mAb-bl + C-Dox combinations resulted in no apparent acute toxicities or significant weight losses at the early stages in the experiment, many of the 96.5-bl treated mice that received C-Dox at 60 mg/kg/injection developed a disorder around day 54 that resulted in partial hindlimb paralysis. This led to delayed weight loss (approximately 20% at day 54) and, eventually, to 4 of 5 deaths by day 70. Clearly, this was above the maximum tolerated dose. The toxicity was probably doxorubicin related, since similar neurological effects have been reported in rats treated with doses of doxorubicin that were above the maximum tolerated dose (28). There was no apparent toxicity associated with lower doses of C-Dox. Better antitumor activities than those shown in Fig. 5 could be obtained in 96.5-bl-treated animals that received higher prodrug doses (80 mg/kg), but this resulted in earlier systemic toxicity and deaths (data not shown). Thus, although tolerated doses (40 mg/kg, the maximum tolerated dose in this experiment) of C-Dox in 96.5-bl-treated mice resulted in better therapeutic activities than doxorubicin, further improvements in efficacy were limited due to treatment-related toxicities.

Therapy with CCM. We next determined whether better therapeutic effects could be obtained using a prodrug other than C-Dox. The rationale behind this was that prodrugs with different pharmacological and biochemical properties than C-Dox might not be subject to the same dose-limiting toxicities. This would be particularly likely if the released drug has a completely different toxicity profile than doxorubicin. Therefore, studies were undertaken with CCM in mAb-bl-treated mice. It has been demonstrated previously that bl mediated-hydrolysis of CCM leads to the release of the alkylating agent PDM (14), which is mechanistically different in activity than doxorubicin (29). As reported previously, CCM was much less toxic to mice than PDM (14). It was possible to administer CCM i.v. at a dose of 175 mg/kg/injection weekly for three rounds without any weight loss or apparent toxic side effects. Under this dosing schedule, the maximum tolerated dose of PDM was approximately 3 mg/kg. This represents at least a 26-fold difference in toxicity on a molar basis between the two agents.

In vivo experiments were performed using mice that had s.c. 3677 tumors of approximately 125 mm³ at the initiation of therapy. It was found that CCM (175 mg/kg) and PDM (3 mg/kg) had only modest antitumor effects (Fig. 6). Pronounced antitumor activity was obtained in mice that received 96.5-bl 72 h prior to treatment with CCM. The combination of 96.5-bl and CCM (125 mg/kg, maximum tolerated dose in this experiment) resulted in regressions of five of five tumors by day 44 after tumor implant, leading to cures past day 120 (the end of the experiment) in four of five mice. These effects were not accompanied by any apparent adverse side effects. The antitumor effect was immunologically specific (P = 0.008 at day 40), since very little activity was obtained with the nonbinding control conjugate, P1.17-bl, prior to treatment with CCM. Antitumor activity was also obtained at a 40% lower dose of CCM (75 mg/kg) in combination with 96.5-bl (P = 0.008 at day 40), but four of five tumors started to grow back around day 50. In this group, there was a single cure past day 120. No cures were obtained in any of the animals not receiving the 96.5-bl + CCM combination. CCM at 175 mg/kg in conjugate-treated mice proved to be toxic.

An additional study was performed to test the efficacy of the 96.5-bl + CCM combination in mice that had large tumors at the initiation of treatment. In this experiment, 96.5-bl was administered when the 3677 tumors averaged approximately 500 mm³. CCM was...
established melanoma xenografts and can induce the regression combination with CCM on both large (Fig. 7) and smaller (Fig. 6) 800 mm³ (Fig. 7). After three rounds of therapy, partial regressions given three days later when the tumors had reached approximately therapeutic effects were compared to an untreated control group. Data represent the mean; followed by CCM (100 or 125 mg/kg) on days 17, 24, and 31 (arrow on the X-axis). The therapeutic effects were compared to an untreated control group. Data represent the mean; bars, SEM.

given three days later when the tumors had reached approximately 800 mm³ (Fig. 7). After three rounds of therapy, partial regressions were obtained in all of the treated animals, and one of four of the mice receiving 125 mg/kg CCM was cured. It may have been possible to continue treating the animals and increase the number of cures, since there were no apparent toxicities associated with the three rounds of therapy. We chose not to do this, so that the effects of 96.5-bL in combination with CCM on both large (Fig. 7) and smaller (Fig. 6) tumors could be compared under the same conditions. The studies indicate that 96.5-bL in combination with CCM can lead to cures of established melanoma xenografts and can induce the regression of tumors averaging 800 mm³ in volume.

**DISCUSSION**

It has been shown previously that mAb-enzyme conjugates in combination with prodrugs can lead to significant antitumor activities (reviewed in Refs. 1–3), and there is a growing body of evidence that this targeting strategy results in intratumoral drug generation (4–7). Much of the work in this field centers around the use of enzyme conjugates that are capable of generating a variety of drugs having different modes of activity (reviewed in Refs. 1–3). With such enzymes, it should be possible to tailor the therapy so that the released drug is highly active against the particular tumor being treated. The studies described here were designed to find out if a human melanoma model might be differentially sensitive to doxorubicin and PDM, two mechanistically distinct cytotoxic agents that were generated from their respective cephalosporin derivatives by mAb-bL conjugates. Melanomas may be a good target for mAb-enzyme/prodrug therapy, since these tumors are particularly difficult to treat in the clinic (30), and improved therapeutic approaches are needed. The human melanomas we have used, 3677 and SK-MEL 28, bind the mAb 96.5 with high avidity. This antibody was chosen, since its targeted antigen, p97, is present on most human melanomas as well as on some carcinomas (19, 20, 27, 31). Furthermore, 96.5 has been shown to localize in patients with disseminated melanoma (32). Normal tissue reactivity has been observed in benign nevi (31) and, to a small extent, in uterine, vaginal, and bladder tissues (19).

Both C-Dox and CCM were found to be less cytotoxic compared to their respective parent drugs and could be activated in an immuno logically specific manner by 96.5-bL (Fig. 3). This is consistent with previous studies, in which it was shown that C-Dox and CCM could be selectively activated on human lung adenocarcinoma cells (7, 14). In light of the fact that mAbs localize poorly to human solid tumors (33), it may be significant that prodrug activation was achieved on 3677 cells, which have low levels of p97 antigen expression (2 X 10⁴/ cell). It should be noted that some other drug/prodrug systems described for this targeting strategy display greater cytotoxicity differences that those reported here (1, 6, 34–36). How this translates into therapeutic efficacy in vivo is still an open question, particularly since significant therapeutic effects have been reported with a Vinca alkaloid prodrug that is almost as toxic as its corresponding drug (9).

An in vivo therapy study demonstrated that established 3677 tumors were not responsive to treatment with doxorubicin at the maximum tolerated dose (Fig. 5). In contrast, a pronounced therapeutic effect was obtained if C-Dox was administered to 96.5-bL-treated mice. There was, however, a major drawback to therapy with 96.5-bL in combination with C-Dox, in that dose-limiting toxicities resulted in suboptimal therapeutic effects. No cures or long-term regressions were obtained in animals receiving this conjugate + prodrug combination. Since it was considered possible that the toxicities associated with the 96.5-bL + C-Dox treatment might be dependent on the particular characteristics of the prodrug and its corresponding drug, we chose to investigate the antitumor effects using CCM in place of C-Dox. The therapeutic effects resulting from treatment with 96.5-bL + CCM were superior to those obtained using C-Dox as the prodrug and were not accompanied with any noticeable toxic side effects. When therapy was initiated with tumors that were approximately 150 mm³ in size, 96.5-bL + CCM led to cures in four of five mice treated (Fig. 6). With this regimen, it was even possible to induce regressions of tumor masses that were 800 mm³ at the initiation of prodrug treatment (Fig. 7).

One possible explanation for the enhanced activity of CCM compared to C-Dox in this targeting strategy is that the 3677 melanoma is inherently more sensitive to PDM than to doxorubicin. This is consistent with in vitro experiments in which it was found that in a 1-h exposure assay, PDM (IC₅₀, 0.2 µM) is 2.5 times more potent than doxorubicin (Fig. 3). In addition, 3677 tumors appear to be more sensitive to treatment with PDM (Fig. 6) than with doxorubicin (Fig. 5), when both agents were administered at their maximum tolerated doses. Interestingly, alkylating agents are generally more active than other chemotherapeutics for the clinical treatment of melanoma (30). Another important factor that may have contributed to the relative benefits of CCM versus C-Dox in 96.5-bL-treated mice is that compared to their respective drugs, it was possible to administer more CCM than C-Dox. Pharmacological studies, such as those reported for C-Dox (7), are needed to determine whether this would lead to correspondingly higher intratumoral drug concentrations.

A potential drawback to the approach described here is that bL is a bacterial enzyme and might be expected to be immunogenic in humans, in spite of the fact that the released drugs are by nature immunosuppressive. The extent to which this will limit therapeutic efficacy in the clinic can only be determined experimentally. It should be noted, however, that there are a number of methods available to modulate immunological responses to foreign proteins (37).

The therapeutic effects described here illustrate the potential of targeted prodrug activation for tumor therapy. We have demonstrated that the antitumor effects using this targeting strategy can vary depending on the drug that is released, and that by using an appropriate prodrug for a given tumor type, it is possible to induce regressions of large tumor masses.
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