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## CORRESPONDENCE

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### Specificity of BCR-ABL Antisense Oligonucleotides

To the Editor:

Recently in a very interesting publication in *Blood*<sup>1</sup> 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 p210<sup>bcr-abl</sup> protein. A Western blot was screened with the 8E9 monoclonal antibody. This antibody is specific to the SH2 domain of the c-abl protein<sup>2</sup> and detects the p210<sup>bcr-abl</sup> as well as the p145<sup>c-abl</sup>. The investigators show a decrease of the p210<sup>bcr-abl</sup> expression relative to the p145<sup>c-abl</sup> expression and conclude that the antisense strategy results in selective inhibition of expression of p210<sup>bcr-abl</sup>. The cell lines used were BV173 and K562. Both showed the same phenomenon (Figs 4 and 5 in Tari et al<sup>1</sup>).

However, the presence of the p145<sup>c-abl</sup> in cell line BV173 is surprising because this cell line lacks the normal chromosome 9<sup>3</sup> and, as a result of that, does not express the normal c-abl protein. Dikstein et al<sup>4</sup> 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 transcriptase-polymerase chain reaction assay was also confirmed.<sup>5,6</sup>

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.<sup>7</sup> We fully agree with Tari et al<sup>1</sup> 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 al<sup>1</sup> find that there is a selective decrease of p210<sup>bcr-abl</sup> relative to p145<sup>c-abl</sup> in BV173 when the cell line is p145<sup>c-abl</sup> 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 p210<sup>bcr-abl</sup> levels.<sup>6</sup>

We would like these questions to be addressed. If the observation by Tari et al<sup>1</sup> 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.

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#### RESPONSE

We thank Drs Smetsers and Mensink for their very kind comments on our work published in the July 15, 1994 issue of *Blood*.<sup>1</sup> 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 p210<sup>bcr-abl</sup> 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 p210<sup>bcr-abl</sup> relative to p145<sup>c-abl</sup> in BV173 when the cell line is p145<sup>c-abl</sup> negative, we point out that the clones cited by them were negative, whereas others have found clones that are positive.<sup>2,3</sup> 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 p210<sup>bcr-abl</sup> 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 p210<sup>bcr-abl</sup> 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 Smetsers and Mensink for raising these important questions.

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### Guidelines for Management of Hemophilia A and B

To the Editor:

Furie et al<sup>1</sup> have provided us with an excellent summary of commonly accepted guidelines for management of the common hemophilias. Unfortunately, advances in our understanding of the molecular biology of the clotting disorders<sup>2</sup> have not been paralleled by equivalent development of clinical studies defining optimum management strategies for many clinical care situations in hemophilia. Accordingly, even careful review of the literature does not allow one to formulate a comprehensive set of management guidelines that follows the principles of "evidence-based" medicine.<sup>3</sup> Most of the current clinical "guidelines" for intensity and duration of coagulation factor replacement represent consensus opinions based on the individual experience of experts such as Furie et al.<sup>1</sup> Our own interpretation and application of the currently available literature on treatment differs in some respects from those given in this review and has been summarized in algorithmic form elsewhere.<sup>4</sup>

We particularly want to comment on the management of dental extractions in patients with hemophilia. We do not agree with the implication that factor replacement for 3 or more days is commonly needed in this situation. In hemophilia A, replacement therapy with a single dose for factor VIII in conjunction with tranexamic acid, as described by Sindet-Pederson et al,<sup>5</sup> has not been complicated by delayed bleeding in our practice. In hemophilia B, only very limited data on combined treatment with factor IX and antifibrinolytics have been presented. The report by Walsh et al<sup>6</sup> cited by Furie et al<sup>1</sup> contains data on only three patients with Christmas disease that were treated by combined therapy (prothrombin complex concentrate with epsilon-aminocaproic acid).

We have recently reported preliminary results with a protocol designed for dental extraction in hemophilia B that provides a single preoperative dose of purified factor IX (mononine, 60 U/kg) followed by oral antifibrinolytic agents for 10 days.<sup>7</sup> In nine patients to date (mean number of extractions per patient, 4; range, 1 to 17) hemostasis was excellent with no patients requiring additional replacement therapy. Four of the nine did have nausea with epsilon-aminocaproic acid, which resolved with substitution of tranexamic acid. No clinical thrombotic complications occurred, nor was there evidence of an induced hypercoagulable state as judged by molecular markers (pro-

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thrombin fragment F1 + 2, fibrinopeptide A, and fragment B $\beta$  15-42). Although this series represents the largest to date on dental extraction in hemophilia B using combined therapy, the results must be regarded as preliminary because of the small number of patients.

Additional well-designed clinical trials are needed for most clinical management situations in hemophilia before we can confidently assert that our current practice guidelines represent anything more than expressions of personal opinion.

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### RESPONSE

We agree with the comments made by Djulbegovic and Goldsmith that in formulating guidelines for hemophilia care, "a careful review of the literature does not allow one to formulate a comprehensive

set of management guidelines" and that "most of the current clinical guidelines . . . represent consensus opinion."

In regards to dental extractions, minimal data are available to