Alnylam® PHARMACEUTICALS

6 June 2008

Mr. Nicolas Rossignol
European Commission
DG Enterprise & Industry
Unit F2 'Pharmaceuticals'
45 Avenue d'Auderghem, Office 10/128
B-1049 Brussels
BELGIUM

Re: Comment on Public Consultation Paper
PROPOSALS TO AMEND ANNEX I TO DIRECTIVE 2001/83/EC AS REGARDS ADVANCED THERAPY MEDICINAL PRODUCTS
Version: 8 April 2008

Dear Mr. Rossignol,

Enclosed please find comments to the Public Consultation Paper entitled "Proposals to amend Annex I to Directive 2001/83/Ec as regards Advanced Therapy Medicinal Products (Version: 8 April 2008)". Alnylam is a pharmaceutical company that is developing therapeutics based on the mechanism of RNA interference (RNAi), employing small interfering RNAs (siRNAs) targeting specific messenger RNAs that encode disease causing proteins. As elaborated in the attached response, we outline why siRNAs should not be classified as gene therapy products, and the potential consequences if this were to occur.

We would appreciate it if you would take into consideration our scientific rationale for this objection and appropriately make exceptions to the definition, or modify it suitably.

Please do not hesitate to contact us should you require any further commentary or input in this matter.

Thank you.

Sincerely,

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RESPONSE FROM ALNYLAM PHARMACEUTICALS TO THE ADVANCED THERAPEUTICS AMENDMENT

siRNA is not gene therapy

Summary

The new definition of gene therapy products included in the Amendment for Advanced Therapeutics is overly broad and now includes previously excluded technologies such as oligonucleotide and small interfering RNA, also termed siRNA, therapeutics whose composition, structure and mechanism of action are unrelated to gene therapy. For siRNA based therapeutics there is no expression of an exogenous gene or potential for replication in cells. siRNA alters the function of messenger RNA (mRNA) and does not interact with, or alter, the genome itself. Exogenously administered siRNA has properties typical of small molecule drugs, and most importantly the effects are reversible as the siRNA is cleared by metabolism. The pharmacologic effect of an exogenously administered siRNA therapeutic is unrelated to effects on the genome, and such products should therefore not be categorized as gene therapy products solely on the basis of their chemical composition or their sequence specific activity. Alnylam agrees with the concept that novel therapeutic agents may need to be regulated differently from small molecules, but characterizing them broadly as gene therapy ignores their mechanism of action and will not help to appropriately address issues of safety.

RESPONSE FROM ALNYLAM PHARMACEUTICALS TO THE ADVANCED THERAPEUTICS AMENDMENT

RNA Interference Using siRNAs is Not Gene Therapy

We at Alnylam Pharmaceuticals applaud the efforts of the European Commission to highlight specific issues relating to Advanced Therapeutics. As the leader in the development of RNA interference (RNAi) therapeutics using small interfering RNAs (siRNAs), we acknowledge that there are differences between traditional low molecular weight drugs and novel biotechnology derived therapeutic agents like oligonucleotides. However there are more fundamental similarities between siRNA and low molecular weight drugs than there are differences, with the foremost being the reversible nature of the pharmacologic effect. More importantly there are critical differences between gene therapy agents and siRNA. Small interfering RNAs cannot replicate, express a protein, or become integrated into the genome. The action of siRNA is therefore unrelated to gene therapy.

What is Gene Therapy?

Current Definition:

A gene therapy medicinal product shall mean a product obtained through a set of manufacturing processes aimed at the transfer, to be performed either *in vivo* or *ex vivo*, of a prophylactic, diagnostic or therapeutic gene (i.e. a piece of nucleic acid) to human/animal cells and its subsequent expression *in vivo*. The gene transfer involves an expression system contained in a delivery system known as a vector, which can be of viral, as well as non-viral origin. The vector can also be included in a human or animal cell.

Proposed Definition: gene therapy means a medicinal product:

1) that contains or consists of a nucleic acid sequence used in or administered to human beings, in vivo or ex vivo, with a view to regulating, repairing or replacing a targeted genetic sequence;
2) whose therapeutic, prophylactic or diagnostic effect relates directly to the nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

The current definition of a gene therapy product is appropriate in that it specifies that the nucleic acid is expressed or is associated with a viral vector that delivers the genetic material in such a way as to allow its expression or integration into the genome; however, the proposed definition broadly classifies any therapeutic that is a nucleic acid as gene therapy merely by virtue of its oligonucleotide-based composition or its sequence dependent effect. Why this latter definition is inappropriate for siRNA and the scientific reasoning for that conclusion is provided below.

The Mechanism of Action of siRNA is Cytoplasmic and Unrelated to DNA and the Genome

Since the discovery of RNA interference[1] (RNAi), the mechanism of action and associated molecular biology have been delineated in great detail[2, 3];. With siRNA therapeutics, a short piece of linear, double stranded RNA (dsRNA; 20-30 base pairs in length) is the therapeutic agent. The siRNA is administered like a typical drug (not by a viral vector), is taken up by cells and acts on a cytoplasmic target, messenger RNA (mRNA). This dsRNA is designed such that one of the strands of the duplex is complementary to a segment of mRNA that encodes a disease-related protein. The dsRNA is bound by an enzyme complex known as RNA Induced Silencing Complex (RISC). RISC selects one strand of the dsRNA, and then the enzyme complex efficiently seeks out and binds to mRNAs containing a sequence that is complementary to the RNA strand loaded in RISC. When a complementary mRNA strand is found, RISC cleaves the target mRNA at a defined location, and is then free to bind and cleave another mRNA. Cleavage leads to mRNA degradation which ultimately reduces the expression of the (disease-related) protein encoded by the target mRNA. All of these actions affect RNA, not genomic DNA, and they occur outside the nucleus in the cytoplasm. The enzymatic complex involved in RNAi is capable of cleaving RNA, but is incapable of cleaving deoxyribonucleic acids (DNA) that comprise the genome. Thus the mechanism of action of siRNA does not involve effects on the genome.

siRNAs Cannot Integrate in the Host DNA

One of the concerns when administering an oligonucleotide is that there could be integration of the oligonucleotide fragment into the genome. Can genomic DNA be an unintended target for siRNA as a result of integration of the siRNA into the genome? This is highly unlikely if not impossible because there is no precedence for direct

integration of an RNA molecule into genomic DNA. While it is well known that retroviruses, a class of RNA-genome viruses, can become stably integrated into the host genome, this requires the creation of a DNA copy of viral genomic RNA[4, 5]. This highly complex process is dependent upon virally-expressed factors, including reverse transcriptase, and specific sequence elements within the viral genome. If by some chance one of the strands of the siRNA pairs with a complementary sequence on DNA, there are cellular mechanisms that destroy RNA-DNA duplexes and prevent them from being incorporated in the genome. Thus, integration into the genome cannot be an unintended consequence of administering siRNA.

siRNA Duplexes are Neither Expressed nor Amplified by Cells

In contrast to siRNAs, currently gene therapy agents are intended to augment the expression of a protein and are administered via an expression vector. The siRNAs do not have the necessary genetic information required to cause the cellular machinery to create additional copies of the siRNA nor do these siRNA drugs have the necessary sequence information that would enable them to be translated into proteins. In this regard siRNAs are very much more like drugs that interact with RNA. They are not functional pieces of genetic material with expression and replicative properties. In fact, the siRNAs that are in development at Alnylam are cleared like typical drugs with pharmacologic effects that reverse when the drug is cleared by excretion or metabolism. For example, ALN-RSV01, an siRNA therapeutic for the treatment of respiratory syncytial virus (RSV) infection that has been studied in a Phase 2 clinical trial in the UK, has a serum half-life of 12 minutes (EudraCT 2006-006902-27).

Unlike Gene Therapy Agents, siRNAs Exhibit Drug-Like Properties

In the siRNA mechanism, all of the classic principles of pharmacology apply. The target mRNA is the receptor and the ligand is the antisense strand of the siRNA. The consequence of the ligand-receptor interaction is cleavage of the target mRNA. Like all pharmacologic agents the concentration of the ligand delivered to the cell determines the magnitude of the response, and the pharmacologic response is reversed as the ligand (siRNA) is cleared as a result of metabolism. Reversal of siRNA activity has been repeatedly demonstrated in animal models including studies in non-human primates[6].

Targeting RNA and Transcription/Translation do not Constitute Genetic Manipulation

Small interfering RNAs target RNA. Is it appropriate to classify all drugs that target RNA as gene therapy? In our opinion, the answer is no. For example, another class of therapeutics that targets disease-related mRNAs is antisense oligonucleotide drugs. One such drug,fomiversen, is registered in the European Union. That these drugs do not impact the genome has been thoroughly demonstrated with multiple sequences and chemical modifications in *in vitro* genotoxicity assays. In fact those assays are no longer required by the EMEA[7]. More importantly thousands of human subjects have been treated with antisense oligonucleotide drugs, and their safety profile is well established[8].

In addition to oligonucleotide therapeutics, there is also precedence for several types of low molecular weight drugs that bind to and interfere with RNAs as their mechanism of action. The macrolides, aminoglycosides and other RNA-binding antibiotics all interact with RNA[9,10] but are not considered gene therapies involving genetic manipulation.

Steroids and fat soluble vitamins A and D, and histone deacetylase (HDAC) inhibitors are examples of drugs that act directly to influence gene expression but none of these agents is considered gene therapy. In fact, histone deacetylase inhibitors alter the biochemistry of chromatin proteins leading to modulation of gene transcription[11], yet, it would be inappropriate to characterize HDAC inhibitors as gene therapy.

Oligonucleotide therapeutics deserve to be classified as advanced therapeutics, but having an effect due "directly to the nucleic acid sequence it contains, or to the product of genetic expression of this sequence" (as in the proposed definition) does not make them gene therapy products.

RNAi Induced by Gene Expression Vectors IS Gene Therapy

Alnylam's comments above are limited to exogenously administered, chemically synthesized siRNAs. It is important to distinguish these from gene-based RNAi approaches (for example short hairpin RNAs or shRNAs) that depend on expression vectors designed to be transcribed in cells and tissues. Typically, the expression vector is a modified adeno- or lenti-viral system. Unlike siRNAs, these shRNA expression vectors are dsDNA, possess motifs that allow integration, and sequence elements (e.g. promoters and enhancers) that interact with host machinery to permit expression of the shRNA

precursor from the incorporated gene. Integration occurs in the nucleus, and the expressed shRNA is exported to the cytoplasm where it is then processed into an siRNA. As such it is clear that shRNA-based expression vectors are gene therapy and should be classified accordingly.

The distinction between expression-vector-based-RNAi and RNAi induced by the administration of exogenous siRNAs exemplifies why the current definition of Gene Therapy is scientifically correct and the proposed definition is flawed. The existing definition distinguishes between these two forms of oligonucleotide therapy because interference induced by the administration of exogenous siRNAs is like any other pharmacologic effect, except that the composition of the drug happens to be an oligonucleotide.

Public Policy Issues

Public safety as a whole has been well served by regulatory authorities world-wide who have understood and appreciated that novel agents require scientifically based regulations. Failures have resulted when the best science was not used in assessment and regulation. Advanced biotechnology therapeutics are a rapidly expanding source of new drugs and new types of therapies are constantly being developed. Regulation of this rapidly changing field has been successful because authorities have depended on high quality scientific judgment and case by case approaches when regulating these novel agents. These careful approaches have led to important new therapeutic agents in a wide variety of disease indications. The boundaries between biologics and advanced therapeutic agents are getting less clear and oligonucleotide therapeutics are in that middle ground. They behave like drugs, but have some of the chemical characteristics of biologics. With all that is known and appreciated about the mechanism of action of these agents, ignoring this information to impose artificial labels and regulations that disregard the science is not in the best interest of patients who will be served by new therapies. Inappropriately labeling oligonucleotide therapeutics as gene therapy could and will cause undue concern in patients, and they can conceivably reject important therapies based on this misperception.

It is the responsibility of both regulators and the developers of these drugs to understand their biologic effects. Like the regulatory guidelines governing small molecule drugs, regulations concerning siRNA drugs should require that these agents demonstrate safety

and efficacy using the appropriate science as a guide. Burdening the compounds with an inappropriate set of hurdles will ultimately not serve the best interests of patients nor the innovators. Public policy should be set with the most correct and most appropriate science. As such, we therefore request that the current definition of gene therapy not be altered as proposed.

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