How Do Antisense Oligodeoxynucleotides Inhibit the Growth of Chronic Myelogenous Leukemia Cells?

To the Editor:

In a recent issue of Blood Vaerman et al 1 describe the sequence-dependent, apparently nonantisense-mediated, growth inhibition of chronic myeloid leukemia (CML) cells by synthetic oligodeoxynucleotides (ODNs). Although this phenomenon has been described before, 2,4 Vaerman et al describe a novel TAT consensus sequence that is apparently responsible for mediating growth inhibition in their study. In discussing this process Vaerman et al state that the relationship between ODN sequence and growth inhibition remains to be elucidated. We would like to point out that work on this matter has been previously reported: both Smetsers et al 2 and Bergan et al 3 have shown that antisense ODNs, in a sequence-dependent fashion, inhibit the growth of CML cells in the face of sustained levels of p210(bcr-abl) protein. We have also shown that a GGC consensus sequence (contained within antisense ODNs) can directly inhibit p210(bcr-abl) autophosphorylation kinase activity in vitro. 4 In vitro inhibition of kinase activity was shown to correlate with in vivo decreases in total cellular phosphotyrosine content as well as with growth inhibition in the K562 CML blast cell line, all in the face of sustained p210(bcr-abl) protein levels.

It is interesting to note that some (not all) of the ODNs used by Vaerman et al contain GGC consensus sequences. Our investigations have shown that GGC containing ODNs inhibit p210(bcr-abl) kinase activity in vitro in a manner, which is noncompetitive with respect to adenosine triphosphate (ATP) (a substrate for p210(bcr-abl)). 5 Although we have not tested whether the TAT sequence may be directly inhibiting p210(bcr-abl) kinase activity, it is interesting to speculate whether the presence of adenosine in the TAT sequence may be acting as an active site competitive inhibitor of ATP.

In discussing their results, Vaerman et al allude to the "nonspecific" effects of antisense ODNs on cell growth in an effort to explain why nonantisense ODNs inhibit cell growth in their study. However, a presumption of nonspecificity does not appear to be warranted. Antiproliferative effects were shown to be highly dependent on the ODN sequence in their study, in the studies referenced, 2,4 and in the study from our laboratory. 2 Though not mediated through classical antisense mechanisms, such sequence dependence suggests that a nonspecific effect is unlikely.

The current dilemma with the antisense approach is that the outcome does not conform to our preconceptions of mechanism. The discovery of endogenously synthesized antisense RNA in the early 1980’s defined the mechanistic model which has characterized the antisense approach (for review see ref 6). While many investigators have tried this approach, these systems were, and still are, unpredictable. The rules which governed these so-called nonspecific effects remained elusive until a few years ago when reports of sequence-dependent interactions between specific ODNs and proteins began to emerge. 2 These aptameric effects are sequence dependent but are mechanistically distinct from antisense mediated effects. Aptameric effects are presumably mediated through the formation of specific secondary and tertiary structures that form as a function of primary ODN base sequence; the final structures of these aptamers possess specific functional characteristics. This model is directly based on that which is applied to protein chemistry, wherein function is derived from structure, which is ultimately based on primary amino acid sequence.

Defining the mechanism of action of ODNs in these CML-based systems is a critical issue in terms of finding the operative mechanism for growth inhibition in this well characterized disease entity stemming from a single chromosomal translocation. In the absence of a well characterized mechanism of action, we must grapple with the future application of antisense ODNs. Although Vaerman et al strongly question the use of antisense ODNs as purging agents in CML, we feel that efforts to develop them should continue in light of their activity in preclinical systems. The recognition of other mechanisms of ODN action (separate from classical antisense), though potentially confounding at our current levels of understanding, add to the breadth of applicability. Indeed, the elucidation of other mechanisms of action is serving to explain previously inexplicable effects. 5 Furthermore, the lack of a mechanism, or our inability to place it in the pigeon hole of "antisense mediated," should in no way prevent the development of an effective purging agent for this (or any other) malignant entity. 8 No matter how hard we wish otherwise, the history of the development of anticancer agents is more correctly characterized by the development of effective agents, whose mechanisms are subsequently elucidated, than by the converse.

Raymond Bergan
Leonard Neckers
Clinical Pharmacology Branch
National Cancer Institute
Bethesda, MD

REFERENCES

Response

In two recent papers, Bergan et al. have published that oligodeoxynucleotides (ODNs) of specific sequences (containing for instance the GGC consensus sequence) inhibit the p210BCRABL tyrosine kinase activity in vitro and in electroporated K562 leukemia cell line. This inhibition is an example of the aptameric properties of ODNs.

We think that the published ODN induced antiproliferative effect we analysed is not directly comparable to the interesting observations of Bergan et al. Therefore, we want to make the following comments:

1. In their letter, Bergan et al. insist that chronic myelogenous leukemia (CML) cell growth inhibition by synthetic ODNs has been described previously. In our report, we never claimed to be the first to observe this effect, but rather (as said in our introduction) that its interpretation was questionable and not elucidated yet.

2. Bergan et al. are discussing about synthetic ODNs. In our work, we use phosphodiester ODNs, whose physico-chemical as well as biological properties may be different from those of phosphorothioate or methylphosphonate ODNs, as already largely described in the literature.

3. The authors of the letter claim that the relationship between the ODN sequences and the antiproliferative activity had been already assessed. Indeed Smetsers et al. or O’Brien et al. have already analysed this problem with phosphorothioate or chimeric phosphorothioate/phosphodiester ODNs. However, they did not show a "consensus" sequence of toxicity.

4. Bergan et al. point out that some (not all) of the ODNs we used contain a GGC consensus sequence and suggest that this might explain the observed effect. Among all the active ODNs we tested (ending with a TAT at the 3′ position), 4 contain a GGC, 4 do not, but all have the same antiproliferative activity. Moreover, all the tested ODN containing a GGC triplet but no terminal TAT have no effect. We show that the TAT is necessary and sufficient to induce the antiproliferative effect.

5. Bergan et al. also argue that we allude to "nonspecific" effects of antisense ODNs in an effort to explain the observed antiproliferative effect. We disagree. We referred in our discussion to the different nonantisense effects of ODNs already reported in the literature. We clearly said in our conclusions that, although some sequence-specific and nonsequence-specific effects of ODNs had already been described, the terminal TAT induced cytotoxicity is a novel example of a sequence specific but nonantisense effect of phosphodiester ODNs.

6. Bergan et al. believe that efforts to develop antisense ODNs as purging agent in CML should continue in light of their activity in preclinical systems. We would not disagree at all with such a statement, provided there was some evidence of leukemia specificity of this non antisense effect. Unfortunately, this is not the case, as we showed it in our report: the effect we observed is not CML specific. This very fact renders the use of these ODNs as purging agents questionable.

We mentioned in our report that the interpretation of the TAT induced antiproliferative effect and the extrapolation of our conclusions to phosphorothioate ODNs were under investigation. This work has now been completed and the results will soon be submitted for publication.

Jean-Luc Vaerman
Philippe Martiat
Laboratoire de biologie moléculaire hématologique
Cliniques St Luc, Université Catholique de Louvain
Bruxelles, Belgium

REFERENCES


7. Smetsers TFCM, van de Locht LTF, Pennings AHM, Wessels HMC, de Witte TM, Mensink EJB: Phosphorothioate BCR-ABL antisense oligonucleotides induce cell death, but fail to reduce cellular bcr-ab1 protein levels. Leukemia 9:118, 1995

How do antisense oligodeoxynucleotides inhibit the growth of chronic myelogenous leukemia cells? [letter; comment]

R Bergan and L Neckers