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Editorial

To Discover, Develop and Deliver a Right Drug: A Showcase for Antisense Technology

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Editorial

In genetic diseases, the information from a defective gene is passed from DNA as an RNA sense strand, also known as messenger RNA (mRNA), which causes disease when aberrant proteins are made in the body or when a required protein cannot be produced. The basis of antisense technology is that if antisense oligomers bind defective mRNAs and deactivate or usefully alter the mRNAs, these illnesses can then be treated. Scientists speculate that antisense technology can be applied to treat many infections, genetic diseases, inflammation or cancers with negligible side effects.

Antisense drugs have sequences of nucleic acid bases which are complementary to the sense strand, can bind and deactivate or alter the targeted mRNA, and so can impact protein expression or generate alternate splice forms. Such a simple and sound principle has been well established in model systems and accepted in the scientific community for more than quarter century. One would naturally expect that this technology should have come to fruition as therapeutics. The reality, however, is that so far only two antisense drugs have been approved by the United States Food and Drug Administration (FDA). Some scientists doubt the activity of the first drug, the 21-base phosphorothioate oligo Vitravene (fomivirsen, ISIS 2922) for ocular cytomegalovirus retinitis, is due to a classic antisense mechanism [1]. The second drug, Kynamro (mipomersen, ISIS 301012), is a 10-base phosphorothioate DNA oligo with 5 bases of 2'-O-methoxyethyl phosphorothioate RNA at each end, for a total of 20 bases. Kynamro interferes with apolipoprotein B-100 synthesis and is approved for treatment of homozygous familial hypercholesterolemia [2]. However, Kynamro causes serious side effects, including hepatic toxicity.

What are the problems with antisense technology that have prevented its widespread use in medicine? This short article will describe the current state of antisense technology in the line of drug discovery, development and delivery.

Discovering the Right Drug

For an antisense drug to achieve high sequence specificity and lack off-target effects, the chemistry of the agent is fundamental. Three such agents are in use in most current clinical trials. A comprehensive comparison has been published [3]. Brief highlights are reiterated below:

(1) S-DNAs (phosphorothioate-linked DNA) cause multiple off-target effects, largely because their backbone sulfurs bind to many different proteins. S-DNAs also achieve poor sequence specificity because S-DNA/RNA duplexes as short as 7 base-pairs are cleaved by RNase H. The related 2'-alkyl phosphorothioate RNA do not because RNase-H mediated cleavage of their RNA targets, but retain the backbone sulfur like S-DNA. Sometimes chimeras of several backbone types are used, as is the case for Kynamro.

(2) siRNAs (short interfering RNA) also suffer from off-target effects, particularly in stimulating Toll-like receptor activation [4]. siRNAs also provide only limited sequence specificity as short guide sequences largely determine which gene transcripts will be blocked or cleaved, and those guide sequences appear to recognize insufficient sequence information to uniquely target a selected gene transcript [5]. This specificity limitation is inherent in their mechanism of action and probably cannot be greatly improved.

(3) Morpholinos are virtually free of off-target effects likely because their uncharged backbone does not interact electro statically with proteins. Morpholinos also achieve very good sequence specificity mostly because they must bind about 14 contiguous bases or more to significantly block a gene transcript. In addition, Morpholinos are completely stable in biological systems [6,7] and highly predictable in targeting a specific gene [8,9].

These respective properties, uncovered with research conducted over many years, should clearly direct principal investigators and decision-makers to choose the optimal chemistry accordingly along the course of drug discovery.

Developing the Right Drug

A scan of the literature and a look at the current approach of Pharma companies to antisense suggest that little has changed in recent years toward a focus on the better drug. Academicians who have devoted their careers in the research of S-DNAs and /or siRNAs have invested tremendous time in establishing their scientific paths. They have been observing severe off-target effects [10] and poor sequence specificity [11], but they continue to work on solving those inherent problems of their chosen chemistries and they are "almost there". Pharmaceutical companies who have convinced investors that their technology is going to succeed in clinical trials resist changing their course because firstly, the FDA would not allow them to do so once an IND (Investigational New Drug) has been filed, and secondarily, investors would not happily support switching to a new chemistry when they were painstakingly convinced that the initial one held such great promise.

A good example of staying the course in the face of much better chemistries involves the treatment of Spinal Muscular Atrophy (SMA). SMA is an autosomal-recessive disorder characterized by α-motor neuron loss in the anterior horn of the spinal cord. SMA

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results from deletion or mutation in the *Survival Motor Neuron* 1 gene (SMN1). Humans harbor a nearly identical gene called SMN2 derived from a genetic duplication event, but a single base difference results in exclusion of exon 7 from the majority of SMN2 transcripts, and this gene product cannot complement the critical functions of the SMN1 protein. Exon 7 of SMN2 can be forced to remain in the mRNA using an antisense-mediated strategy. Testing this strategy has clearly shown that, among several chemistries of antisense oligos, Morpholinos are superior for prolonging splice modulation and correcting the disease state [12]. One would expect the best drug candidate would be pursued for further development once discovered. Unfortunately, the real scenario is that a huge sum of money is in place to start a clinical trial for a suboptimal drug, and at least one optimal sequence has been patented, thwarting development of that sequence with a better antisense oligo chemistry by other pharmaceutical companies.

Delivering the Right Drug

You may now be asking: If Morpholino oligos are great, why is there no FDA approved Morpholino oligo-based drugs?

Morpholinos provide all the desired properties of stability, nuclease resistance, high efficacy, long term activity, water solubility, low toxicity and very good specificity. However, like other types of antisense oligos, bare Morpholino oligomers have very poor cell permeability which limits access to the cytosol / nuclear compartment of cells of living animals. In consequence, the broad application of Morpholino oligos in animal research and therapeutics has been hindered.

There is currently a clinical trial of a Morpholino-based drug, eteplirsen (AVI-4658), for treatment of Duchene Muscular Dystrophy (DMD). DMD is caused by disruption of the dystrophin gene (*dmd*) resulting in a severe muscle wasting disorder. In many patients a mutation disrupts the *dmd* mRNA reading frame resulting in an out-of-frame *dmd* transcript and a non-functional dystrophin protein. While the trial shows some therapeutic benefit, it has slowed but not halted muscular degeneration. Morpholinos do not readily enter healthy muscle cells [13]. Somewhat surprisingly with DMD, bare Morpholino oligo administered intravenously (i.v.) can initially enter diseased "leaky" muscle cells [13]. As the treated cells produce functional dystrophin, delivery of the Morpholino oligo into muscle cells may be hampered by reduced leakiness of treated muscle tissue until the muscles degenerate and allow oligo entry again. These cycles of improvement and degeneration may be a factor compromising the therapeutic outcome. Heart damage and eventual failure is a hallmark of DMD, however, bare oligos do not enter cardiac cells as efficiently as most other muscle cells [14]. A delivery technology is, therefore, crucial to improve the treatment of DMD with Morpholino oligos.

Most diseases do not create a "leaky" cell scenario like DMD and would be even more difficult to treat. To overcome the last and most difficult challenge for safe and efficient *in vivo* delivery of Morpholino antisense oligos and to enable their application in a broad range of diseases, delivery-enabled Morpholino conjugates have been developed, mostly containing arginine-rich peptides [15,16] and oligoguanidine moieties [17]. Among those are Vivo-Morpholinos, octa-guanidine dendrimers coupled with Morpholino oligos, which have demonstrated highly efficient delivery and antisense activity [18]. Outstanding results can be achieved systemically with i.v. injection and modest systemic delivery can be achieved by intraperitoneal (i.p.) injection. Efficient localized delivery can be achieved by injecting the Vivo-Morpholino directly into the area of interest.

However, the extremely low toxicities of bare Morpholinos raise the benchmark for delivery-enabled Morpholinos, especially for Vivo-Morpholinos in animal studies. Toxicity becomes an issue for high doses of Vivo-Morpholinos and higher doses are often applied in model organisms in pursuit of rapid and complete gene-knockdown results.

To alleviate Vivo-Morpholino toxicity, a few approaches have been explored. For example, use of a low dosage of Vivo-Morpholino has shown extensive and prolonged effect in restoration of Dystrophin expression in a dystrophic dog model with no toxic side effects reported [19]. Another interesting approach to minimize toxicity was to design and use an alternative antisense oligo sequence to avoid possible oligo hybridization through self-complementary base moieties. It is speculated that any oligo dimerization of Vivo-Morpholinos would cause an over-exposure to the guanidinium dendrimer which is doubled in the dimerization [20].

A search for a less toxic or ideally non-toxic delivery moiety for Morpholino antisense application is the frontier of antisense research and development. Use of currently available Vivo-Morpholinos with techniques such as the aforementioned low dosing, or formulation for controlled release [21,22], may expedite the development of Morpholino antisense drugs for safe and effective treatments for most viral diseases and multiple infectious or genetic diseases.

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