Therapeutic Oligonucleotides—The Road Not Taken

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Summary: Antisense oligonucleotide therapeutics have been in development for almost 25 years without a single USFDA approved product in cancer. The reasons, for this stem, in part, from a deep lack of understanding of how to deliver these molecules to cancer cells in vivo.

In this issue of Clinical Cancer Research, Hong, et al (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 MTD and biologic effective dose was set at 1000 mg, due to a DLT of grade 3 fatigue at the 1200 mg dose, and target inhibition was assessed by serial tumor biopsies. At first look, the ASO appeared to achieve target silencing, but closer inspection reveals that the housekeeping genes (e.g., β-actin) were also downregulated (by 64%, compared to 80% for the target mRNA), despite in vitro results suggesting excellent target specificity (2). But even so, why (since the target was in fact silenced) 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 oft 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 though this point has been understood for decades, we still do not have a single FDA-approved approved ASO therapeutic agent in cancer. What has happened? The answer is that each clinical trial in cancer of an ASO, in addition to its own unique problems, also suffers from the problems shared by all ASO therapeutics, including RNAi. Unfortunately, despite the passage of near 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 18mer antisense molecule such as, for example, the anti-Bcl-2 ASO Oblimersen (4), there are 17 negative charges. Substitution of a sulfur for an oxygen atom at each phosphorus, forming a phosphorothioate ASO, the type of ASO employed in virtually all clinical cancer trials, maintains the negative charge. However, the melting temperature (Tm) of the duplex formed between the target mRNA and a phosphorothioate antisense ASO will almost always be significantly depressed (5). In addition, while 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’-methoxyethoxyoligoribonucleotide gapmers, as done by Hong, et al (1), both greatly enhances nuclease resistance, and increases Tm). The combination of insufficient in vivo nuclease resistance and the diminution of Tm after phosphorothioate substitution have probably done much to vitiate the efficacy of phosphorothioate ASOs in earlier clinical trials in cancer. These problems were not apparent in experiments performed in tissue culture, because the ASOs were delivered into cells by lipofection, which provided extremely high nuclear concentrations. Further, data from in vivo experiments was often interpreted as resulting from antisense gene silencing, when in fact it 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 ultra-complex world of phosphorothioate ASOs, Oblimersen is an outlier, as its Tm with its target Bcl-2 mRNA (codons 1-6), for unclear reasons, is significantly higher than predicted. However, Oblimersen contains two CpG motifs and is highly immunostimulatory (6). While earlier work suggested that Bcl-2 was an important target in melanoma, later work challenged this idea (7), 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 performed in combination with DTIC was successful (9), and thus was launched the GM301 trial (4). This non-blinded, randomized, trial in 775 patients compared Oblimersen + DTIC vs. DTIC alone. Patients were also pre-stratified by baseline LDH. A continuous improvement in overall survival (OS) was observed in the patients receiving Oblimersen as a function of baseline LDH. Patients whose LDH was less than or equal to 0.8 times the upper limit of normal (ULN) showed the greatest benefit in OS, and those with LDH at >1.1 x ULN demonstrated no difference in OS (10). Based on these results, a randomized, phase 3 trial of Oblimersen + DTIC vs. DTIC alone was performed in 300 patients with LDH < 0.8 x ULN (GM307). Here, however, no difference in OS was observed. Thus ended clinical trials of Oblimersen, which had also failed in myeloma and whose development in CLL was also halted.

Why was the data in the earlier trial not reproduced? The probable reason was that the GM301 trial was not blinded, unlike GM307, and patients received an average of 5 cycles of DTIC (a minimally active anti-melanoma drug) in GM307 (vs. 3.2 cycles in the GM301 study). This difference was sufficient to eliminate the observed increase in OS in the low LDH population in GM301.

But would have a successful GM307 constituted that elusive in vivo proof of principle? The answer in our opinion is no. Even ignoring the issue of whether Bcl-2 is a target in melanoma, the question of whether sufficient ASO enters melanoma cells, or any other cancer cell clinically to reproducibly and robustly silence any target has never been answered. This contention is also true of LY2275796 in this trial, which appears to silence its target and down-regulate housekeeping genes with similar potency and must thus be suspected of accomplishing both here non-specifically. Despite huge expenditure on a vast array of delivery strategies, carrier molecules, etc., all of which suffer from cost issues, toxicity, poor delivery to tumors, or a combination, the foremost technologic hurdle blocking clinical progress for therapeutic ASOs (antisense and siRNA) in cancer is delivery. ASOs administered to patients in phase I and II trials can be found at high concentration in liver, kidney and small intestine, but whether sufficient concentrations for gene silencing are found in tumors, and critically, intracellularly, is unknown. Further, optimal concentrations may differ dramatically depending on tumor type, ASO chemistry, route of administration, and dose schedule, none of which are
usually rationally optimized for a cancer indication. In human tumors, what factors determine the rate of delivery of ASOs to cells? Or the rate at which ASOs enter or leave endosomes, where they reside in cells? Where in the cell does antisense silencing occur, and how do polar ASOs transit hydrophobic endosomal membranes? All are unknown (Figure 1).

We do know that for some newer modified ASO gap-mers that produce nuclease resistance and increased \( T_m \) (e.g., those containing 3' and 5' locked nucleic acids), no carriers are required, at least in vitro, for gene silencing (11). However, it is unclear if silencing can be produced in human tumors with these ASOs, despite some unpublished successes in human tumor xenograft models in mice. This is the road not taken in oligonucleotide therapeutics for cancer: In the search for the most rapid path to the market, the difficult, time-consuming and costly work of meticulously studying the delivery process has never been undertaken. And for this lack of knowledge, the field of oligonucleotide therapeutics, despite the occasional glimmer of hope as described by Hong, et al., (1) continues to pay the price in its lack of clinical activity in cancer.

REFERENCES

**Figure Legend:** Features of ASO delivery to cells that are not well understood. ASOs bind to cell surface proteins (1), which are then internalized into endosomes (2) with variable rates and mechanisms, depending on cell types. The hydrophobic ASO probably must transit the endosomal membrane (3), though how this is accomplished is not understood. The location of target mRNA cleavage, nucleus or cytoplasm, is also uncertain, (4) although RNAse H does appear to be the enzyme responsible for mRNA cleavage.
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