CORRESPONDENCE

Specificity of BCR-ABL Antisense Oligonucleotides

To the Editor:

Recently in a very interesting publication in Blood,1 it was shown that antisense treatment of Philadelphia-positive cell lines resulted in growth inhibition of chronic myelogenous leukemia (CML) cells. Liposomes containing methylphosphonate oligodeoxynucleotides complementary to specific regions of the bcr-abl mRNA were used. The investigators claim that growth inhibition resulted from selective inhibition of the expression of the p210bcr-abl protein. A Western blot was screened with the 8E9 monoclonal antibody. This antibody is specific to the SH2 domain of the c-abl protein2 and detects the p210bcr-abl as well as the p145bcr-abl. The investigators show a decrease of the p210bcr-abl expression relative to the p145bcr-abl expression and conclude that the antisense strategy results in selective inhibition of expression of p210bcr-abl. The cell lines used were BV173 and K562. Both showed the same phenomenon (Figs 4 and 5 in Tari et al). However, the presence of the p145bcr-abl in cell line BV173 is surprising because this cell line lacks the normal chromosome 9 and, as a result of that, does not express the normal c-abl protein. Dikstein et al3 used Western blotting with the same monoclonal antibody and indeed demonstrated that BV173 is c-abl negative. Absence of c-abl mRNA in a reverse transcription-polymerase chain reaction assay was also confirmed.4,5

The rationale behind antisense studies is that antisense oligos are effective because they inhibit translation of the mRNA to which they are targeted. The specificity of BCR-ABL antisense oligonucleotides is still a controversial issue.6 We fully agree with Tari et al1 that protein studies are the best way to determine the effectiveness of an antisense approach in CML. In fact, we think that this is crucial.

How can Tari et al1 find that there is a selective decrease of p210bcr-abl relative to p145bcr-abl in BV173 when the cell line is p145bcr-abl negative? What does this mean for the experiments performed in K562 and the specificity of the BCR-ABL antisense oligonucleotides in general? Using the same monoclonal antibody in a flow cytometric analysis of BV173 cells after BCR-ABL antisense phosphorothioate treatment in which only viable cells were analyzed, we observed a growth inhibitory effect but no decrease in cellular p210bcr-abl levels.6

We would like these questions to be addressed. If the observation by Tari et al1 holds, it may well be that BCR-ABL methylphosphonates are the best alternative antisense approach in CML. This would be an important contribution to this field of research.

REFERENCES


RESPONSE

We thank Drs Smetsers and Mensink for their very kind comments on our work published in the July 15, 1994 issue of Blood.1 In our report, we studied the effect of liposomal-MPs (MPS, methylphosphonate antisense bcr-abl oligonucleotides) on the growth inhibition of CML cell lines. Exposure of K562 and BV173 cells to L-MPs targeted to the breakpoint junction of the bcr-abl mRNA inhibited specifically the growth of the CML cells and was associated with a decrease in the level of p210bcr-abl protein. This effect was specific and selective and did not affect the transcript of the nonrearranged gene.

In response to the question posed by Drs Smetsers and Mensink regarding how we find that there is a selective decrease of p210bcr-abl relative to p145bcr-abl in BV173 when the cell line is p145bcr-abl negative, we point out that the clones cited by them were negative, whereas others have found clones that are positive.5,6 This variability in the relative expression of single-copy genes has been commonly obtained in established cell lines maintained in long-term culture.

They also ask what this means for experiments performed in K562 and the specificity of the BCR-ABL antisense oligonucleotides in general. In our experiments, we found the antiproliferative effect of the antisense oligonucleotides to be specific for the junctional sequence. These L-MPs are homologous to the mRNA transcript of bcr-abl but not the nonrearranged abl allele. The clear implication is that we observed a sequence specificity downregulation of the p210bcr-abl protein product that led to the inhibition of cell growth.

They also remark that, using the same monoclonal antibody in a flow cytometric analysis of BV173 cells after BCR-ABL antisense phosphorothioate treatment in which only viable cells were analyzed, they observed a growth inhibitory effect but no decrease in cellular p210bcr-abl levels. This is a common problem with antisense oligonu-
cleotides. In many cases, the antiproliferative activity generated by antisense may be related to unexpected homologies with other transcripts.

Once again, we thank Drs Smetters and Mensink for raising these important questions.

G. Lopez-Berestein
A. Tari
A. Deisseroth
Section of Immunobiology and Drug Carriers
M.D. Anderson Cancer Center
University of Texas
Houston, TX

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Specificity of BCR-ABL antisense oligonucleotides [letter; comment]
TF Smetsers and EJ Mensink

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