

## Session Summaries

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### Introduction

*Scientific Organizing Chair: Brett P. Monia, PhD, Isis Pharmaceuticals*

I am pleased to report on the 9<sup>th</sup> Annual Meeting of the Oligonucleotide Therapeutics Society, which was held recently in Naples, Italy. Staged on the beautiful backdrop of the Bay of Naples, this year's conference proved to be an outstanding forum of scientific exchange and discussion on cutting edge basic research and therapeutic applications in the world of oligonucleotide science. This three day meeting, which included two Keynote Presentations, 50 oral talks, and more than 130 poster presentations, broadly covered the major aspects of the oligonucleotide field, including basic mechanisms underlying RNA function, the role of noncoding RNAs in normal and disease processes, mechanisms of action underlying aptamers and immunomodulatory oligonucleotides, RNAi, splice switching, RNaseH, and oligonucleotide medicinal chemistry, and of course therapeutic applications across preclinical studies and Phase III clinical trials. All of this culminating in a most enjoyable and fun post-meeting social event involving Italian wine tasting and music! This year's meeting was another huge success for the OTS, thanks of course to the quality of our Scientific Organizing Committee, our session chairpersons, our speakers and poster presenters, our attendees and our sponsors. Following is a short summary of the oral scientific sessions from this year's meeting. I hope you enjoy!

Arrivederci Napoli!

### Day One – Sunday, October 6, 2013

#### Session I: Aptamers: Mechanisms & Applications

*Chair: Vittorio de Franciscis, PhD, National Council of Research, IEOS*

**Nebojsa Janjic**, Science officer at SomaLogic in Boulder Colorado, described the theoretical basis for the design of synthetic nucleotide modifications that confer protein-like diversity on a nucleic acid scaffold, resulting in a new generation of binding reagents called SOMAmers (Slow Off-rate Modified Aptamers). At SomaLogic, the crystal structure of SOMAmer-protein pairs was determined revealing the unique binding properties of these nucleic acids as compared to traditional aptamers. The crystal structure of a SOMAmer bound to its target, platelet-derived growth factor B (PDGF-BB) has shown that, in addition to known shape complementarity usually observed in protein- aptamer interactions, interactions include hydrophobic contacts that mimics the interface between PDGF-BB and its receptor. The modified nucleotides expands the structural possibilities of nucleic acid ligands and open new possibilities for the development of this emerging new class of ligands.

**Sven Klussmann**, Science officer at Noxxon Berlin described the advantage to synthesize non-natural (mirror-image) aptamers to target proteins involved in different pathological diseases. These aptamers, named Spiegelmers consist of nucleotides in L-configuration that renders the molecule very stable. At Noxxon Klussmann employed this approach to generate spiegelmer ligands to the chemokine monocyte chemoattractant protein-1 (MCP-1, CCL2), a protein involved in several inflammatory diseases. The best Spiegelmer molecule, named NOX-E36, showed excellent pharmacokinetic and pharmacodynamic properties, and efficiently inhibits the target MCP-1. In phase 1, NOX-E36 showed an excellent safety profile and it is currently being assessed in a double-blind phase 2a study.

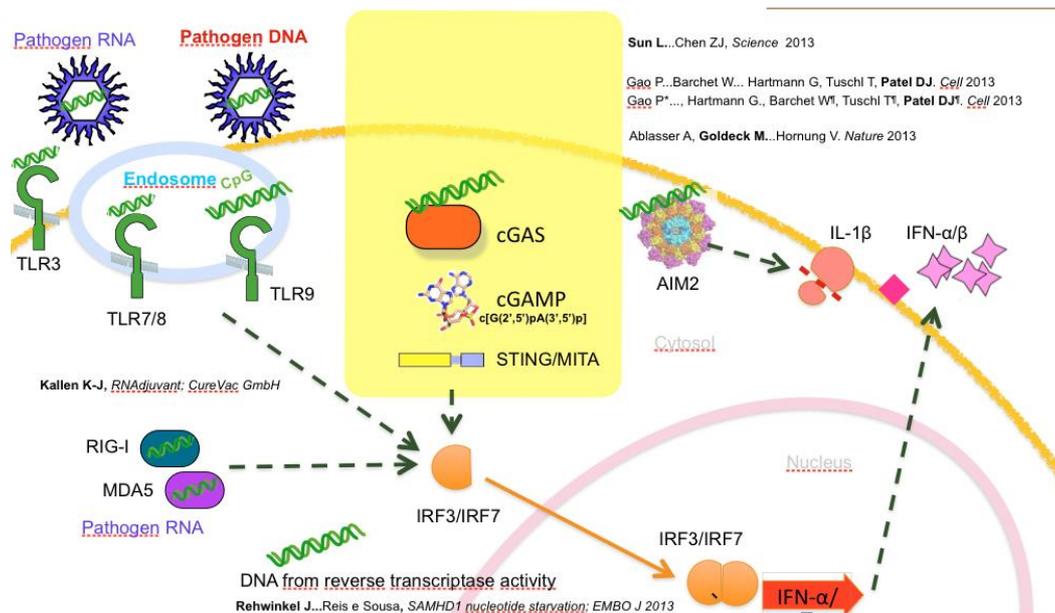
**Bruce Sullenger** from the Duke University reported on the evaluation of aptamers targeting various coagulation factors to act synergistically as well as synergize with small molecule anticoagulant agents. A phase 3 clinical trial of factor a IXa RNA aptamer open to has been initiated which offers new hope for the control of thrombosis by targeting coagulation factors and platelet proteins

**Abdullah Ozer**, from the Cornell University described a new scheme, RNA Aptamer isolation. The Dual-cycles SELEX (RAPID-SELEX or RAPID for short), which combines the efficiency of a previously described, Non-SELEX, with the robustness of conventional SELEX and provides a generalized approach for accelerating the rate of aptamer selections. Further, Ozer reported that multiplex selections of aptamers for various targets, if combined to HTS, identifies specifically enriched aptamer clusters for each given target with a procedure that takes only a third of the time required for conventional SELEX.

## Session II: Immunomodulatory Oligonucleotides

Chair: Gunther Hartmann, MD, PhD, University Hospital Bonn, Germany

A short introduction to the topic was given by Gunther Hartmann from the University of Bonn, the chair of the session. He pointed out that in 2013 there was tremendous progress in the specific understanding of the molecular mechanism of immune sensing of DNA in the cytoplasm of cells (see introductory slide). He introduced the talks of Lijun Sun, Dinshaw Patel and Marion Goldeck to provide novel insights to the recently discovered and characterized cGAS-STING pathway. He continued that in this session, Jan Rehwinkel will contribute new information about a closely related topic on, the DNA metabolism in the cytosol via SAMHD1, and that Karl-Josef Kallen will talk about the use of a proprietary RNA from CureVac used as vaccine adjuvants. Gunther Hartmann highlighted that specifically the new DNA sensing pathway is highly relevant for the application of different forms of DNA oligonucleotides in the field, and contributes to the understanding of so far undefined immunological properties of DNA oligonucleotides which may substantially advance the application of DNA for therapeutic purposes.



### Introductory Slide (with permission of Winfried Barchet, University of Bonn, Germany):

Innate immune sensing receptors involved in the recognition of nucleic acids include endosomal Toll-like receptors (TLR, TLR3, TLR7/8, TLR9), cytoplasmic helicases RIG-I and MDA5, cytoplasmic AIM2 of the inflammasome pathway and the novel cytoplasmic

DNA sensing pathway of cGAS. The cGAS generates cGAMP from GTP and ATP upon binding of dsDNA which binds to STING resulting in type I IFN production.

**Lijun Sun** (UT Southwestern Medical Center): He reported that the presence of pathogen-derived or self DNA in the cytoplasm of cells is detected by the DNA sensor cyclic-GMP-AMP (cGAMP) synthase (cGAS), which binds to dsDNA, and subsequently catalyzes the production of cGAMP. The cGAMP in turn represents a second messenger that induces type I IFN and other inflammatory responses via the signaling molecule STING. Endogenous cGAMP in mammalian cells contains a phosphodiester linkage between 2'-OH of GMP and 5'-phosphate of AMP, and a second linkage between 3'-OH of AMP and 5'-phosphate of GMP. Sun presented data showing that the DNA sensor cGAS is an innate immune sensor of HIV and other retroviruses. Furthermore he demonstrated that cells from cGAS-deficient mice lack activation upon DNA transfection or DNA virus infection. He continued by demonstrating that cGAMP is an adjuvant, boosting antigen-specific T cell activation and antibody production in mice.

**Dinshaw J. Patel** (Memorial Sloan-Kettering Cancer Center) presented studies that combined structural, chemical, biochemical, and cellular assays to demonstrate that the second messenger cGAMP which is produced by cGAS upon dsDNA binding contains G(2',5')pA and A(3',5')pG phosphodiester linkages. With these results the group and collaborators were the first to provide evidence of the existence of 2',5'-containing cyclic heterodinucleotide second messengers in the metazoan system which are distinct from bacterial 3',5' cyclic dinucleotides. He continued to demonstrate that both human and mouse STING adopt a closed conformation upon binding of c[G(2',5')pA(3',5')p], while the antiviral agent DMXAA only adopts a closed conformation in mouse STING. Specifically in human STING, 2',5'-linkage-containing cGAMP isomers were more specific triggers of the IFN pathway compared to the all-3',5'-linkage isomer. A unique point mutation was identified (S162A) within the cyclic-dinucleotide-binding site of human STING that rendered it sensitive to the otherwise mouse-specific drug DMXAA.

**Jan Rehwinkel** (University of Oxford): Jan presented a project on SAMHD1 which is a triphosphohydrolase that depletes the cellular pool of deoxynucleoside triphosphates. In the literature SAMHD1 is thought to prevent reverse transcription of retroviral genomes, but there is no formal proof in vivo. The Rehwinkel group generated SAMHD1 null mice that display a type I interferon signature but do not develop autoimmune disease. Although SAMHD1 knockout cells show increased deoxynucleoside triphosphate (dNTP) levels, the group found that infection with HIV-1 vectors was not enhanced. However, a HIV-1 vector mutant with a reverse transcriptase that has a lower affinity for dNTPs was sensitive to SAMHD1-dependent restriction in cultured cells and in mice in vivo. He argued that these data support the concept that SAMHD1 indeed restrict lentiviruses in vivo and that nucleotide starvation is an evolutionarily conserved antiviral mechanism. He pointed to the possibility that the generation of endogenous retroviral DNA in the cytoplasm may also be restricted by SAMHD1, and a defect in SAMHD1 as seen in tumor cells may trigger activity of endogenous retroviruses leading to cGAS activation.

**Karl-Josef Kallen** (CureVac GmbH) presented results showing that a proprietary RNA molecule of undisclosed structure (RNAdjuvant) has adjuvant activity and improved the antigen specific response to an approved influenza vaccine and to HPV-derived recombinant peptides. He claimed that the stimulatory mechanism of this RNA is independent from established recognition mechanisms such as TLR or RIG-I-like helicases, but no data were shown to support this notion.

**Marion Goldeck** (University Hospital Bonn) presented studies in which the group used the combination of mass spectrometry, enzymatic digestion, NMR analysis and chemical synthesis to demonstrate that cGAS produces a cyclic GMP-AMP dinucleotide containing a 2'-5' and a 3'-5' phosphodiester linkage. The data shown confirm the conclusions drawn from structural and functional results of Patel and coworkers.

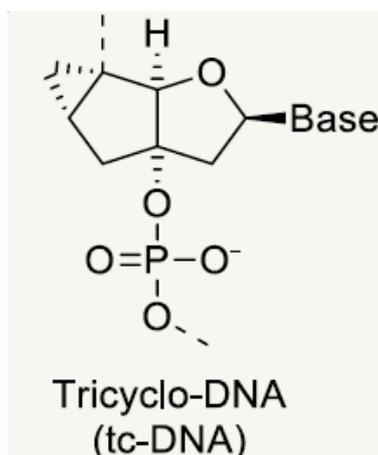
### Session III: Promoting RNA Degradation I

Chair: Muthiah Manoharan, PhD, Alnylam Pharmaceuticals

**David Corey**, UT Southwestern Medical Center, “RNAi in Cell Nuclei”

In this provocative talk, Professor Corey presented evidence for RNAi mechanism and machinery in cell nuclei using a combination of techniques including cellular and biochemical fractionation and microscopy. In the mammalian cell nucleus, RNAi machinery functions similar to cytoplasmic RNA-induced silencing complex (RISC) and induce specific cleavage of target nuclear RNAs. RNAi can influence transcription and splicing using this nuclear activity. Examples of therapeutic applications of nuclear RNAi based on transcription and splicing modulation were presented.

**Christian Leumann**, University of Bern, “The Therapeutic Potential of Tricyclo DNA”



Professor Leumann described Tricyclo DNA (tcDNA), which is a conformationally constrained nucleic acid analog exhibiting high binding affinity for RNA with exceptional biostability. Applications of this chemistry in RNAi, ASOs and splice-altering technologies were summarized. As siRNAs, better tolerance in the sense strand with tcDNA and improved antisense strand activity when placed opposite to the cleavage site of mRNA due to unique structural features were observed. As an antisense oligonucleotide construct, in a 5-10-5 gapmer P=S ASO, similar in vivo activity to a 2'-O-methoxyethyl MOE gapmer for the SR-B1 gene, but improved extra-hepatic distribution than MOE gapmers was observed. As Splice altering oligonucleotides: for DMD (exon 23 skipping) and SMN2 (exon 7 inclusion), sc administration, rescued phenotype of mice with 5-10% gain of activity in brain. Evidence for a better safety profile than 2'-OMe/P=S and PMOs at 200 mpk in rodents was also presented. Finally, this tricyclic chemistry is being extended to synthesize RNA analogs (tcRNA).

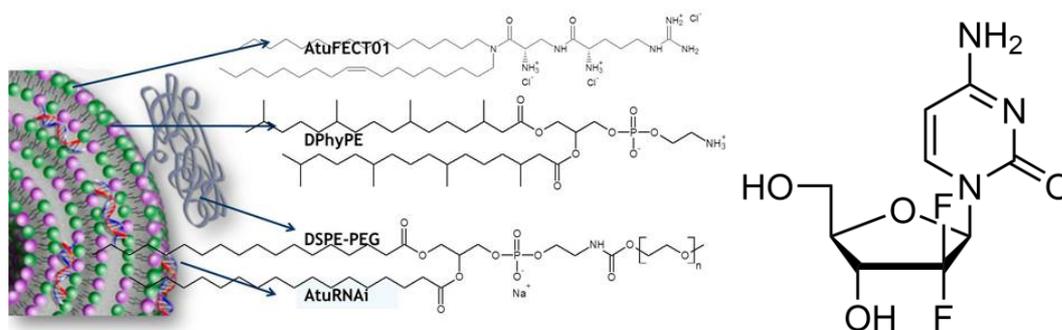
**Qinghua Liu**, UT-Southwestern Medical Center “Assembly of the Catalytic Engine of RNAi, a RISCy Business”

Professor Liu described the biophysical and biochemical studies of the RNA-induced silencing complex (RISC), the catalytic engine of RNAi, wherein the endoribonuclease argonaute and single-stranded siRNA direct target mRNA cleavage. He described reconstituted long dsRNA- and duplex siRNA initiated RISC activities using recombinant *Drosophila* Dicer-2, R2D2 and Ago2 proteins. Employing this core reconstitution system to purify an RNAi regulator-component 3 promoter of RISC (C3PO), a complex of Translin and Trax was identified. C3PO is a Mg<sup>2+</sup>-dependent endoribonuclease that promotes RISC activation by removing siRNA passenger strand cleavage products. Both RNA-binding and catalytic residues are facing the inside of the C3PO barrel, suggesting C3PO binds and cleaves RNA at its interior surface.

**Jessica Cohen**, University of Massachusetts Medical School  
“Peptide-Conjugated Glucan Particles for Delivery of Therapeutic siRNA”

Jessica Cohen et al. reported the development of a platform for the delivery of small interfering RNA (siRNA) to phagocytic macrophages and dendritic cells in vivo. The novel siRNA delivery system is denoted as glucan-encapsulated RNAi particles (or GeRPs) and is based on hollow microspheres composed primarily of  $\beta$ -1,3-D-glucan derived from Baker's yeast. They demonstrated that the GeRPs are able to mediate gene silencing specifically in macrophages, both in vitro and in vivo, in lean or obese mice. In addition, Cohen et al. simplified the GeRP delivery system by covalently attaching weakly basic peptides to the glucan shell. The new peptide-modified GeRPs can efficiently deliver siRNA to macrophages in healthy mice upon intraperitoneal injection. They also demonstrated that the modified GeRPs can reduce expression of inflammatory cytokines in a model of inflammation in adipose tissue--obesity. These data obtained with this simplified GeRP formulation suggests a useful technology for the delivery of therapeutic siRNAs for alleviation of inflammation in a variety of disease indications.

**Klaus Giese**, Silence Therapeutics, “Atu027 Plus Gemcitabine in Advanced or Metastatic Pancreatic Cancer (Atu027-I-02)”



Dr. Geise discussed advances in targeting the PKN3 gene, using an siRNA delivered by their lipid platform along with the standard chemotherapeutic gemcitabine. PKN3 is a downstream effector of PI-3 kinase signaling in endothelial cells. A summary of Phase I results and plans for the Phase II studies, which has been initiated covering first-line patients with advanced or metastatic pancreatic cancer treated with Atu027 (left) in combination with standard chemotherapeutic gemcitabine (the structure on right) were presented.

### **Keynote Presentation I** **Sequence-defined Carriers for Targeted Nucleic Acid Delivery**

Ernst Wagner, PhD, Ludwig-Maximilians University (LMU) of Munich

*Moderator: Mike Gait, PhD, MRC Laboratory*

**Ernst Wagner's** lab in Munich, Germany, specializes in synthetic construction of defined polymeric carriers for nucleic acids (such as siRNA and plasmid DNA). He described the principles of construction of bio-responsive polymers based on solid phase synthesis using building blocks of diaminoethane, branched Lys residues and amino acids such as His, Arg and Lys to create proton sponges and structures of different character for different nucleic acid payloads. He showed how these sequence- and structure-defined polymers were much better than undefined polyethyleneimine and similar commercial polymeric complexing agents both for cellular and in vivo delivery, to help the controlled uptake and release into cells. Many recent publications from his lab were outlined demonstrating the utility of various polymers, including addition of cell targeting peptides (eg to the hepatocyte growth factor

receptor and the folate receptor) to give good targeted gene silencing using siRNA in tumours.

## Day Two – Monday, October 7, 2013

### Session IV: Promoting RNA Degradation II

*Chair: David Corey, PhD, UT Southwestern Medical Center*

The first speaker of Session 4 was **Dr. Muthiah Manoharan**. Dr. Manoharan described progress at Alnylam Pharmaceutical on using GalNAc-siRNA conjugates as a delivery platform. These conjugates achieve potent uptake without the need for nanoparticle formulation and will form the basis of future liver-targeted clinical programs at Alnylam. The next speaker was **Dr. Beverly Davidson**. Huntington's disease is a devastating neurological disorder and is caused by expression of mutant huntingtin protein. Dr. Davidson described viral approaches to inhibiting expression of huntingtin, using non allele-selectivity and allele-selectivity approaches, and showed promising outcomes in animal studies.

The final talk was from **Dr. Jyoti Chattopadhyaya** (University of Uppsala). He described the intriguing hypothesis that chemical modifications could affect water organization at a relatively long distance from the modification. Such modification might affect enzyme activity in unexpected ways and provide a new rationale for optimizing activity.

**Brian Johnston** (SomaGenics) described the identification of short synthetic hairpin RNAs to block Hepatitis C replication in mice. Anti-HCV activity in mice was observed at doses as low as 0.5 mg/kg and compounds were well tolerated. Resistance was generated, demonstrating an on-target in vivo effect and that combinations of shRNAs would be most effective clinically.

**Peter Hagedorn** (Santaris) concluded the session by reporting on studies that aim to improve the ability to predict toxicity. He used a database of results from 206 locked nucleic acid (LNA) oligomers in mice. He found that single-base changes can affect toxicity, and that the occurrence of some dinucleotides correlates with hepatic effects. In a validation set, the toxicity of 17 out of 23 LNAs was predicted correctly. This result demonstrates the usefulness of the model, but also that the complexity of oligonucleotide sequence makes perfect predictions difficult.

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### Session V: Long Noncoding RNAs

*Chair: Shuling Guo, PhD, Isis Pharmaceuticals*

**Claes Wahlestedt** from University of Miami opened the session with an extensive overview of the long noncoding RNA field. He walked the audience through the understanding of the transcriptional landscape from the original central dogma to the revelation by the FANTOM and ENCODE projects that the majority of the genome is transcribed and most of the transcripts are noncoding RNAs. After describing the different RNA species, he focused on cis-acting antisense transcripts, including BDNF-AS which suppresses BDNF expression. Reducing BDNF-AS up-regulates BDNF expression, alters chromatin marks at the BDNF locus, and induces neuronal growth and differentiation in vitro and in vivo. Thus modulating antisense transcripts provides a novel method for locus-specific up-regulation of protein-coding gene expression.

**Kevin Morris** from the Scripps Research Institute presented new findings from his lab on HIV-encoded antisense long non-coding RNA. This HIV derived antisense lncRNA functions as an epigenetic brake to modulate viral transcription. Suppression of this antisense lncRNA results in activation of viral expression, correlating with a loss of silent state epigenetic marks at the viral promoter. These data provide insight into one of the mechanisms by which HIV regulates its own gene expression and potentially enters into a state of latency.

**Amanda Ward** from Isis Pharmaceuticals presented her research on targeting Ube3a-ATS for the treatment of Angelman Syndrome. By reducing the antisense transcript of Ube3a (Ube3a-ATS) with antisense oligonucleotides (ASOs), she was able to activate paternal Ube3a expression, which could compensate for the function of the deficient maternal copy. She also demonstrated that Ube3a-ATS likely represses Ube3a expression via a transcriptional interference mechanism. Human-specific ASOs for reducing Ube3a-ATS are being identified for treating Angelman Syndrome.

**Art Krieg** from RaNA Therapeutics discussed selective upregulation of gene expression by blocking specific PRC2-lncRNA interactions. Using single strand antagonist oligonucleotides, KLF1 could be up-regulated by 50 fold. He also demonstrated up-regulation of SMN2 using a similar method. Interestingly, the same oligonucleotide also induces exon 7 inclusion which would allow for the translation of the full-length SMN protein. The mechanism of this simultaneous up-regulation and splicing modulation is being investigated. These technologies are used to develop new treatments for rare genetic disorders, oncology, metabolic and neurodegenerative diseases.

**David Frendewey** from Regeneron Pharmaceuticals shared his experience in generation of knockout mouse models for 20 lincRNAs using LacZ reporter knock-in strategy. These knockout mice exhibit a wide spectrum of tissue specific expression profiles in adults and unique developmental spatiotemporal patterns in embryos. After breeding to homozygosity for 18 lines, 2 showed perinatal lethality and another 4 showed discernible phenotypes, including skeletal morphologies. LincRNAs in this pilot study includes Linc-p21, linc-MKLN1, HOTAIR, and HOTTIP.

**John Burnett** from the Beckman Research Institute at the City of Hope presented his research on the role of lncRNA in small RNA-triggered gene activation. He established a cell system where a single copy of a self-inactivating lentiviral vector that encodes the CMV promoter and EGFP reporter was integrated. Using this model, he observed transcription of lncRNA in the antisense direction of the CMV promoter and EGFP sequence. Several small RNAs that target this lncRNA can up-regulate EGFP expression via transcriptional gene activation (TGA) mechanism. This regulation is cis-acting and orientation dependent, which may involve small RNA-Ago2 interactions and chromatin modifying enzymes.

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## **Keynote Presentation II**

### **Micro RNAs and Cancer**

**Carlo Croce, MD, The Ohio State University Comprehensive Cancer Center**

*Moderator: Vittorio de Franciscis, PhD, National Council of Research, IEOS*

**Carlo Croce** is Professor and Chair of the Department of Molecular Virology, Immunology and Medical Genetics at the Ohio State University. While at Jefferson University, he discovered the role of microRNAs in cancer pathogenesis and progression, implicating a new class of genes that cause cancer. Indeed, investigating a chromosomal region, on chromosome 13q14, that is frequently deleted in most CLLs, he found that the deletion encompasses a region encoding two microRNAs (miR-15a and miR-16-1). This was the first evidence that non coding genes can be involved in different human diseases, particularly in cancer. A main challenge will be to identify the miRNAs regulated by pathways that are consistently

dysregulated in various types of human cancers. This will open new possibilities for the development of ODN-based targeted therapies.

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## **Session VI: Micro RNAs**

*Chair: David Blakey, PhD, AstraZeneca*

**Mauro Giacca** (ICGEB, Trieste) talked about microRNAs for inducing cardiac repair and regeneration to treat patients with myocardial infarction and heart failure. The view that cardiac muscle cells are not replaced after birth is now in doubt since C-14 dating suggests approx 1% of cardiomyocytes are replaced annually and so stimulation of this population of cells might help contribute to repair after cardiac injury. A search was conducted to identify microRNAs that could stimulate postnatal cardiomyocytes to proliferate and 4 microRNAs were highlighted (miR-590-3p, miR-199a-3p, miR-1825 and miR-33b) that caused significantly increased proliferation. 199a-3p and 590-3p induced fully differentiated cardiomyocytes to re-enter the cell cycle. Mauro showed that delivering 590-3p microRNA via an Adeno-Associated Viral (AAV) vector locally into the heart resulted in an increase in heart size (although morphologically normal) and evidence of increased proliferation of cardiomyocytes. For further information see Eulalio, A. et al. *Nature* 492, 376–381 (2012).

**Carla Nervi** (University of Rome “La Sapienza”) talked about transcriptional gene targeting by micro-RNA-Polycomb complexes in normal and leukemic haematopoiesis as a novel mechanism by which microRNAs can modulate transcription. As an example she described how miR-223 plays a key role in regulating granulocyte differentiation by entering the nucleus, binding to NF1A promoter DNA sequences which are complimentary to the miR-223 seed sequence and this leads to recruitment of a protein complex involving Ago1 and Ago2, Dicer1 and polycomb family members YY1 and Suz12. The complex then regulates transcription via impacting methylation of the DNA. In HL60 leukaemic cells induction of differentiation by Retinoic Acid (RA) leads to up regulation of miR-223 and the differentiation also involves recruitment of this protein complex.

**Anders Naar** (Harvard Medical School/MGH, Boston) talked about the role of microRNAs in regulating Cholesterol/Lipid Homeostasis. He initially described work on mir33 and in particular its role in regulating ABCA1 (involved in reverse cholesterol transport). Inhibition in mice and primates led to raised HDL and in mice in reduction of atherosclerotic plaques. In the primate studies a tiny 8-mer was used as the seed sequence to provide modulation of both miR33a and b isoforms which was required for elevation of HDL (inhibition of individual isoforms did not lead to a HDL increase). He then went on to describe identification of other promising microRNA targets via a meta-analysis of data from genome wide association studies (GWAS) linking SNPs to altered blood lipids. Two microRNAs were discussed (mir128-1 and miR148a) which impacted both the LDL receptor and ABAC1 protein. In addition mir128-1 impacted key proteins (IRS1, Foxo1, SIRT1) involved in insulin sensitivity making it a very interesting target that could have multiple impacts benefiting patients with cardiovascular disease.

**Jennifer Broderick** (University of Massachusetts Medical School) talked about the silencing of gene expression by recruiting RISC. She described a novel approach utilising a synthetic oligo containing one region complimentary to the target mRNA and another to an abundant microRNA in the target cell of interest. The molecule brings the microRNA Argonaute complexes to the target mRNA leading to inhibition of translation of the mRNA. As an example a molecule designed to recruit let-7 to luciferase mRNA resulted in >90% reduction of luciferase expression in let 7 microRNA expressing cells compared to a control tether directed towards a microRNA not present in the cells. A similar approach was successfully

used with miR-122. An attraction of this approach is the oligonucleotide can be designed to silence mRNA that are not regulated by the microRNA present in a particular cell type broadening the potential of microRNA based, cell specific, modulation of therapeutic targets.

**Iddo Magen** (Weizmann Institute of Science, Israel) described the causes and consequences of deregulated microRNAs in Amyotrophic Lateral Sclerosis (ALS) also known as Motor Neurone Disease or Lou Gehrig's disease which leads to loss of motor Neurones. He reported that many microRNAs are down-regulated in human ALS motor neurones and that impairment of Dicer-complex activity is a primary reason for loss of microRNA expression in ALS. Dicer KO mice resulted in ALS like symptoms and phenotype. The studies suggest that microRNA based replacement therapies may have utility in ALS and miR-9 was highlighted as an interesting candidate to explore.

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### Special Session

#### Business Aspects of Starting a Biotech Company

Mick McLean, PhD, ABeterno Ltd and Link Technologies

*Moderator: Marc Lemaitre, PhD, Avecia*

**Dr. Mick McLean** [CEO, ABeterno Ltd.] gave a presentation on "Setting your business up - Getting started". This presentation aimed at informing candidate entrepreneurs about the path to innovation using real-life experience. Having a great idea is for sure important when setting up a business. However it is also an adventure that requires very specific preparation that Dr. McLean described very clearly using examples taken from his extended experience in setting up new companies. Covering topics like IP and freedom to operate, building a team able to convince investors, ways to raise money, differences between a scientific presentation and a successful "road-show" presentation, Dr. McLean exposed the "what not to do, what not to say" that every founder must keep in mind. He concluded: Don't set up a business without thinking through what it will mean for you, your family and your lifestyle, and what you want from it, do take advice, be patient and at the end, "Enjoy the ride". It can be far better and far more rewarding than being a "salaryman".

### Day Three – Tuesday, October 8, 2013

#### Session VII: RNA Modulation

*Chair: Frank Rigo, PhD, Isis Pharmaceuticals*

**Włodzimierz Krzyzosiak** from the Polish Academy of Sciences described his efforts in the area of triplet repeat diseases. First, enzymatic probing and crystallography experiments provided insights into the secondary structure of CNG repeats. Then, Krzyzosiak showed that expression of CNG repeats results in the formation of nuclear foci and the sequestration of RNA binding proteins with downstream alternations in alternative splicing and microRNA biogenesis. Finally, he used siRNAs targeted to expanded CAG repeats to demonstrate allele specific silencing of the mutant HTT allele, without disrupting expression of other genes that contain shorter CAG repeats.

**Gene Yeo** from the University of California at San Diego presented how his lab is using next generation sequencing technologies to decipher the function of many RBPs that have been implicated in neurodegeneration. To achieve this, he used oligonucleotides in collaboration with Isis, to reduce the levels of RBPs in human cultured cells and in the CNS of rodents. Changes in gene expression and alternative splicing were measured by RNA-seq. Furthermore, the RBP binding sites in the mouse and human transcriptomes were determined by using CLIP-seq. This work has furthered our understanding of how mutations in RBPs such as TDP43 and FUS/TLS result in ALS, in addition to providing mechanistic insights into

how these RBPs regulate gene expression and alternative splicing.

**Michelle Hastings** from the Chicago Medical School/Rosalind Franklin University shared her work on the use of splice-switching oligonucleotides to treat Usher syndrome in a mouse model of the disease. The hearing impairment and vestibular dysfunction of the Usher mice is caused by a mutation in an exon of USH1C that activates a cryptic splice site and results in the production of a truncated harmonin protein. Hastings, in collaboration with Isis, identified a splice-switching oligonucleotide targeted to the cryptic splice site that partially corrects USH1C splicing and increases harmonin production in the ear. ASO treatment protects cochlear hair cells from degeneration and rescues hearing to normal levels, in addition to eliminating the vestibular dysfunction.

**Jeannie Lee** from the Massachusetts General Hospital/Harvard Medical School focused her presentation on how Xist RNA spreads across the X chromosome during X-inactivation. To gain insights into the mechanism of spreading, she mapped the Xist binding sites on the X chromosome using CHART-seq over a developmental window of several days. Lee found that Xist is initially targeted to gene-rich islands before spreading to intragenic regions, pointing to a two-step mechanism of spreading. The pattern of Xist binding is mirrored by the binding of PRC2 and the deposition of H3K27me3 suggesting co-migration of Xist and PRC2. Lee also investigated dynamics of Xist spreading after depleting Xist in cultured cells where X-inactivation had already been established. In this scenario, Xist re-occupies its binding sites in the order of hours. This suggests that after the establishment of X-inactivation, the X chromosome is primed for rapid Xist spreading.

**Takanori Yokota** from the Tokyo Medical and Dental University elaborated on his efforts to develop a new class of antisense oligonucleotides called double-stranded antisense oligonucleotides. These consist of a gapmer-ASO hybridized to a complementary RNA that is conjugated to vitamin E. These duplexes silence gene expression in cultured cells and in the liver when administered to mice. Furthermore, in mice, these duplexes display 10 to 50-fold greater activity compared to the corresponding single-stranded gapmer-ASOs. Vitamin E is thought to drive duplexes to hepatocytes by virtue of their binding to lipid particles. Yokota provided evidence that once inside the cell, cleavage of the complementary RNA in a duplex liberates the gapmer-ASO which is then free to engage its target.

**Luca Monfregola** from the University of Colorado at Boulder closed the session by discussing the synthesis and biological properties of new DNA analogs containing lysine and boranephosphonate linkages. He showed that DNA oligonucleotides with these modifications are stable to exonucleases and incur a penalty on hybridization affinity of  $\sim 0.6^{\circ}\text{C}$  per modification. Monfregola also demonstrated that these modified oligonucleotides are taken up by cells without lipid transfection reagents and can inhibit microRNAs by acting as anti-miRs.

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## **Session VIII: Therapeutic Applications: Preclinical I**

*Chair: Rachel Meyers, PhD, Alnylam Pharmaceuticals*

**Stefano Rivella** from Cornell Weill shared his data on the consequences of ASO-modulation of the hepcidin regulatory protein TMPRSS6 in models of thalassemia intermedia and hemochromatosis. He beautifully demonstrated that decreases in TMPRSS6 resulted in increased hepcidin and had a positive impact on a variety of disease parameters. He demonstrated decreased levels of iron in the liver, improved spleen pathology as evidenced by reduced splenomegaly, improved anemia with increasing circulating Hb levels, and, restored red cell function by normalizing the balance of Hb $\alpha$  and Hb $\beta$ . These studies suggest that ASO

targeting of TMPRSS6 may have a significant clinical benefit in diseases where chronically low levels of hepcidin lead to iron overload.

**Kevin Petrecca** from McGill shared his provocative data on the role of DRR in glioblastoma, an aggressively invasive brain cancer. Kevin first described his efforts to identify targets relevant to invasion in neuronal cells and ultimately identified DRR as a key protein upregulated in grade 2-4 brain tumors. He demonstrated that DRR expression results in the misregulation of AKT thereby driving tumor invasion. In collaboration with Masad Dahma, he showed that reduction of DRR using a FANA-ASO leads to decreased glioma invasion and represents a promising approach for treating this challenging disease.

**Jasper van den Borne** from University of Bonn is studying the potential of RIG-I activation (and subsequent type I interferon induction) to treat melanoma. He showed the characterization of oligos that specifically upregulate RIG-I leaving TLR pathways unstimulated. Such compounds are characterized by a unique 5'PPP as well as additional sequence and chemical motifs. Using a mouse model of melanoma, he showed that direct injection of a RIG-I ligand could lead to tumor regression which was mediated by NK cells. He proposed broader application across tumors in combination with standard chemotherapy.

**Sue Murray** from Isis Pharmaceuticals described the use of ASOs to inhibit rhodopsin for the treatment of retinitis pigmentosa, a disease caused, in some patients, by aggregation of mutant rhodopsin in the eye. She demonstrated, via intravitreal injection, good distribution of ASO to all cell layers in the eye. She further showed that ASO injection resulted in good inhibition of rhodopsin mRNA and protein in photoreceptor cells. The effect was long lasting suggesting that infrequent injections might be sufficient to treat this disease and opens the possibility of generalizing this strategy to other diseases of the retina.

**Rachel Meyers** from Alnylam is using siRNAs to KD aminolevulinate synthase (ALAS1) for the treatment of porphyria. POC studies in a mouse model of disease, using siRNAs in lipid nanoparticles (LNPs), showed that KD of the enzyme leads to decreases in the toxic metabolites ALA and PBG in either a prophylaxis or treatment setting. Further, using a GalNAc-siRNA conjugate against ALAS, she showed protein and mRNA KD in a non-human primate (NHP) by evaluating circulating RNA from serum. Finally she showed that Alnylam had identified their development candidate GalNAc-siRNA with activity in a rat model of disease and she outlined preliminary plans for clinical development of the compound in porphyria patient populations.

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## **Session IX: Therapeutic Applications: Preclinical II**

*Chair: Mike Gait, PhD, MRC Laboratory*

**Matthew Wood**, University of Oxford, UK, outlined an approach for treating the devastating disease of young boys, Duchenne muscular dystrophy, through exon skipping of mutations in the dystrophin gene. The approach utilized the well-known PMO oligonucleotides but covalently conjugated (in collaboration with Mike Gait's lab in Cambridge) with a class of peptides called Pip. These PPMOs were shown to have exceptionally high activity in an mdx mouse model of DMD in all muscle types in vivo including diaphragm and heart, the latter being hitherto hard to reach. Recent data in collaboration with Nic Wells's lab at the Royal Veterinary school in London for Pip6a-PMO showed amazing restoration of certain muscle force parameters in treated mouse muscle compared to diseased mice suggesting that high dystrophin restoration has the potential for muscle strength restoration as well as halting the disease. These PPMOa are being developed for clinical use. In addition, Wood described progress in defining microRNA serum biomarkers of disease and treatment which may prove of value in clinical use.

**Jürgen Soutschek** from Ugichem (Austria) showed how various side-chain modified PNA oligonucleotides (called Ugimers) showed potential in exon skipping applications, particularly in inflammation. He described how the nature and number of the modifications drastically affects the biodistribution in vivo in mice models and allows the possibility of new applications for gene silencing in the immune system, although the exact nature of the PNA modifications was not revealed.

**Valérie Robin** from the University of Versailles showed the exceptional promise of TricycloDNA (described in a talk by Christian Leumann from Bern) in exon inclusion of the SMN2 gene in a mouse model of spinal muscular atrophy. She showed how weekly s.c. treatments for 12 weeks rescued the phenotype in type III mice and prevented necrosis of tails, ears and toes. TricycloDNA is now being exploited for a range of neuromuscular diseases.

**Joe Bolen** (Moderna) described proof of concept studies in a range of animal models for efficient delivery of mRNA constructs containing chemical modifications (not defined) that could produce remarkably high levels of both intracellular and secreted therapeutic proteins, for example Epo. The methodology has good prospects for impacting patient treatments in a variety of genetic disorders and diabetes.

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## **Session X: Therapeutic Applications: Clinical Development**

*Chair: Art Krieg, MD, RaNA Therapeutics, Inc.*

**Kim Chi** (University of British Columbia) opened the session with an overview of heatshock protein 27 (hsp27) and its association with cancer, leading to the development of the 4-12-4 antisense MOE gapmer against this target, OGX-427. Phase 2 randomized clinical trial results were presented showing a positive trend to increased responses (including a CR) in the patients receiving OGX-427 + prednisone compared to those receiving prednisone alone. Adverse events included chills and rigors, especially during the loading dose infusions and grades 3 to 4 leukopenia.

**Frank Bennett** (Isis) gave an update on the development of intrathecal infusion and bolus antisense oligos for Huntington's disease, amyotrophic lateral sclerosis, and spinal muscular atrophy (SMA), and systemic administration for myotonic dystrophy. One of the surprises in these programs has been the remarkably durable PK and biologic effects of single dose oligos in the CNS and muscle. In preclinical studies oligos show broad distribution through most of the grey matter including essentially all types of neurons and cells except for cerebellar granular cells. In the SMA program, children receiving a single bolus (9 mg) dose of a splice-correcting oligo showed sustained presence of detectable oligo in the CNS, but more importantly, showed evidence of clinical benefit and improvement with no dose-limiting toxicities over the subsequent 9-14 months!

**Rachel Meyers** (Alnylam) presented the evolution of RNAi therapeutics to prevent the synthesis of transthyretin (TTR). The program has recently expanded from the initial clinical trials that used an RNAi formulated in SNALP and infused IV to now also include a conjugate with GalNAc (TTRsc: described by Mano in a separate talk) which is given SC. In 40 normal subjects the TTRsc was dosed from 1.25 to 10 mg/kg given SC daily X 5 (loading dose) and then weekly X 5. The high dose in the multiple dose portion of the trial achieved >90% suppression of serum TTR levels with excellent tolerability and no evidence of significant immune stimulation nor elevated liver function tests. Results from the phase II trial in patients, and the initiation of a phase 3 trial in this program are expected next year.

**John Kraus** (GSK) joined via Skype to provide details of the first phase 3 clinical trial of a splice-correcting 2'OMe 20-mer oligo (6 mg/kg/wk) for Duchenne muscular dystrophy (DMD), which recently failed to achieve the primary or secondary endpoints. The trial in

186 DMD patients did show a significant decrease in the abnormally elevated basal serum level of the muscle protein CPK, but unfortunately there was only a very modest nonsignificant trend to improvement in the primary outcome, 6 minute walk time (6MWT), at the end of the 48 wk randomized controlled trial. In general the placebo and treated subjects were well matched, although the placebo subjects in the trial were generally younger and had a higher mean 6MWT at the start of the study, both of which could indicate a slightly better prognosis, though the differences were not significant. Subgroup analyses that were shown (did not include baseline 6MWT) did not alter the conclusion that the trial showed no significant benefit. The major adverse events were reversible proteinuria and transient injection site reactions.

**Ryszard Kole** (Sarepta) provided an update on the treatment of 12 boys with DMD, who have now been followed for nearly 2 years in a trial with 3 arms, including placebo, or Morpholino splice-correcting oligo at either 30 mg/kg or 50 mg/kg. The placebo boys were crossed over to the treatment arms after 24 wks, and 2 of the boys in the low-dose treatment arms were removed from analysis for early disease progression to non-ambulation, as they could no longer be evaluated by 6 MWT. At the start of the study the boys had 6MWT >350m, which generally is associated with a better prognosis. Although the boys randomized to placebo had initially progressed with a deterioration in their 6 MWT, they stabilized after being crossed over to treatment and have since remained relatively stable, like the boys on the treatment arm. In contrast to the much larger GSK/Prosensa Phase 3 trial, the 6 MWT of the treated boys have remained stable. In addition, clear increases in dystrophin staining were observed on muscle biopsy. The safety profile of the PMO was excellent.



### Scientific Organizing Committee

The Scientific Organizing Committee built an outstanding program while securing funding for this year's annual meeting. We thank each of you for your perseverance and commitment to the Oligonucleotide Therapeutics Society and for sharing your expertise.

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# The Oligo Meeting

10<sup>th</sup> Annual Meeting of the Oligonucleotide Therapeutics Society

October 12-15, 2014  
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**Announcing the 11<sup>th</sup> Annual Meeting in 2015, to be held in The Netherlands.**

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