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DRUG DISCOVERY FALL 2013

COMPASS

A Publication Dedicated To Research Updates

Antisense Therapeutic Approach for SMA

The Ins and Outs of Antisense Drugs

ABC's of antisense drugs

Antisense drugs are small snippets of synthetic genetic material that bind to RNA (short for *ribonucleic acid*). The main factor driving where the antisense drug binds to the RNA is the nucleic acid sequence used. Nucleic acids consist of a chain of linked units called nucleotides. Antisense drugs are typically 12-21 nucleotides that are chemically modified to bring about the appropriate drug response. (Figure 1)

How does the antisense approach work for SMA?

SMA is caused by a loss of, or defect in, the survival motor neuron 1 (SMN1) gene. The SMN1 gene produces most of the SMN protein, which is critical to the health and survival of the nerve cells in the spinal cord responsible for muscle function. The severity of SMA is correlated with the amount of SMN protein in the cell. A second closely related back-up gene called SMN2 exists that normally produces a truncated, low-functioning form of SMN protein.

Antisense therapy could be used to change the functioning of the SMN2 gene by utilizing alternative RNA splicing. Alternative splicing is a normal mechanism that the cell uses to produce different, but closely related proteins from a single gene.

How do antisense drugs cause the SMN2 gene to produce more functional SMN protein?

To understand alternative splicing, it's important to understand how DNA creates messenger RNA (mRNA).

DNA carries the genetic information of a cell and consists of thousands of genes. Each gene serves as a recipe on how to build a protein molecule. mRNA is created when the entire DNA strand is copied. This includes the sequences that encode for proteins. and regions that are unnecessary for making proteins. Before mRNA can function, the regions that are unnecessary for making proteins are deleted from the RNA strand.

Figure 1. This figure shows the structure of the DNA double helix through Watson-Crick nucleotide base pairing.
It is comprised of four different types of building blocks called nucleotides. These are designated A, T, C, and G. Note that in DNA, nucleotide adenine (A) in a DNA molecule always pairs with the nucleotide thymine (T), while cytosine (C) always pairs with guanine (G).

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How does the antisense therapeutic approach work for SMA?

- In SMA, the survival motor neuron (SMN) protein level is reduced due to the loss of the SMN1 gene. The low level of this protein causes the motor neuron nerves to not function correctly.
- A second closely related gene called SMN2 – the back-up gene - exists that normally produces a shorter, low-functioning form of the SMN protein.
- Antisense drugs are *small snippets of synthetic genetic material or RNA* that utilize a natural RNA processing mechanism to increase the production of the SMN protein.
- It is called "anti-sense" technology, because the pieces of synthetic material run in a *"mirror-image"* or "reverse" or "anti" order from the natural RNA that it binds to.
- Antisense drugs can be designed to bind to very specific regions of the SMN2 back-up gene to enhance the production of the full length, fully-functioning SMN protein.
- This increase in the amount of normal SMN protein produced by the SMN2 gene should provide the necessary amounts of SMN protein needed for proper muscle function and development.

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The natural process that removes these regions and re-forms the finished mRNA is called "splicing." Through the splicing process, the cell can create many diverse proteins from a single gene and splicing accounts for most of the diversity in proteins in the cell. In fact, of the approximately 25,000 genes in the human genome, approximately 90% have alternative splice forms.

Antisense drugs can be designed to bind specific regions of the RNA and change the splicing of the SMN2 gene to increase the production of functional SMN protein. The antisense drug binds and promotes the inclusion of a specific nucleotide sequence (exon 7), correcting the known pre-mRNA processing defect in splicing in the SMN2 gene. This allows the SMN2 back-up gene to express more full-length SMN protein, rather than a truncated, low-functioning protein.

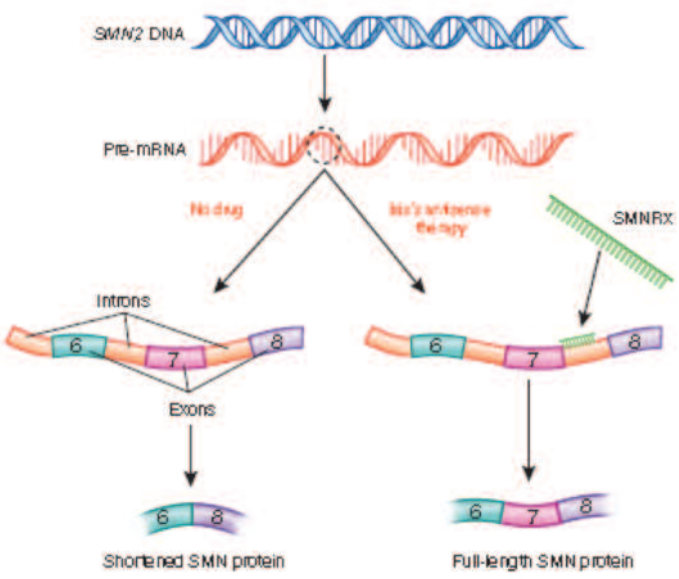

Figure 2. This figure shows splicing of RNA and where the antisense drug changes the splicing to produce full-length SMN protein.

What exactly does the antisense drug do at the molecular level to the SMN2 gene?

Normally SMN2's mRNA undergoes incorrect splicing and encodes for an unstable SMN protein, known as Δ7SMN. The production unstable SMN protein is the result of exon 7 being skipped during the splicing process of the SMN2 pre-mRNA (Figure 2). The clinical result is low amounts of full length SMN protein and ultimately decreased motor function in SMA patients. The use of anti-sense oligonucleotides, targeting sequences in the SMN2 pre-mRNA, can correct the spicing defect and promote exon 7 inclusion, leading to higher SMN protein levels.

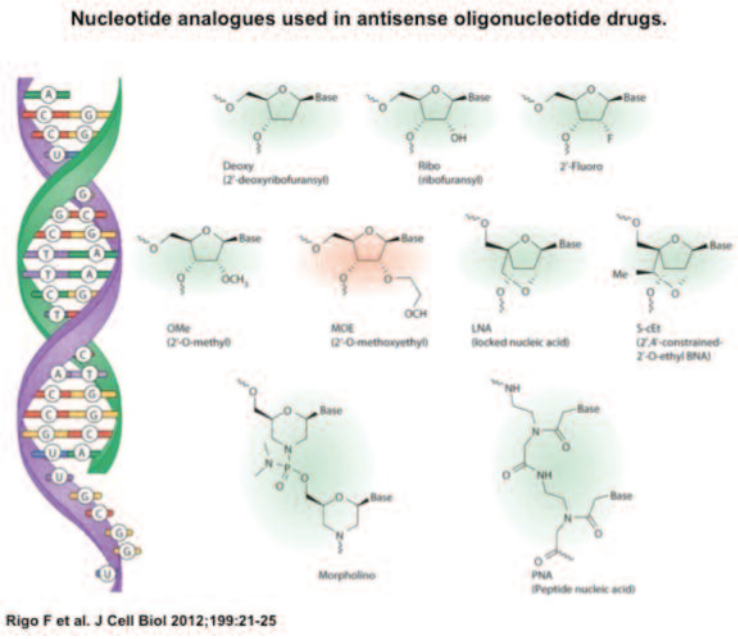

Figure 3. Nucleotide analogues used in antisense oligonucleotide drugs. Structures of various nucleotides or nucleotide analogues commonly used in antisense drugs are shown. The antisense oligonucleotide developed by the ISIS group to improve SMN2 splicing has the 2′-MOE modification (red). The one being used by the Burghes group is called a Morpholino and is seen in the bottom left. From Rigo et al., J Cell Biol. (2012) 199(1):21-5.

Influence of backbone chemistry

A secondary factor, in addition to the nucleic acid sequence, that influences the effectiveness of an antisense drug is the backbone chemistry used to hold the nucleotides together. See Figure 3. The different chemistry can influence

many factors, including: **1**) how the drug distributes throughout the body; **2**) the length of time the drug persists in a given tissue; **3**) the efficiency of drug uptake into cells; **4**) how strongly the drug binds to the target RNA; and **5**) the likelihood of non-specific interactions with other RNAs.

Drugs for SMA need to cross the blood brain barrier

For a treatment for SMA to be effective a drug has to be active in motor neurons and other cell types in the central nervous system (CNS). This will require the drug to be present in cerebrospinal tissues.

Antisense drugs do not cross an intact blood–brain barrier, but there are several approved methods and devices available for delivery of drugs into the cerebrospinal fluid including direct injection into the intrathecal space. Antisense drug injected in this manner distributes broadly into CNS tissues.

Intrathecal delivery refers to the administration of a drug directly into the cerebral spinal fluid (CSF), a fluid that surrounds the brain and spinal cord tissue. The drug is administered through an injection in the lower back into a fluid-filled space below the end of the spinal cord, resulting in broad distribution of the drug in various brain regions and the spinal cord. This is a common procedure in hospitals frequently used for pain relief.

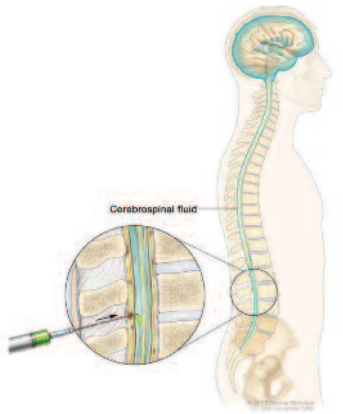

Figure 4. Illustration of intrathecal delivery.

Isis-SMN$_{Rx}$ Program Update on Ongoing and Future Clinical Trials

Infant Program Update: Isis Opens Cohort 2 of the Clinical Study in Infants with SMA

This study is evaluating the investigational compound, ISIS-SMN$_{Rx}$, in infants with Type 1 Spinal Muscular Atrophy (SMA). This Phase 2 study is an open-label, multiple-dose, dose-escalation pilot study, designed to examine the safety and tolerability of ISIS-SMN$_{Rx}$ in infants with SMA and to provide data to define the optimal dose for future studies in infants. To meet enrollment criteria, infants must be between the ages of three weeks and seven months, live in close proximity to a study site and pass additional screening evaluations conducted at the study site. The study is being conducted at 4 centers in the United States and Canada who are looking for eligible subjects -

- Stanford University Medical Center in Stanford, California
- Nemours Children's Hospital in Orlando, Florida
- Columbia University Medical Center in New York, New York
- The Hospital for Sick Children (SickKids) in Toronto, Ontario, Canada

For further study information and contact information for the study sites, please visit www.clinicaltrials.gov and search for ISIS-SMN$_{Rx}$.

Next Steps:

A controlled Phase 3 study in infants is planned to begin early in 2014. This study will be larger than the current ongoing study and will be conducted in the US and internationally.

Child (Type 2 and Type 3) Program Update: Isis Completed Dosing and Enrollment in the Multi-Dose Phase 2 Dose Escalation Study in Children with SMA

This Phase 2 study is a multiple-dose, dose-escalation study designed to examine the safety and tolerability of multiple doses of the drug given over a longer period of time. This study is being conducted in children age 2–15 with Type II and Type III SMA. The study is fully enrolled, has completed dosing, with data expected once the follow up is completed early next year.

Next Steps:

A larger controlled Phase 3 study in children is planned to begin in 2014. This study will be larger than our current ongoing study and will provide more opportunity for enrollment at an increased number of participating sites, including Canada and other international sites.

If you would like further information, please contact your study site or you can contact Isis directly at: Kristina Lemonidis (klemonidis@isisph.com).

Please go to **www.clinicaltrials.gov** for info on participating in ongoing SMA clinical trials.

Families of SMA Awards $150,000 to Dr. Hua in the Laboratory of Dr. Adrian Krainer at CSHL to Assess the Influence of Backbone Chemistry on Antisense Drugs for SMA.

This is the fourth drug discovery project funded by Families of SMA in 2013 with a total investment of $550,000. This program will systematically assess the effect of backbone chemistry on the therapeutic efficacy of Antisense Oligonucleotides (ASO) that target the ISS-N1 region of the SMN2 RNA. ASOs that bind to the ISS-N1 region will be compared. This is the binding region of the drug ISIS-SMN_{Rx} that is currently being tested by Isis Pharmaceuticals in SMA clinical trials. This funding is being awarded to Dr. Yimin Hua in the laboratory of Dr. Adrian Krainer at Cold Spring Harbor Laboratory. Both scientists are long-term collaborators with Isis Pharmaceuticals and actively participated in the pre-clinical development and characterization of the mechanism of action of ISIS-SMN_{Rx}.

Program Description:

Comparison of efficacy mechanisms of MOE and PMO antisense splicing therapy in type I SMA mice.

Objective: The project goal is to compare the ISS-N1 antisense oligonucleotides using two alternate backbone chemistries called MOE and Morpholino (PMO). Please see the first article in this edition of Compass for more information on backbone chemistry of ASOs. Both chemistries have shown great promise in treating SMA by correcting SMN2 splicing. In this project, the team will systematically explore parameters that influence the efficacy of each type of ASO.

Research Strategy: We will use a severe SMA mouse model to compare the therapeutic effects of antisense oligonucleotides with two types of chemical backbones, delivered either to the central nervous system or under the skin. We will then perform detailed pharmacological studies to compare their distribution and efficacy in different tissues and cell types. This project will provide important information for further developing the antisense oligonucleotide drug, ISIS-SMN_{Rx}, an antisense oligonucleotide drug candidate that is currently in clinical trials by Isis Pharmaceuticals. This project may also help understand which tissues or cells are most important in SMA pathogenesis in this mouse model.

"As a Research Investigator in my lab at Cold Spring Harbor Laboratory, and as part of our long-standing collaboration with Isis, Dr. Hua has been doing pioneering work towards the development and characterization of the mode of action of ISIS-SMN_{Rx}. I am grateful for the support from Families of SMA, which will allow Dr. Hua to study key aspects of ASO efficacy in SMA mouse models, with relevance to the clinic," said Dr. Adrian Krainer, Ph.D., Professor at Cold Spring Harbor Laboratory.

"ASO10-27 (ISIS-SMN_{Rx}) and its longer derivatives, which restore SMN expression through correction of SMN2 splicing, hold great promise to treat spinal muscular atrophy. ASOs must be chemically modified to enhance their stability and facilitate their binding to the target RNA. ASOs with different chemical modifications have been tested in various SMA mouse models; two types of modifications, MOE and morpholino, showed striking improvement including long-term survival. However, there were apparent differences among various studies, in terms of optimal ASO dose and sites of delivery. We will conduct a comprehensive side-by-side comparison of MOE and morpholino ASOs in a severe mouse model, and study the mechanisms underlying any observed differences in ASO potency and efficacy," said Yimin Hua, Ph.D., Research Investigator at Cold Spring Harbor Laboratory.

"We have a long term collaboration with Drs. Adrian Krainer and Yimin Hua to identify an antisense drug to treat spinal muscular atrophy. This has been a very productive collaboration resulting in the identification of ISIS-SMN_{Rx}, which is currently in clinical trials. We would be delighted to continue to work with Dr. Hua to investigate the mechanistic differences between the different olignucleotide chemistries. Dr. Hua has a strong track record of successfully completing projects and making important contributions to our understanding of SMA and antisense drugs," said Dr. C. Frank Bennett, Ph.D., Senior Vice President of Research, Isis Pharmaceuticals.

Families of SMA Awards $150,000 to Dr. Burghes and Dr. Lorson to Investigate New Antisense Therapies for Spinal Muscular Atrophy.

Families of SMA is dedicated to creating a treatment and cure for Spinal Muscular Atrophy by funding and advancing a comprehensive research program, including drug discovery programs to make practical new therapies. This new program will assess the therapeutic potential of second-generation Antisense Oligonucleotides (ASO) sequences for SMA. This funding is being awarded to Co-Principal Investigators Dr. Arthur Burghes at Ohio State University and Dr. Christian Lorson at University of Missouri.

Program Description:

New Morpholino Antisense Oligonucleotides for the Treatment of SMA.

Objective: Antisense oligonucleotides (ASOs) targeted to the ISS-N1 region have been shown to be effective in mice and are now being tested in the current clinical trials conducted by Isis Pharmaceuticals. The major goal of this new program is to explore the therapeutic potential of two new sequences, which are located in different regions of the SMN2 gene. Importantly, the possibility exists to use these different ASOs in combination. The ASOs in this project will be generated using a different morpholino backbone chemistry.

Research Strategy: First, the Burghes and Lorson groups will determine the optimal nucleic acid sequence. They will then establish the best dose of the optimized ASOs to provide maximal rescue in the severe Delta7 mouse model of SMA, developed by Dr. Burghes. Knowing this information in mice will help design subsequent studies in primates to assess whether these ASOs reach the correct tissues including motor neurons, when delivered into the cerebral spinal fluid (CSF) in larger animals. Dr. Ravindra Singh at Iowa State University will be collaborating on the project.

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"There are now a series of exciting therapies for induction of SMN from the back-up gene SMN2, with the antisense oligonucleotides being one. In essence, the best way to increase SMN protein from SMN2 remains a critical issue, and this will be investigated in this new project," stated Arthur Burghes Ph.D., Professor at Ohio State University.

"We are very grateful to Families of SMA for funding our ASO project as we hope we can continue to develop therapeutics that target an additional genetic element within SMN2. SMA is a very complex disease and it is possible that more therapeutic options will be required to effectively combat this disease," stated Chris Lorson, Ph.D., Professor at University of Missouri.

A portion of this new research program is being generously funded by Stop SMA. Stop SMA was created to raise awareness and funding for research to treat and end SMA. http://www.stopsma.org

Families of SMA Funding Strategy and the SMA Drug Pipeline

There are now 15 new SMA drug programs in development, including 3 in clinical trials. This pipeline has expanded from just 4 programs 5 years ago. Families of SMA has funded about two thirds of all the ongoing drug programs for SMA. The FSMA research approach funds programs at early stages, and then partners with companies to take them through clinical trials. Supporting multiple programs gives different approaches for a SMA therapy, which increases the chances of success and accelerates the timeline to a treatment and cure. In 2013 alone, FSMA has funded four new drug programs. See our website at www.curesma.org for the 2013 SMA Drug Pipeline.

Updated Data from the Completed Phase I Study Evaluating the Safety of ISIS-SMN$_{Rx}$ in Children with SMA

Isis has completed a Phase 1 study evaluating the safety of ISIS-SMN$_{Rx}$ in children with SMA ranging in age from 2 to 14 years old. In this study, ISIS-SMN$_{Rx}$ was well tolerated at all dose levels tested with no safety or tolerability concerns. The compound is delivered by an injection into the lower back (an 'intrathecal injection') into the space containing cerebral spinal fluid below the spinal cord in order to best distribute the drug to spinal cord motor neurons. The intrathecal injection procedure was also well tolerated in the children. All patients who participated completed the study.

ISIS-SMN$_{Rx}$ drug levels were measured in body fluids in these children (cerebral spinal fluid and blood) and were consistent with levels that were expected. Along with data from animal studies indicating that the drug stays in the nervous system tissue for a long time, this indicates that dosing once every 6 to 12 months is feasible.

The study also showed encouraging early data demonstrating a possible improvement in muscle function in the highest dose group using the Hammersmith Functional Motor Scale-Expanded (HFMSE). Although encouraging, these data are early and these results need to be confirmed in a placebo controlled study.

"SMA represents a serious unmet medical need with no currently available treatments. Based on its mechanism of action and encouraging preclinical and clinical data, ISIS-SMN$_{Rx}$ could be an effective treatment for these very sick children, though additional work still needs to be done.

The rapid advancement of this drug to this stage in development reflects the support from the SMA community and the success of the collaboration between Isis and Biogen Idec. Isis and Biogen Idec are committed to advancing the program for children with SMA," said C. Frank Bennett, Ph.D., senior vice president of research at Isis. **"ISIS-SMN$_{Rx}$ is our first drug designed to intervene in the splicing of RNA to increase the production of a normal protein, SMN. Antisense drugs could offer novel new therapeutics for a number of severe neurodegenerative diseases. The encouraging safety data from this program and our preclinical and clinical experience in other neurodegenerative diseases support the broadening of our efforts to develop antisense drugs to treat such diseases."**

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