Therapeutic Oligonucleotides: The Road Not Taken

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Antisense oligonucleotide therapeutics have been in development for almost 25 years without a single U.S. Food and Drug Administration–approved product in cancer. The reasons for this absence stem, in part, from a deep lack of understanding about how to deliver these molecules to cancer cells in vivo. Clin Cancer Res; 17(20); 6369–72. ©2011 AACR.

In this issue of Clinical Cancer Research, Hong and colleagues (1) report on the safety and tolerability of LY2275796, a second generation antisense oligonucleotide (ASO) targeted to the eukaryotic translational initiation factor eIF-4E mRNA, a gene that has been reported to play a role in tumor initiation.

In the study, ASO maximum tolerated dose and biologic effective dose were set at 1,000 mg, because of a dose-limiting toxicity of grade 3 fatigue at the 1,200-mg dose, and target inhibition was assessed by serial tumor biopsies. At first look, the ASO seemed to achieve target silencing, but closer inspection reveals that the housekeeping genes (e.g., β-actin) were also downregulated (by 64%, compared with 80% for the target mRNA), despite in vitro results suggesting excellent target specificity (2). Although the target was silenced, why didn’t “target inhibition” achieve “tumor inhibition” in this phase I trial? The problem, in part, is that human cancer is a multitude of processes, pathways, and “cross-talk” with profound redundancy. Cells can often “bypass” the inhibited pathway with minimal detriment to their malignant potential (3). LY2275796, therefore, fails in part because the preclinical models in which it was evaluated do not accurately recapitulate human clinical cancer. Mice and their xenografts, it has often been suggested, are not men.

Nevertheless, as the authors suggest, combining gene-targeted with cytotoxic therapy is clearly the way forward. We strongly agree, but although this point has been understood for decades, we still do not have a single U.S. Food and Drug Administration–approved ASO therapeutic agent in cancer. What has happened? The answer is that each clinical cancer trial of an ASO, in addition to its own unique problems, suffers from the problems shared by all ASO therapeutics, including RNAi. Unfortunately, despite the passage of nearly 25 years for ASOs, our level of understanding about fundamental processes that govern in vivo efficacy of therapeutic ASOs, particularly in cancer, is almost nil.

DNA is negatively charged, and in an 18-mer antisense molecule such as, for example, the anti-Bcl-2 ASO oblimersen (4), there are 17 negative charges. Substitution of a sulfur atom for an oxygen atom at each phosphorus atom, forming a phosphorothioate ASO, the type of ASO employed in virtually all cancer clinical trials, maintains the negative charge. However, the melting temperature ($T_m$) of the duplex formed between the target mRNA and a phosphorothioate antisense ASO will almost always be significantly depressed (5). In addition, whereas first generation phosphorothioate ASOs (those without any additional chemical modifications) have been believed to be exonuclease resistant, they are probably insufficiently so for in vivo gene silencing. [The use of 2‘-methoxyethyloligoribonucleotide gapmers as done by Hong and colleagues (1) both greatly enhances nuclease resistance and increases $T_m$.] The combination of insufficient in vivo nuclease resistance and diminution of $T_m$ after phosphorothioate substitution has probably done much to vitiate the efficacy of phosphorothioate ASOs in earlier clinical trials in cancer. These problems were not apparent in experiments done in tissue culture, because the ASOs were delivered into cells by lipofection, which provided extremely high nuclear concentrations. Further, data from in vivo experiments were often interpreted as resulting from antisense gene silencing when, in fact, they resulted from CpG sequence motifs in the phosphorothioate ASO binding to TLR9 receptors on mouse plasmacytoid dendritic cells, with the resulting “cytokine storm” leading to inhibition of tumor growth (6).

In the ultracomplex world of phosphorothioate ASOs, oblimersen is an outlier, because its $T_m$ with its target Bcl-2 mRNA (codons 1–6) is, for unclear reasons, significantly higher than predicted. However, oblimersen contains 2 CpG motifs and is highly immunostimulatory (6). Although earlier work suggested that Bcl-2 was an important target in melanoma, later work challenged this idea.
and it is difficult to understand how silencing of this gene can meaningfully chemosensitize a virulent tumor with such extensive redundant signaling pathways (8).

Nevertheless, a small phase II trial done in combination with dacarbazine (DTIC) was successful (9), and thus, the GM301 trial was launched (4). This nonblind, randomized
References


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