ISIS PHARMACEUTICALS AND COLLABORATORS AT THE 7TH ANNUAL MEETING OF THE OLIGONUCLEOTIDE THERAPEUTICS SOCIETY
SEPTEMBER 8-10, 2011
COPENHAGEN, DENMARK

Session Type	Presentation
Session Title	Session 1: Hot Topics: Short Talks Selected from Abstracts
Date	**Thursday, September 8, 2011**
Time	**9:30 a.m. – 9:45 a.m. CEST**
Presentation	**ACTIVATION OF RNA INTERFERANCE IN ANIMALS WITH SINGLE STRANDED OLIGONUCLEOTIDES**

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Abstract

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The use of double stranded oligonucleotides to inhibit gene expression via the RNA interference (RNAi) pathway has generally required a complex (usually lipid based) formulation for delivery of the oligonucleotide to the desired compartment of a cell. Unfortunately, this requirement has extended from cell culture to animals, which severely limits the potential of harnessing the RNAi pathway for therapeutic approaches. Unlike double stranded oligonucleotides, single stranded antisense oligonucleotides have shown activity in multiple species (including humans) without formulation vehicles. Furthermore, the double stranded RNA (dsRNA) structure is not required for RNAi, as single stranded RNA (ssRNA) delivered to cells using cationic lipids has been shown to activate the RNAi pathway. \This suggested to us that the dsRNA structure could be simplified to a single stranded oligonucleotide that would activate RNAi in cells and in animals, and provide significant benefits over double stranded structures for the potential development of human therapeutic agents.

To achieve this objective, we have engaged in an extensive chemical structure activity relationship (SAR) study of ssRNAs, and coupled this to biochemical studies on the mechanism of activation of the RNA induced silencing complex (RISC). This SAR has led to an understanding of key structural features required for a ssRNA activity, and allowed us to achieve potency within 5-fold of the corresponding double stranded structures in cells with multiple fully modified, partially phosphorothioated ssRNA oligonucleotide designs. Our initial attempts to show activity in animals with these early designs failed due to pharmacokinetic limitations. These limitations were overcome with further chemical stabilization achieved via an *in vivo* SAR optimization cycle. Sequence optimization of the optimal chemical motif provided highly potent compounds in cell culture which

were shown to function via an argonaute-2 dependent mechanism. These ssRNAs are active in cell culture without cationic lipids, and this activity translated to activity in animals at pharmacologically relevant doses with subcutaneous administration in saline formulations. These studies provide a framework for further optimization of the ssRNA structure for the potential development of human therapeutic agents.

Session Type	Poster Presentation
Session Title	Attended Poster Session
Date	**Thursday, September 8, 2011**
Time	**1:15 p.m. – 2:00 p.m. CEST**
Abstract Number	**007**
Abstract	

A UNIQUE MOE-DNA CHIMERIC OLIGONUCLEOTIDE INDUCES MDA-5 DEPENDENT INDUCTION OF TYPE I INTERFERON RESPONSE

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Second generation 'Gapmer' antisense oligonucleotides (ASO) containing 2'-O-methoxyethylribose (MOE) modifications have been shown to possess both excellent pharmacokinetic properties and robust pharmacological activity in several animal models of human disease. These beneficial properties have translated to human therapeutics. Gapmer ASOs are generally well tolerated displaying minimal to mild proinflammatory effect caused by the release of cytokines via the activation of monocyte and or dendritic cells compared to phosphorophioate deoxy oligonucleotides at doses far in excess of expected therapeutic doses. While the vast majority of 2'-MOE ASOs are largely void of hepatotoxicity as characterized by the absence of serum transaminases increase, a very small subset of ASO with a propensity for producing acute hepatotoxicity in mice has been identified. The mechanism for these findings is not clear at this point, but the effects are clearly sequence-specific. Intense effort has been made to understand the mechanism underlying these effects.

One of those ASO, ISIS 147420, was found to cause profound hepatotoxicity characterized by increased ALT that was atypical of this class of oligonucleotides. In addition to increased ALT, subcutaneous injection of ISIS 147420 was associated with extensive hepatocyte apoptosis and necrosis, as well as mononuclear cell infiltrate in liver at 72 hours. Liver morphology and ALT levels were normal at 24 or 48 hours. Whole genome gene expression profiling was performed on livers collected at 8, 24, 48 and 72 hours. A large number of interferon stimulated genes (ISGs) were significantly upregulated as early as 24 hours. Administration of ISIS 147420 to Stat1 or IFNAR1 deficient mice showed no evidence of hepatotoxicity and no induction of ISGs up to 96 hours post treatment. We speculated that a specific sequence motif might cause ISIS 147420 to be mistaken for viral RNA or DNA thus triggering an innate immune response ultimately resulting in severe hepatotoxicity. ISIS 147420 toxicity was independent of Toll-like receptors as there was no decrease in ALT in TRIF or Myd88 deficient mice. The involvement of the

cytosolic pattern recognition receptors, RIG-I and MDA-5, were also investigated. Pretreatment of mice with ASOs inhibitors of IPS-1, an adaptor protein critical to the function of RIG-I and MDA-5, reduced mRNA level down to 8% of control and prevented the toxicity induced by ISIS 147420 (reduced ALT and interferon-b levels). Unlike ASO pretreatment with RIG-I inhibitors that was unable to reduce the severity of hepatotoxicity, pretreatment with MDA-5 antisense inhibitors reduced MDA-5 mRNA level down to 7% of control and prevented the toxicity induced by ISIS 147420 (reduced ALT and interferon-b levels). These results revealed a novel mechanism of oligonucleotide mediated toxicity requiring both MDA-5 and IPS-1 and resulting in an adverse activation of the innate immune response.

Session Type Poster Presentation
Session Title Attended Poster Session
Date **Thursday, September 8, 2011**
Time **1:15 p.m. – 2:00 p.m. CEST**
Abstract Number **055**
Abstract

ANTISENSE APPROACHES TO STUDY REST/NRSF TRANSCRIPTIONAL REGULATION AS A THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISORDERS

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The repressor element-1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) is a master regulator of neuronal gene expression. REST functions through binding the genomic loci that contain the repressor element-1 (RE1) binding motif and recruits a series of epigenetic and regulatory cofactors to its N- and C-terminal domains. Various studies have characterized the specific roles played by REST during neuronal lineage specification and maturation. Among the REST targets BDNF has emerged as a candidate of interest as a potential neuroprotective and functionally restorative treatment in psychiatric and neurological disorders, including Huntington's disease (HD). In HD, BDNF levels are significantly attenuated as a result of impaired BDNF transport from the cortex to the striatum in the presence of mutant HTT. Wild-type HTT also regulates transcription of BDNF through REST, by sequestering REST in the cytoplasm, therefore restricting its access to the nucleus, which leads to the transcription of target genes such as BDNF.

In this study, we were interested in down-regulation of REST as a potential therapeutic strategy to both slow down cell death and maintain the functional state of remaining neurons. This could be possible through the neuroprotective and functional effects of BDNF by activation of signaling in neuronal pro-survival pathways. In order to specifically target REST, we applied 2nd generation Antisense Oligonucleotides (ASOs) to reduce levels of REST mRNA in the liver and the CNS in both normal (wild type) and HD mice and characterized the effects on BDNF expression and gene expression more globally, as determined by microarray analysis.

We have demonstrated specific reduction in REST levels in both liver and CNS

following treatment by ASOs, which resulted in the induction of a number of neuronal genes including BDNF and Synapsin1 at the mRNA and protein levels. Gene array expression analysis was performed on BALB/c mouse liver and R6/2 brain, a mouse model of HD, following systemic and ICV administration of REST ASO, respectively. Samples were analyzed by hybridization to the MouseWG-6 v2 Expression BeadChip array (Illumina), to gain insight into putative pathways affected by REST suppression. Array data analysis was performed using GeneSpring, followed by Gene network prediction using Ingenuity™ Pathway Analysis (IPA). 416 genes were found to be up regulated and 119 genes down regulated following REST ASO treatment. Numerous novel genes were identified that were affected by REST suppression that are predicted to play a role in cancer, genetic disorders, neurological diseases, cell-to-cell signaling, and tissue development.

Our findings suggest that REST may be an important target for neurodegenerative diseases like HD, that it is also involved in the regulation of a broad range of cellular pathways outside of the CNS, and that the antisense approach is a viable strategy for selectively modulating REST activity in both the CNS and in the periphery.

Session Type	Presentation
Session Title	Session 6: Targeting Coding RNA
Date	**Friday, September 9, 2011**
Time	**11:00 a.m. – 11:30 a.m. CEST**
Presentation	**MODULATION OF GENE EXPRESSION BY OLIGONUCLEOTIDE CHEMISTRY-DEPENDENT RECRUITEMENT OF PROTEINS TO RNA TRANSCRIPTS**
	Frank Rigo, Ph.D.
	Isis Pharmaceuticals, Carlsbad, CA, USA

Session Type	Presentation
Session Title	Session 6: Targeting Coding RNA
Date	**Friday, September 9, 2011**
Time	**11:30 a.m. – 12:00 p.m. CEST**
Presentation	**CLINICAL DEVELOPMENT OF MIPOMERSEN**
	Erik Stroes, M.D., Ph.D.
	AMC Research Institute, Amsterdam

Session Type	Presentation
Session Title	Session 7 : Targeting microRNA
Date	**Friday, September 9, 2011**
Time	**1:30 p.m. – 2:00 p.m. CEST**
Presentation	**THERAPEUTIC TARGETING OF MICRORNAS**

Neil Gibson, Ph.D.
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Abstract

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microRNAs act as master regulators in biological pathways, and are dysregulated in disease areas including cancer, metabolism, fibrosis, and inflammation. Their ability to modulate disease pathways makes targeting microRNAs an exciting new approach for drug discovery. Oligonucleotides that inhibit microRNA function have been termed anti-miRs. Critical to the development of anti-miRs as a therapeutic modality are chemical modifications to enhance stability and target affinity, and an understanding of functional biodistribution of anti-miRs to cells and tissues of therapeutic interest. Systemic and local delivery of unformulated antimiRs enables broad distribution for targeting microRNA function in a diverse range of tissues and cell types. We present recent advances in our use of anti-miRs against two specific targets – miR-33 in metabolic disease and miR-21 in oncology.

miR-33a and b are intronic microRNAs located within the SREBF2 and SREBF1 genes, respectively. This microRNA family suppresses the expression of the ABCA1 cholesterol transporter and lowers HDL levels. LDL receptor-deficient mice treated with anti-miR-33 showed an increase in circulating HDL levels as well as enhanced reverse cholesterol transport to the plasma, liver, and feces. The anti-miR-33-treated mice had reduced plaque size and lipid content, increased markers of plaque stability, and decreased inflammatory gene expression. The systemic delivery of an antisense oligonucleotide that targets both miR-33a and miR-33b increases hepatic expression of ABCA1 and induces a sustained increase in plasma HDL cholesterol in African green monkeys. These data suggest the modulation of microRNA function as a promising strategy to treat atherosclerotic vascular disease.

miR-21 is frequently over-expressed and has been shown to correlate with poor outcome in multiple cancer types. We have used a publically available data set from 86 patients to show that miR-21 is over-expressed in hepatocellular carcinoma (HCC). Short term treatment with the anti-miR-21 oligonucleotide in a genetically engineered mouse model of HCC led to a reduction in tumor formation and an increased survival advantage. Furthermore, inhibition of miR-21 was clearly demonstrated by analysis of genome wide mRNA expression data from treated versus untreated tumors. Our findings suggest that miR-21 is a promising candidate for the therapeutic intervention of liver cancer and further highlights the potential of antimiR-mediated inhibition of microRNAs in cancer.

Overall our data suggest the therapeutic utility of anti-miRs targeting microRNAs involved in human disease pathogenesis.

Session Type	Presentation
Session Title	Session 10: Targeting the Central Nervous System
Date	**Saturday, September 10, 2011**
Time	**9:45 a.m. – 10:15 a.m. CEST**
Abstract	**SYSTEMATIC VERSUS CNS DELIVERY OF MOE ANTISENSE OLIGONUCLEOTIDE TO CORRECT DEFECTIVE SPLICING IN A SEVERE MOUSE MUSCULAR ATROPHY**

Adrian Krainer, Ph.D.
Cold Spring Harbor Laboratory