Antisense Oligodeoxynucleotide Combination Therapy of Primary Chronic
Myelogenous Leukemia Blast Crisis in SCID Mice

By Tomasz Skorski, Małgorzata Nieborowska-Skorska, Paweł Włodarski, Gerald Zon, Renato V. Iozzo, and Bruno Calabretta

The proliferation of chronic myelogenous leukemia (CML) cells and the transformation of normal hematopoietic cells by BCR-ABL appear to require the expression of a functional MYC protein, suggesting an approach to treatment of Philadelphia + leukemias based on simultaneous targeting of BCR-ABL and c-MYC. To test this hypothesis, CML-blast crisis (CML-BC) primary cells were treated in vitro with bcr-abl and c-myc antisense phosphorothioate oligodeoxynucleotides (SIODNs), individually or in combination. Compared with antisense ODNs targeting of individual oncogenes, downregulation of both BCR-ABL and c-MYC by specific antisense SIODNs resulted in a synergistic antiproliferative effect. Colony formation of normal bone marrow cells was not affected by either treatment. To assess the therapeutic potential of multiple oncogene downregulation, SCID mice injected with CML-BC primary cells were treated systemati-}

THE ABILITY of the p210BCR/ABL tyrosine kinase to transform hematopoietic cells rests in the activation of downstream effectors that transmit the oncogenic signal from the cytoplasm to the nucleus. One such effector is the protooncogene c-myc, which is required for BCR-ABL or v-ABL transformation of hematopoietic cells. The identification of downstream effectors of p210BCR/ABL has raised the possibility of developing a combination therapy of chronic myelogenous leukemia (CML) based on targeting genes with distinct roles in the process of transformation. Antisense oligodeoxynucleotides have been extensively used in the past years in vitro and in animal models of human malignancies as a biological tool to assay oncogene function and as potential therapeutic agents. Thus, we undertook experiments to compare the effects of phosphorothioate bcr-abl and c-myc antisense oligodeoxynucleotides, alone and in combination, in vitro and in immunodeficient SCID mice carrying primary blast cells from a patient with CML-blast crisis (CML-BC). Primary CML-BC cells were used because of our previous studies indicating that colony formation of these cells was inhibited more efficiently than that of CML chronic phase cells by bcr-abl ODNs. Systemic injection of phosphorothioate bcr-abl antisense oligodeoxynucleotides into SCID mice carrying the BV173 Philadelphia + cell line retarded the disease process and prolonged the survival of leukemic mice. However, the proliferation of BV173 and other Philadelphia + cell lines was affected in vitro by certain bcr-abl oligodeoxynucleotides designed to serve as controls. Although the nonspecific effects were more evident when BV173 cells were cultured at low density in the presence of bcr-abl SIODNs, it remains possible that some of the in vivo effects might have been sequence-independent. Such sequence-independent effects of SIODNs might be caused by sequence-independent binding to a variety of intra- and extracellular proteins in mono- or multimeric forms. We report here that only bcr-abl oligodeoxynucleotides specifically targeting bcr-abl hybrid transcripts suppress the disease process in mice injected with CML-BC primary cells. Furthermore, the combination with c-myc antisense ODNs further enhances the survival of these leukemic mice, showing the potential of such an approach for the treatment of Philadelphia + leukemias.

MATERIALS AND METHODS

Cells. Normal bone marrow (BM) and CML-BC cells were obtained by aspiration from the iliac crest of healthy volunteers and from the peripheral blood (PB) of patients (Department of Hematology-Oncology, Thomas Jefferson University, Philadelphia, PA), respectively, after informed consent. Cells were processed as described. Patient cells used for injection in SCID mice (see below) were karyotypically characterized and found to contain, in addition to the Philadelphia chromosome (b2a2 breakpoint), a 16q- abnormality with breakpoint in the q12.2-q13 region in 75% of the metaphases. Trisomy of chromosomes 4, 8, and 13 was found in 15% of the metaphases. Sequence analysis of the p53 coding sequence (cDNA and exon-intron junction amplification) did not reveal any point mutation.

Oligodeoxynucleotides (ODNs). ODNs were prepared on an Applied Biosystems model 3808 or 3902 automated synthesizer (Lynx Therapeutics Inc, Hayward, CA). The following phosphorothioate SIODNs were used for the in vitro antisense experiments: (1) 16 mer b2a2 antisense sequence (AGG)GCTCTTCTCTTA), containing 8 nucleotides each complementary to the BCR exon 2 and the ABL exon 2 portion, respectively; (2) 16 mer b3a2 antisense sequence (AGG)GCTTTGAAGCT), containing 8 nucleotides each complementary to the BCR exon 3 and the ABL exon 2 portion,
respectively; (3) 16 mer b2/a2 sense sequence (TAAGGAA-GAGGCCC), complementary to the translation initiation codon and downstream sequences of the human c-myc mRNA; (5) 15 mer c-myc sense sequences (AGGCGCCCTCAAGGCT), corresponding to the translation initiation codon and downstream sequences of c-myc mRNA; (6) 15 mer c-myc scrambled sequence (AAGCATCGG-TGTG), containing the "G quartet" motif; (7) 18 mer myeloperoxidase antisense sequence (AGAGAAAGGGGGGACCCC), complementary to nucleotides for codons 2-7 of the myeloperoxidase mRNA. Oligodeoxynucleotides used for reverse transcription-polymerase chain reaction (RT-PCR) detection of BCR-ABL and β-actin transcripts in mouse tissues are as follows: (1) bcr exon 2, 5′ primer (CACAGCTATGCGGTAGGACT), corresponding to nucleotides 3273-3294 of BCR mRNA; (2) c-abl exon 2, 3′ primer (GCT-TCACACCATCCCCATTG-GTGT), corresponding to nucleotides 431-452 of c-abl mRNA; (3) β-actin 5′ primer (TGGGAATGG-GTCAGTGTCGACT) corresponding to nucleotides 224-244 of β-actin mRNA; (4) β-actin 3′ primer (TTTTCAGGTGTCCTTAGGT) corresponding to nucleotides 411-433 of β-actin mRNA.

[5]ODNs treatment of normal BM and CML cells in vitro. For clonogenic assay, cells (10⁴/400 µL/well) were plated in 24-well tissue culture plates (Costar Corp., Cambridge, MA) in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% fetal bovine serum (FBS) (GIBCO-BRL, Grand Island, NY), L-glutamine, and penicillin/streptomycin. [5]ODNs were added at 40 µg/mL when the cells were seeded; two additional doses of 20 µg/mL each were added 24 and 48 hours after seeding. Control cells were incubated without [5]ODNs. Recombinant human interleukin-3 (IL-3) (generous gift of the Genetics Institute, Cambridge, MA) was added (10 U/mL) to the cell cultures, when indicated, together with the third dose of the [5]ODNs. Cells were incubated for 120 hours and then plated in semisolid methylcellulose medium (HCC 4230; Terry Fox Laboratory, Vancouver, Canada) in duplicate 35-mm Petri dishes, without washing. Colonies were scored after 9 to 12 days. For protein studies, 5 × 10⁶ cells/mL of medium were placed in 175-cm² LUX tissue culture flasks (Nunc, Inc., Naperville, IL). [5]ODNs were added at the beginning of the culture (40 µg/mL) and again (at 50% of the initial dose) 24 and 48 hours later. For protein analysis, cells were centrifuged on Histopaque-1077, washed, and solubilized in RIPA lysis buffer containing 1% deoxycholate, 2% NP-40, 0.2% SDS, and 10% glycerol, in Tris-buffered saline (TBS), pH 7.2. Proteins (from 10⁶ cells/sample) were separated on 7.5% SDS-PAGE, containing the "G quartet" motif; (7) 18 mer myeloperoxidase antisense sequence (AGAGAAAGGGGGGACCCC), complementary to nucleotides 2-7 of the myeloperoxidase mRNA. Oligodeoxynucleotides used for reverse transcription-polymerase chain reaction (RT-PCR) detection of BCR-ABL and β-actin transcripts in mouse tissues are as follows: (1) bcr exon 2, 5′ primer (CACAGCTATGCGGTAGGACT), corresponding to nucleotides 3273-3294 of BCR mRNA; (2) c-abl exon 2, 3′ primer (GCT-TCACACCATCCCCATTG-GTGT), corresponding to nucleotides 431-452 of c-abl mRNA; (3) β-actin 5′ primer (TGGGAATGG-GTCAGTGTCGACT) corresponding to nucleotides 224-244 of β-actin mRNA; (4) β-actin 3′ primer (TTTTCAGGTGTCCTTAGGT) corresponding to nucleotides 411-433 of β-actin mRNA.

RESULTS

Effects of bcr/abl and/or c-myc antisense [5]ODNs on CML-BC primary cells. In vitro colony formation of CML-BC primary cells was partially inhibited after incubation with bcr/abl junction-specific antisense [5]ODNs (b2/a2 or b3/a2) or by c-myc antisense [5]ODNs at optimal concentrations (80 µg/mL each) (Table 1); in combination, the bcr/abl + c-myc antisense [5]ODNs, used in a final concentration identical to that of single antisense [5]ODNs, inhibited proliferation 2.5 to 7 times more effectively than the individual antisense [5]ODNs (Table 1). c-myc sense [5]ODNs and bcr/abl junction-nonspecific antisense [5]ODNs were noninhibitory at the concentrations tested, and when used in combination did not exert any synergistic antileukemic effect. CML-BC marrow cells from UPN1 (selected for injection in SCID mice) were also exposed to a 16 mer b2/a2 sense [5]ODN, a 15 mer c-myc scrambled "G quartet" [5]ODN, or an 18 mer myeloperoxidase antisense [5]ODN, individually or in combination with either the c-myc or the bcr-abl b2/a2 antisense sequence. None of these sequences had a toxic effect at the concentrations tested (Table 1). c-myc antisense [5]ODNs, alone or in combination with bcr/abl [5]ODNs, had no effect on colony formation from normal marrow progenitors (Table 2). Inhibition of CML-BC cell proliferation by bcr/abl junction-specific or c-myc antisense [5]ODNs was accompanied by downregulation of BCR/ABL and c-Myc protein levels, respectively (Fig 1). The combined treatment downregulated both BCR/ABL and c-Myc protein (Fig 1). Levels of HSP 72/73 and Myb protein were unaffected. Since the half-life of c-Myc protein is very short (10 to 30 minutes), the lack of effects on c-Myc levels argues against a nonspecific inhibition of cell growth.

Effects of bcr/abl, c-myc, or bcr/abl + c-myc antisense [5]ODNs on leukemia progression in SCID mice. We assessed the antileukemic effects of bcr-abl and c-myc [5]ODNs, alone and in combination, in immunodeficient SCID mice (males, 7 to 9 weeks old, 20 to 22 g) injected intrave-
nously (IV) with $10^7$ CML-BC primary cells (UPN1). Primary cells from UPN1 were selected because of their ability to form a high number of growth factor-independent colonies, and the sensitivity to treatment with specific S[ODNs (Table 1). Injection of $10^7$ cells induced, reproducibly, a disease process reminiscent of that in humans, with initial infiltration of hematopoietic tissues and subsequent spread to nonhematopoietic organs. Twenty one days later, mice were systemically injected for 12 consecutive days with $1$ mg/d/mouse of b3/a2 AS and c-myc S (6 days each, every other day), b2/a2 AS, c-myc AS, or b2/a2 AS and c-myc AS (6 days each, every other day). Control mice were injected with diluent only. Eight weeks after injection of leukemic cells, the disease process was examined in three mice from each group by immunofluorescence, colony assay, and RT-PCR using RNA of various tissues. Immunofluorescence assay detected CD45+ cells in BM of control, b3/a2 junction-nonspecific AS, and c-myc S-treated mice, and also at very low frequency in c-myc AS S[ODN-treated mice, but not in mice treated with b2/a2 junction-specific AS or b2/a2 AS and c-myc AS (Table 3). The more sensitive clonogenic assay revealed the development of leukemic colonies from spleen suspensions, and, with higher frequency, from BMC of control and c-myc sense + b3/a2 AS S[ODN-treated mice; in contrast, cell suspensions of c-myc or b2/a2 antisense-treated mice contained far fewer clonogenic cells (Table 3). Only one of the mice treated with both b2/a2 + c-myc AS S[ODNs contained detectable clonogenic leukemic cells. RT-PCR amplification of bcr/abl transcripts in RNA isolated from various tissues of control and c-myc sense + b3/a2 antisense S[ODN-treated animals (three mice/group) revealed bcr/abl transcripts in each of these tissues (Fig 2). Bcr-abl transcripts were also detected in all examined tissues of mice treated with individual antisense S[ODNs, but the signal was much weaker than that observed in the tissues of control mice. Even weaker signals or no signals were detected in the RNA isolated from all of the examined organs of mice injected with b2/a2 + c-myc AS S[ODNs, suggesting that the leukemic cell load in mice treated with the S[ODNs combination was reduced as compared with that of mice treated with individual ODNs. Comparable amounts of β-actin detected in each group of organs indicated the integrity and the similar loading of the amplified products. These results are consistent with those obtained by immunofluorescence and clonogenic assays. Such differences among the groups of mice were reflected in their mortality rates (Fig 3); all 10 control and 10 b3/a2 antisense + c-myc sense S[ODN-treated mice died with diffuse leukemia, as confirmed by necropsy (Fig 4), 13 to 21 weeks after IV injection of $10^7$ CML-BC cells (mean survival time 15.5
were treated with the same concentration (80 pg/mL) of bcr/abl (b2/a2 or b3/a2) or c-myc antisense (AS), or bcr/abl b2/a2 antisense [SIODNs and 10 c-myc antisense [SIODN-liquid culture for 5 days in the presence of IL-3. Cells were then plated in methylcellulose and colonies were counted 12 days later. Results represent mean ± SD from two independent experiments.

<table>
<thead>
<tr>
<th>Donor</th>
<th>bcr/abl</th>
<th>c-myc</th>
<th>Colonies* IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>233 ± 20</td>
</tr>
<tr>
<td></td>
<td>AS (b3/a2)</td>
<td>AS</td>
<td>241 ± 35</td>
</tr>
<tr>
<td></td>
<td>AS (b2/a2)</td>
<td>—</td>
<td>231 ± 16</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>AS</td>
<td>253 ± 42</td>
</tr>
<tr>
<td></td>
<td>AS (b2/a2)</td>
<td>AS</td>
<td>238 ± 13</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>282 ± 28</td>
</tr>
<tr>
<td></td>
<td>AS (b3/a2)</td>
<td>AS</td>
<td>236 ± 21</td>
</tr>
<tr>
<td></td>
<td>AS (b2/a2)</td>
<td>—</td>
<td>257 ± 18</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>AS</td>
<td>223 ± 16</td>
</tr>
<tr>
<td></td>
<td>AS (b2/a2)</td>
<td>AS</td>
<td>296 ± 35</td>
</tr>
</tbody>
</table>

* BMC depleted of adherent cells and T lymphocytes as described,4 were treated with the same concentration (80 μg/mL) of bcr/abl (b2/a2 or b3/a2) or c-myc antisense (AS), or bcr/abl + c-myc ODNs in liquid culture for 5 days in the presence of IL-3. Cells were then plated in methylcellulose and colonies were counted 12 days later. Results represent mean ± SD from two independent experiments.

± 2.1 and 17.2 ± 1.6 weeks, respectively). In contrast, the 10 b2/a2 antisense [S]IODNs and 10 c-myc antisense [S]IODN-treated mice died after 23 to 26 and 23 to 28 weeks, respectively, of leukemia growth (mean survival time 23.0 ± 1.1 and 23.3 ± 1.4 weeks, respectively; P < .001 compared with control or sense-treated groups). Ten mice treated with both antisense [S]IODNs survived significantly longer than mice treated with either antisense ODNs (mean survival time 32.5 ± 6.9 weeks; P < .001). Injection of b3/a2 antisense ODNs alone did not exert any antileukemic effect in SCID mice bearing CML-BC cells carrying b2/a2 junction of bcr-abl (not shown).

Thus, the combination of bcr-abl junction-specific (b2/a2) antisense ODNs and c-myc antisense ODNs inhibited the expression of BCR/ABL oncogenic tyrosine kinase and c-MYC protooncogene, and exerted synergistic antitumor effects that were much more effective than those achieved by the same doses of individual antisense ODNs.

In vitro effects of bcr/abl and/or c-myc antisense ODNs on CML-BC cells recovered after in vivo antisense ODNs therapy. The inability of the [S]IODNs, individually or in combination, to completely eradicate leukemia in SCID mice most likely reflects the insufficient delivery of these agents to the leukemic cells in vivo, but could also be due to distinct biologic properties of leukemic cells that could result in the emergence of clones with an ODN-resistance phenotype.

To assess the latter possibility, terminally ill mice (2 per group), previously treated with antisense ODNs, were killed and the sensitivity of BM and spleen cells to the antisense ODNs treatment was analyzed in vitro in clonogenic assays conducted in the absence of hematopoietic growth factors to allow growth factor-independent colony formation from human leukemic cells only.17 As indicated in Table 4, colony formation from leukemic cells that survived the in vivo antisense therapy was inhibited by treatment with c-myc and/or bcr-abl antisense [S]IODNs.

DISCUSSION

In the present study we assessed whether antisense ODNs targeting the bcr-abl transcript junction could delay the leukemic disease process in SCID mice injected with CML-BC primary cells, and whether the combination with c-myc AS ODNs enhances the therapeutic potential of the antisense strategy in the treatment of Philadelphia1 leukemias.

We recently reported that systematically injected 26-mer b2/a2 antisense [S]IODNs suppress temporarily the disease process in SCID mice harboring BV173 Philadelphia1 cells.11 However, the use of a 26-mer sequence able to hybridize partially with the normal c-ab1 mRNA (manuscript in preparation) and the reported sequence-independent effects of certain bcr-ab1 ODNs on the BV173 line,12 prompted us to investigate the therapeutic effect of junction-specific bcr-ab1 ODNs on CML-BC primary cells growing in SCID mice. In agreement with our previous studies,13 we found that the disease process was delayed by the junction-specific 16-mer b2/a2 ODNs, but not by the 16-mer b3/a2 ODNs, as reflected by reduction of the leukemic load in mouse tissues and prolongation of survival. Such effects correlated with the ability of the b2/a2 16-mer ODNs (but not the b3/a2 16-mer sequence) to downregulate the expression of the p210bcrl protein in vitro (Fig 1). The partial therapeutic effect of the 16-mer b2/a2 ODNs likely reflects the inefficient uptake of ODNs by leukemic cells in vitro and in vivo20,21; furthermore, [S]IODNs are not uniformly distributed in mouse tissues, with a preferential recovery of intact [S]IODNs from liver and kidney but, at much lower levels, from hematopoietic tissues such as bone marrow and spleen.11,22 We also considered the possibility that the prolonged treatment with systemically injected ODNs might have induced the emergence
Table 3. Number of CD45+ Cells and Leukemic Colonies in SCID Mice Injected With CML-BC Primary Cells (UPN1) and Treated With the Indicated Antisense (AS) or Sense AS (S)ODNs

<table>
<thead>
<tr>
<th>Group of Mice</th>
<th>% of CD45+ in BMC</th>
<th>SPL Colonies/10^3 Cells</th>
<th>BMC Colonies/10^3 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.5, 4.5, 4.8 (4.9 ± 0.5)</td>
<td>42, 17, 24 (28 ± 13)</td>
<td>837, 669, 713 (740 ± 87)</td>
</tr>
<tr>
<td>b2/a2 AS + c-myc S</td>
<td>8.4, 3.5, 5.6 (6.0 ± 2.3)</td>
<td>45, 51, 21 (39 ± 16)</td>
<td>728, 649, 615 (644 ± 58)</td>
</tr>
<tr>
<td>b2/a2 AS</td>
<td>0.0, 0.0</td>
<td>7, 5, 3 (5 ± 2)</td>
<td>29, 13, 25 (22 ± 8)</td>
</tr>
<tr>
<td>c-myc AS</td>
<td>0.0, 0.1, 0.2 (0.1 ± 0.1)</td>
<td>3, 5, 4 (4 ± 1)</td>
<td>19, 33, 9 (20 ± 12)</td>
</tr>
<tr>
<td>b2/a2 AS + c-myc AS</td>
<td>0.0, 0.0</td>
<td>0, 0, 0</td>
<td>0, 2, 0 (1 ± 1)</td>
</tr>
</tbody>
</table>

Results represent the data from individual mice; in parentheses, mean ± SD.

of leukemic clones with intrinsic resistance to such agents. However, colony formation of leukemic cells isolated from terminally ill mice after the full course of [S]ODNs treatment was still greatly diminished by b2/a2 antisense ODNs (Table 4), suggesting that potential development of "ODN resistance" is not a primary factor in determining the therapeutic response in systematically treated leukemic mice, at least after a single course of therapy. Thus, repeated courses of ODN therapy might have enhanced antileukemia effects. Consistent with this possibility, we have recently shown a dose-dependent effect of systematically injected c-myc antisense ODNs in nude mice bearing a melanoma tumor.23 The second objective of our studies was to take advantage of the role of c-myc as a downstream effector of p210(Bcr-Abl)-mediated oncogenic signals for therapeutic purposes. As expected, [S]ODNs targeted to the human c-myc mRNA inhibited CML-BC colony formation in vitro and suppressed the disease process in SCID mice. Because the c-myc antisense [S]ODN contains the "G tetrad," a motif shown to exert nonspecific antiproliferative effects on cells growing in monolayer,24,25 we used as a control a c-myc scrambled [S]-ODN preserving the G tetrad and found that it does not inhibit colony formation of CML-BC primary cells (Table 1). It has been also reported that BCR-ABL tyrosine kinase autophosphorylation is inhibited by [S]ODNs containing closely spaced repeats of the consensus sequence GGC.26 This raises the possibility that [S]ODNs act in an aptameric manner to interfere with a function that might be required...
Fig 4. Light micrographs depicting (arrows) the tissue distribution of human CML-BC cells in SCID mouse. (A) Lung, interstitium; (B) lung, tumor nodule in the subpleural region; (C) diffuse infiltration by blast cells in spleen; (D) small focus of leukemic cells in liver; (E) tumor cell infiltration in the subcapsular region of liver; asterisk indicates the presence of adjacent necrotic tissue. Original magnification: A = 125, B-E = 250.

for some aspects of BCR/ABL-mediated in vitro transformation of hematopoietic cells. However, a single GGC motif is present only in the 16 mer b3/a2 [S]ODN, which had no effects in vitro or in vivo. Both c-myc S and AS ODNs contain the hexamer palindromic sequence AACGTT known to induce IFN and augment NK cell activity, and yet only the c-myc AS [S]ODN had antileukemic effects in vivo. Accordingly, it appears unlikely that this antitumor mechanism is an explanation for all of the in vivo effects described here. [S]ODNs containing the CpG motif may also stimulate interferons and augment NK cell activity. Once again, it is unlikely that our findings can be explained by postulating the involvement of this mechanism, as the CpG motif is not present in the bcr/abl ODNs, whereas it is present in both the c-myc sense and antisense sequences. Nevertheless, nonspecific antiproliferative effects might be due to as yet unknown motifs, as pointed out recently.

However, the nonspecific effects we have considered as
LEUKEMIA TREATMENT BY ANTISENSE ODNs COMBINATION

Table 4. Sensitivity of CML-BC (UPN1) Cells, From SCID Mice With Relapsed Leukemia After Antisense [SI]ODNs Therapy, to b2/a2, b3/a2, and/or c-myc AS [SI]ODNs In Vitro

<table>
<thead>
<tr>
<th>Mouse Treatment</th>
<th>Cell Treatment*</th>
<th>No. of Colonies</th>
<th>% Inhibition (P1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b2/a2 AS</td>
<td>—</td>
<td>177 ± 11</td>
<td>—</td>
</tr>
<tr>
<td>b3/a2 AS</td>
<td>153 ± 31</td>
<td>75 (P &lt; .001)</td>
<td>—</td>
</tr>
<tr>
<td>b2/a2 AS</td>
<td>38 ± 11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c-myc AS</td>
<td>—</td>
<td>178 ± 15</td>
<td>—</td>
</tr>
<tr>
<td>c-myc S</td>
<td>160 ± 49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c-myc AS</td>
<td>16 ± 4</td>
<td>90 (P &lt; .001)</td>
<td>—</td>
</tr>
<tr>
<td>b2/a2 AS</td>
<td>188 ± 57</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>b3/a2 AS</td>
<td>181 ± 42</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c-myc AS</td>
<td>192 ± 26</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c-myc AS</td>
<td>41 ± 7</td>
<td>77 (P &lt; .001)</td>
<td>—</td>
</tr>
<tr>
<td>b2/a2 AS</td>
<td>27 ± 7</td>
<td>89 (P &lt; .001)</td>
<td>—</td>
</tr>
<tr>
<td>b2/a2 AS + c-myc AS</td>
<td>10 ± 2</td>
<td>95 (P &lt; .001)</td>
<td>—</td>
</tr>
</tbody>
</table>

* Mononuclear populations of splenocytes and BMC from antisense-treated mice were isolated on Histopaque-1077, and 10^6 cells/0.4 mL of IMDM supplemented with 2% human AB serum, Heps buffer, L-glutamine, and peni/strepto were treated with junction-specific 16-mer bcr/abl (b2/a2), 15-mer c-myc AS or bcr/abl (b3/a2) + c-myc sense (S) or antisense (AS) [SI]ODNs (40 μg/mL added on day 0, 20 μg/mL on day +1, and 20 μg/mL on day +2; the [SI]ODNs doses were equally divided in the case of combination) in liquid culture for 5 days. Cells were then plated in methylcellulose without growth factors and colonies were counted after 9 days. Results represent mean ± SD from two different experiments.

† Percent of inhibition of colony formation (statistical significance by t-test).

a potential explanation of the results reported here are usually evident where [SI]ODNs are used at relatively high concentrations (>5 μmol/L); such concentrations might not be reached in vivo, especially in the hematopoietic tissues.11,22 The bcr/abl + c-myc [SI]ODN combination exerted greater therapeutic effects than the individual agents used at the same dose, consistent with the antileukemic effects of bcr/abl and c-myc antisense [SI]ODNs in SCID mice carrying BV173 cells.23 This synergistic effect may rest in the higher likelihood of achieving growth arrest in cells escaping the treatment with either bcr/abl or c-myc antisense [SI]ODNs by simultaneous targeting of both oncogene-encoded mRNAs. Alternatively, the downregulation of gene expression by individual antisense [SI]ODNs at the relatively low concentrations reached in vivo11,22 might be insufficient to inhibit cell proliferation, whereas “partial” inhibition of two cooperating oncogenes might induce a more permanent block in the ability to proliferate.

It is unlikely that such a combination ODN approach will be restricted to the targeting of bcr/abl and c-myc. Because several downstream effectors of p210^bcr/abl have been recently identified,14 it would be interesting to compare therapeutic responses associated with the targeting of either cytoplasmic and nuclear effectors, or cytoplasmic or nuclear effectors only.

In principle, a combination antisense ODN therapy of Philadelphia's leukemias should be based on targeting bcr/abl transcripts because of the pathogenetic role of the encoded protein31-34 and its selective presence in leukemic cells. The downstream effectors of p210^bcr/abl are not leukemia-specific and therefore the consequences of targeting such genes for the proliferation of normal cells must be considered. Antisense oligodeoxynucleotides targeting c-myc had no apparent effect on the colony-forming ability of normal progenitor cells32 (Table 2). In agreement with these findings, use of a dominant negative c-myc construct blocked proliferation of bcr/abl-transformed fibroblasts, but did not affect normal cells.2 Finally, control mice injected with murine c-myc antisense [SI]ODNs revealed no toxicity-associated morphologic changes in the organs examined and no decrement in the proliferation capacity of BMC as indicated by clonogenic assays (not shown). Together, these studies suggest that the targeting of bcr/abl and c-myc spares most or all normal cells, while affecting leukemic cells.

The synergistic antileukemic effect shown here is similar to that observed for combined chemotherapy, in which different drugs are designed to target tumor cells as they progress through distinct cell cycle stages. However, the promise of antioncogene therapy rests in the potential selectivity for disease-inducing agents, an essential condition for devising more effective anticancer treatments.

ACKNOWLEDGMENT

We thank Larry DeDionisio, Laura Christensen, and Annette Rable of Lynx Therapeutics, Inc for their skilled preparation of the phosphorothioate oligodeoxynucleotides. We thank Dr. R. Arlinghaus of MD Anderson Cancer Center, University of Texas (Houston, TX) for the generous gift of the 8E9 anti-ABL antibody. We thank Marina Hoffman for critical reading of the manuscript.

REFERENCES


From www.bloodjournal.org by guest on September 13, 2017. For personal use only.
mouse model with c-myb antisense oligodeoxynucleotides. Proc Natl Acad Sci USA 89:11823, 1992
27. Elefanty AG, Hariharan IK, Cory S: bcr-abl, the hallmark of chronic myeloid leukemia in man, induces multiple hematopoietic neoplasms in mice. EMBO J 9:1069, 1990

From www.bloodjournal.org by guest on September 13, 2017. For personal use only.
Antisense oligodeoxynucleotide combination therapy of primary chronic myelogenous leukemia blast crisis in SCID mice

T Skorski, M Nieborowska-Skorska, P Wlodarski, G Zon, RV Iozzo and B Calabretta