Identification of B-Cell Growth Factors (Interleukin-14; High Molecular Weight–B-Cell Growth Factors) in Effusion Fluids From Patients With Aggressive B-Cell Lymphomas

By Richard Ford, Archito Tamayo, Barbara Martin, Keyi Niu, Kent Claypool, Fernando Cabanillas, and Julian Ambrus, Jr

The molecular basis of neoplastic B-cell growth is complex and poorly understood. Cytokines have been postulated to contribute to neoplastic cell growth, and many in vitro studies have confirmed this prediction, but little is known about the in vivo role of these growth factors. We have examined the production of interleukin-14 (IL-14) (high molecular weight [HMW], B-cell growth factor [BCGF]) by aggressive intermediate (diffuse large cell) lymphomas of the B-cell type non-Hodgkin's lymphoma (NHL-B) in four patients with lymphomatous effusions. In these studies, IL-14 was detected in the effusion fluids by Western blots and IL-14 mRNA was constitutively expressed in the freshly isolated lymphoma cells that also expressed the receptor for IL-14 (IL14R). Lymphoma B cells placed at low serum and cell density proliferated in vitro to either purified IL-14 or IL-14 derived from effusion fluids. Antibodies to IL-14 removed the growth-stimulating cytokine(s) from the effusions. Cell lines developed from these patients produced IL-14 in vitro and antisense oligos to IL-14 blocked their growth in vitro. Thus, autocrine or paracrine production of IL-14 may play a significant role in the rapid proliferation of aggressive NHL-B. Interrupting this pathway could be a useful goal of therapy for patients resistant to conventional chemotherapy.

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lymphocytes. For these studies, a microbead methodology was used (Quantum Simply Cellular [QSC]; Flow Cytometry Standards, Research Triangle Park, NC) to determine the number of bound antibody molecules per cell. A calibrated standard curve is used to convert linear histogram channels into molecular equivalents of soluble fluorochrome (MESF). Cell surface indirect labeling was performed using BA1 (1 μg/20 μL) and a goat F(ab')2, antimouse IgG fluorescein isothiocyanate (Caltag, San Francisco, CA) secondary label. Cells were incubated at 4°C for 45 minutes. Fifty microliters of QSC beads was labeled with the primary and secondary antibodies under the above conditions. A standard curve was constructed from the quantities of BA5 (MoAb bound to the calibrated beads). The number of BA5 molecules bound per NHL-B cell was determined using Flow Cytometry Standards software. Anti-μ-activated normal B cells were used as positive controls, and peripheral blood T cells as negative controls.

Establishment of lymphoma cell lines. Freshly obtained lymphoma cells were cultured at varying concentrations in RPMI 1640 and 20% fetal calf serum (FCS) (Hyclone, Ogden, UT) at 37°C in blood T cells as negative controls.

Fractions with BCGF activity were collected, pooled, and subjected to Western blot analysis and immunoprecipitation with monospecific anti-IL-14 antibodies. L14 from cultured NHL-B cells was used to convert linear histogram channels into molecular equivalents of soluble fluorochrome (MESF). Cell surface indirect labeling was performed as previously described, using exogenously provided purified IL-14 to overcome spontaneous apoptosis occurring in the initial weeks of autocrine (or paracrine) growth factors in the effusion fluids. Fresh NHL-B cells pelleted from effusion fluids were E-rosetted and spun through Ficoll/Hyphaque (F/H) (Sigma) gradients, washed, and cultured for 48 hours in the presence or absence of PHA (0.75% vol/vol) (GIBCO, Grand Island, NY) in RPMI 5% FCS for 48 hours. Control NHL-B cells were cultured in FCS, 5% in RPMI. Established cell lines from NHL were created similarly and used as positive controls. Supernatants from cell cultures were clarified through 0.22-μm filters and concentrated in an Amicon concentrator using YM-10 membranes. Samples were then passed through a PD-10 column (Pharmacia) and subsequently tested for BCGF activity, as described below.

In vitro production of growth factor by NHL-B cells from effusion fluids. Fresh NHL-B cells pelleted from effusion fluids were E-rosetted and spun through Ficoll/Hyphaque (F/H) (Sigma) gradients, washed, and cultured for 48 hours in the presence or absence of PHA (0.75% vol/vol) (GIBCO, Grand Island, NY) in RPMI 5% FCS for 48 hours. Control NHL-B cells were cultured in FCS, 5% in RPMI. Established cell lines from NHL were created similarly and used as positive controls. Supernatants from cell cultures were clarified through 0.22-μm filters and concentrated in an Amicon concentrator using YM-10 membranes. Samples were then passed through a PD-10 column (Pharmacia) and subsequently tested for BCGF activity, as described below.

In vitro growth factor assays. Growth factor assays for BCGF activity were performed as previously described, using anti-μ-stimulated B lymphocytes obtained from normal donor peripheral blood mononuclear cell populations. B cells were purified using SRBC rosetting and separation on F/H, followed by plastic adherence to remove monocytes. Residual T cells or monocytes were removed magnetically, if necessary, by further treatment with cocktails of MoAb (eg, CD2, 4, 14), followed by magnetic beads attached to antimouse Ig (Dynal, Great Neck, NY). B cells used were greater than 95% CD19 and slg positive. Trilobate microtiter wells containing 1 × 106 anti-μ B cells were exposed to different concentrations of growth factor containing fluids or column fractions. 1H-Thymidine uptake was determined over the last 18 hours of a 96-hour culture period, as previously described. Freshly obtained patient NHL-B cells were used in similar assays to assess response to exogenously provided growth factors, including IL-14, IL-2 (rIL-2; Cetus-Chiron, Emeryville, CA), IL-4, IL-6 (rIL-6), Gensyme, Cambridge, MA.

Specific antibody binding of HMW-BCGF (IL-14) activity in effusion fluids and in vitro culture supernatants. Partially purified BCGF activity (pooled DEAE active fractions) from freshly obtained effusion fluids were concentrated 10× through a 10,000 molecular weight cutoff membrane (Amicon Centriprep 10) and desalted into RPMI media with a PD 10 column (Pharmacia). Conditioned media (CM) from cultures of freshly obtained NHL-B cells were concentrated 10× in a similar fashion. Anti-IL-14 polyclonal IgG antibody (4 μg) or preimmune control rabbit IgG was bound to 500 μL bed volume of RPMI-equilibrated Protein A Sepharose beads (Sigma). A total of 500 μL of the concentrated active fractions (effusion fluids or CMs) were incubated with the antibody bound or control IgG-bound beads for 12 hours at 4°C. The supernatants were then passed through microfuge filter units (Ultrafrite MC, 0.45 μm; Millipore, Bedford, MA), and immediately assayed for growth factor activity.

IL-14 gene expression analysis using reverse transcriptase-poly-
merase chain reaction (RT-PCR). Freshly obtained NHL-B cells from effusion fluids were processed as described above, and total or poly A RNA was extracted and cDNA synthesized with reverse transcriptase, using GIBCO BRL (Gaithersburg, MD) SuperScript PreAmplification Kit. Oligonucleotide primers based on the IL-14 cDNA sequence were synthesized as follows: forward (147) TCC-TGACCAGCACATV and reverse (R1412) AGCAAGGACCTA-CAACAGAGCCTC. PCR analysis was performed using a Thermo Cycler 480 (Perkin-Elmer-Cetus, Norwalk, CT), Taq polymerase, and dNTPs. Thirty-five cycles of amplification were used for 1 minute 94°C denaturation, 1 minute at 55°C annealing, and 2 minutes at 72°C. The amplified material was run on a 1.2% agarose gel, stained with ethidium bromide and photographed under ultraviolet (UV) illumination. Amplified bands were confirmed by Southern blot analysis using a radioactively labeled IL-14 cDNA probe.

Antisense inhibition of B-cell lymphoma cells in vitro. Cell lines derived from the patients' NHL-B cells were seeded at densities of 5 to 20 × 10^5/100 μL in triplicate wells of 96 flat bottom-well microculture plates (Falcon, B-D, Oxnard, CA) in RPMI 5% FCS with L-glutamine. Antisense 3'-T TAC TATTCCGAT GAC TCT-T-5' and control (sense, scrambled antisense, unrelated (irrelevant), etc) phosphorothioate oligonucleotides (Oligos Etc. Wilsonville, OR) were selected from the 5' ORF of IL-14 sequence. Oligos were added to NHL-B cell cultures in the dose range between 3 to 20 μmol/L, and incubated at 37°C in 5% CO₂ for 48 hours, exposed to 0.5 μCi of tritiated thymidine for an additional 12 hours, harvested, and counted in a liquid scintillation counter.

RESULTS

Characterization of tumor cells from lymphomatous effusions in patients with aggressive NHL-B. Effusion fluids from NHL-B patients were processed. The tumor cells were initially pelleted from the effusion fluids with the fluid and cellular components analyzed separately. Microscopically, all cases were diagnosed as large cell, non-Hodgkin's lymphoma on hematoxylin and eosin (H&E)-stained cyto spin preparations (Fig 1A). Immunophenotyping by flow cytometry confirmed that the lymphoma cells were in each case aggressive (diffuse large cell) NHL-B (Table 1). Controls consisted of effusions from patients with low-grade, NHL-B (Fig 1B), or from patients with nonhematologic neoplasms (eg, breast carcinoma). Cytogenetic analysis of NHL-B cells from the effusions showed aneuploid karyotypes of the hyperdiploid or hypertetraploid type, with abnormalities present in chromosome 14 of the nonrandom type (t14; 18) (Fig 2A) or indeterminate type in 3 of 4 patients and deletions in chromosome 6q in 2 of 4 patients (Fig 2B). Multiple random structural abnormalities were also observed in each of the cases. Cell lines were established from the NHL-B cells recovered from the effusion patients in each of the four patients who had identical immunophenotypic and cytogenetic profiles to the originally obtained lymphoma cell specimens.

IL-14 (HMW-BCGF) activity in effusion fluids from aggressive NHL-B patients. In vitro growth factor activity for autochthonous NHL-B cells was present in the chromatographically fractionated effusion fluids, as shown in Fig 3A. BCGF activity determined on anti-μ stimulated peripheral blood B lymphocytes was found in the same effusion fluids when DEAE fractions of NHL-B patients’ fluids were analyzed in Fig 3B. No BCGF activity was present in the control patients’ fluids (Fig 3B, patients a through c). These findings suggest that the same factor(s), might be stimulating both the normal and lymphoma cells.

Presence of IL-14R on cultured NHL-B cells. To further characterize the possible involvement of IL-14 in the growth of NHL-B from our patients, we analyzed lymphoma cells for the presence of IL-14R from the cell lines that we had established. To identify the IL-14R, we used the MoAb BA5,
Summary of Cytogenetic Abnormalities in Aggressive NHL-B

<table>
<thead>
<tr>
<th>Lymphoma Patient</th>
<th>Karyotypic abnormality</th>
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<tbody>
<tr>
<td>1. JMcA</td>
<td>67 chromo; del 6 (q13); t(14:18) (q32;q21), 17p-, mult. random abnorm.</td>
</tr>
<tr>
<td>2. MS</td>
<td>109 chromo; two t (14:18) (q32;q21) 17p-; multiple random abnormal.</td>
</tr>
<tr>
<td>3. JK</td>
<td>47 chromo; t (6: ?) (q21: ?) multiple random abnormalities.</td>
</tr>
<tr>
<td>4. JM</td>
<td>51 chromo; t (14: ?) (q32: ?) multiple random abnormalities.</td>
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that has been shown to identify these receptors on normal (activated peripheral blood lymphocyte [PBL] B cells) and neoplastic (chronic lymphocytic leukemia [CLL], acute lymphocytic leukemia, etc) B cells. In Fig 4, it can be seen that NHL-B cells from three of our patients expressed 789-5989 IL-14R when analyzed by flow cytometry using a microbead methodology for determining and quantifying the numbers of binding sites for the BA5 MoAb. Insufficient numbers of cells were available to study the fourth patient, J.K. Normal activated B cells expressed 100 IL-14 receptor sites per cell, whereas T cells expressed no detectable IL-14, as has been previously described.

Production of IL-14 activity by freshly obtained NHL-B cells from lymphomatous effusions. To further verify that the growth factor activity present in the effusion fluids was produced by the NHL-B cells present in these fluids and not by a contaminating accessory cell (eg, mesothelial cells), the pelleted lymphoma cells were extensively washed, and the contaminating T lymphocytes were removed by E-rosetting. Monocytes/macrophages, and mesothelial cells (which were particularly scarce in these patients fluids) were deleted by plastic adherence. NHL-B cells accounted for >98% of the final lymphoma cell population by flow cytometric and cytopathologic analysis. The NHL-B cell population was then cultured in 5% FCS with or without PHA for 48 hours, concentrated, and assayed for growth factor activity as above. The B-cell proliferative activity contained in the supernatants of the patients NHL-B cell populations is shown in Fig 5A. Patients whose fluids contained BCGF activity also had NHL-B cells that produced BCGF activity in vitro. In Fig 5B, similar results are shown for established NHL-B cell lines that we had derived from other patients with similar aggressive (intermediate or high-grade) NHL-B, as well as the Namalva (BL) cell line, from which IL-14 was originally described.

In vitro growth stimulation of NHL-B cells from effusion fluids. To determine the responsiveness of the freshly-obtained NHL-B cells from the effusion fluids to putative autocrine growth factor(s)/BCGFs produced by these lymphoma cells, we exposed the tumor cells to purified IL-14 in vitro, obtained from one of the patient’s cell lines (patient MS) (Fig 6A).

In Fig 6B, it can be seen that in two of the patients, NHL-B cells responded actively to exogenously provided IL-14,
but not to other putative B-cell growth promoting cytokines, such as IL-6, IL-2 and IL-4 (rIL-2 and rIL-4), which are also putative BCGFs, also failed to stimulate the NHL-B cells (Fig 6C). One of the patients had a very high spontaneous proliferation that was not significantly increased with the addition of exogenous IL-14 or other putative B-cell stimulatory cytokines, but was inhibited by rIL-4 (data not shown).

**Immunologic identification of IL-14 in patient effusions by Western blot and immunoprecipitation analysis.** To confirm that the proliferation-inducing biologic activity present in the effusion fluids was IL-14, we analyzed the fluids before and after immunoprecipitation by Western blot using a monospecific polyclonal antiserum to IL-14, as described previously.

As shown in Fig 7, the anti-IL-14 antiserum recognized an ~60-kD band in both untreated or anti-IL-14 immunoprecipitated effusion fluids known to have BCGF activity. Control effusion, lacking BCGF activity, did not contain IL-14. The broadness of the IL-14 bands is most likely secondary to variable glycosylation.

**Removal of BCGF activity from effusion fluids and NHL-B conditioned media with anti-IL-14 (HMW BCGF) antibodies.** If autocrine growth factor activity mediated by IL-14 is responsible for lymphoma cell growth, as we have hypothesized, it should be possible to block growth stimulation with specific antibodies. To address this point, we treated the freshly obtained effusion fluids and CM, obtained after in vitro culture of purified NHL-B cells, with anti-IL-14 antibody attached to sepharose beads. In Fig 8, it can be seen that when effusion fluids (8A) and CM (8B) were treated with antibody-bound beads for 12 hours at 4°C, greater than 70% of the growth factor activity was removed in both instances. Effusion fluids and CM treated with control (nonimmune) rabbit IgG-conjugated beads, had less than 5% of the growth factor activity removed.

**Fig 3. B-cell growth factor (IL-14) activity of ion-exchange chromatographic fractions from freshly obtained NHL-B patient effusion fluids.** The fractions were assayed on (A) autologous purified NHL-B patient cells (10^6 cells/well) for 72 hours or (B) on freshly prepared, purified human peripheral blood B cells from normal donors (10^5 cells/well) for 96 hours in vitro, and pulsed with tritiated thymidine for the final 12 hours. Data shown are the mean of triplicate wells (SEM ± 10%).
Expression of the IL-14 (HMW-BCGF) gene in NHL-B cells isolated from effusion fluids. Because we were able to confirm that the cytokine growth factor present in the patient's effusions had the biologic and immunologic characteristics of IL-14, we examined expression of IL-14 mRNA in the lymphoma cells. Using RT-PCR analysis, IL-14 mRNA (1.3-kb band) was identified in each of the patient's NHL-B cells (Fig 9). Controls included the Namalva Burkitt's lymphoma cell line, which secretes IL-14, and normal peripheral blood monocytes, which do not secrete this cytokine. Amplification controls used the β-actin gene, shown in Fig 9B. Southern blot analysis confirmed that the bands obtained by PCR were IL-14.

Inhibition of lymphoma cell growth in vitro by IL-14 antisense oligonucleotides. To further confirm the role of IL-14 in mediating the cell growth in these aggressive large cell NHL-B, we tested the efficacy of a 20-mer antisense phosphorothioate oligonucleotide (PT-ASO) based on the IL-14 cDNA, on recently established lymphoma cell lines derived from our patients. In Fig 10, it can be seen that IL-14 PT-ASO could block greater than 95% of the thymidine incorporation of the NHL-B cell cultures from patients J.M. and M.S. A 50% inhibition occurred at less than 5 μmol/L IL-14 PT-ASO. Sense IL-14 PT-ASO controls from the same region resulted in less than 12% inhibition even at a 30 μmol/L dose. Similarly, irrelevant PT-ASO controls or randomly
scrambled IL-14 PT-ASO showed less than 20% inhibition at a dose of 30 μmol/L (Fig 10). The viability of the NHL-B cells was greater than 95% at time zero, but diminished to 50% to 60% in IL-14 PT-ASO treated cultures at 72 hours, whereas media controls were greater than 80% viable after 72 to 96 hours.

**DISCUSSION**

Regulation of tumor cell growth in patients with malignant lymphoma is clearly an area of central importance, both biologically and therapeutically. A number of studies, particularly in aggressive intermediate (large cell) and high-grade NHL-B, where cell kinetics were evaluated by flow cytometry, have shown that this parameter relates directly to patient prognosis. Our previous studies have shown that both low and HMW BCGF cytokines were capable of stimulating cell proliferation in NHL-B cells in vitro, and that cell lines from aggressive NHL-Bs secreted cytokine molecules with properties similar or identical to IL-14. This study extends the previous findings in a small, but interesting, group of NHL-B patients with high tumor cell burdens present in lymphomatous effusion fluids (a common finding in relapsing or widespread NHL-B). These NHL-B cases were quite typical of progressive or late stage aggressive intermediate and high-grade lymphoma (although one patient was studied untreated at presentation), and had cytogenetic abnormalities consistent with nonrandom chromosomal abnormalities characteristic of these lymphomas. Other cytokines with
putative B-cell stimulating activity (IL 2, 4, 6, tumor necrosis factor [TNF], etc) have been studied and shown to have no significant proliferative activity in vitro on the lymphoma cells. Also, recent studies have shown that IL-4 has a marked inhibitory effect on NHL-B cell growth when analyzed in vitro.28 In our experience with well over 100 cases of aggressive intermediate and high-grade NHL-B in recent years, only rare cases (<1%) have failed to respond with significant proliferative responses to either purified natural product IL-14, such as that used in these studies, or low molecular weight BCGF (LMW-BCGF) in vitro.1,2,12 These studies were performed before recombinant IL-14 (rIL-14) was available, but recent studies on similar patients with rIL-14 have yielded similar results. Other cytokines, such as IL-6, with putative human B-cell growth or differentiation stimulating activity have been hypothesized as autocrine growth factors for several NHL-B cell lines29 or for Epstein-Barr virus (EBV)-positive lymphoblastoid B-cell lines,30 but we have not observed significant stimulation or any consistent pattern of reactivity with any of these cytokines on freshly obtained NHL-B cells from patient biopsy specimens or in our NHL-B cell lines. The presence of IL-14R on the NHL-B cells is consistent with IL-14 being a growth factor for these cells. The level of IL-14 receptor expression correlates

Fig 8. Abrogation of B-cell growth factor activity by antibody to IL-14. Treatment of NHL-B patient effusions. Active DEAE effusion fluid fractions were reacted with anti--IL-14 IgG overnight at 4°C. These fractions were then incubated with protein A Sepharose beads to precipitate the 60-kD growth factor. Bound protein (beads) and unbound proteins (supernatants) were run on SDS-PAGE gels and blotted onto nitrocellulose membranes. An alkaline phosphatase conjugated antirabbit Ig was then added for detection. Namalva cell derived IL-14 was used as the positive control. The negative controls were derived from the control patient fluids (a, b, and c) from low-grade NHL-B (a) or nonlymphoid malignancies (b and c). Equal amounts of protein (150 ug) were added to experimental and control lanes.
B-CELL GROWTH FACTORS IN LYMPHOMAS

Fig 9. RT-PCR analysis of IL-14 expression in purified NHL-B cells isolated from patient effusion fluids. The predicted 1.3-kb band for IL-14 is found in the cDNAs from patients’ cells (MS, JMcA, JM, and JK), as well as the Namalva Burkitt’s lymphoma cell line (positive control). Freshly prepared human peripheral blood monocytes (Mon), which do not produce IL-14, were used as a negative control. (A) IL-14 (exp. size: 1,371 bp). (B) β-Actin (exp. size: 838 bp). Lane 1, master negative control; 2, IL-14 (210B) positive control; 3, monocyte; 4, Namalva; 5, MS; 6, JMcA; 7, JM; 8, JK.

with radiolabeled IL-14 binding and is similar to that observed on CLL B cells, reported in previous studies by Uckun et al.11,22 Those studies demonstrated similar numbers of IL-14R using either Scatchard analysis of radiolabeled IL-14 binding or flow cytometric analysis of BA5 binding. The level of IL-14 receptor expression correlated with the number of CLL-B colonies formed in vitro.

Cell lines were derived from these aggressive B-cell lymphomas, consistent with our previous experience. Earlier stage high or intermediate grade (large cell), B-cell lymphomas are less amenable to the establishment of cell lines at initial presentation.12 This has suggested that an autocrine growth factor might be present in the aggressive intermediate (large cell) and high-grade NHL-B. The cell lines derived from our patients were similar to those that we have previously reported showing phenotypic concordance with the patient’s freshly obtained biopsy cells and density dependence for in vitro cell growth. Cell lines from effusions do not appear to be different from similar histotypes present in lymph nodes by immunophenotypic or genotypic analyses. Most of our patients (including those reported here) begin with nodal lymphomas that progress to lymphomatous effusions, often after relapse postchemotherapy. These cell lines, like the freshly isolated NHL-B cells from the patients, express IL-14 mRNA, secrete IL-14, and proliferate in response to IL-14. This suggests that IL-14 may be an autocrine growth factor for these lymphomas in vivo, although other factors could also be involved with growth regulation. Because exogenous IL-14 also stimulated NHL-B cell proliferation in vitro, use of IL-14 may be paracrine, as well as autocrine. The demonstration of a possible autocrine/paracrine growth factor (AGF) for aggressive (intermediate and high-grade) NHL-B suggests that a variety of potential biologic approaches to therapy may be tried for these lymphomas. One might be the use of antisense oligonucleotide based on IL-14, as demonstrated in this study. This is of particular importance for patients, such as those described here, as these patients were refractory to further chemotherapy or other salvage treatments, resulting in an extremely poor prognosis and consequently very short survival times.

The antisense studies (AS) involving the IL-14 gene, may be of significant interest in regard to the pathogenesis of these NHL-B cases, particularly when considered together with the other studies showing the involvement of IL-14 in stimulating NHL-B cell proliferation. They used IL-14 PT-ASO that were designed for efficient uptake11,23 optimal oligonucleotide size, and sequence site (initiation codon) specificity,34 as well as resistance to nuclease activity (sulphur substitution in phosphate backbone).35 Specific inhibition of

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Fig 10. Inhibition of aggressive (diffuse large cell) NHL-B cell lines from effusion fluids with IL-14 antisense oligonucleotides. Lymphoma cells were cultured at 10^6 to 10^7/cells/µL for 48 hours in the presence of IL-14 PT-ASO or control oligos. A total of 0.5 µCi of ^3H-TdR was added for the final 12 hours in vitro. Media only controls ranged from 1 to 2 x 10^4 cpm. Data shown are the mean of triplicate wells for a representative assay of three experiments performed. SEM was ≤10%.
in vitro NHL-B cell growth by IL-14 ASO, may indicate downregulation of the IL-14 gene, possibly through inhibition of mRNA translation. Such a mechanism would support the involvement of the IL-14 gene in the modulation of growth in actively growing NHL-B cells that constitutively express the IL-14 gene. Inhibition of about 40% by a scrambled ASO inpatient, JM, is of interest, but may reflect non-antisense effects, perhaps by degradation products of the ASO. Other AS approaches to tumor cell growth inhibition in the NHL-B, have targeted protooncogenes believed to be involved in lymphomagenesis, including c-myc in Burkitt's lymphoma cell lines, and bcl-2 in NHL-B cell lines containing the t(14;18) translocation. The bcl-2 studies were recently extended to a severe combined immunodeficiency (SCID) human lymphoma model, where it was shown that a bcl-2 AS oligomer delivered by diffusion chamber, resulted in specific inhibition of lymphoma development.

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