Potent and Specific Antitumor Efficacy of CMC-544, a CD22-Targeted Immunoconjugate of Calicheamicin, against Systemically Disseminated B-Cell Lymphoma

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ABSTRACT

Purpose: CMC-544 is a CD22-targeted immunoconjugate of calicheamicin and exerts a potent cytotoxic effect against CD22+ B-cell lymphoma. This study evaluated antitumor efficacy of CMC-544 against systemically disseminated B-cell lymphoma.

Experimental Design: Scid mice received i.v. injections of CD22+ Ramos B-cell lymphoma cells for their systemic dissemination. CMC-544, G5/44, CD33-targeted CMA-676 (control conjugate) or rituximab were given i.p. 3, 9, 15, or 21 days after B-cell lymphoma dissemination. Diseased mice were monitored daily for hind-limb paralysis and death. Histopathological examination of CMC-544-treated and vehicle-treated diseased mice was also performed.

Results: Mice with disseminated B-cell lymphoma developed hind-limb paralysis within 35 days. When given up to 15 days after B-cell lymphoma dissemination, CMC-544 extended survival of the diseased mice to >100 days, and these mice were considered cured. CMC-544 was efficacious when given during both the early initiation phase and the late established phase of the disease. A single dose of CMC-544 was effective in delaying the occurrence of hind-limb paralysis. In contrast, neither CMA-676 nor unconjugated G5/44 was effective. Rituximab was effective when given early in the disease process but not when the disease was established. Histopathological analysis revealed B-cell lymphoma infiltration in brain, spinal cord, bone marrow, and kidney in vehicle-treated but not in CMC-544–treated diseased mice. Consistent with its efficacy against the disseminated B-cell lymphoma, CMC-544 also caused regression of established Ramos B-cell lymphoma xenografts in scid mice.

Conclusions: CMC-544 confers strong therapeutic activity against systemic disseminated B-cell lymphoma and protects mice from hind-limb paralysis and death. These results support clinical evaluation of CMC-544 in the treatment of CD22+ lymphoid malignancies.

INTRODUCTION

Combination chemotherapy with cytotoxic agents that have distinct mechanisms of actions is a common strategy in the treatment of metastatic cancers. Although this approach is widely used in clinical practice, cumulative toxicity, narrow therapeutic windows, and low therapeutic indices often hamper its continued use. This realization has led to a strategy that involves the use of monoclonal antibodies (mAbs) to target potent cytotoxic agents to tumor cells (1–3). In this strategy, commonly referred to as an antibody-targeted chemotherapy, a mAb specific for a tumor-associated antigen (TAA) is covalently linked to a potent cytotoxic agent (4). Antibody-targeted chemotherapy involves specific binding of the targeting mAb-drug conjugate to the TAA followed by internalization of the TAA-mAb complex to ensure focused delivery and release of the conjugated cytotoxic drug inside the tumor cells. Tumor-targeted delivery of the cytotoxic drug not only maximizes its antitumor efficacy but also significantly reduces its exposure to normal tissues, thereby improving its therapeutic index (1, 2, 4).

This strategy of tumor-targeted chemotherapy can be therapeutically more advantageous than the traditionally used chemotherapeutic strategy. Similar tumor-targeted therapies involving diphtheria toxin (denileukin diftitox/Ontak) and radioimmunotherapeutics (tositumomab–iodine-131/Bexxar and ibritumomab tiuxetan/Zevalin) have shown clinical benefit and are licensed for clinical use by the United States FDA.4,5,6

Antibody-targeted chemotherapy is now a clinically validated strategy, exemplified by gemtuzumab ozogamicin (Mylotarg/CMA-676), the first antibody-targeted chemotherapeutic agent approved by the United States Food and Drug Administration (5–7). The cytotoxic moiety in gemtuzumab ozogamicin is N-acetyl gamma calicheamicin dimethyl hydrazide (CalichDMH), which is a derivative of the potent cytotoxic natural product, gamma calicheamicin (8). Gamma calicheamicin binds DNA in the minor groove and causes double-strand DNA breaks in a thiol-dependent manner, leading to apoptosis and cell death (9). Gemtuzumab ozogamicin is a conjugate in which Cali-
chDMH is covalently linked via an acid-labile AcBut linker to a humanized IgG4/k anti-CD33 monoclonal antibody, hP67.6 (10, 11), and is indicated for the treatment of elderly (>60 years of age) acute myeloid leukemia patients in first relapse who are not candidates for other therapies (7). Clinical efficacy of the CD33⁺ myeloid lineage-targeted gemtuzumab ozogamicin has provided impetus to apply this strategy to malignancies of the CD22⁺ B lymphoid lineage with CMC-544 (12).

CMC-544, generically known as inotuzumab ozogamicin, is an immunotoxin conjugate of a humanized IgG4/k anti-CD22 antibody, G5/44, covalently linked to CalichDMH via the same acid-labile AcBut linker used in gemtuzumab ozogamicin (10, 11). CMC-544 binds human CD22 with subnanomolar affinity and causes potent cytotoxicity against human B-cell lymphoma cell lines with subnanomolar IC₅₀ values (12, 13). In the present study, we evaluated preclinical antitumor efficacy of CMC-544 in scid mice with disseminated Ramos B-cell lymphoma. In this murine model of disseminated B-cell lymphoma, B-cell lymphoma infiltrates various organs, including the central nervous system, and causes hind limb paralysis and death of the diseased mice (14–16). Our results demonstrate that CMC-544 protects scid mice with disseminated B-cell lymphoma from hind-limb paralysis and death. CMC-544 may provide a therapeutic alternative for the treatment of patients with CD22⁺ leukemias and lymphomas.

**MATERIALS AND METHODS**

**Antibodies, Conjugates, and Cell Line.** Humanized anti-CD22 mAb, G5/44, an IgG4 isotype, was derived by complementarity-determining region–grafting from murine anti-CD22 mAb m5/44 by Celltech, Slough, United Kingdom (13) and expressed at Wyeth Biopharma (Andover, MA). G5/44 was conjugated to CalichDMH with an acid-labile AcBut (4-(4’-acetylphenoxy) butanoic acid) linker and the resulting conjugate was termed CMC-544 (12). The quantity of CalichDMH present in CMC-544 was 73 μg per mg of G5/44. CMA-676 (Mylotarg/ gemtuzumab ozogamicin) is an immunotoxin conjugate in which a humanized anti-CD33 antibody, hP67.6, an IgG4 isotype, is conjugated to CalichDMH via the same AcBut linker described for CMC-544 (10–13). Chimeric human IgG1 anti-CD20 mAb, rituximab (Rituxan, Biogen-Idec Pharmaceuticals, San Diego, CA, and Genentech, South San Francisco, CA), was purchased from Med World Pharmacy (Chesnut Ridge, NY). All conjugates were endotoxin free (<5.0 endotoxin units/mL) as determined by a modified Limulus amebocyte assay test (Biowhittaker, Walkersville, MD). Doses of calicheamicin conjugates are expressed as equivalents of CalichDMH and that of unconjugated antibody are expressed as antibody protein.

**CD22⁺ CD33⁻ B-cell lymphoma-derived cell line Ramos (CRL-1923) was obtained from the American Type Culture Collection (Manassas, VA). This cell line was determined to be Mycoplasma free by a PCR Mycoplasma detection assay (American Type Culture Collection). Ramos cells were maintained as suspension cultures in RPMI 1640 plus 10% fetal bovine serum, 10 mmol/L HEPES, 1 mmol/L sodium pyruvate, 0.2% glucose, 100 units/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate.

**Mice.** Six to 8-week-old male scid mice (CB17 scid; body weight, 20 to 25 g) were obtained from Charles River Laboratories (Wilmington, MA). All mice were housed in microisolator units and provided with sterile food and water ad libitum throughout the studies. Studies were conducted in laminar flow cabinets. All experimental procedures involving mice were approved by the Wyeth Animal Care and Use Committee and carried out according to established guidelines.

**Assessment of Antitumor Efficacy against Disseminated B-Cell Lymphoma.** Male scid mice received i.v. injections of 1 × 10⁶ Ramos B-cell lymphoma in a volume of 0.2 mL in the tail vein. Dissemination and growth of B-cell lymphoma was allowed to occur for either 3 or 9 days before the initiation of drug therapy. In some studies, the treatments were initiated 15 or 21 days after the B-cell lymphoma dissemination. Scid mice that received i.v. injection of Ramos B-cell lymphoma are hereafter referred to as the diseased mice. In this report, stages of the disseminated B-cell lymphoma disease were temporally defined as minimal disease (tumor growth occurring within 3 days after the i.v. injection of B-cell lymphoma), established disease (tumor growth within 9 to 15 days after the intravenous injection of B-cell lymphoma), and advanced disease (tumor growth within 21 days after the i.v. injection of B-cell lymphoma). Mice with disseminated B-cell lymphoma (8 to 11 mice/treatment group) were given vehicle (PBS), unconjugated G5/44 mAb, rituximab, or calicheamicin conjugates (CMC-544 or CMA-676) for a maximum of three doses given i.p. 4 days apart starting on days 3 or day 9 (q4d×3). In some experiments, the q4d×3 administration of the conjugates was delayed until days 15 or 21 after the initiation of B-cell lymphoma dissemination. The effect of a single dose (given on day 9) or two doses (given on days 9 and 13) of CMC-544 (40 μg of conjugated CalichDMH/kg) was also investigated. In additional studies, unconjugated G5/44 was given 1 hour before CMC-544 to investigate its effect on the antitumor efficacy of CMC-544.

Mice with disseminated B-cell lymphoma were monitored daily for the presence of hind-limb paralysis and/or death. Mice exhibiting hind-limb paralysis were euthanized by CO₂ asphyxiation according to institutional regulations. Some mice died without exhibiting hind-limb paralysis. The average survival time (days ± SD) was calculated for each group. The percentage of mice surviving throughout the observation period (percentage cured) and the percentage with hind-limb paralysis were also recorded. The difference in survival distribution between groups was determined by using nonparametric methods comparing the survival distribution of the diseased mice. Multiple comparisons were done with the rank transformation procedure. The rank transformation procedure consists of replacing the survival times with their ranks and applying the usual parametric F test to the ranks. Multiple comparisons were done with Tukey’s method on the ranks. Tukey’s method indicates the difference in survival times among mice with significance reported at the 0.05 level. The survival curves were constructed with the Kaplan-Meier method (17).

**Assessment of Antitumor Efficacy against s.c. B-Cell Lymphoma Xenografts.** Male scid mice (CB17 scid) received s.c. injections of 1 × 10⁷ Ramos cells in Matrigel
(diluted 1:1 in RPMI medium; Collaborative Biomedical Products, Belford, MA) in the dorsal, right flank. When the tumors reached an average mass of ~0.4 g, the tumor-bearing mice were randomized into various treatment groups. The treatments included three doses of vehicle, CMC-544, or CMA-676 (both conjugates given were 160 μg of conjugated CalichDMH/kg i.p., q4d×3). Tumors were measured at least once a week and calculated as tumor mass (g) = [(tumor width^2) × (tumor length)]/2. Average tumor mass for each group (mean ± SE) was calculated and recorded up to 31 days or until either a mouse died (which disrupted the group mean) or the tumors grew too large (>3.5 g), in which case the large tumor-bearing mice had to be euthanized as per institutional regulations. The number of tumor-free mice at the end of the study for each treatment group was also recorded.

Histopathologic Evaluation. Histopathological evaluation of various organs from mice with systemically disseminated B-cell lymphoma was carried out upon treatment with either CMC-544 (40 or 160 μg/kg, q4d×3, n = 3 and n = 7, respectively) or vehicle (n = 7). Mice were killed by CO2 asphyxiation when the presence of hind-limb paralysis was noted (day 22 through day 35 after the initiation of B-cell lymphoma dissemination for the vehicle-treated group). CMC-544–treated mice were killed on day 32 after the initiation of B-cell lymphoma dissemination. Various tissues (kidneys, femur, spinal cord, brain, liver, small and large intestine, heart, and sciatic nerve) were collected from these mice and placed in 10% buffered neutral formalin. The fixed tissues were embedded in paraffin, sectioned, and the sections were stained with H&E before their histopathological evaluation. At least three sections from each organ were evaluated by a board-certified veterinary pathologist. The number of mice with tumor cell infiltration in the selected organ was recorded. Representative photomicrographs of the sections illustrating the degree of tumor-cell infiltration in the organs were made.

RESULTS

Effect of CMC-544 on Disseminated B-Cell Lymphoma in scid Mice. The effect of CMC-544 on the survival of scid mice with disseminated B-cell lymphoma was examined. CMC-544 or the isotype matched nonbinding control conjugate, CMA-676, was given i.p. to scid mice 9 days after the initiation of the B-cell lymphoma dissemination. CMC-544 or control conjugate CMA-676 was given in the dose range of 10 to 160 μg of conjugated CalichDMH/kg (0.25 to 4 μg of conjugated CalichDMH/mouse) with each dose being repeated three times, 4 days apart (q4d×3). In the vehicle-treated group, 100% of the diseased mice developed hind-limb paralysis by day 35 at the latest (average survival time 27.9 ± 1.5 days) and had to be euthanized (Fig. 1). For CMC-544–treated mice, the lowest dose (10 μg/kg q4d×3 or 250 ng of conjugated CalichDMH/mouse × 3) given caused survival of 75% of the diseased mice over the observation period of 92 days with a mean survival time of 83.1 days. The two intermediate doses (40 and 80 μg/kg) caused survival of 94 and 89% the diseased mice, respectively, over the course of this study. Additional studies not shown here confirmed that almost complete protection of the diseased mice can be achieved at the CMC-544 dose of 40 g/kg q4d×3. In a separate study, where the observation period was extended to 175 days, 90% of the diseased mice treated with CMC-544 (40 μg of CalichDMH/kg) survived for 175 days, at which time the study was terminated (data not shown). The highest dose of CMC-544 (160 μg/kg)
Calicheamicin conjugates were given at 40 mg/kg of CalichDMH/kg survived, and the remaining 65% of the diseased and treated mice either became moribund and had to be killed or died spontaneously. It is noteworthy that none of the CMC-544–treated diseased mice, at all dose levels given, developed hind-limb paralysis. In contrast, similar treatment with a nonbinding control conjugate, CMA-676, was ineffective at all doses in prolonging survival of the diseased mice as on an average 75% of the diseased mice treated with CMA-676 developed hind-limb paralysis (Fig. 1).

The ability of CMC-544 to protect scid mice with either the minimal B-cell lymphoma disease or the established B-cell lymphoma disease states was examined. CD20-targeted rituximab, a widely used antibody therapeutic agent in the treatment of non-Hodgkin’s B-cell lymphoma (18, 19), was included in this evaluation. Ramos B-cell lymphoma cells were injected i.v. in scid mice on day 1 and various i.p. treatments were initiated on day 3 (minimal disease state), day 9 (established disease state), day 15 (established disease state), or day 21 (advanced disease state). CMA-676 (nonbinding control) and unconjugated anti-CD22 mAb G5/44 were also included in this evaluation. Calicheamicin conjugates were given at 40 µg/kg q4d ×3, and the unconjugated antibodies were given at 20 mg/kg q4d ×3. The dose of 20 mg/kg rituximab was chosen because this dose was reported to be effective in delaying Nalmital B-cell lymphoma growth in NOD/scid mice (18). When given on day 3, rituximab conferred complete protection against the disseminated B-cell lymphoma (minimal) disease as shown in Fig. 2.

Ninety percent of these mice survived for >100 days. However, delaying the initiation of rituximab treatment until day 9 or beyond resulted in the loss of its protective effect. In contrast with rituximab, CMC-544 was protective regardless of whether it was given earlier (3 or 9 days after i.v. injection of B-cell lymphoma cells) or later (15 or 21 days after i.v. injection of B-cell lymphoma cells) during the disseminated disease process. When CMC-544 was given 3, 9, or 15 days after the i.v. injections of B-cell lymphoma cells, 100, 100, and 80%, respectively, of these mice survived for 100 days and were therefore considered cured. However, when the initiation of the treatment with CMC-544 was delayed until 21 days (advanced disease state), ≥90% of the diseased mice survived for 60 days but only 40% of these mice survived for 100 days and beyond (data not shown). Both CMA-676 and G5/44 were ineffective in protecting the diseased mice against the disseminated disease regardless of when the treatment with these agents was initiated.

In the experiments described above, three doses of CMC-544 were given to demonstrate its antitumor efficacy. Whether a single dose or two doses of CMC-544 could exert similar antitumor activity was explored with scid mice with the disseminated B-cell lymphoma. As shown in Fig. 3, a single dose of CMC-544 at 40 µg of CalichDMH/kg was able to significantly prolong the survival of 64% of the diseased mice (P < 0.05). Furthermore, two doses of CMC-544 (40 µg of CalichDMH/kg) were sufficient to confer long-term protection against the disseminated disease as both q4d ×2 and more commonly used q4d ×3 dosing schedules produced identical protection against the disseminated B-cell lymphoma with the diseased mice surviving beyond 100 days.

**Histopathological Analysis.** Histopathological analysis of sections derived from select organs from vehicle-treated scid mice with disseminated B-cell lymphoma indicated the presence of B-cell lymphoma in the brain (meninges, seven of seven mice), femur (around the femur and in the marrow in seven of seven mice and often associated with focal necrosis in the marrow), kidneys (around the renal pelvis in five of seven mice), and in the spinal cord (meninges of three of seven mice; Table 1, Fig. 4). Tumor cells were uniform in appearance, size, color, and shape with regard to their nuclei, nucleoli, and cytoplasm having round to ovoid, dark basophilic prominent nuclei and scant round, pale basophilic cytoplasm. Normal bone marrow cells presented a morphologic appearance of hematopoietic (erythropoietic and granulopoietic) cells that appeared in clusters in varying stages of development interspersed with megakaryocytes and lymphocytes (Fig. 4). The hind-limb paralysis observed in the diseased mice may be caused by the presence of B-cell lymphoma in the brain and/or the spinal cord. Infiltration of B-cell lymphoma was not observed in other organs examined, including small and large intestines, heart, and liver (data not shown). There was no evidence of B-cell lymphoma infiltration in any of the organs from the diseased mice that had been treated with CMC-544 at the dose of either 40 or 160 µg/kg. These results indicate that effective elimination of disseminated B-cell lymphoma from various organs by CMC-544 was primarily responsible for its therapeutic effect.

**Effect of CMC-544 on the Growth of s.c. Ramos B Lymphoma in scid Mice.** Our previous study demonstrated the efficacy of CMC-544 against s.c. B-cell lymphoma x-
nografts in nude mice with long-term tumor-free survival achieved at 160 μg/kg CMC-544 (12). In the present study, the same dose of CMC-544 was less efficacious in any of the disseminated B-cell lymphoma model in scid mice, even if there was no evidence of the presence of B-cell lymphoma in any of the organs from the treated mice. This apparent lack of efficacy of the CMC-544 at the highest dose could be a consequence of the adverse effects of CMC-544. This possibility was experimentally tested by evaluating the effect of the dose of 160 μg/kg CMC-544 on the growth of s.c. Ramos B-cell lymphoma xenografts established in scid mice. CMC-544 or the nonbinding conjugate, CMA-676, was given at 160 μg of CalichDMH/kg, i.p., q4d×3 to scid mice with s.c. Ramos B-cell lymphoma xenografts (average initial tumor mass of 0.4 g). CMC-544 completely suppressed B-cell lymphoma xenograft growth for 31 days (Fig. 5), at which time the measurement of tumors was terminated. The nonbinding control conjugate, CMA-676, had no effect on the growth of B-cell lymphoma xenografts and these mice had to be euthanized by day 9 because of excessive tumor growth. These results indicate that CMC-544 is efficacious against both localized subcutaneous B-cell lymphoma as well as systemically disseminated B-cell lymphoma.

In the above experiments, one of the eight CMC-544–treated s.c. B-cell lymphoma-bearing scid mice died on day 32 posttreatment. The studies with the disseminated B-cell lymphoma model described in Fig. 4 showed that even if there was no histologic evidence of the presence of B-cell lymphoma in various organs, including the central nervous system of scid mice treated with CMC-544 at a dose of 160 μg/kg q4d×3, only 30% of mice within this treatment group survived the entire observation period. These two independent observations prompted us to monitor survival of non–tumor-bearing scid mice (n = 10) given CMC-544 at the doses of either 80 or 160 μg/kg q4d×3 i.p. All scid mice receiving CMC-544 at the dose of 80 μg/kg survived the entire period of evaluation of 84 days. In contrast, ~20% of scid mice given CMC-544 at 160 μg/kg died within 50 days and 50% were dead at the end of the evaluation period. Thus, the minimum nonlethal dose of CMC-544 in scid mice is >80 and <160 μg/kg.

Effect of Unconjugated mAb G5/44 on Antitumor Activity of CMC-544. To evaluate the impact of unconjugated G5/44 on the antitumor efficacy of CMC-544 against disseminated B-cell lymphoma, G5/44 was given i.p. 1 hour before each administration of a fixed dose of CMC-544 (40 μg/kg) to the diseased mice. This dose of CMC-544 corresponds to 548 μg/kg CalichDMH-conjugated G5/44 antibody protein and has consistently protected mice against the disseminated disease. Four different dose levels of unconjugated G5/44 (164, 548, 1,644, and 5480 μg/kg) were given corresponding to the G5/44:CMC-544 antibody protein ratios of 0.3:1, 1:1, 3:1, and 10:1. In addition, two separate controls were used in this evaluation: unconjugated G5/44 given i.p. at 5480 μg/kg without CMC-
544, and 5480 μg/kg hP67.6 (anti-CD33 antibody) given as a nonbinding isotype-matched antibody control 1 hour before the administration of CMC-544. As shown in Fig. 6, nonbinding control antibody, hP67.6, even at a 10-fold higher dose, had no effect on the anti–B-cell lymphoma protective activity of CMC-544 with average survival period of 100 days. In contrast, a similar 10-fold higher dose of G5/44 caused a significant inhibition (P < 0.05) of the anti–B-cell lymphoma-protective effect of CMC-544 with the average survival period of 83.2 days with only 60% of the treated mice surviving the entire observation period. The 3-fold higher dose of G5/44 reduced the average survival time of treated mice to 89 days with 80% survival. The two lowest doses of G5/44 (0.3- and 1-fold the dose of CMC-544) had no effect on the anti–B-cell lymphoma-protective effect of CMC-544 with 100% survival. As observed before, vehicle or G5/44-treated diseased mice in the absence of CMC-544 treatment survived an average of 28.7 and 29.5 days, respectively. These results indicate that at least 10 fold higher antibody protein doses of G5/44 are required to inhibit significantly the protective effects of CMC-544 against disseminated B-cell lymphoma.

DISCUSSION

CMC-544 is a CD22-specific antibody-targeted chemotherapeutic agent that binds human CD22 with high affinity and causes potent cytotoxic activity against malignant CD22⁺ B cells (12, 13). CMC-544 can prevent the establishment of subcutaneous human B-cell lymphoma xenografts and also can cause regression of established small and large B-cell lymphoma xenografts in nude mice (12, 13). The present study demonstrates the inhibitory effect of CMC-544 against systemically disseminated B-cell lymphoma in scid mice. The systemically disseminated B-cell lymphoma model studied here represents dissemination, infiltration, and growth of B-cell lymphoma in various organs, including the central nervous system, leading to hind-limb paralysis and subsequent death (14, 15). This model mimics a number of clinicopathological attributes of disseminated extranodal non-Hodgkin’s B-cell lymphoma and has been used extensively to show antitumor activities of various antibody-based therapeutic agents (14–16).
CMC-544 was effective in protecting diseased mice against both the early (minimal disease) and late (established or advanced disease) stages of the B-cell lymphoma-disseminated disease. Diseased mice treated with either an isotype-matched nonbinding control conjugate, CMA-676, unconjugated anti-CD22 mAb G5/44, or vehicle succumbed to the systemic disease within 35 days. In contrast, the antitumor efficacy of CMC-544 was clearly evident at all stages of the disseminated disease, even when its administration was initiated 21 days after the dissemination of B-cell lymphoma.

Rituximab is a chimeric human IgG1 antibody targeted to another B-lymphoid lineage-specific molecule, CD20, and has now become a mainstay of various combination therapies currently used in the treatment of B-cell lymphomas (19, 20, 21). It was used as a benchmarking immunotherapeutic agent in this study. Because of the differences in the mechanisms underlying their antitumor effects, a comparison of antitumor efficacy of rituximab and CMC-544 in the preclinical models may not be appropriate. When examined for its therapeutic effect on scid mice with disseminated Ramos B-cell lymphoma, rituximab was effective in preventing hind-limb paralysis in the diseased mice only if given early during the establishment of the disseminated disease (minimal disease) but was ineffective against the established or advanced disseminated disease state. Rituximab’s therapeutic effects are often attributed to its ability to facilitate effector cell-mediated antibody-dependent cellular cytotoxicity and/or complement-mediated cytotoxicity against rituximab-bound malignant B cells (22–25). In addition to its ability to facilitate antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity against CD20+ targets, rituximab can also exert a direct antiproliferative and apoptotic effect (26, 27).

The overall B-cell lymphoma burden is expected to be lower during the early phase of the disseminated disease (within 3 days postdissemination) than that during the advanced phases of the disease (9 to 21 days postdissemination). Scid mice used in this study possess FcR+ effector cells capable of mediating antibody-dependent cellular cytotoxicity (28–30). If the therapeutic effect of rituximab in this model is dependent on the participation of effector cells then it is likely that, with the increasing B-cell lymphoma burden, the available effector cells are unable to effectively eliminate all B-cell lymphoma, thereby allowing progression of the systemic disease. Consistent with this notion is the study reported by Bertolini et al. (18) wherein rituximab was shown to be effective in preventing the development of B-cell lymphoma but was inactive against established or bulky disease. In mouse models, the above mechanisms seem to be sufficient to inhibit the growth of smaller, developing tumors but not that of larger, more established tumors (30). We have used, in our preclinical model of disseminated B-cell lymphoma, this differential antitumor activity of rituximab to distinguish minimum residual disease state from the established disease state of B-cell lymphoma. Similar differential antitumor effects have been reported with anti-CD45 and anti-CD52 antibodies, suggesting that the observed effects against the minimal residual state of the disseminated B-cell lymphoma are not rituximab-specific but are a more general feature of B-cell lymphoma-targeted effector function-competent antibody therapeutics (31).

In contrast with the effects of rituximab, the therapeutic benefit conferred by CMC-544 can be attributed to the cytotoxic effect of calicheamicin, whose specific intracellular delivery in B-cell lymphoma is facilitated by its physical linkage to anti-CD22 mAb. CD22 is one of the better internalizing receptors on
the surface of B-lymphoid cells (32, 33) and is able to deliver, via its binding of CMC-544, the conjugated calicheamicin inside B-cell lymphoma (12, 13). The internalizability of CD22 has been successfully used to show preclinical therapeutic activities of CD22-targeted immunotoxins (34, 35), and one such toxin has exhibited strong antitumor activity in hairy-cell leukemia (36). The unconjugated anti-CD22 antibody carrier, G5/44, did not possess any intrinsic anti–B-cell lymphoma activity and was ineffective even against minimal residual disease. The lack of effect of G5/44 in this model is not surprising because G5/44 (human IgG4 isotype) cannot mediate antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity activities (37). It is also unlikely that the lack of effect of rituximab against the established or advanced disease state was due to inadequate access to disseminated B-cell lymphoma because rituximab, used at a dose of 20 mg/kg antibody protein, was ineffective, whereas CMC-544 given at a dose of 600 μg/kg conjugated antibody protein (40 μg/kg conjugated CalichDMH) was almost completely protective against the same disseminated disease.

Histopathological analysis of selected tissues from the vehicle-treated diseased mice showed tumor cell infiltration of the brain and spinal cord meninges, bone marrow, and around the hilus of the kidney. The tumor cell infiltration of the spinal cord was most likely the cause of the hind-limb paralysis that was observed in all vehicle-treated mice. Similar analysis of CMC-544–treated diseased mice did not reveal the presence of B-cell lymphoma in any of the organs examined, consistent with the observed lack of hind-limb paralysis in any of the CMC-544–treated mice. Unless effective therapy is given, disseminated B-cell lymphoma infiltrate and populate various organs. Previous studies on the extravasation of systemic tumor cells suggest that the malignant cells from within the peripheral blood bind to the vascular wall and extravasate within 72 hours (37). Whether CMC-544 targeted B-cell lymphoma while these cells were still in the intravascular compartment was not investigated in our study. However, it seems unlikely that the therapeutic effect of CMC-544 was primarily due to targeting of B-cell lymphoma that were still in the intravascular compartment as CMC-544 given 21 days after the B-cell lymphoma dissemination was still effective in retarding the hind-limb paralysis. Duration of survival of diseased scid mice treated with the highest dose of CMC-544 (160 μg/kg q4d×3) was shorter than that of mice treated with the lower doses of CMC-544. The reduction in the survival duration at the highest dose of CMC-544 investigated was not due to B-cell lymphoma infiltration of organs because none of the organs examined showed any histopathological evidence of B-cell lymphoma infiltration, nor did any of these mice exhibit hind-limb paralysis. In scid mice with s.c. B-cell lymphoma xenografts, CMC-544, at 160 μg/kg (q4d×3), strongly inhibited tumor growth (Fig. 5) but also induced death of 50% of the treated mice within 50 days. When examined against s.c. B-cell lymphoma xenografts in nude mice, the same dose of 160 μg/kg CMC-544 (q4d×3) is completely efficacious (curative) and nonlethal (12, 13). The minimum nonlethal dose of CMC-544 in scid mice was determined to be between 80 and 160 μg/kg q4d×3. In the nude mice, no deaths were recorded at CMC-544 doses up to 240 μg/kg q4d×3 (12). Thus, scid mice appear to be more sensitive to the adverse effects of the CMC-544 than nude mice. Similar increased sensitivity of scid mice to radioimmunoconjugates (38) and ionizing radiation (39) has been demonstrated and can be attributed to a deficiency in repairing DNA double-strand breaks in scid mice (39). Thus, the diseased scid mice treated with 160 μg/kg CMC-544 in the current study were freed of B-cell lymphoma but probably died from drug-mediated adverse effects. The reasons underlying the deaths of mice treated with high dose of CMC-544 remain unexplored as histopathological analysis of various organs from these mice was not carried out in this study.

Clinical applications of CD20-targeted radioimmunotherapeutic agents, 131I-labeled tositumomab (Bexxar) and yttrium-90–labeled ibritumomab tiuxetan (Zevalin), both involve predosing with the unconjugated anti-CD20 antibody.5,6 This step is deemed necessary to block normal B-cell CD20 antigenic sinks (40, 41) to focus the effects of radiolabeled therapeutic antibody on B-cell lymphoma. The source of these potential normal B-cell sinks is in the circulation, the spleen, and the bone marrow. Similar CD22-targeted radioimmunotherapeutic strategies have also used the use of unlabeled (cold) anti-CD22 mAb to block the normal B-cell CD22 antigenic sinks (42, 43). It is unclear whether a predosing clinical strategy would be required for CD22-targeted immunoconjugates such as CMC-544. However, if a similar strategy were to be applied to CMC-544, it would be important to determine the impact of unconjugated anti-CD22 mAb treatment on the antitumor efficacy of CMC-544. As shown in this study, predosing of G5/44 before CMC-544 caused a significant reduction in the antitumor activity of CMC-544 when the amount of preadministered G5/44 was 10-fold higher than the amount of G5/44 present in the conjugate. Similar results were observed in vitro where G5/44 was able to inhibit in a concentration-dependent manner the cytotoxic activity of CMC-544. Both CMC-544 and G5/44 bind human CD22 but not CD22 from mice, rats, dogs, and cynomolgus monkeys. Hence, the above experimental conditions preclude the potential contribution from endogenous murine CD22 to bind CMC-544. Whether similar predosing with unconjugated anti-CD22 mAb would be necessary and its potential impact on the clinical efficacy of CMC-544 in patients remains to be determined. In summary, CMC-544, a CD22-specific antibody-targeted chemotherapy, causes long-term survival of mice with systemically disseminated B-cell lymphoma. Additionally, unlike rituximab, CMC-544 was effective even when given during the advanced phase of the disseminated disease. These results support the clinical application of CMC-544 as a targeted chemotherapy in the treatment of CD22+ B-lymphoid malignancies, including pre–B-cell and B-cell acute lymphocytic leukemia and non-Hodgkin’s B-cell lymphoma. CMC-544 is currently being investigated in phase I clinical trials in non-Hodgkin’s B-cell lymphoma.

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5 L. Kalyandrug, unpublished observations.

9 Unpublished observation.
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