Superior Antitumor Activity of SAR3419 to Rituximab in Xenograft Models for Non-Hodgkin’s Lymphoma
Ayad M. Al-Katib,1 Amro Aboukameel,1 Ramzi Mohammad,1 Marie-Christine Bissery,2 and Claudia Zuany-Amorim2

Abstract
Purpose: To investigate the activity of SAR3419, a novel humanized anti-CD19 antibody (huB4), conjugated to a cytotoxic maytansine derivative N2'-deacetyl-N2'- (4-mercaptop-4-methyl-1-oxopentyl) maytansine, in preclinical xenograft models for non-Hodgkin’s lymphoma.

Experimental Design: Antitumor activity of SAR3419 was assessed as a single agent and in comparison with conventional therapies using a subcutaneous model for diffuse large B-cell lymphoma (WSU-DLCL2) and a systemic model for follicular small cleaved cell lymphoma (WSU-FSCCL) in mice with severe combined immune deficiency.

Results: Our results showed that in these chemotherapy-resistant models, SAR3419 was more effective than CHOP (cyclophosphamide-Adriamycin-vincristine-prednisone) regimen or rituximab. Only treatment with SAR3419 led to survival of the whole group of animals to the end of the experiment (150-155 days) in both models. Higher doses of SAR3419 (15 and 30 mg/kg) were more effective than lower dose of 7.5 mg/kg. The immunoconjugation was necessary because neither huB4 nor DM4 alone had significant activity. Treatment with rituximab resulted in antitumor activity in both models comparable with the low dose of SAR3419. Cyclophosphamide-Adriamycin-vincristine-prednisone alone showed modest activity in both models. Necropsy and tissue staining in the WSU-FSCCL systemic model revealed that all deaths featured leptomeningeal lymphoma in the control and treated groups. Interestingly, some of the animals that survived to the end of the experiment and seemed healthy at time of euthanasia did show microscopic evidence of lymphoma.

Conclusions: Overall, SAR3419 is a very active immunotoxin in preclinical models for human B-cell lymphoma and holds promise as a novel and well-tolerated therapy in B-cell non-Hodgkin’s lymphoma.

Non-Hodgkin’s lymphoma (NHL) is the fifth most common cancer in the United States where >66,000 cases are expected to be diagnosed in 2008 (1). An increasing incidence of NHL at a rate of 3% to 4% per year was observed world-wide for the 1970s and 1980s and 1% to 2% in the 1990s, resulting in almost doubling the NHL incidence (2). Although there are geographic variations in the distribution of different histologic subtypes of NHL, the most common ones are the diffuse large B-cell and the follicular lymphomas (3). The addition of anti-CD20 antibody (rituximab) to cytotoxic chemotherapy has been the most notable improvement of NHL treatment since the introduction of Adriamycin in the 1970s (4). Overall, the 5- and 10-year relative survival of NHL has improved from 50.4% and 39.4% (1990-1992) to 66.8% and 56.3% (2002-2004), respectively (5). Improvements were seen in all age groups, in both sexes, in both nodal and extranodal disease, and in both low-grade and high-grade lymphomas. There are a number of limitations to rituximab therapy, however. In the pivotal clinical trial that led to its approval for clinical use in the United States, only 48% of patients with relapsed follicular lymphoma responded (6% complete and 42% partial responses; ref. 6). Moreover, almost 60% of initially responding patients will not respond to subsequent therapy (7). The success (and limitations) of rituximab presents clear opportunity for exploring additional monoclonal antibody–based therapy in lymphoma. To that end, different approaches are being explored including targeting different cell surface antigens.
conjugating antibodies with radioisotopes, chemotherapeutic drugs, or toxins (reviewed in ref. 8). The most developed among these is the radio-immunotherapy where a radioisotope is conjugated to a monoclonal antibody. Two anti-CD20 IgG--radioconjugates, 109V-ibritumomab tiuxetan (Zevalin) and 131I-tositumomab (Bexxar), have been approved by the U.S. Food and Drug Administration for the treatment of relapsed/refractory, indolent, or transformed B-cell NHL. Several studies have shown that radio-immunotherapy is well-tolerated, has the highest single-agent activity observed in lymphoma therapy, and can provide durable responses even in patients who had failed previous treatments, including rituximab therapy (9).

Antibody-targeted chemotherapy is another therapeutic strategy that involves the use of a cytotoxic agent chemically linked to a monoclonal antibody recognizing a tumor-associated antigen. The monoclonal antibody delivers the cytotoxic agent preferentially to tumor cells that express the targeted antigen, hence limiting systemic toxicity. In this study, we investigated the preclinical efficacy of anti-CD19 humanized monoclonal antibody (huB4) conjugated with a maytansine derivative [N2-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl) maytansine, DM4] in two xenograft models representing human diffuse large B-cell lymphoma and follicular lymphoma in comparison with rituximab and standard cytotoxic chemotherapy regimen (cyclophosphamide-Adriamycin-vincristine-prednisone, CHOP).

Maytansine is a highly cytotoxic natural product that has failed previous treatments, including rituximab therapy (9).

**Materials and Methods**

**Lymphoma cells**

Two established cell lines were used in this study: WSU-DLCL2 and WSU-FSCCL. Both cell lines were established at Wayne State University (WSU), are EBV negative and were previously published (11, 12). The WSU-DLCL2 line is established from a patient with relapsed and resistant diffuse large B-cell lymphoma. The WSU-FSCCL was established from a patient with follicular lymphoma, grade I according to the WHO classification [follicular small cleaved cell (FSCCL) by the Revised European American Lymphoma classification; ref. 13]. Both cell lines grow as suspension in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% l-glutamine, penicillin, and streptomycin. Cultures are main-lined in a humidified incubator at 37°C and 5% CO2. Culture medium is changed every third day. Cells are checked periodically for immunophenotypic or karyotypic changes.

**Xenograft models**

The two xenograft models used in the study were based on the WSU-DLCL2 and WSU-FSCCL cell lines grown in mice with severe combined immune deficiency (SCID). ICR-SCID mice, age 3 to 4 wk, were purchased from Taconic Farms, housed in special protective cages, and left to adapt to the new environment for 1 wk before beginning the experiments. All in vivo studies were conducted according to Animal Investigation Committee–approved protocol by WSU.

**WSU-DLCL2-SCID subcutaneous model**

For initial establishment of the xenograft, 10⁷ WSU-DLCL2 cells in serum-free RPMI 1640 are injected s.c. bilaterally in each flank of 2 ICR-SCID mice. Within 2 wk, animals developed palpable tumors at injection sites and were euthanized when tumor size reached 1,000 to 1,500 mg. Tumors were then dissected into 20 to 30 mg pieces that were re-implanted into 56 naïve ICR-SCID mice s.c. in the flanks. Using this method, 100% of animals developed tumors. Tumor-bearing animals were randomly assigned to either control or a treatment group as shown below.

**WSU-FSCCL-SCID intravenous (systemic) model**

10⁷ WSU-FSCCL cells in serum-free RPMI 1640 were injected i.v. via tail vein into each of 56 ICR-SCID 3 to 4-wk-old female mice. Based on previous experience with this model (14), animals develop disseminated disease involving bone marrow, liver, spleen, lymph nodes, and leptomeningeal involvement of the central nervous system. There is no secondary propagation in this model. Animals injected with the WSU-FSCCL were randomly assigned to control or a treatment group.

**Preclinical efficacy trial design**

Treatment of tumor-bearing animals in both models prepared as described above was started 1 wk after transplantation of tumors (WSU-DLCL2) or i.v. injection of cells into mice (WSU-FSCCL). There were 8 treatment groups, 7 animals in each treated as follows: group 1, vehicle (5% glucose in water), every fourth day for 2 doses (q4dx2); group 2, SAR3419 at 30 mg/kg/injection, q4dx2; group 3, SAR3419 at 15 mg/kg/injection, q4dx2; group 4, SAR3419 at 7.5 mg/kg/injection, q4dx2; group 5, free DM4 at 0.6 mg/kg/injection, q4dx2, which is comparable with the amount of DM4 detected in the immunoconjugate SAR3419 at the high dose; group 6, naked huB4 at 30 mg/kg/injection, q4dx2; group 7, CHOP at maximum tolerated doses as previously determined in our laboratory (cyclophosphamide, 40 mg/kg i.v.; doxorubicin, 3.3 mg/kg i.v.; vincristine, 0.5 mg/kg i.v.; and prednisone, 0.2 mg/kg orally daily for 5 d; all iv drugs were given via tail vein once on day 1; ref. 15); group 8, rituximab at 40 mg/kg/injection, q4dx2. This dose/schedule is believed to provide sustained blood level and saturate CD20 antigens of lymphoma cells.

**Treatment agents**

CHOP components were obtained from commercial sources: Cyclophosphamide USP from Bristol-Myers Squibb Co; Doxorubicin HCl from Bedford Laboratories; Vincristine sulfate from Mayne Pharma, Inc., and Prednisone from Schein Pharmaceutical, Inc. Rituximab was obtained from IDEC Pharmaceutical Corp. HuB4 is a humanized mouse monoclonal antibody targeting CD19. DM4 is a cytotoxic compound derived from maytansine (10). SAR3419 is huB4-DM4 immunoconjugate (patent # US 2004/0235840, November 25, 2004). The ratio of DM4 to huB4 antibody is 3.4 moles of DM4 per mole of huB4. The antibody is linked to DM4 by an optimized linker incorporating hindered disulfide bond [N-Succinimidyl-4-(2-pyridyldithio)butanonic acid (SPDB)]. Free drug (DM4) is <1% (by high performance liquid chromatography). The immunoconjugate is stable in plasma and in aqueous formulation and is cleavable inside cells. HuB4,
DM4, and SAR3419 are provided by ImmunoGen, Inc., through a license agreement with sanofi aventis. Structure of SAR3419 is shown in Fig. 1.

Animal monitoring

Animals were observed for changes in weight, side effects of therapy, measurement of subcutaneous tumor size (WSU-DLCL2 model), or signs of any sickness. They were euthanized when their tumor burden reached ~10% of the body weight, lost ~20% of body weight, or became moribund due to treatment toxicity or systemic disease in the case of WSU-FSCCL model. The experiment was designed to terminate 150 d after tumor cell injections (16). Some animals in this study were euthanized due to treatment toxicity or systemic disease (WSU-FSCCL) because tumor-free animals at that point are considered disease free and “cured”. This duration of experiment exceeds most standard protocols where experiments are usually terminated 90 to 120 d after tumor cell injections (16). Some animals in this study were euthanized after 155 d from transplantation of tumor fragments. Necropsy was done on selected animals from each treatment group. Animals that were tumor free at that point are considered cured of their lymphoma.

Determination of response

Different criteria were used to determine treatment efficacy in the WSU-DLCL2 model from those used for the WSU-FSCCL model. For the WSU-DLCL2-SCID model, tumor growth inhibition, tumor growth delay, and log_{10} cell kill were used, which are standard measures of tumor response evaluation in subcutaneous xenograft models (16). For the WSU-FSCCL-SCID systemic model, median day of death for each group and number of animals alive at the end of the experiment were used as end points. This method is applicable to systemic diseases, such as leukemia, that disseminate in internal organs of animals but do not have measurable subcutaneous tumors (16).

Data processing

Estimation of Tumor doubling (Td) of subcutaneous (WSU-DLCL2) tumors. Td is the time for the tumor in the control group to reach twice a given tumor weight. Given that tumor weight follows a log-normal distribution, a base 10 logarithmic transformation is done on this variable. Individual Td values were estimated in the time zone between day 13 and day 25 posttransplantation of tumor in the control group. Tumor growth was fairly linear in this zone for all animals and comprised at least two measurements by animal. For each animal in the control group, a linear regression is done on the base 10 logarithm of the tumor weights (LOGTUM) according to time as follows: LOGTUM = slope* time + constant. Individual Td is calculated according to the following formula by dividing the base 10 logarithm of the 2 value by the slope estimated from the considered linear regression: Td = log_{10} (value 2)/slope. The control group Td is then calculated by taking the median of the control group individual Td.

T and C estimation. Defined as the time to reach 800 mg tumor weight for the control (C) group and for the treated (T) group. Tumor weights were analyzed after log_{10} transformation. T and C values were estimated for each animal by linear interpolation between the tumor weight values surrounding the 800-mg threshold. To illustrate this formula, we use a hypothetical tumor weight target of 1,000 mg: tumor weight of animal 1 in the control group was 905 mg (value 1) at day 31 (time 1) and 1,050 mg (value 2) at the next observation, day 34 (time 2); these 2 values surround the 1,000 mg threshold value. A linear interpolation between these 2 observations allows estimating the time for the tumor to reach 1,000 mg (Time 1000) as follows: Time 1000 = time 2 + [(Log_{10}(1000) – Log_{10}(value 2)) × (time 2 – time 1)]/[Log_{10}(value 2) – Log_{10}(value 1)]. So, for animal 1 of the control group, Time 1000 = 34 + [(Log_{10}(1000) – Log_{10}(1050)) × (34–31)]/[Log_{10}(1050) – Log_{10}(905)] = 33 d. The medians of the T and C individual values of the treated and control groups, respectively, are then calculated taking into account censored values in animals where tumors did not reach threshold value by the end of their observation period.

T − C calculation: T minus C values were calculated from T and C estimates as above.

Log_{10} cell kill (lck): calculated using the following formula, lck = T − C/(3.32 × Td) where T is the median of individual time-to-reach 800 mg for the treated group; C is the median of the individual time-to-reach 800 mg in the control group and Td is the control group doubling time.

Statistical methodology

Td and Log_{10} cell kill were calculated as descriptive parameters. Time to reach 800-mg threshold for the subcutaneous tumors was determined for each mouse, and then the treated groups were compared with vehicle one. However, for the systemic model (WSU-FSCCL), the log-rank test was applied and P value for all experimental groups was determined.

![Fig. 1. Structure of SAR3419 used in this study.](image-url)
Necropsy and histopathologic examination

All euthanized animals were dissected and examined macroscopically for tumors or signs of gross tissue or organ abnormalities. Histopathologic examination of organs was conducted on selected animals in each group. Tissue sections were stained with H&E and examined by two investigators (AMK and AA) with previous experience in this model (14).

Immunophenotyping of cells

Expression of CD19 and CD20 on WSU-DLCL2 and WSU-FSCCL was determined using direct immunofluorescence staining with anti-CD19 and anti-CD20 monoclonal antibodies (DAKO A/S) as previously described (11). Stained cells were analyzed by flow cytometry using FACScan (BD Biosciences). Background fluorescence is determined by staining cells with normal mouse IgG. The proportion of positive cells is determined by subtracting positive cells in the control from the specific monoclonal antibody. Channel of peak fluorescence (CPF) is visually determined as the peak of the flow cytometry-generated curve of fluorescence where 20,000 cells per test are analyzed. CPF reflects the intensity of fluorescence signal and is an indirect measure of the density of antigen expression.

Results

CD19 and CD20 expression by lymphoma cells. As shown in Fig. 2, both cell lines expressed CD19 and CD20. In the WSU-DLCL2, 99% and 99.9% of cells were positive for CD19 and CD20, respectively. CD20 expression was brighter in this cell line as indicated by a higher CPF (238.2 versus 10.8, respectively). Similar trend was observed in the WSU-FSCCL line but with lower values; 81.4% and 93.6% of cells expressed CD19 and CD20, respectively, with CPF values of 9.5 and 35.1, respectively. CD19 intensity of expression was comparable in both cell lines, whereas CD20 expression was ~7-fold brighter in the WSU-DLCL2 compared with WSU-FSCCL as indicated by CPF.

Efficacy of SAR3419 in WSU-DLCL2-SCID model. Td time in this experiment is estimated to be 5.5 days. As shown in Table 1, SAR3419 at the higher dose levels (30 and 15 mg/kg) was effective in eradicating the xenograft tumors. At the lower dose level (7.5 mg/kg), there was significant antitumor activity (tumor growth inhibition of 0.0, tumor growth delay of 60 days, and ≥3 Log_{10} cell kill). There were 4 delayed deaths (days 113-130) in the highest dose of SAR3419 (30 mg/kg) where necropsy and histopathologic examination did not show evidence of tumor. We therefore believe that such deaths may represent treatment-related toxicity. In the intermediate dose level of 15 mg/kg, all animals survived to the end of experiment (day 155) and were tumor free. Unconjugated (naked) huB4 antibody showed no significant activity. DM4 alone was toxic after 1 or 2 injections when given at a concentration of ~214 μg/mL and resulted in severe toxicity (weight loss, dehydration, and lethargy) or death of all animals within few days of treatment. The same dose (0.6 mg/kg) was tolerable when given in a more diluted form (~93 μg/mL). CHOP regimen resulted in modest efficacy consistent with previous observations in this model (15, 17, 18). However, activity score of (+) is not considered clinically relevant. The WSU-DLCL2 is considered a chemotherapy-resistant model since it was established from a patient with aggressive lymphoma refractory to therapy (11). Activity score in xenograft models of (+++) or (++++) corresponds with clinical partial and complete response, respectively (16). For rituximab, four of the seven animals survived to the end of the experiment and were tumor free. In the other 3, tumors reappeared between days 99 and 103 after transplantation and reached ~10% of body weight between days 116 and 123. Tumor response calculations could not be done because data were censored in more than half of the animals due to activity of rituximab.

Efficacy of SAR3419 in WSU-FSCCL-SCID model. All animals in the control group died of disseminated lymphoma between days 60 and 75 (median day of death is 70; Table 2). The pattern of lymphoma was typical of that previously described in this model (14), i.e., lymphoma involves bone marrow, liver, spleen, lymph nodes, and central nervous system (Fig. 3). There was a clear dose-response relationship of SAR3419 in this model. The highest dose (30 mg/kg) was most effective where all animals survived to the end of the experiment (Fig. 4 and Table 3). In contrast, 4 of 7 and 2 of 7 animals in the intermediate dose (15 mg/kg) and the low dose (7.5 mg/kg) survived to the end of the experiment, respectively. Unconjugated huB4 alone had very little activity in this model where the median survival for the whole group was 75 days compared with control. DM4 showed negligible activity with median day of death of 75 and no animal survived beyond 91 days after cell dose injection. When compared with SAR3419, rituximab at 40 mg/kg had antitumor activity comparable with the low dose of 7.5 mg/kg with 2 of 7 animals survived to the end of the experiment. Median day of death for the rituximab group was 110 compared with 96 days for SAR3419 low dose. CHOP chemotherapy showed modest activity in this model with median day of death of 147 days (range, 65-150). However, only three of seven animals survived to the end of the experiment. Necropsy examination was done on 37 of the 56 total animals in this experiment (66%). All animals that died before the end of the experiment (day 150) had tumors. Most common sites of involvement were lymph nodes (93%), followed by central nervous system (83%), liver (21%), spleen (10%), and bone marrow (8%). The pattern of central nervous system involvement was leptomeningeal (Fig. 3), which we believe is the cause of death whenever it occurred. An unexpected finding in this study is the presence of microscopic disease in animals that

Fig. 2. Expression of CD19 and CD20 on lymphoma cells used in this study. Numbers shown on top of each flow peak represent the CPF. Vertical axis, relative cell number (total 20,000 per test); horizontal axis, log fluorescence intensity.
survived to the end of the experiment and seemed healthy at time of euthanasia (seen in six of seven animals examined).

Discussion

In this article, we provide evidence that anti–CD19-DM4 conjugate has superior antilymphoma activity compared with unconjugated anti-CD19 antibody (huB4), the drug conjugate alone (DM4), or with unconjugated anti-CD20 (rituximab). Findings in this study show the value of immunoconjugation and support their further development for clinical use. The strategy can be used to deliver otherwise prohibitively toxic chemotherapy, maytansine derivative in this case, selectively to tumor tissue without undue host toxicity.

Maytansine was originally isolated from the bark of the African shrub Maytenus ovatus (19). It is the first in class of benzoansamacrolide antibiotics known as maytansinoids (20); antimitotic agents that bind to tubulin and inhibit the microtubule assembly in a manner similar to but 100- to 1,000-fold more potent than Vinca alkaloids (21). Maytansine was evaluated by the U.S. National Cancer Institute in phase I and phase II clinical trials in the 1970s. The compound was found to be very effective with complete and partial responses seen in a variety of tumors such as NHL, melanoma, thymoma, acute lymphoblastic leukemia, ovarian, and breast cancers (22-26). However, severe toxicity including nausea, vomiting, diarrhea, elevated liver enzymes, weakness, and lethargy precluded further development in favor of other antimicrotubule agents. The compound, therefore, is ideal for antibody conjugation that enables selective delivery to CD19-positive cells, hence limiting toxicity. CD19 (B4) is a B-cell–restricted signaling molecule that functions as a positive regulator of the B-cell receptor signaling pathway. Lack of CD19 leads to arrest in B-cell development and hyporesponsiveness to B-cell receptor stimulation (27). Binding of anti-CD19 antibody leads to internalization of its target (28). However, the process is negatively influenced by CD21 expression on target cells (29). Binding of naked anti-CD19 to its target does not necessarily lead to cell death or antitumor activity. In one study where mouse antihuman CD19 antibody was used to treat six patients with B-cell lymphoma, antibody-coated cells remained in the circulation of some patients for several days (30). The CD19 pathway can involve Src family kinases, lead to amplification of B7-1 and B7-2 costimulatory molecules, and activate T cells (31). A defect in any of these steps might explain the lack of activity of the naked antibody.

Developing antibody-drug conjugates (ADC) that recognize tumor-associated antigens has been an active area of research in this decade (32) and can circumvent some of the limitations of naked antibodies. Some of these agents have gained approval for clinical use as standard therapy in selected cancers. As often is the case, clinical success in this area is first achieved

<table>
<thead>
<tr>
<th>Table 2. Response data in WSU-FSCCL-SCID xenograft model by treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment Group</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Control (PBS)</td>
</tr>
<tr>
<td>SAR (30 mg/kg)</td>
</tr>
<tr>
<td>SAR (15 mg/kg)</td>
</tr>
<tr>
<td>SAR (7.5 mg/kg)</td>
</tr>
<tr>
<td>CHOP (MTD)</td>
</tr>
<tr>
<td>Rituxan (40 mg/kg)</td>
</tr>
<tr>
<td>huB4</td>
</tr>
<tr>
<td>DM4</td>
</tr>
</tbody>
</table>

Abbreviations: 95% CI, 95% confidence interval; no. alive at end of exp., no of alive at end of experiment.
in hematologic malignancies; calicheamicin conjugated to anti-CD 33 (Mylotarg) was approved for acute myeloid leukemia in 2000 (33), followed by approval of 2 radio-immunoconjugates: 90Yttrium (Zevalin) and 131I (Bexxar); isotopes conjugated to anti-CD20 in 2002 and 2003, respectively (34, 35). Both Zevalin and Bexxar are proven to be more effective than the unlabeled anti-CD 20 antibody (rituximab). The cytotoxic agent calicheamicin was also conjugated to anti-CD22 (CMC-544) and has shown antilymphoma effect in preclinical animal models (36, 37). Given the encouraging results of ADCs, there are attempts now to develop rituximab-based ADCs (monomethyl auristatin E) to enhance its efficacy (38). Monomethyl auristatin E was also conjugated to anti-CD30 and found to be effective against animal models for Hodgkin lymphoma (39).

Two different linkers have been used to conjugate maytansinoids to antibodies, one is the disulfide bond used in this study (SPDB-DM4; Fig. 1), which is cleavable, and the other is the thioether linker (SMCC-DM1; ref. 40). Lysosomal processing is required for the activity of antibody-maytansinoid conjugates, irrespective of the linker. SPDB-DM4 degrades in the lysosomes releasing intact maytansinoid drugs and linkers attached to lysine. The lysine-SPDB is then reduced and S-methylated to yield lipophilic and potent cytotoxic metabolite, S-methyl-DM4. DM1 conjugated to a human cancer antigen expressed on a number of solid tumors (huC242) was evaluated in preclinical animal models and in a phase I clinical trial (41, 42). The compound was also conjugated to CD79 and CD138 and evaluated in lymphoma and multiple myeloma, respectively (43, 44). Our study is the first one evaluating the DM4-anti-CD19 conjugate. It is noteworthy that ADCs with the disulfide linker (SPDB-DM4) were shown by Kovtun et al. (45), to have a “bystander” effect where antigen-positive and antigen-negative tumor cells are killed effectively. Presence of antigen-positive cells is required for such effect (Table 3).

Our results presented in this study show the efficacy of the anti-CD19-DM4 conjugate against subcutaneous and systemic models of B-cell NHL representing diffuse large B-cell and
follicular lymphoma. Both models are EBV negative and were established from patients with resistant disease to standard chemotherapy. Standard chemotherapy regimen (CHOP) therefore has only modest activity in these models. The demonstration that SAR3419 has more significant activity in these models compared with CHOP is clinically significant and illustrates the promise of ADCs as new therapeutic agents for lymphoma. Our systemic model, the WSU-FSCCL-SCID, has predilection for central nervous system involvement, which is the major cause of death of animals (Fig. 3). SAR3419 was effective against this model when given 7 days after a large cell dose (10^7) inoculum. Similar results were reported using CMC-544-DM1 (anti-CD22 ADC) in the Ramos lymphoma xenograft model (37). In that study, therapy was effective when given up to 15 days after cell dose injection, although the number of cells used was lower than ours (10^6). Like our study, rituximab showed some activity in the xenograft models supporting earlier findings that SCID mice possess FcR-positive effector cells capable of mediating antibody-dependent cell-mediated cytotoxicity (46). SAR3419 exhibited more significant activity than rituximab when given at optimal doses despite the fact that both models used in this study showed brighter expression of CD20 than CD19 (Fig. 2).

We conclude that hu84-DM4 conjugate (SAR3419) is extremely active in both subcutaneous and systemic preclinical xenograft models for diffuse large cell and follicular lymphomas. In addition to further development for clinical use, future studies can explore its use in combination with other antilymphoma agents.

### Disclosure of Potential Conflicts of Interest

A.M. Al-Katib, commercial research grant, Sanofi-aventis.

### Acknowledgments

We thank Bertrand Monneau and Noëlle Boussac-Marlèire for excellent biostatistical support.

### References


Superior Antitumor Activity of SAR3419 to Rituximab in Xenograft Models for Non-Hodgkin's Lymphoma

Ayad M. Al-Katib, Amro Aboukameel, Ramzi Mohammad, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/12/4038

Cited articles
This article cites 45 articles, 25 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/12/4038.full#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/15/12/4038.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 AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/15/12/4038.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.