Montréal June 5th, 2008

Contribution to the public consultation to the Proposed Amendment on Advanced Therapeutics (Regulation (EC) No 1394/2007)

Differences between antisense therapeutics and gene therapy

Executive Summary

The proposed change to the definition of gene therapy medicinal products appears to have been expanded to include any oligonucleotide that acts as a result of its sequence. With the proposed definition there will be no distinction between technologies like antisense drugs and therapies that use virally or vector-mediated transformation of cells by the administration of functional genetic material. This blurring of the boundaries between agents designed and known to act solely on the product of gene transcription, RNA, and those agents that act directly to introduce genes or change the genome itself is based on the incorrect assumption that any nucleic acid drug represents genetic material. Thus the proposed broad reclassification is based on the composition of the agent, or chemistry rather than an understanding of the science. The new definition would ignore differences in the sizes of the gene therapy sequences, the sites of action (RNA vs. DNA), the reversibility and potential heritability of the activity, and absence of functional genes in the antisense and siRNA based drugs. For these reasons we would respectfully request that the proposed definition be modified to distinguish between vector or virally mediated nucleic acid therapies and technologies that employ short stretches of nucleic acids as drugs to target RNA (siRNA and antisense) or nucleic acids designed to interact with specific sequences (aptamers).

Any definition of gene therapy should only include therapies that administer functional genetic material - antisense therapeutics do not

The short sequences used in antisense therapeutics do not constitute a gene product. Antisense therapeutics consist of a chemically synthesized short sequence of nucleobases (20 -30 bases in length) that are complementary to small region on its target mRNA. The mRNA target selected usually encodes for a disease related protein. The antisense drug hybridizes to its cognate sequence and then either through steric hindrance or through enzymatic activity, the drug prevents that RNA from being translated into a functional protein. The antisense drug itself is not a functional gene. Antisense drugs are single-stranded and do not have protein binding regions (promoters or ribosome binding sites) that would enable them to be transcribed and translated. There is no mechanism for these drugs to self propagate like vector driven therapies, and as such they are more similar to traditional small molecule drugs that are active as a result of exogenous administration and the magnitude of the effect is determined by the administered dose and the concentration in the target organ. In contrast, vector-driven gene therapy agents can be self propagating. The activity of vector-driven gene therapy agents is dependent on cellular expression of the desired gene products which is in turn dependent on the gene therapy agent using cellular machinery to produce a gene product.

Antisense drugs reduce the expression of an endogenous protein product, but gene therapy agents produce a gain of function by the addition of genetic material and its amplification.

Unlike gene therapy, the activity of antisense oligonucleotides is through a loss rather than a gain of function. Dosing with the antisense drug does not add competent genetic information to the cell and there is no possible gain of function. Unlike vector-driven gene therapy, the antisense drug is incapable of being amplified and its activity is totally dependent on the amount of drug administered. Furthermore, nor is it capable of being amplified. More importantly its activity is diminished over time as the antisense drug is metabolized and cleared from the body. In contrast, gene therapy agents are competent, are amplified by cellular machinery, and their activity is equally dependent on the amount delivered and the degree of amplification; a situation very different than that for antisense drugs.

Antisense drugs do not integrate into the genome

While it is impossible to demonstrate the negative, there has not been any evidence of integration of antisense drugs into cellular DNA in both prokaryotic and eukaryotic cells in *in vitro* genetic toxicity assays. In *in vitro* assays vast numbers of cells are exposed to antisense drugs at near toxic concentrations and the cells are allowed to replicate multiple times over the course of these experiments. Despite the fact that the *in vitro* assay incubation conditions are overwhelmingly in favor of inducing some integration, these assays have been uniformly negative in both bacteria and in mammalian cell lines, including human cell lines. Recently data compiled from across the industry indicate that dozens of sequences of phosphorothioate or phosphodiester antisense drugs that contain different chemistries (modified bases) have been negative in bacterial and mammalian genotoxicity assays. Because of the

uniform negativity in these assays, EMEA no longer requires *in vitro* genetic toxicity testing of some antisense drugs (*CHMP/SWP Reflection paper on assignment of the Genotoxicity potential of antisense oligonucleotides., London, January 2005*). What is more important is that these *in vitro* experiments represent vast numbers of potential integration events that are readily amplified if they occur: yet the tests on dozens of sequences show no evidence of this phenomenon. Taken together, these data support the conclusion that antisense agents neither contain sufficient genetic information to be functional genes nor do they insert into the genome to produce heritable genetic changes or damage.

Antisense sequences behave like drugs in that their effects are transient and that the reversibility of their effects is dependent on metabolism and clearance of the metabolites.

Antisense drugs, like traditional low molecular weight drugs, share similar principles of activity. The most critical for this discussion are the dose and concentration relationships. The activity of antisense drugs is completely dependent on the dose of drug administered and the concentrations of the drug in the target tissue. When the antisense drug is metabolized (by nucleases) the concentration of the antisense drug decreases and the pharmacologic effect is diminished to the baseline no effect level. This is in contrast to vector driven gene therapy where integration, expression and amplification of a functional gene can permanently affect changes in target cells and result in gain of function effects in daughter cells or even heritable changes.

Antisense drugs target RNA not genomic DNA

The target for antisense drugs is RNA not the genomic DNA. Antisense drugs inhibit the functional activity of mRNA. These drugs work through an RNAse H based mechanism. This mechanism is dependent on a natural process in which the RNAse H enzyme destroys any RNA (in this case target mRNA) that is hybridized to DNA (the antisense drug). The enzyme is incapable of cleaving DNA so that it is not possible to affect the genome through this mechanism.

Can antisense drug target genomic DNA?

It is possible that antisense drugs interact with DNA, but the effects are transient. Some antisense drugs can nestle into the DNA helix and form triplex structures with DNA. This process has been thoroughly evaluated as a potential therapeutic modality, but like other antisense activity, it is epigenetic in that the interaction is transient and does not affect the fidelity of transcription nor does it induce changes in the genomic sequence. It simply blocks transcription. Triplex formation does not induce heritable changes. In theory, in triplex formation, the antisense drug would binds to its cognate sequence in genomic DNA to sterically block transcription. This type of interaction is not favored thermodynamically for a number of reasons and it is unclear if it is possible at physiologic pH and temperature. However the most critical point is that like all other forms of exogenously administered antisense drugs, the effects will be transient and reverse as the antisense drug is metabolized and cleared. Again like the other mechanisms of antisense activity these interactions should not be classified as gene therapy because they do not alter the genome.

Antisense drugs are advanced therapeutics

Although synthetically produced antisense agent behave, and have transient effect like small molecule drugs, there is little doubt that they represent a novel or advanced therapy and warrant regulatory pathways that are different from normal drugs yet different still from biologics. Considering that these differences will be key to regulate this class of drugs effectively and rationally without keeping critical drugs from the patients who need them. It is noted of course that antisense drugs have already been approved and are in patient use. The stakeholders in these therapeutic areas include industry, academia, and the patients. With the growing level of sophistication among these groups it may be worthwhile to exploit this expanding knowledgebase to come up with regulatory definitions and solutions that satisfy scientific rigor, but still insure patient safety. We would support efforts to increase the level of scientific rigor in the design and promulgation of these regulations which may guide the effective development of other drug in this class.

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