mRNA

RISC

# R N A   I N T E R F E R E N C E :

**AN INTRODUCTION TO RNA INTERFERENCE (RNAI) AND DRUG DEVELOPMENT**

## Alnylam®
PHARMACEUTICALS

# Contents

## An Introduction to RNA Interference (RNAi) and Drug Development

Drugs based on RNA interference (RNAi) are believed by many to be the next major class of human therapeutics. This potential has been recognized with the 2006 Nobel Prize for Medicine awarded to Craig Mello and Andrew Fire for their discovery of RNAi, and has resulted in major investments in RNAi-based drug development by large pharmaceutical and biotech companies. These investments include a series of transformative drug discovery alliances considered to be among the biggest in biotech history.

*Recent breakthroughs in the understanding of the central role for RNA in a variety of cellular mechanisms have further facilitated the development of RNAi therapeutics.*

The development of RNAi therapeutics has greatly benefited from an unprecedented advancement of the basic science relating to the role of RNA in an ever expanding spectrum of biological processes. While RNA had long been regarded to merely facilitate gene expression as a passive intermediate and structural component of the protein expression apparatus – or operating in a 'messenger' capacity – it is now clear that RNA has many of the genetic, regulatory, and catalytic properties formerly thought to be possessed only by DNA and proteins.

The original function of the RNAi mechanism, which still operates in many invertebrates today, was that of a cellular RNA-based immune system designed to 'interfere' with the uncontrolled production of aberrant RNA molecules, which could be interpreted by the body as an opportunistic viral

## Timeline of RNAi Discovery

| | 1990 | 1998 | 1999 |
|---|---|---|---|
| **Event** |  Jorgensen observes "PTGS" in petunias |  Fire and Mello discover RNAi in C. elegans |  Kreutzer/Limmer show RNAi mediated by short dsRNA |
| **Publication** | Napoli et al., Plant Cell 2, 279-289 (1990) | Fire et al., Nature 391, 806-811 (1998) | None |
| **Patents (US/EU)** | None | Dec., 1997 Carnegie Institute "Fire and Mello" US 6,506,559 EP, Pending | Jan., 1999 Alnylam "Kreutzer-Limmer I" US, Pending EP 1144623 EP 1214945 |

infection. Like our natural adaptive immune response, the RNAi mechanism is extremely specific in targeting these RNA molecules. In fact, they are identified for degradation by well-defined complementarity rules between the targeting small RNA and target messenger RNA (mRNA).

## From Plants to Worms to Humans: Discovery and Mechanism of RNAi

Much of our understanding of RNAi is based on seminal observations made in model organisms such as the mustard weed *Arabidopsis* and the nematode *worm C.* elegans.

*A failed genetic experiment leads to surprising biological insight*

The first experimental observation of RNAi began with attempts by Jorgensen and colleagues to create more attractive petunia flowers by genetically engineering additional flower pigmentation genes into the plant genome. To their surprise, instead of producing plants with more colorful flowers, many had lost most or all pigmentation, and as a result, became white. Intriguingly, when they looked at the expression of genes involved in the natural pigmentation biosynthesis pathway, they found that the mRNA level of the gene that corresponded to the newly introduced designer pigment gene had been strongly reduced. Thus, the concept of co-suppression was born whereby sequence-related genes could negatively regulate each other.

*Long double-stranded RNAs induce specific gene silencing in a nematode worm*

The first major breakthrough in understanding the mechanism of this

| 2000 | Kreutzer/Limmer start Ribopharma AG (now Roche Kulmbach AG) as 1st RNAi Therapeutics company | Tuschl leaves Whitehead Institute to join Max Planck Institute | 2001 |

Tuschl/Zamore/Bartel/Sharp isolate short dsRNAs from ?D. melanogaster

Zamore et al., Cell 101, 25-33 (2000)

March, 2000
WI/MIT/UMass/MP
"Tuschl I"
 US, Pending
 EP, Pending

Tuschl shows that siRNAs have defined structure and can be made to elicit RNAi in mammalian cells

Elbashir et al., Nature 411, 494-498 (2001)

Dec., 2000
Max Planck (Garching)
"Tuschl II"
 US 7,056,704
 US 7,078,196
 EP 1407044

type of gene silencing came from studies conducted by Andrew Fire and Craig Mello. By introducing various forms of long RNA molecules into the nematode worm *C. elegans*, Fire and Mello observed that it was double-stranded RNAs (dsRNAs) that were the actual inducers of the RNAi gene silencing phenomenon.

dsRNAs are similar in structure to the double-stranded DNA helix that makes up our genome, only that DNA is replaced by its chemical cousin, RNA. While Fire and Mello's work represented a major advance in understanding the mechanism of RNAi, it also provided for a simple and reproducible method by which long dsRNAs could be used to induce specific gene silencing in lower organisms commonly used in genetic research such as *C. elegans* and the fruitfly, *D. melanogaster*. Because of this work and the impact it would have on biomedical research, Andrew Fire and Craig Mello were awarded the 2006 Nobel Prize in Physiology or Medicine, even though they did not show or expect the RNAi mechanism to work in mammalian systems when their early work was performed.
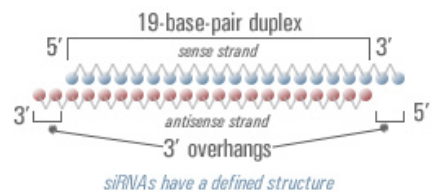
### Short double-stranded RNAs (dsRNAs) mediate RNAi in human cells

Shortly after Fire and Mello's discovery, dsRNAs were found to induce similar gene silencing in a variety of other organisms. However, it was still not believed that RNAi could also work in humans. This is because long dsRNAs, typically larger than 30 base pairs in length, induce an innate cellular response, including production of interferons (often called the interferon response).
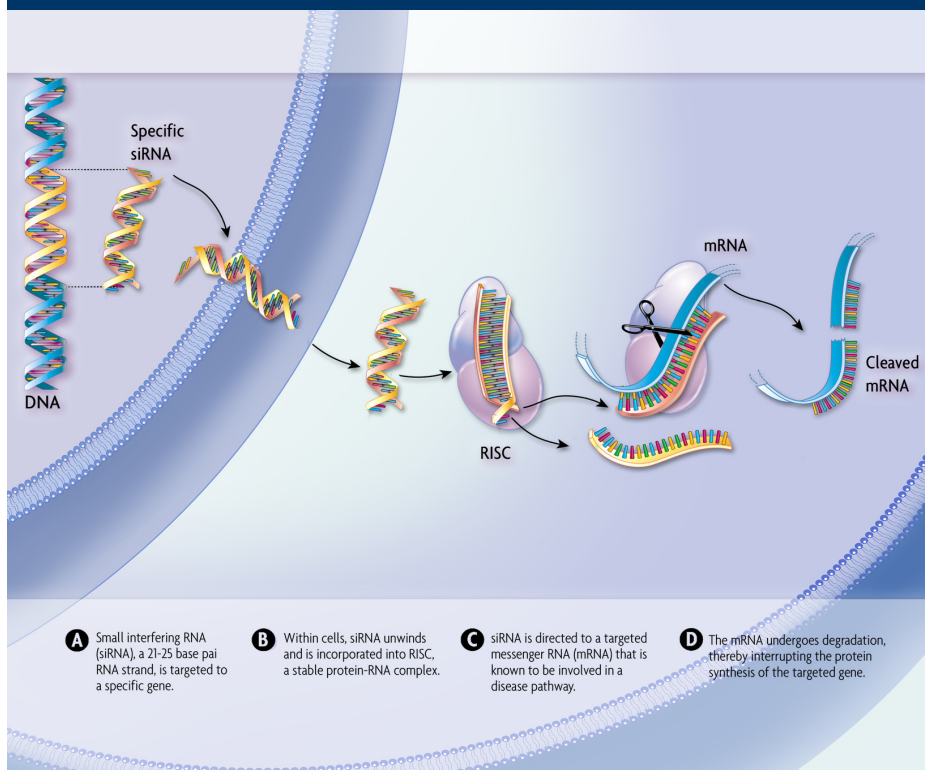
The first demonstrations that RNAi also functions in humans came from work by two separate groups in Germany.

At the University of Bayreuth, Roland Kreutzer and Stefan Limmer reasoned that short fragments of dsRNA might mediate the RNAi response triggered by the long dsRNAs as observed by Fire and Mello. Kreutzer and Limmer showed these short fragments worked in mammalian cells, and while they did not publish their findings, they did file key patents around the discovery, and left their academic positions to start a company called Ribopharma AG. In June, 2000, Ribopharma AG, which later became Alnylam Europe AG and then Roche Kulmbach AG, thus became the first company formed to focus on the development and commercialization of RNAi therapeutics.

Around the same time, Thomas Tuschl, and his colleagues at the Max Planck Institute would show in a seminal publication that synthetic versions of short double-stranded RNA molecules result in potent RNAi gene silencing in mammalian cells without inducing the interferon response. Moreover, Tuschl found that structurally defined 21 to 23 base-pair small RNAs, known as small interfering RNAs (siRNAs), with 2 nucleotide unpaired overhangs at the 3' ends were the most efficient mediators of RNAi. He also made and tests a wide range of siRNA design features, e.g.



*siRNAs have a defined structure*

## The RNAi Natural Process

**A** Small interfering RNA (siRNA), a 21-25 base pai RNA strand, is targeted to a specific gene.

**B** Within cells, siRNA unwinds and is incorporated into RISC, a stable protein-RNA complex.

**C** siRNA is directed to a targeted messenger RNA (mRNA) that is known to be involved in a disease pathway.

**D** The mRNA undergoes degradation, thereby interrupting the protein synthesis of the targeted gene.

"blunt ends," length parameters, and chemical modifications. This work was published in the journal Nature (2001) 411:494-8. and became the scientific content for a key patent in the RNAi field called "Tuschl II".

Targets from the work of Tuschl, and his discovery that siRNAs can be used to silence virtually any human gene this technology has immediately changed the practice of biomedical research.

### The naturally occurring RNA gene silencing pathway in humans

As research progressed, it became evident that the reason siRNAs, but not long dsRNAs, are able to induce efficient gene silencing in humans is because they feed into an endogenous RNA silencing pathway. This pathway is also known to process microRNAs. MicroRNAs are a large class of small RNAs, typically 19-24 nucleotides in length, and are believed to regulate a significant portion of our genes with diverse roles ranging from development to cell signaling.

While this RNA gene silencing pathway is used by both siRNAs and microRNAs, there exist some important differences. The siRNA pathway begins with cytoplasmic cleavage of long double-stranded RNA by the Dicer

enzyme that results into short double-stranded RNA duplexes. The short double-stranded RNAs (called siRNAs) are then incorporated into the RNA-induced silencing complex (RISC). If the siRNA strand loaded into RISC has perfect sequence complementarity with its target mRNA sequence, then site-specific mRNA cleavage takes place. This cleavage is a catalytic event, involving repeated cleavage of multiple copies of the target mRNA, leading to reduced copies of target mRNA and ultimately to inhibition of the target protein. Therefore, RNAi therapeutics developed to harness the siRNA pathway typically involves delivery of sufficient synthetic siRNA into the cell cytoplasm to trigger this catalytic process.

In contrast to the siRNA pathway, the mRNA pathway begins with endogenously encoded primary miRNA transcripts in the cell nucleus that are processed into precursor microRNA and then exported from the nucleus into the cytoplasm. Here, the Dicer enzyme complex further processes them for RISC loading. Unlike siRNAs, mature miRNAs typically have imperfect sequence complementarity to their RNA target sites which are typically limited to the 3' untranslated regions of the mRNA. Furthermore, interaction of a microRNA with its RNA target usually leads to direct translational inhibition rather than specific site-directed cleavage of the mRNA. Regardless of translational inhibition or cleavage, both microRNAs and siRNAs are able to regulate protein production at the RNA level in a very specific sequence-based manner.

## The RNAi Therapeutic Mechanism



Specific

siRNA

DNA

mRNA

RISC

Cleaved mRNA

**A** Short interfering RNA (siRNA) designed to correspond to gene target

**B** siRNA synthesized with drug-like properties: stability and conjugation for delivery

**C** Modified siRNAs penetrate the cell membrane and harness the RNAi mechanism for gene silencing

**D** Gene silencing achieves a therapeutic effect
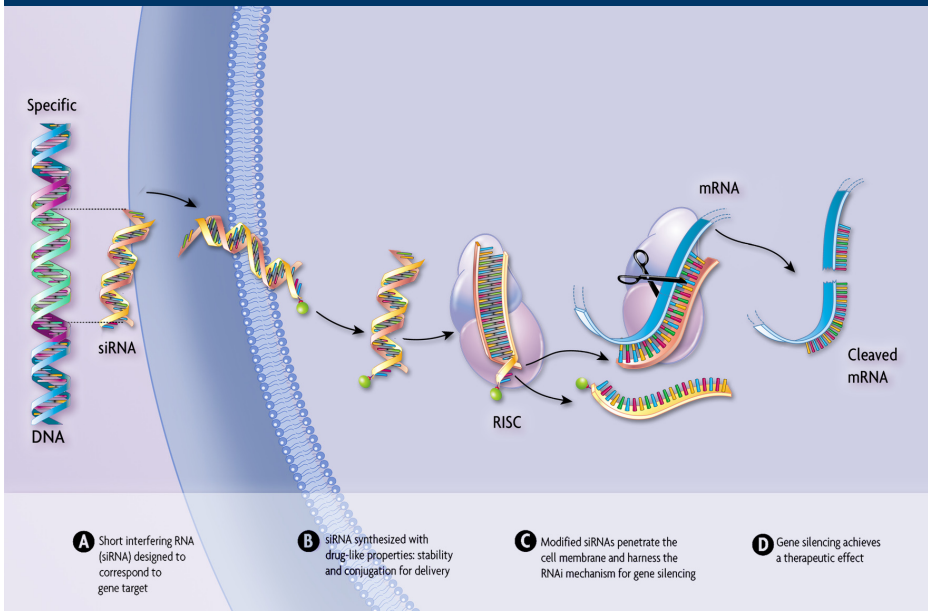
### Advantages of an RNAi Therapeutics Platform

Compounds operating via the RNAi mechanism have been demonstrated numerous times to specifically silence genes with the expected biological outcome not only in cultured cells ex vivo, but also in animal models of human disease. In fact, RNAi is already being widely exploited by drug developers for drug target validation purposes. It is therefore not surprising that there is growing interest in using RNAi as a therapeutic modality itself.

### RNAi therapeutics target gene expression upstream of protein production

It is known that proteins are responsible for much of the physical and dynamic properties of a cell, and defects in their function or regulation contribute to - if not cause - most diseases. The majority of drugs today are therefore designed to inactive proteins; with most attempting to neutralize disease-associated proteins through direct binding without removing them.

Much of the interest in RNAi is based on the fact that the RNAi mechanism operates upstream of protein production by eliminating the mRNAs coding for such proteins. Hence, instead of trying to stop a flood by mopping up the floor, with RNAi we can stop the flood by turning off the faucet. Moreover, as each mRNA is the template for the translation of multiple proteins, comparatively low amounts of an RNAi therapeutic should be effective in ablating protein function.

Due to the sequence specificity of double-stranded RNAs used to exploit the RNAi mechanism, it is now possible to address many of the conditions where RNA and its processing play a central role. In addition, their unique mechanism of action may allow RNAi therapeutics to work synergistically with other drug and treatment regimens, for example in conjunction with chemotherapy in cancer or statins for the treatment of hypercholesterolemia.

### RNAi is an endogenous biological pathway, thus allowing for the development of safe and efficacious drugs

RNAi occurs in all human cells. As with other major biotechnologies before, like recombinant proteins and monoclonal antibodies, the fact that RNAi is an endogenous biological pathway lead many to believe that it could make a particularly safe and effective therapeutic platform. Indeed, one siRNA mediates the silencing of many mRNAs as part of the catalytic RISC complex. The catalytic nature of the RNAi mechanism allows for the use of very low concentrations of siRNAs, which minimizes the risk of adverse side effects often caused by the use of large amounts of drugs.

Another major advantage that comes from harnessing an endogenous biological pathway is the ability to translate knowledge on normal function into the development of even safer and more efficacious therapeutics. Indeed, due to their central importance in many cell biological processes, an unprecedented research effort is aimed at elucidating additional RNA biology.

### RNAi opens up many new targets for drug development

Drug development has traditionally focused on the so called "druggable genome." This includes genes coding for extracellular proteins, cell-surface receptors, and enzymes with well-defined catalytic sites which are physically accessible and have structures that allows them to be bound with sufficient strength and specificity. As these represent only a small subset of our genome, the identification of suitable druggable targets has become a major bottleneck in the development of new, innovative drugs.

RNAi is different. As dsRNAs can be designed to target essentially any protein-coding mRNA independent of protein structure and localization, it opens up the many genetically validated, yet previously deemed "undruggable targets" for RNAi-based drug development. One example is Huntington's disease where the genetic cause has long been known, but efforts to neutralize the harmful effects of the mutated huntingtin protein have failed. By being able to suppress huntingtin expression, drugs based on RNAi promise to be the first modality that may slow or even prevent the progression of this devastating disease.

## Key Milestones in the Development of RNAi Therapeutics

**1990**
First scientific observation of the "co-suppression" phenomenon in plants, today known to be caused by RNAi.

**1998**
Double-stranded RNAs shown to trigger gene silencing in the nematode worm *C. elegans*.

**1999**
RNAi is mediated by small dsRNA fragments including in mammalian cells leading to the formation of Ribopharma AG.[*]

**2001**
siRNAs of 21-25 base pair length with 3' overhangs shown to induce efficient RNAi in mammals.[*]

**2002**
RNAi shown to inhibit viral replication, including HIV and HCV.[*]

**2004**
RNAi following systemic administration of siRNAs in adult mammals.[*]

**2006**
RNAi following systemic administration of siRNAs in non-human primates.[*]

**2008**
An RNAi therapeutic demonstrated clinical efficacy in the double-blind, placebo-controlled, randomized GEMENI study.[*]

[*]*Contributions made by scientists affiliated with Alnylam Pharmaceuticals, Inc.*

To be sure, the discovery of RNAi comes at a good time. Less than five years after the sequencing of the human genome, genetic research is booming with a record number of potential drug targets being uncovered, all of which can theoretically be addressed through RNAi.

An example of one such gene that has consequently attracted interest for the treatment of hypercholesterolemia is PCSK9, a gene that encodes for a protein involved in the metabolism of LDL-cholesterol. Genetic studies have shown that individuals who lack this protein are far less likely to develop cardiovascular disease without any overt side effect, while those that have a genetic disposition that causes an increase in PCSK9 production are at an elevated risk of cardiac disease.

As a result, Alnylam and others are currently developing drugs aimed at reducing PCSK9 mRNA levels in order to treat hypercholesterolemia. Such a rapid progression from genetic validation to clinical-stage drug targeting is quite unusual in the context of traditional drug development. The development of PCSK9-targeting small molecules for example would likely have been much more time-consuming and suffered from insufficient target specificity given the presence of a number of structurally very similar, yet functionally distinct proteins.

Knowing all the human genes as a result of the Human Genome Project further helps in designing siRNA sequences with optimized target specificities. As many investigational drugs fail due to off-target toxicities, RNAi, due to its high target selectivity, offers the promise for a safer class of human therapeutics with reduced development risk.

### RNAi is a robust platform for drug development

In sharp contrast to small molecules which have very diverse chemical properties and therefore pharmacological challenges that are very specific to each drug candidate, the chemical uniformity of siRNAs as a class means that the know-how from the development of one siRNA drug candidate can be largely applied across the whole platform. This may include medicinal chemistries to enhance the pharmacology of siRNAs, maximize target efficacy and specificity, manufacturing expertise, and ultimately allows for the rapid expansion to generate RNAi-based drugs to multiple distinct targets.

The identification of siRNA-based drug candidates to a given target is particularly rapid and reproducible. All that is needed is the sequence information of the gene of interest, readily available from the Human Genome Project, and a number of siRNA candidates can then be rapidly designed based on simple sequence complementarity rules and chemically synthesized for initial testing. All this should facilitate fast and economical drug development attractive not only to major pharmaceutical and biotech companies, but also public agencies concerned about preparedness in the face of potential biological threats such as Ebola and pandemic flu.

## Delivering RNAi: The Challenges and Strategies

Arguably the biggest challenge in translating the breakthrough science of RNAi into a broad new class of human therapeutics is delivering siRNAs to the appropriate tissues within the body. This is not an entirely new problem to drug development and similar obstacles apply to related oligonucleotide therapeutics (e.g. antisense technologies). In fact, considerable experience gained from years of developing nucleic acid delivery methods can now be applied to the development of RNAi therapeutics, while new, innovative technologies are being devised due to the immense interest in RNAi. It is expected that depending on the therapeutic application and target tissue, different delivery strategies will ultimately be used in the clinic.

### An RNAi therapeutic has to cross the cellular membrane barrier

In an unmodified form, siRNAs are negatively charged molecules, and to reach their site of action (e.g. the cytoplasm), they must overcome the equally negatively charged outer cell plasma membrane and the "grease-like" barrier of the cell membrane. It is therefore surprising that efficient delivery of unformulated siRNAs can be obtained for a few tissues, particularly mucosal epithelia. Still, for most purposes, delivery formulations are required to ensure cellular entry. Cationic liposomes and polymers are some of the more popular methods to overcome electrostatic repulsion by binding and neutralizing the negative charge of a number of siRNAs at once. Following incorporation of the siRNAs, the liposomes can favorably interact with the plasma membrane and deliver their siRNA cargo into the cytoplasm to harness the endogenous RNAi machinery and effect gene silencing.

Conjugation approaches have also shown promise in facilitating cellular entry of siRNAs. This may involve conjugating siRNAs to small peptides that can penetrate cellular membranes, or to simple small molecules such as a cholesterol moiety. Cholesterol-conjugation of siRNAs allowed Alnylam scientists to demonstrate for the first time functional *in vivo* delivery of an siRNA following systemic administration in mice. Cholesterol-conjugation has the added benefit of functioning as a carrier for the siRNA in the blood thereby improving its pharmacokinetic properties. This is of particular importance for the systemic delivery of RNAi therapeutics.

*Cholesterol Conjugated siRNA*

### From Direct to Systemic RNAi delivery

Many of the current RNAi therapeutics development programs employ direct delivery strategies whereby the siRNA is applied directly at the site of disease. This increases the likelihood that the siRNA is taken up before it can either be degraded or secreted from the body, in addition to limiting unnecessary systemic drug exposure.
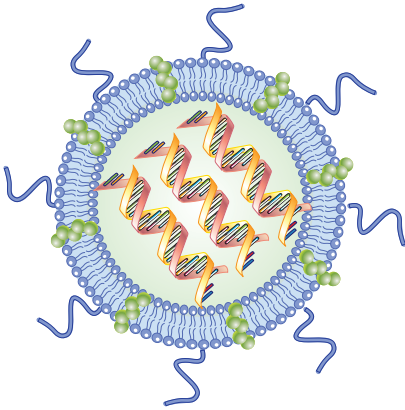
*Liposome formulated siRNA*

The eye and the lung are two organs which are amenable to such approaches since appropriate direct delivery technologies have been well established. As a result, siRNAs delivered by inhalation with a nebulizer or intraocular injection are under study for treating conditions such as respiratory syncytial viral (RSV) infection and age-related macular degeneration (AMD), respectively.

While the number of possible indications that can potentially be addressed with current direct delivery capabilities is significant, the successful application of systemic routes of administration such as intravenous or subcutaneous injection would open up RNAi for a vast number of additional diseases representing significant unmet medical needs.

The liver is a natural first frontier for exploration of systemic delivery approaches due to its natural role in maintaining blood homeostasis, which means that most drugs will pass through and enter this organ. Most progress in systemic delivery has been achieved in targeting siRNAs to the liver following intravenous administration of a number of different formulations. In many cases, potent gene silencing has been observed, including demonstration of potent silencing via RNAi in non-human primates. The first clinical trials using these formulations are now beginning for a number of liver-associated conditions such as primary and metastatic liver cancers and hypercholesterolemia.

For other tissues, however, active targeting strategies and improved pharmacological attributes are likely required. As it will logically take the siRNA longer to accumulate in tissues that are deep inside the body, the risk for premature enzymatic degradation of the siRNA increases when these tissues are the drug target. Stability of the siRNA within the therapeutic composition is therefore an important consideration in the design of systemic RNAi therapeutics. For example, just packaging the siRNA into nanoparticles will not only enhance cellular uptake, but also significantly stabilize the siRNA. Additionally, siRNA modification with medicinal chemistries, many of which have already been used in FDA-approved drugs, can further increase their stability without negatively affecting their gene silencing
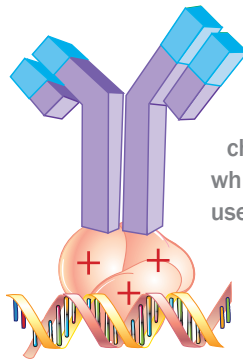


*Antibody-Protamine Complexed siRNA*

activity. Importantly, once the siRNA has reached the cytoplasm of the cell, it is typically active for a period of weeks.

Targeting siRNAs by exploiting tissue-specific features is another major area of research yielding promising systemic siRNA delivery solutions. One example is linking siRNAs to antibodies that recognize tissue-specific cell surface proteins. An additional example is RNA itself in the form of so called RNA aptamers. Aptamers are RNAs that assume 3-dimensional structures that when appropriately selected will allow them to bind to defined structures with high specificity and affinity.

### RNAi in the Clinic

The speed with which RNAi therapeutics are progressing towards the clinic only six years after Tuschl and colleagues published on the first synthetic siRNAs is impressive. The number of RNAi-based clinical development programs continues to grow rapidly. While most of these programs utilize direct RNAi delivery applications, the first systemically delivered RNAi drug candidates are beginning to enter the clinic.

Confirming pre-clinical data, clinical experience has shown that siRNA-based drugs are safe and well tolerated over a wide range of dosages. In the largest safety study of its kind, Alnylam and their collaborators have treated nearly a hundred volunteers with an siRNA drug candidate for RSV infection and found it to be comparable to placebo controls in terms of safety and tolerability.

Trials in progress for the treatment of AMD further suggest clinical activity of RNAi therapeutics, and first definitive proof-of-concept of an RNAi drug candidate may well emerge in the

## Biogenesis of MicroRNAs
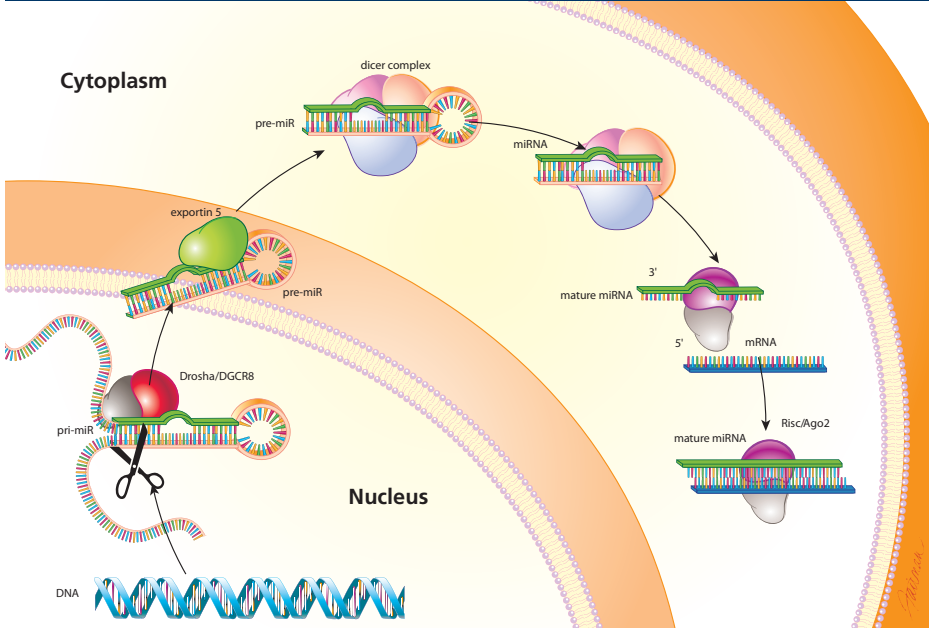
# Clinical and Advanced Pre-clinical RNAi Therapeutics Programs
**(as of June 2008; partial list)**

| Disease | Site | Stage |
|---|---|---|
| wet AMD | eye | phase III |
| diabetic retinopathy | eye | phase II |
| RSV infection | lung | phase II |
| HBV infection | liver | phase I |
| HIV infection | hematopoietic stem cells | phase I |
| Acute kidney injury | kidney | phase I |
| Cancer | Solid tumors | phase I |
| hypercholesterolemia | liver | pre-IND |
| cancers | liver, various others | pre-IND |
| neurodegenerative diseases | CNS | pre-IND |
| bioterrorism and pandemic flu preparedness | various | pre-IND |

near future from randomized, placebo-controlled, double-blind studies of RNAi therapeutics for RSV infection or hypercholesterolemia as well as in other indications. Recently, a double-blind, placebo-controlled clinical study of Alnylam's RNAi therapeutic targeting RSV demonstrated statistically significant anti-viral efficacy; the so-called "GEMINI" study thus represents the first evidence for the ability of RNAi therapeutics to work in man.

Clearly, the prospect of RNAi therapeutics to address diseases of significant unmet medical need, but for which there are genetically well validated targets not druggable by the traditional approaches is quite exciting. RNAi drug candidates for the treatment of a number of previously intractable neurodegenerative diseases and cancers are expected to soon undergo human testing. Accelerated progress towards the clinical use of RNAi may further be due to public initiatives to harness RNAi for protecting the public from emerging viral threats such as Ebola and pandemic flu.

### New Frontiers: MicroRNAs and Human Disease

MicroRNAs are a large class of small RNAs, typically 19-24 nucleotides in length, and are believed to regulate a significant portion of our genes with diverse roles ranging from development to cell signaling. The natural counterparts of therapeutic small RNAs or siRNAs, microRNAs are an abundant class of regulatory RNAs thought to control about one third of our genes.

It is therefore not surprising that during disease, changes in the pattern of microRNAs will occur with some being

indicative of treatment outcome and disease progression. Arguably even more exciting than their diagnostic value is the mounting evidence that directly implicates microRNAs in a number of diseases, most notably cancer. There is, consequently, increased interest in manipulating microRNA function for therapeutic purposes.

One approach is to mimic microRNA activity by introducing microRNA "mimics"containing the same genetic information as the natural mcroRNA. This should be of therapeutic value where the loss of microRNA function contributes to or causes a disease, or where the addition of a "good" microRNA will improve a condition.

For example, by adding more of a microRNA known as let-7, it has been possible to halt cancer cells from further multiplying. Since microRNA mimics in many ways resemble an RNAi therapeutic, their development as a therapeutic modality will be greatly benefitted from progress in the RNAi therapeutics field.

Another complementary approach to using microRNAs for therapy is to inhibit the activity of disease-associated microRNAs. This can be achieved by employing antisense oligonucleotides that, based on sequence complementarity, will bind to and consequently inactivate microRNA function.

Studies from Alnylam and those of others have supported the viability of this strategy by demonstrating efficient and specific microRNA inhibition using "antagomirs" in animal models. Such studies show that inhibiting a highly abundant miRNA in the liver, microRNA-122, not only lowered the level of LDL-cholesterol in the blood, but did so without any adverse effects on the animal. High LDL-cholesterol is a major risk factor for cardiovascular disease and additional strategies to lowering it are urgently needed for patients that do not achieve their cholesterol goals.

MicroRNA-122 inhibition also holds promise for the treatment of Hepatitis C Virus (HCV) infection. Underlining the central importance of non-coding RNAs in many cellular processes, some viruses of major medical importance such as HCV and HIV have learned to make use of and even come to depend on microRNAs found in the host cell. Accordingly, antisense-mediated inhibition of microRNA-122 negatively impacts HCV replication and this result may lay the foundation for novel HCV antivirals. Still other viruses encode and produce their own microRNAs, usually as a means to avoiding recognition by the immune system. Sometimes, as is the case for some Herpes viruses, these microRNAs are the only visible manifestation of an infection and therefore the only therapeutic targets.

Clearly, the exponential growth of knowledge on microRNA function as part of a global research effort combined with the development of potent therapeutic technologies will make drug development centered on microRNAs a vibrant field in the years to come. To this end, Alnylam and Isis Pharmaceuticals Inc. have formed Regulus Therapeutics LLC, a joint venture focused on the discovery, development and commercialization of miRNA therapeutics.

# Glossary

**Antisense technology:** Oligonucleotides designed to bind target RNAs (e.g. mRNA, microRNA, etc.) based on sequence complementarity in order to block their natural function. Antisense technology can also describe a general approach that includes RNAi and siRNAs.

**Monoclonal antibody:** A single type of purified antibody derived from a clone of antibody-producing cells that can be generated in the laboratory and used for drug development where a therapeutic benefit is expected from the binding of the antibody to a protein target.

**Bioinformatics**: Computational biology concerned with the management and analysis of biological data.

**Conjugation**: The addition of chemical groups to an active drug ingredient with the aim of improving drug performance.

**Dicer**: Central enzyme in the natural RNAi pathway that generates the active small RNAs by cleaving longer dsRNA precursors.

**DNA**: Deoxyribonucleic acid; genetic material consisting of any sequence of deoxyribonucleotides containing bases of adenine (A), thymine (T), guanine (G), and cytosine (C).

**dsRNA**: Double-stranded RNA; matching of a chain of ribonucleic acid, RNA, by a complementary strand of RNA. In this conformation, an adenine nucleotide present in one strand bonds, or base-pairs, with a uracil nucleotide on the complementary strand, and a guanine nucleotide to a cytosine.

**Gene**: Basic genetic unit; DNA sequence that carries all the information for the functional production of a protein or non-coding RNA.

**Genome**: The entire DNA sequence of an organism.

**microRNA**: Endogenous small RNAs, functionally related to siRNAs, involved in negatively regulating the expression of a large number of genes.  microRNAs can also be targets for new drug discovery.

**mRNA**: Messenger RNA functioning as the template in protein translation. They are generated by the transcription of protein-coding genes.

**Pharmacokinetics**: Study on the absorption, distribution, metabolism, and elimination of a drug in the body over time.

**Protein**: Amino acid chain generated following the translation of an mRNA. Proteins have a central structural, regulatory, and catalytic function in every living cell.

**RiSC**: RNA-induced silencing complex which when guided by an incorporated small RNA cleaves complementary target mRNA during RNAi.

**RNA**: Ribonucleic acid; genetic information consisting of any sequence of ribonucleotides containing bases of adenine (A), uracil (U), guanine (G), and cytosine (C).

**RNAa:** RNA activation, double-stranded RNAs that target promoter regions in chromosomal DNA resulting in transcriptional activation of genes. The transcriptional activation, or up-regulation, of genes results in an increase in mRNA and protein production.

**RNAi:** RNA interference, a natural cellular process where small interfering RNAs or microRNAs are used to control the normal expression of genes or are used to pharmacologically target disease-casuing genes.

**shRNA**: Small 'hairpin' RNA expressed from a DNA template and processed into small RNAs to guide RNAi-mediated target mRNA degradation.

**siRNA**: Small interfering RNA. Optimally, two strands of RNA, each 19-25 nucleotides long with a central region of complementarity for the formation of a dsRNA region and optionally nucleotide overhangs at one or both of the the 3' ends. siRNAs are the molecules that mediate RNAi. They are incorporated into RISC and used to form complementary pairing with a target mRNA, resulting in its enzymatic cleavage.

Canonical siRNA: Two strands of RNA, each 21 nucleotides long with a central region of complementarity that is 19 base-pairs long for the formation of dsRNA and two nucleotide overhands at each of the 3 'ends'.

**Small molecule**: A small chemical, usually drug that due to its small size may be absorbed through the gut. Term often used in contrast to "large" protein therapeutics which have to be injected to bypass the gut.

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Elbashir, S.M., Lendeckel, W. & Tuschl, T. (2001). RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*. **15**: 188-200. **Pair of papers demonstrating that long double-stranded RNAs are processed into small RNAs in fly cell extract and that these guide mRNA cleavage during RNAi.**

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Soutschek, J. et al. (2004). Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* **432**: 173-8. **RNAi in mice following the systemic administration of siRNAs.**

Zimmermann, T.S. et al. (2006). RNAi-mediated gene silencing in non-human primates. *Nature* **441**: 111-4. **RNAi in a non-human primate following the systemic administration of siRNAs.**

## MicroRNA

Lee, R.C., Feinbaum, R.L. & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**: 843-54.

**Discovery of the first miRNA.**

Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. *Science* **26**: 797-9. **MiRNAs represent an abundant class of small RNAs found in most organisms, including humans.**

Kruetzfeld, J., Rajewski, N., Braich, R., Rajeev, K.G., Tuschl, T., Manoharan, M. & Stoffel, M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. *Nature* **438**: 685-9. **Functional miRNA inhibition by antisense technology in an adult mammal.**

## Additional Resources

Introduction to RNAi by PBS Nova:
http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html

Wikipedia online encyclopedia entry on RNAi:
http://en.wikipedia.org/wiki/RNA_interference

Animation of RNAi by Nature:
http://www.nature.com/focus/rnai/animations/index.html

Press release by the Nobel Assembly on the 2006 Nobel Prize in Physiology or Medicine to Andrew Z. Fire and Craig C. Mello for their discovery of RNAi:
http://nobelprize.org/nobel_prizes/medicine/laureates/2006/press.html

Science magazine 2002 Breakthrough of the Year special issue:
http://www.sciencemag.org/content/vol298/issue5602/#special

RNAi.net portal on RNAi:
http://www.rnai.net/