Bcl-2 Antisense Oligonucleotides Are Effective against Systemic but not Central Nervous System Disease in Severe Combined Immunodeficient Mice Bearing Human t(14;18) Follicular Lymphoma

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ABSTRACT

The t(14;18) is present in 85–90% of follicular lymphomas. It results in overexpression of the Bcl-2 protein, which inhibits apoptosis and plays a role in lymphomagenesis. Bcl-2 antisense oligonucleotides (ODNs) down-regulate Bcl-2 expression and inhibit growth of the follicular lymphoma cell line WSU-FSCCL. In this study, we have established a human lymphoma xenograft model in severe combined immunodeficient (SCID) mice using the WSU-FSCCL cell line. s.c., i.v., or i.p. injection of WSU-FSCCL cells into SCID mice results in the development of disseminated tumors, with the liver, spleen, bone marrow, and lymph nodes as major sites of disease. Tumors were fatal in 7–14 weeks, depending on cell inoculum and route of administration. Immunohistochemistry, flow cytometry, and cytogenetic analysis confirmed the human B-cell origin of tumor cells in the xenograft. Phosphorothioate ODNs against the translation initiation site of bcl-2 mRNA in the antisense and mismatched antisense sequences were administered i.v. or i.p. to the xenograft models three times a week for 2 weeks, starting on day 7 after tumor injections. Antisense-treated animals had significantly longer survival (mean, 11.6 weeks) compared with 7.6 weeks for the control group and 7.5 weeks for the mismatched antisense-treated animals (P = 0.002 and 0.004, respectively). More significantly, a pathological examination showed no tumor in the liver, spleen, or bone marrow of the antisense group. However, subsequent experiments showed that the central nervous system was involved, causing mice to die although other sites were disease free. We conclude that bcl-2 antisense ODN therapy is effective against systemic FSCCL disease in SCID mice xenografts; however, it does not prevent disease dissemination into the central nervous system causing animal death.

INTRODUCTION

Follicular lymphoma is the most common type of low-grade non-Hodgkin’s lymphoma. Low-grade lymphomas remain incurable with standard chemotherapy, and over time, many of them transform into aggressive high-grade lymphomas with diffuse morphology (1). Approximately 85–90% of follicular lymphomas have the t(14;18)(q32;q21) chromosomal translocation (2, 3) in which the bcl-2 oncogene at 18q21 is translocated to the joining region (JH) of the immunoglobulin heavy-chain locus at 14q32 (4). As a consequence, the bcl-2 gene comes under control of the IgH promoter. This results in the production of high levels of a chimeric mRNA that encodes a normal, yet overexpressed, Bcl-2 protein. Bcl-2 protein functions as an antidote to programmed cell death (apoptosis; Refs. 5, 6). Overexpressed Bcl-2 in follicular lymphoma promotes cell survival and plays a key role in lymphomagenesis. Down-regulation of Bcl-2 expression, therefore, can be a therapeutic target in t(14;18)-carrying lymphomas (7, 8). Using molecular approaches such as antisense ODNs3 is one way of achieving this goal.

Antisense ODNs are sequence-specific inhibitors of gene expression and have been used to study the consequences of oncogene inhibition (reviewed in Refs. 9–11). Antisense bcl-2 ODNs were found to inhibit the growth of a leukemia cell line that expresses Bcl-2 protein (12). In another study, it inhibited Bcl-2 protein expression and decreased cell survival of acute myelogenous leukemia cells in culture (13). Several studies have examined the prognostic importance of Bcl-2 expression in patients with non-Hodgkin’s lymphoma. Bcl-2 was found to be an independent poor prognostic factor in these patients (14–16). Recently, a Phase I clinical trial of Bcl-2 antisense ODNs in patients with non-Hodgkin’s lymphoma showed that there were 1 complete response and 2 minor responses, 9 patients with stable disease, and 9 patients with progressive disease (17).

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3 The abbreviations used are: ODN, oligodeoxynucleotide; WSU, Wayne State University; FSCCL, follicular small-cleaved cell lymphoma; CNS, central nervous system.
Bcl-2 is an antiapoptotic gene involved in the regulation of apoptosis pathway (15, 16) and can be used as a therapeutic tool to target the Bcl-2 protein.

We tested the efficacy of bcl-2 antisense ODNs against a human t(14;18) lymphoma cell line. WSU-FSCCL is established in our laboratory from the peripheral blood of a patient with FSCCL in leukemic phase (18). The cell line exhibits t(14;18)(q32;q21), and PCR analysis confirmed the juxtaposition of the major breakpoint region of bcl-2 to the immunoglobulin heavy chain (JH) gene. Antisense ODNs directed against the translation initiation site of bcl-2 mRNA inhibits WSU-FSCCL growth in vitro in a dose-dependent manner, where 40 μg/ml (6.9 μmol/l) resulted in complete growth inhibition (19).

Other in vivo studies have shown that the use of a combination of bcl-2 antisense ODNs and cytotoxic agents may have great therapeutic benefits (20, 21).

To extend our results and confirm the activity of bcl-2 antisense ODNs in vivo, we set the goal of establishing a t(14;18) human low-grade lymphoma xenograft model. Mice with SCID, preconditioned with cyclophosphamide, were injected with WSU-FSCCL cells s.c., i.v., or i.p. The animals developed disseminated tumors and died at various intervals (7–14 weeks), depending on the cell dose and route of administration. Nuclease-resistant, phosphorothioate-modified bcl-2 ODNs in the antisense and mismatched antisense sequences were used to test the in vivo effects of antisense treatment.

**MATERIALS AND METHODS**

**Tumor Cell Line.** The human follicular lymphoma cell line WSU-FSCCL was used as a source of tumor cells (18). It carries the translocations t(14;18)(q32;q21) and t(8;11)(q24; q21) and overexpresses Bcl-2 protein (19). Tumor cells grow as a single cell suspension in tissue culture. The cell suspension is maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 1% L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cell line expresses cytoplasmic and surface IgM k, CD20, and CD10 and lacks T-cell and myeloid/monocytic markers. It is negative for EBV nuclear antigen.

**Establishment of the Xenograft Model.** Four-week-old female Fox Chase CB 17 SCID mice were obtained from Taconic Laboratories (Germantown, NY). The animals were kept in a sterile environment in the animal facility at WSU. After adaptation, the animals were conditioned with 150 mg/kg cyclophosphamide s.c. (22). Seventy-two h later, WSU-FSCCL cells suspended in RPMI 1640 were injected into each animal. At first, 5 × 10^6 cells were injected s.c. into each flank of five animals. The animals were examined three times a week for the development of s.c. tumors, general activity, and weight.

The following tumor cell inocula were injected i.v. into five mice each: 2.5 × 10^6, 5 × 10^6, 1 × 10^7, and 2 × 10^7. A dose of 2 × 10^7 was injected i.p. into five mice. Animals’ activity, weight, and survival were monitored three times a week. Mice were sacrificed when they developed hind region paralysis, had decreased activity and weight loss of 15% or more, and death was felt to be imminent. Necropsy was carried out. The extent of macroscopic disease was identified, and all major organs were taken for microscopic pathological examination, including the liver, spleen, kidney, pancreas and retroperitoneal fat, heart and lungs, femur (for bone marrow), and brain. Peripheral blood smears were examined for evidence of circulating lymphoma cells.

The 10^7 i.v. tumor cell inoculum was chosen as the standard cell dose for all subsequent experiments. To determine the earliest date of pathologically demonstrable tumors after injection, five SCID mice received injections of 10^7 tumor cells, and one mouse was subsequently sacrificed each week on days 8, 12, and 16.
15, 22, 29, and 35 after tumor injection; the liver, spleen, and bone marrow were examined pathologically.

**Phenotypic and Karyotypic Analysis.** To document the human B-cell origin of the tumor in our xenograft, flow cytometry and immunohistochemistry were performed. Immunohistochemistry was performed on the tissue sections of involved organs using antihuman monoclonal antibodies to IgM, κ, λ, CD20 (Becton-Dickinson Immunodiagnostic, San Jose, CA), and Bcl-2 (Dako Corp., Carpinteria, CA). Cells for flow cytometry were obtained from the liver, spleen, and bone marrow of the xenografts. Portions of the liver and the spleen were mechanically minced into a single cell suspension using a steel mesh. Cells were suspended in RPMI 1640, and Ficoll-Hypaque density centrifugation was used to separate them from red cells and other tissue debris. Cells from the bone marrow were obtained by flushing the femur with RPMI 1640 using a 23-gauge needle and a 3-ml syringe. The cell suspension was collected in a centrifuge tube, and Ficoll-Hypaque density centrifugation was used to separate the cells from bone and tissue debris. Some of the cells obtained were suspended in complete RPMI 1640 and subjected to cytogenetic analysis. The cells were stained for flow cytometry using antihuman monoclonal antibodies to CD10, CD19, CD20, CD22, κ, and λ (Becton-Dickinson Immunodiagnostics), as routinely performed in our laboratory (23), to determine the expression of human B-cell markers. Flow cytometric analysis was carried out on the Becton-Dickinson FACS 440.

Cytogenetic analysis was performed on cell suspension obtained from the bone marrow of the femurs. Both G- and Q-banding techniques were applied, similar to those used in the characterization of the WSU-FSCCL line in culture (18).

**ODNs.** ODNs were synthesized on Applied Biosystems Model 394 using standard β-cyanethyl phosphoramide chemistry at the Macromolecular Core Facility of WSU School of Medicine. Phosphorothioate ODNs directed against the translation initiation site of bcl-2 mRNA in the antisense and mismatched antisense orientation were made according to the following sequences: antisense, 5’-ACCCTGTGTTCACCGTGCG-3’; and mismatched antisense, 5’-CCCTTGTGCTACCCCGTGCAGA-3’.

The crude deblocked ODNs were precipitated in isopropanol, suspended in Tris-EDTA, and stored at −20°C. Before injection into the mice, ODNs were thawed and diluted in RPMI 1640 to a final concentration of 1 mg/ml.

**Treatment Plan.** A tumor cell inoculum of 10^7 was chosen to test the effects of bcl-2 antisense ODN on the lymphoma xenograft model. Fifteen SCID mice received injections of 10^7 WSU-FSCCL cells each on day 0, given i.v. via a tail vein. On day 7 after tumor cell injection, the animals were randomly divided into three groups: control untreated, antisense, and mismatched antisense-treated groups.

ODNs, diluted in RPMI 1640, were administered i.v. via a tail vein at a dose of 10–15 mg/kg/mouse three times a week for 2 weeks. Because of the quantity of bcl-2 antisense ODN available to us at the time of the animal experiment, two mice (in each experimental group) received 10 mg/kg of antisense and of the mismatched antisense. Animals were monitored three to five times a week for activity, weight, and survival. The animals were euthanized when they had hind region paralysis, lost weight of 15% or more, and death was imminent. Necropsy was carried out on euthanized animals with sampling of the liver, spleen, and bone marrow, because these were the major sites of disease in the xenograft model.

In another experiment, a set of 10 SCID mice was used to address the question of why ODN-treated animals died although necropsy results of the first experiments showed no significant systemic disease. In this experiment, necropsy of the CNS in control and ODN-treated mice was conducted.

**Statistical Analysis.** The end point of study was survival duration as determined from the day of tumor injection. The t test and the Wilcoxon rank sum test were used to determine significance of survival differences. *P* < 0.05 was considered significant. Analysis was performed on SAS statistical software.

### RESULTS

**WSU-FSCCL-SCID Xenograft Model.** After s.c. tumor injection of 5 × 10^6 WSU-FSCCL cells into the flanks of five animals, s.c. tumors failed to develop. At about 10–12 weeks after tumor injection, the animals were inactive and lost weight. All five animals died in 12–14 weeks from tumor injection. Postmortem examination revealed an enlarged, pale-yellow, tumor-studded liver, enlarged spleen, and enlarged retroperitoneal lymph nodes. Histopathological sections of the liver showed infiltration by tumor cells of the perportal areas and around the central vein (Fig. 1A). The bone marrow and spleen showed diffuse infiltration and replacement by tumor cells (Fig. 1B). The lungs, kidney, and pancreas were focally involved by tumor in a perivascular pattern. Retroperitoneal lymph nodes were diffusely replaced by tumor cells. The heart muscle was not involved by tumor; however, skeletal muscles attached to the femur were infiltrated by tumor cells. Morphologically, they

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose</th>
<th>No. of animals</th>
<th>s.c. tumor*</th>
<th>Lymph nodes*</th>
<th>Liver*</th>
<th>Spleen*</th>
<th>Bone marrow*</th>
<th>Survival (wk)</th>
<th>CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>5 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12–14</td>
<td>ND</td>
</tr>
<tr>
<td>i.v.</td>
<td>2.5 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10–12</td>
<td>ND</td>
</tr>
<tr>
<td>i.v.</td>
<td>5 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10–12</td>
<td>ND</td>
</tr>
<tr>
<td>i.v.</td>
<td>1 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7–9</td>
<td>+</td>
</tr>
<tr>
<td>i.v.</td>
<td>2 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6–7</td>
<td>ND</td>
</tr>
<tr>
<td>i.v.</td>
<td>2 × 10^6</td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6–7</td>
<td>ND</td>
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*+, tumor involvement; −, no tumor; ND, not done.
were cleaved lymphoma cells with prominent nucleoli. The cell size was 1.5–2 times the size of WSU-FSCCL cells in culture.

After i.v. tumor cell injection at 2.5 × 10^6 and 5 × 10^6 into each mouse via the tail vein, the animals became inactive with hind region paralysis and died in 10–12 weeks. At necropsy, pathological examination of the liver, spleen, bone marrow, and lymph nodes revealed the same changes seen with the s.c. tumor inoculation. Examination of the central nervous tissue revealed tumor infiltration of the brain and cerebrospinal fluid.

Increasing tumor cell inoculum to 1 × 10^7 cells i.v. resulted in shortening animal survival to 7 weeks (Table 1). Further increases in tumor cell dose to 2 × 10^7 i.v. or i.p. resulted in a survival of 6–7 weeks. The earliest time to the development of a pathologically demonstrable tumor after i.v. injection of 10^7 tumor cells was 14 days. The first organ of involvement was the liver, followed by the spleen and bone marrow. Other organ involvement and peripheral circulation of tumor cells (leukemic phase) were delayed.

Animal death within 24 h of tumor cell injection i.v. was encountered on some occasions, probably as a result of complications of the i.v. injection. A pathological examination of the lungs in animals that died acutely within 10 hours of tumor cells injection revealed pulmonary vascular congestion, without tumor cell emboli. Blood cultures from these animals showed no evidence of infection.

**Phenotype and Karyotypic Characteristics of the Xenograft.** Immunohistochemical staining of pathological sections showed the tumor cells to stain positive with antihuman monoclonal antibodies to κ and to Bcl-2 (Fig. 2). Flow cytometric analysis of tumor cells obtained from the xenograft model demonstrated positive staining for CD10 (48%), CD20 (59%), CD22 (65%), κ (45%), and IgM (63%). The staining pattern was consistent with WSU-FSCCL cell line staining pattern *in vitro* (Table 2), thus confirming the human B-cell origin of tumor cells in the xenograft model. Analysis of 10 metaphase cells derived from the bone marrow of WSU-FSCCL-bearing SCID mice showed human chromosomes with the same aberrations of WSU-FSCCL cells in culture, including t(14;18)(q32;q21) and t(8;11)(q24;q21).

**Effects of ODNs on the Xenograft Model.** Fifteen SCID mice were conditioned with cyclophosphamide (150 mg/kg) s.c. on day −3. On day 0, 10^7 WSU-FSCCL cells were injected i.v. into each animal. One animal died acutely within 24 h. On day 7 after tumor cell injection, the remaining 14 animals were randomly assigned to three groups: 5 animals to receive *bcl-2* antisense ODN treatment, 4 animals to receive *bcl-2* mismatched antisense ODN treatment, and 5 were untreated controls. ODNs were given i.v. or i.p. three times a week for 2 weeks starting on day 7. There were no acute adverse effects related to ODN administration.

The mice in the control and the *bcl-2* mismatched antisense ODN-treated groups behaved similarly, developing decreasing activity, weight loss, and lethargy by 5 weeks after tumor injection. Control animals had a mean survival of 7.6 weeks (SD, 0.9) with a range of 7–9 weeks. There was no difference in the survival duration of these two groups (*P* = 0.85). A pathological examination of animals in the control and mismatched antisense-treated groups demonstrated tumor infiltration and replacement of the liver, spleen, and bone marrow with cleaved lymphoma cells (Fig. 1). Necropsy of the CNS was negative.

On the other hand, the *bcl-2* antisense ODN-treated group started to become less active at ∼7 weeks. The disease process in this group was rather indolent, weight loss in this group was not as fast as the other two groups, and survival was moderately prolonged. Animals in the antisense-treated group survived a mean of 11.6 weeks (SD, 1.8), with a range of 10–14 weeks (Table 3). The survival difference between the antisense-treated group on one hand and the control and mismatched antisense-

### Table 2 Phenotypic characters of the tumor cells derived from the WSU-FSCCL-SCID xenograft model compared with the original cell line, WSU-FSCCL

<table>
<thead>
<tr>
<th>Marker</th>
<th>Xenograft tumor cells (% positive cells)</th>
<th>WSU-FSCCL cell line (% positive cells)</th>
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</thead>
<tbody>
<tr>
<td>CD20</td>
<td>58.5</td>
<td>53.7</td>
</tr>
<tr>
<td>CD22</td>
<td>64.8</td>
<td>75.3</td>
</tr>
<tr>
<td>CD10</td>
<td>48.3</td>
<td>59.9</td>
</tr>
<tr>
<td>κ</td>
<td>45.4</td>
<td>92.0</td>
</tr>
<tr>
<td>λ</td>
<td>12.8</td>
<td>3.3</td>
</tr>
<tr>
<td>IgM</td>
<td>63.0</td>
<td>87.8</td>
</tr>
<tr>
<td>CD5</td>
<td>12.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

![Fig. 2 Immunoperoxidase-stained sections of the bone marrow of xenografts demonstrating positive staining with anti-κ (A) and anti-Bcl-2 (B) antibodies. ×50.](image-url)
treatment groups on the other hand was statistically significant using the *t* test, with *P* values of 0.002 and 0.004, respectively. The survival difference remained significant using the Wilcoxon rank sum test, with *P* values of 0.01 and 0.02, respectively.

On gross inspection, the antisense ODN-treated animals revealed normal liver and spleen. Histopathological examination revealed a hypocellular bone marrow with no evidence of tumor cell infiltration. (Fig. 3). The liver and spleen were normal.

To investigate the cause of death in animals treated with antisense ODNs, we repeated the experiment with 10 SCID mice (5 control mice and 5 antisense ODN-treated mice). In this experiment, necropsy from the CNS of antisense ODN-treated animals revealed tumor infiltration of the leptomeninges (Fig. 1C) and the brain in a focal perivascular pattern.

We have performed immunohistochemistry staining of the liver, spleen, bone marrow, and brain sections of untreated WSU-FSCCL-SCID mice and antisense-bcl-2-treated animals. Results showed that few FSCCL cells were positive in the liver and spleen compared with controls. In addition, in these few positive cells, the expression of bcl-2 was fainter compared with the sections taken from untreated animals. Unfortunately, we could not detect evidence of apoptotic death in the tissue sections of the bcl-2 antisense ODN-treated animals.

**DISCUSSION**

We have successfully established a human follicular lymphoma xenograft using the WSU-FSCCL cell line. This cell line is characterized by t(14;18), which leads to dysregulated *Bcl-2* gene expression and prolongs lymphoma cell survival (18). Regardless of the route (s.c., i.v., or i.p.), injection of WSU-FSCCL cells into SCID mice resulted in the development of a disseminated tumor involving the liver, spleen, bone marrow, brain and meninges, lungs, kidneys, lymph nodes, and retroperitoneal fat. The degree of involvement of different organs by this tumor was variable, with the spleen, bone marrow, and lymph nodes being the most extensively replaced by tumor cells. The liver was the first organ to demonstrate tumor infiltration (at 2 weeks after tumor injection). It was extensively involved with tumor infiltration of the periportal, subcapsular areas and around the central vein. Involvement of the other organs by a tumor was delayed and not as extensive. The lungs, kidneys, pancreas, and the brain revealed perivascular and focal tumor infiltration suggesting a hematogeneous spread from the primary organs of involvement (liver, spleen, bone marrow, and lymph nodes).

The relatively prolonged survival of the xenograft model and the disseminated pattern of tumor involvement is typical of the t(14;18) low-grade lymphoma biology in humans. The *in vitro* characteristics (immunohistochemistry, flow cytometry, and cytogenetic analysis) of the WSU-FSCCL cell line were largely reproduced *in vivo* and documented the human B-cell origin of these tumors. Morphologically, the tumor cells were large in size and have lost the characteristic small-cleaved appearance of the parent lymphoma cell line. It is worth mentioning that, in agreement with previous reports (22), in all our lymphoma xenograft models, the tumor cells attained a larger size compared with their *in vitro* parent cell lines. Similarly, in our well-differentiated Waldenström’s macroglobulinemia xenograft model, the cells attained a larger size compared with

### Table 3

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of animals</th>
<th>Survival, wk</th>
<th>Liver</th>
<th>Spleen</th>
<th>Bone marrow</th>
<th>CNS</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>7.6 ± 0.9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Antisense</td>
<td>5</td>
<td>11.6 ± 1.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mismatched antisense</td>
<td>4</td>
<td>7.5 ± 0.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
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</table>

*Cell inoculum, 10⁷ i.v.*

*Median survival ± SD. +, tumor involvement; −, no tumor; ND, not done.*

*P = 0.002 and 0.004 compared with the control and mismatched antisense groups, respectively.*
the WSU-WM cell line but retained its functional ability of IgM secretion (24).

The WSU-FSCCL cell line has an additional chromosomal translocation, t(8;11)(q24;q21) with activation of the c-myc oncogene. The patient from whom the WSU-FSCCL line was established had a localized transformation in the gastrointestinal tract to the small noncleaved cell lymphoma. On the other hand, the circulating lymphoma cells from the same patient were small cleaved (18). Thus, the t(8;11) might be a signal of transformation to an aggressive lymphoma. Therefore, our WSU-FSCCL xenograft model might serve as a good preclinical model bearing both t(14;18) and t(8;11) to assess the in vivo therapeutic potential of various agents targeting such translocations.

The antisense ODNs have been tested against leukemia cell lines in vitro directed to different oncogenes, including c-myc, c-myc, BCR-ABL (25–28), and Bcl-2 (17, 20–22). Mouse xenograft studies have shown that bcl-2 antisense ODNs combined with cyclophosphamide (20), with free doxorubicin or sterically stabilized liposomal doxorubicin (21), can be very effective. Phosphorothioate-modified ODNs are nuclease resistant and suitable for in vivo use. The pharmacokinetics, biodistribution, and stability of phosphorothioate ODNs have been studied previously in mice (29). ODNs were detected in most tissues up to 48 h after a single dose of 30 mg/kg i.v. or i.p. ODNs have been tested in vivo in a human leukemia SCID mouse model using phosphorothioate-modified c-myc antisense ODNs and were found to be effective (30).

The antisense approach is a promising therapeutic modality against follicular lymphoma, which overexpresses Bcl-2 protein as a survival factor. Our results with bcl-2 antisense ODNs against WSU-FSCCL in vitro were encouraging and led us to test their efficacy in vivo in the preclinical model. Bcl-2 antisense ODNs significantly prolonged animal survival in the WSU-FSCCL-SCID mouse xenograft model. More recently, it was demonstrated that antisense ODNs targeted to immunoglobulin cα sequence against WSU-FSCCL cells were effective as well (22), and that ODNs are active against ascites as well as other sites of systemic disease.

The most significant finding was on pathological examination of antisense ODN-treated animals. Histopathological examination showed no evidence of tumor cell infiltration of the liver, spleen, or the bone marrow (Fig. 3), which we believe is the cause of death. The liver and spleen were morphologically normal; the bone marrow, however, was relatively hypocellular but does not explain animal death. Subsequent experiments carried out on animals treated with antisense ODNs revealed CNS tumor cell infiltration. Examination of animal brains was carried out on animals treated with antisense ODNs revealed CNS tumor infiltration of the leptomeninges (Fig. 1C) with a perivascular pattern. Hence, we speculate that CNS involvement by tumor is the probable cause of death in the antisense ODN-treated animals. This finding suggests that ODNs do not cross the blood-brain barrier, thus allowing tumor growth in the CNS. It is interesting that survival of the bcl-2 antisense ODN group is almost identical to that of control animals given small (2.5–5.0 × 10⁶) inoculum (Table 1). This would indicate that development of CNS disease is a function of time, i.e., animals that survive past 10 weeks in untreated animals that received an injection with a large tumor dose (10⁷ cells), systemic disease involving liver and bone marrow limits survival to 7 weeks. When such a group of animals is treated with antisense bcl-2 ODNs, systemic disease is controlled, leading to increased survival beyond 10 weeks, which allows enough time for development of CNS disease.

We conclude that the WSU-FSCCL-SCID xenograft is a new and exciting model for t(14;18) human lymphoma. It is a valuable preclinical model for the study of the natural history and the effects of inhibition of oncogene expression. In vivo bcl-2 antisense ODN therapy was effective against systemic FSCCL disease. However, ODNs with the ability to penetrate the blood-brain barrier need to be developed. Further improvements in the design of ODNs are possible to enhance their selectivity to the target cells and improve their intracellular delivery. Attaching the ODNs to monoclonal antibodies, e.g., anti-CD19 or CD20, or their incorporation into liposomal particles may improve their intracellular delivery and allow for smaller doses to be used effectively (31).

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