NUCLEIC ACID THERAPEUTICS
Volume 24, Number 6, 2014

DOI: 10.1089/nat.2014.1502

Meeting Report

10th Annual Meeting of the Oligonucleotide Therapeutics Society

San Diego, California | October 12–15, 2014

Session Summaries

**Keith T. Gagnon and Jonathan K. Watts,
on behalf of the Board of the Oligonucleotide Therapeutics Society**

Introduction

The 10th Annual Meeting of the Oligonucleotide Therapeutics Society was held on the edge of Mission Bay in San Diego, California. This year's meeting proved to be an exciting forum for new developments in oligonucleotide and nucleic acid therapeutics. Record attendance, a memorable keynote address, over 150 poster presentations, engaging talks, and impeccable weather all helped to mark its success.

Major topics covered included the mechanisms and applications of aptamers, oligonucleotides for immune system modulation, approaches to modulate RNA including splicing and post-transcriptional regulation, long noncoding RNA biology and targeting, microRNAs as tools and targets, and preclinical and clinical developments of therapeutic nucleic acids. Posters covered a broad range of topics, from chemical modifications to targeted nucleic acid delivery and novel delivery formulations, and insight into disease biology and basic cellular mechanisms to creative early-stage approaches for using nucleic acids as therapeutics.

The meeting was capped with a unique experience of dinner in the hangar of the USS Midway, now a floating museum in San Diego Harbor. We and the entire Society extend our special thanks to the Scientific Organizing Committee, the Hilton San Diego Resort and Spa venue, our sponsors, the session chairs, speakers, and poster presenters, and all those who attended. For those who were unable to attend, we have provided our impressions of the oral sessions here and hope to see you next year in Leiden, the Netherlands!

Keith T. Gagnon
Southern Illinois University
Carbondale, Illinois
ktgagnon@siu.edu

Jonathan K. Watts
University of Southampton
Southampton, United Kingdom
j.k.watts@soton.ac.uk

Day One – Sunday, October 12, 2014

Keynote Presentation: Gene Silencing Therapy for Neurodegenerative Diseases

Don W. Cleveland of the University of California, San Diego, described the opportunities presented by oligonucleotide therapeutics in the brain. He has been an early proponent of the idea that the causes and effects of neurological disease are associated with many contributing cell types, and he presented his talk with this background. From this viewpoint, the use of oligonucleotides to turn off disease gene expression in multiple cell types, instead of targeting individual cell types, is essential.

Cleveland described the history, challenges, and advances of using antisense oligonucleotides as potential therapeutics for ALS, Huntington's disease, and spinal muscular atrophy, all of which are in or about to start clinical development. Three years ago a new genetic mechanism for ALS was discovered, namely a repeat expansion in *C9ORF72*, now seen to be responsible for 34% of ALS cases. This so-called c9FTD/ALS will be able to take advantage of lessons learned in other development efforts for neurodegenerative drugs, with clinical trials predicted as early as 2015.

Day Two – Monday, October 13, 2014

Session I: Aptamers

Co-Chairs: Bruce Sullenger, Duke University Medical Center; Matthew Levy, Albert Einstein College of Medicine

Weihong Tan of the University of Florida presented an update on cell-based SELEX for biomarker discovery. By doing aptamer selection on whole cells, they generally obtain multiple aptamers corresponding to cell surface biomarkers that can differentiate cell types (for example small-cell vs. non-small-cell lung cancers). In one application, they have profiled cells from nearly 600 leukemia patients using 18 aptamers to differentiate the biomarkers found in various leukemias. These aptamers also have potential in targeted

drug delivery. Incorporating the artificial nucleosides Z and P into their aptamers has led to increased binding affinities.

Marcin Kortylewski (City of Hope) described a combination approach based on a CpG immunostimulatory oligonucleotide coupled with an siRNA or ASO to inhibit STAT3. The inhibition of STAT3, which is at the node of an immune network, made the cells vulnerable to immunotherapy. Conjugation of class-A CpG sequences led to improved internalization of the conjugates, to a much greater extent than class-B CpG sequences. In a mouse tumor model, six injections of CpG-STAT3 per day at 5 mg/kg caused a strong antitumor response, which is not accomplished by a CpG-scrambled conjugate.

Samir Patel (Opthotech) described progress on aptamers for ocular applications. Their anti-PDGF aptamer Fovista has completed a large phase 2 trial (449 patients with wet AMD). Patients receiving Fovista showed 62% better vision than those receiving the VEGF antibody Lucentis, with more significant improvement at longer timepoints. Fovista is a 30mer (optimized from 70–80 nucleotides originally) and is PEGylated, though Patel is not convinced that PEG is necessary for ocular applications. They are also developing Zimura as an aptamer targeting the complement pathway, as polymorphisms in the genes coding for complement regulatory proteins may increase the likelihood of developing AMD by 7- to 11-fold.

An interesting insight from Patel's talk is that Macugen is thought to have limited efficacy not because of an inherent failure of aptamer technology, but <rather>, because Macugen only inhibits one isoform of VEGF, while the antibodies that have come to dominate the field inhibit all four VEGF isoforms.

Jean Gariepy of the University of Toronto described the search for agonistic aptamers that modulate the immune response. They have found two CD200 mimics that prolong skin allograft survival. Their results suggest that agonistic aptamers to other immunoreceptors may be able to alleviate additional autoimmune/inflammatory conditions.

Session II: Immunomodulatory Oligonucleotides

Chair: Gunther Hartmann, University Hospital Bonn, Germany

Bhushan Nagar (McGill University) described the structural basis for viral RNA recognition by human IFIT proteins 1 and 5. The IFIT proteins are mainly cytoplasmic interferon-stimulated genes containing multiple TPR repeats. They bind 5′-ppp RNA in a long, positively charged central pocket of the protein. This pocket is of the appropriate dimensions to recognize ssRNA but not dsRNA, and co-crystal structures with ppp-oligo(rC), ppp-oligo(rA), and ppp-oligo(rU) showed that the recognition is sequence independent. There are strong interactions with the 2′OH groups, which might explain why the recognition is inhibited by 2′-O-methylation.

Veit Hornung (University of Bonn) gave an update on the latest work in intracellular DNA sensing. The innate immune system can recognize inappropriate localization, such as DNA in the cytoplasm, but the mechanisms for this have been poorly understood. The nucleotidyltransferase cGAS is now seen to be the dominant cytosolic DNA sensor. It produces the cyclic dinucleotide cGAMP(2′,5′) as a second messenger. This in turn activates a protein called STING (stimulator of interferon genes) in both the same cell and bystander cells, which drives interferon responses.

Stefan Bauer (Philipps-University Marburg) described a process of stimulating the immunoreceptor RIG-I by digesting cellular RNA with RNase A and transfecting it back into cells. The immune response seems to be caused by specific regions of dsRNA derived from 18S rRNA and ITS2. Digested RNA derived from tumor cells and highly proliferating cells is more immunogenic. Transfecting RNase A protein into cells also causes an immune response, and this result depends on the protein retaining its nuclease activity.

Sergei M. Gryaznov (Aurasense) showed that spherical nucleic acids (SNAs) can be made by assembling lipid-conjugated oligonucleotides onto small lipid bilayers 40–60 nm in diameter. This newer approach is in contrast to their earlier work using gold nanoparticles as the scaffold for SNA assembly. To avoid the need for SNAs to escape from endosomes, Aurasense is developing immunostimulatory SNAs that can exert their biological effect from within endosomes. For stimulating multiple cytokines, they see an 80–100× improvement in potency upon tethering oligonucleotides to the core. Presentation on the outside of the lipid bilayer is very important: when 50% of the oligomer is trapped inside of the liposome, the potency of immune stimulation dropped 20-fold. Phosphorothioate backbone oligos were much more potent than their phosphodiester counterparts.

Kerstin Kapp (Mologen AG) presented a new type of immunostimulatory oligomer called EnanDIM (enantiomeric DNA immunomodulator). These consist of four Gs at the 5′ end, which are important for function, several internal CpG dinucleotides, and two enantiomeric deoxynucleotides at the 3′ end. Kapp speculated that the 5′-GGGG moiety might be a multimerisation domain. EnanDIMs are strong inducers of IFN-α.

Session III: RNA Modulation I

Co-Chairs: Muthiah Manoharan, Alnylam Pharmaceuticals; Gunter Meister, University of Regensburg

Many types of bacteria use a CRISPR/Cas9 system for genome and viral defense. **Erik Sontheimer** of the RNA Therapeutics Institute at the University of Massachussetts Medical School described this RNA-guided mechanism and the discovery of a novel version from *Neisseria meningitides*. This new system could prove useful for biotechnology or future therapeutics approaches due to a much smaller Cas9 gene, a different PAM motif, and the potential to be combined with orthologous CRISPR/Cas9 systems.

Matthias W. Hentze of EMBL in Heidelberg, argued for the possibility that the RNA-interaction potential of many proteins, including metabolic enzymes, could contribute to regulation of cellular pathways in unexpected ways. Techniques like cross-linking, sequencing, bioinformatics, and testing in multiple cell types support this hypothesis. Demonstration of regulatory impact on cellular functions for these ''enigmRBPs'' might reveal new roles for RNA binding in biology and disease.

Leemor Joshua-Tor, HHMI investigator at Cold Spring Harbor Laboratory, shared mechanistic insights into the substrate specificity of DIS3L2, an enzyme that degrades polyuridylated pre-let7. Let7 is a miRNA that regulates critical stages in development. A crystal structure of DIS3L2 with a poly-U substrate was presented. Examination of the catalytic site and other structural features may explain why DIS3L2 has a unique specificity for degrading RNAs with 3′ poly-U sequence. Results have implications for the regulation of miRNAs, an important tool and target in nucleic acid therapeutics.

Mechanistic insights and progress in the use of N-acetylgalactosamine (GalNAc) conjugation for targeted siRNA delivery were described by **Martin A. Maier** of Alnylam Pharmaceuticals. When placed centrally, 2′-*O*-GalNAc modifications do not affect siRNA activity and can substantially improve stability. Half-life studies showed target knockdown for over 30 days in mouse liver. GalNAc-siRNAs enter cells through the ASGPR receptor, but it was found that reduced numbers of the receptor has only marginal effects on siRNA uptake.

Georg Sczakiel of the Institute of Molecular Medicine at the University of Lubeck proposed an RNAi model where a complex exists that contains both the sense and antisense small RNA strands and the target RNA. Competitive experiments and other data suggest that it is possible that formation of an Ago2–target RNA complex, or even target RNA cleavage, is required for stable loading of Ago2 with a single small RNA strand. These possibilities highlight the need for deeper investigation of the interplay between Ago2 loading, target RNA recognition and cleavage, and RISC turnover.

Day Three – Tuesday, October 14, 2014

Session IV: RNA Modulation II

Co-Chairs: Marc Lemaitre, ML_Consult; Jonathan K. Watts, University of Southampton

Peter A. Beal of the University of California, Davis, described efforts to minimize immunostimulation by siRNAs using novel chemical modifications. N^2-substituted guanines and N^2-substituted 2-aminopurines effectively reduced immuno-stimulation and maintained efficient RNAi. Using the existing crystal structure of Ago2 and a computational approach, compatible 5′ terminal modifications were identified that could protect siRNAs and retain RNAi activity.

The use of DNA nanostructures for controlled nucleic acid and drug delivery was described by **Hanadi Sleiman** of McGill University. Controlled engineering of structure using nucleic acid hybridization has benefits including target-activated release of drug cargo, nuclease resistance, and cellular penetration. DNA nanotubes and cages showed favorable properties for development as potential nucleic acid therapeutic delivery vehicles.

Meena of WaVe Life Sciences described the controlled synthesis of oligonucleotides containing phosphorothioate backbones of defined *P*-chirality using a stereocontrolled synthesis platform. Incorporation of uniform *Rp* or *Sp* stereoisomers in an oligonucleotide, or five other mixtures including two that were rationally designed based on crystal structures of RNase H, resulted in significant differences in melting temperatures, in vivo stability, lipophilicity, and RNase H cleavage efficiency. The most successful patterns were those based on the crystal structure—namely a repeating pattern of *SpSpRp* or *RpRpSp* linkages—which were dramatically more active in terms of RNase H cleavage than oligomers based on either isomeric linkage or a random mixture. Phosphorothioate chirality affects oligonucleotide performance and its control should provide new therapeutic opportunities.

The effects of incorporating a novel modification, 2′-*O*-methyl phosphorodithioate (MePS2) into RNA was presented by **Xianbin Yang** of AM Biotechnologies. MePS2-modified siRNAs showed significant improvements in potency, which was attributed in part to better loading into RISC, as well as nuclease resistance. It was reported that incorporation of MePS2 into the VEGF165 aptamer improved target affinity 2,000-fold. MePS2 is achiral and straightforward to synthesize, which further lends favor as a modification for nucleic acid therapeutics.

Piotr J. Kamola of GlaxoSmithKline and Imperial College London shared observations of high intronic off-target effects of oligonucleotides associated with LNA modifications. It was found that LNA-modified oligos can inadvertently target and affect pre-mRNA and intron RNA levels, as well as noncoding RNAs. Such off-target effects were much higher than previously anticipated and could lead to a significant liability in nucleic acid drug design. In silico predictions and experimental controls were suggested as methods to mitigate potential off-target effects. These results also indicate that introns may be readily amenable to targeting with antisense oligos. During the question period, Art Levin reminded attendees that all drugs have off-target effects and that oligonucleotide-induced off-target effects are easier to predict and quantitate than those of other types of drugs.

Session V: Long Noncoding RNAs

Co-Chairs: Tariq Rana, University of California San Diego; Michael McManus, University of San Francisco

Understanding the role of noncoding RNA in human biology and disease is an ongoing challenge with direct implications for nucleic acid therapeutics that target or use noncoding RNA. **Michael McManus** of the University of California, San Francisco, described innovative mouse models for studying microRNA expression and function in vivo. Approaches included the coupling of LacZ and microRNA expression in innovative ways to visualize microRNA expression and distribution in mouse tissues and during development or disease.

Mitchell Guttman of the California Institute of Technology presented a mechanism explaining how many chromatin-associated long noncoding RNAs (lncRNAs) might function. RNA affinity purification (RAP) and next-generation sequencing was combined with Hi-C analysis to determine where and when the Xist lncRNA was associated with chromatin and where that chromatin was located spatially. Results suggest a hypothesis whereby lncRNAs pull associated chromatin into local regulatory compartments inside the nucleus for coordinated expression of related genes.

Development of fluorescent RNA aptamers for imaging RNA inside of cells was presented by **Samie R. Jaffrey** of Weill Medical College at Cornell. Using SELEX and cell-based selection techniques, novel properties like photobleaching resistance, minimal sizes, multiple color fluorescence, and enhanced brightness inside of cells can be selected. Mechanistic insight has been provided by high-resolution crystal structures, likening fluorescent aptamers to GFP and its analogs.

Niels M. Frandsen of Exiqon presented a design algorithm to aid in the prediction of effective LNA oligonucleotides for the targeting of lncRNAs using knockdown of nuclear Malat1 lncRNA for validation. Empirical data indicated effective Malat1 knockdown in multiple mouse tissues. Exiqon will provide access to this algorithm and test other lncRNAs to help improve targeting of nuclear noncoding RNAs.

Session VI: miRNAs
Chair: Deidre MacKenna, Regulus Therapeutics

A common feature of tumors is a slightly acidic microenvironment. **Frank J. Slack** (Yale University) presented work with pHLIP [pH (low) insertion peptide] conjugates of an anti-miR to the oncogenic microRNA miR-155. This pHLIP is a 36-aa peptide whose C-terminus inserts across lipid membranes at pH < 7. Making PNA–pHLIP conjugates via a disulfide bond, Slack's group observed localization to tumor and avoidance of the liver. This also led to a survival benefit and a reduction in metastasis.

Stephen Y. Chan (Brigham and Women's Hospital/Harvard Medical School) provided systems-level insights into the roles of miRNAs in pulmonary hypertension (PH). Computational network analysis predicted that miR130/301 would regulate a large number of PH-relevant genes. Experiments confirmed these predictions and showed for example that miR-130/301 is upregulated in seven animal models and three human subtypes of PH. Intratracheal or i.v. injection of miR-130 mimics promoted PH. Collaborating with Regulus, they also showed that 8mer anti-miRs gave strong target derepression and alleviated symptoms of PH.

Continuing at the interface of miRNAs and systems biology, **Sohail F. Tavazoie** of the Rockefeller University then discussed the role of tissue specific miRNAs and pathways in governing metastasis. His group used an in vivo selection approach to identify highly metastatic cells—injecting patient-derived cells into a mouse, then taking the resulting tumors and injecting cells from these into a second mouse and analyzing cells from this second-round tumor. They found miR1908, miR214, miR199-5p, and miR199-3p to be enriched in the metastatic cells compared to the parent. These target DNAJA4 and ApoE, which is a regulatory hub for metastasis. An LNA cocktail targeting the three miRNAs led to a 10-fold reduction in metastasis in mouse at 12 mg/kg.

Vittorio de Franciscis (CNR Naples) described aptamers conjugated to miRNA or anti-miR oligomers. The aptamers target and inhibit Ax1 or PDGFRβ, targets implicated in gliomagenesis. Moreover, they are rapidly internalized and can carry a miRNA or anti-miR cargo with them. Aptamer and oligomer can thus work in synergy to target different aspects of gliomagenesis.

Tim Wagenaar (Sanofi Oncology) focused on the ESCRT-1 complex, an important regulator of oligonucleotide uptake by cancer cells. Knocking down components of the ESCRT-1 complex led to vastly improved uptake of oligonucleotides in various cells lines. The working hypothesis is that ESCRT-1 serves as a toggle between productive and unproductive uptake pathways.

Session VII: RNA Modulation
Chair: Annemieke Aartsma-Rus, Leiden University Medical Center

Aurelie Goyenvalle of the Universite de Versailles St-Quentin described the use of tricyclo-DNA (tcDNA) modified antisense oligonucleotides for therapeutic exon skipping in Duchenne muscular dystrophy. In addition to showing favorable exon-skipping and no acute toxicity, tcDNA was more effective at restoring skeletal muscle and cardiac function than 2′-*O*-methyl and PMO chemistries. Some degree of exon skipping was also observed in the CNS, indicating that tcDNA can cross the blood–brain barrier to correct neurological defects.

Adrian R. Krainer of Cold Spring Harbor Laboratory described the testing of oligonucleotides for switching splice-site usage of the pyruvate kinase PKM2 isoform associated with cancer cell metabolism. Modulation is challenging, requiring the selective exclusion of exon 9 and inclusion of exon 10, which are both very similar in sequence. High levels of PKM1 protein, around 70-fold increase, indicated successful switching from the PKM2 isoform to the PKM1 isoform, which is associated with normal tissues.

A common cause of Timothy syndrome is a missense mutation in a gene called *CACNA1C* that encodes calcium channel CaV1.2, which might be rescued by splice-switching. **Douglas L. Black**, HHMI investigator at the University of California, Los Angeles, presented efforts toward understanding the complex splicing events of CACNA1C pre-mRNA. Splice selection is dictated by two related proteins, PTBP1 and PTBP2. Computational predictions and experiments suggested mechanisms for splice selection that might be modulated with nucleic acids for therapeutic applications.

Burcu Bestas (Karolinska Institutet) described splice-switching as an approach to personalized treatment of X-linked agammagloblulinemia (XLA). This disease arises from mutations in the *BTK* gene, and splice-switching oligos were able to correct the mutation in mice and primary human cells. Chemistries tested included 2′-OMe, LNA, and PMO.

Day Four – Wednesday, October 15, 2014

Session VIII: Preclinical/Clinical I

Chair: Brett Monia, Isis Pharmaceuticals, Inc.

Adam E. Mullick of Isis Pharmaceuticals described therapeutic opportunities in the kidney. ASOs readily distribute to the kidney, particularly the cortex and the PCT. Two of their clinically validated targets are CD36 and CD40. More than 5,000 patients have been dosed without any adverse effects on kidney function (e.g., serum creatinine or GFR). cEt gapmers were more potent than MOE or cEt/MOE mixtures. ASO activity was maintained in animal models of severe kidney damage and macrophage infiltration.

Paloma H. Giangrande of the University of Iowa described the use of RNA aptamers to improve uptake of cancer drugs. For example, in a mouse imaging study with both PMSA+ and PMSA- tumors, their aptamer A9g showed uptake in PMSA+ tumors while a point mutant did not, demonstrating a genuine aptamer-mediated uptake rather than an EPR effect. They are using these aptamers to deliver cytotoxic drugs such as saporin.

Mark E. Davis (Caltech) discussed work on cyclodextrin-based, transferrin-targeted delivery vehicles as well as more recent work on antibody-conjugated delivery systems. On the transferrin-targeted system, there was a one-year gap during their clinical trial, and after this gap they observed many more adverse effects. (Perhaps the transferrin was not sufficiently stable between synthesis and administration 3–4 years later). The complexity of such targeted approaches makes it desirable to get as much impact as possible from a protein delivery agent—would an antibody, for example, be able to show antibody-dependent cell-mediated cytotoxicity (ADCC) effects? They presented results on Herceptin or Cetuximab targeted nanoparticles. However, the ADCC effect shown by the free antibodies was blunted when they were conjugated to the nanoparticles, suggesting that it may be challenging to take advantage of both ADCC and targeted delivery by a single antibody.

Continuing in the theme of delivery, **Marie Didiot** (University of Massachusetts Medical School) described their work on exosome-mediated delivery of siRNAs in the brain. They work with hydrophobic siRNAs (hsiRNA) containing a cholesterol on a 15mer sense strand, and a PS backbone on the single-stranded region of the antisense strand. These show uptake near the site of injection in the brain but do not distribute widely on their own. Didiot's group purified exosomes from U87 glioblastoma cells and loaded these by simply mixing the purified exosomes with hsiRNA for 90 minutes (this gave 80% loading). Injecting these loaded exosomes into one side of the brain gave broad distribution into the neurons on both injected and noninjected sides.

Haifang Yin of Tianjin Medical University described a screen for small molecules that potentiate delivery of PMO splice-switching oligomers. One of their hits, a compound that is already in common clinical use, appears to improve PMO uptake. Its enantiomer is nonfunctional.

Session IX: Preclinical/Clinical II

Chair: Mark A. Kay, Stanford University

Mark A. Kay of Stanford University described the ability of the pre-miRNA of miR151 to engage the 3′-UTR of E2f6—a transcription factor—and regulate its expression. Competition between pre-miR151 and mature miR151 seems to underlie regulation. Pre-miR151 can undergo A-to-I editing in the brain, which was found in vitro to affect its ability to compete with mature miR151 and regulate E2f6 expression. Genome-wide predictions suggest that other competitive networks between precursor and mature miRNAs may exist.

David L. Lewis of Arrowhead Research Corporation presented results of ARC-520 for potential treatment of chronic hepatitis B virus (HBV) infection. ARC-520 is a cholesterol-conjugated siRNA that is coadministered with Arrowhead's proprietary Dynamic Polyconjugate (DPC) formulation. HBV infection was substantially suppressed in a chimp model of chronic HBV infection. Human clinical trials are underway and thus far treatment seems well tolerated. The use of DPC for treatment of antitrypsin deficiency was also described, and phase 1 trials are anticipated.

Ian MacLachlan of Tekmira Pharmaceuticals provided an update on TKM-HBV, an LNP-siRNA for treatment of HBV infection. In particular, models for chronic HBV infection used for studies were described. These included immunocompromised mice with humanized livers and a woodchuck hepatitis virus model. New models would provide better preclinical testing. A multivalent approach using three siRNA triggers was effective and considered a safeguard against HBV adaptation.

Strategies for efficient knockdown of noncoding RNAs can depend on a number of factors. **Mark Behlke** of Integrated DNA Technologies described a systematic analysis comparing siRNAs and LNA-modified ASO gapmers for knockdown of several noncoding RNAs. ASOs seemed to perform better against nuclear targets whereas siRNAs performed better against cytoplasmic targets. Some target noncoding RNAs, such as NRON, seemed impervious to knockdown, presumably due to ribonucleoprotein structure. RNAi and RNase H mechanisms were deemed functional in both nuclear and cytoplasmic compartments but with different efficiencies. Localization and accessibility were determined to be key factors in choosing the appropriate knockdown methods.

Sheena Saayman of the Scripps Research Institute in La Jolla, California, presented the finding that a noncoding antisense RNA regulates cystic fibrosis transmembrane receptor (CFTR) expression. The CFTR antisense transcript appeared to function in trans and involved chromatin modifications. The noncoding RNA localized to its own genomic locus, suggesting an involvement in gene looping. Results suggest that the antisense noncoding RNA could be a target for upregulating CFTR expression for potential treatment of cystic fibrosis.

Session X: Preclinical/Clinical III
Chair: Rachel Meyers, Alnylam Pharmaceuticals

Tracy S. Zimmerman (Alnylam) presented clinical data on their two Transthyretin-mediated Amyloidosis (ATTR) programs in clinical development. They are developing a SNALP-based drug to silence TTR (ALN-TTR02/Patisiran) as well as a GalNAc-targeted drug for the same gene (ALN-TTRsc). The two drugs are designed for different forms of ATTR based on different mutations, but both drugs target the wild type and all mutant forms of TTR. Phase 2 data from Patisiran showed a sustained 80%–90% knockdown of serum TTR and, perhaps of even greater significance for the field, the long-term tolerability of SNALP delivery, with some 20 patients having received doses every 3 weeks for over 6 months. Neuropathy scores showed slight improvement for patients over six months, when natural history studies would have predicted significant worsening in these scores. As for ALN-TTRsc, in phase 1 it achieved a sustained 85% knockdown of serum TTR at doses of 5 mg/kg and above. A phase 2 trial is underway, and a phase 3 trial is to start later this year.

Sanjay Bhanot (Isis Pharmaceuticals) presented an update on their phase 2 antithrombotic drug targeting Factor XI (FXI). While most antithrombotics lead to increased bleeding at high doses, FXI operates at a part of the clotting pathway related to clot propagation rather than initiation, and knockdown of FXI does not appear to cause an increase in bleeding events. It is interesting to compare the absence of immunostimulation by Isis' oligonucleotides early in clinical trials compared with the older generation of oligonucleotide drugs including mipomersen. Bhanot attributes this improvement to the use of more demanding in vitro assays during screening for drugs that begin clinical development today.

Neil W. Gibson (Regulus) described progress in the development of their HCV drug RG-101, which targets host factor miR-122 in the same manner as Santaris' HCV drug miravirsen. RG-101 is a GalNAc-conjugated cEt mixmer, which is 20- to 30-fold more potent than the unconjugated mixmer. In mouse liver, the conjugate has an EC_{50} of 0.19 mg/kg. The phase 1 trial was designed to include a single dose study on HCV patients to assess the impact on viral load. Gibson also mentioned Regulus' work on inhibition of miR-21 in the kidney for Alport syndrome, an orphan disease affecting 20,000–30,000 people in the United States and causing death if kidneys are not transplanted. Inhibition of miR-21 in a mouse model leads to improved survival and kidney function.

Richard Ho (Marina Biotech) gave an update about the use of their delivery platform, called SMARTICLES. This system based on phospholipids, cholesterol, and amphiphilic lipids is designed to release its cargo at low pH, as found in lysosomes. SMARTICLES are being used to deliver a chemically unmodified single-stranded DNA decoy (PNT2258 for ProNai therapeutics, in phase 2), and a double-stranded miRNA mimic (MRX-34, in phase 1) for Mirna therapeutics. In rodents, these particles deliver effectively to the liver, kidney, spleen, tumor, and lung.

Andrew Vaillant (REPLICor) gave the third talk of the conference on the topic of inhibition of hepatitis B with nucleic acids. REPLICor is developing phosphorothioate ''nucleic acid polymers'' which are used in a sequence-independent and size-dependent manner to block assembly of the HBV surface antigen into subviral particles. Clearing the surface antigen in this way allowed patients to mount an immune response, particularly when their oligomers were coupled with immunotherapy. Their oligomers can be delivered as calcium chelates which reduces the class-based toxicity of the phosphorothiate oligonucleotides.

Conference chair **David Corey** (UT Southwestern) closed the meeting by commending the speakers for the quality of their data. The field, he said, has made tremendous progress since the early days of research into oligonucleotide therapeutics. Long may this upward trajectory in quality and depth continue!

Scientific Organizing Committee

Chair

David Corey, PhD, *University of Texas Southwestern Medical Center*

Committee Members

Annemieke Aartsma-Rus, PhD, *Leiden University Medical Center*
Frank Bennett, PhD, *Isis Pharmaceuticals*
Masad Damha, PhD, *McGill University*
Neil Gibson, PhD, *Regulus Therapeutics*
Gunther Hartmann, PhD, *University Hospital, Bonn, Germany*
Ryszard Kole, PhD, *Sarepta Therapeutics*
Marc Lemaitre, PhD, *ML_Consult*
Muthiah (Mano) Manoharan, PhD, *Alnylam Pharmaceuticals*
Gunter Meister, PhD, *University of Regensburg*
Tariq Rana, PhD, *Sanford Burham Medical Research Institute*
Jonathan Watts, PhD, *University of Southampton*

Sponsors

Thank you to all the organizations and individuals who contributed financially to make the 2014 Annual Meeting such a success.

Gold level
Isis Pharmaceuticals
Alnylam Pharmaceuticals

Silver Level
Arrowhead Research Corporation
AstraZeneca
ChemGenes Corporation
Dicerna Pharmaceuticals
Roche
Sarepta Therapeutics

Bronze Level
Agilent Technologies
Axo Labs
Biogen Idec
Idera Pharmaceuticals
Prosensa
Regulus Therapeutics
Shire
Silence Therapeutics
SNBL USA

Friends Level
AUM Lifetech
Integrated DNA Technologies
Link
miRagen
RaNA

Supporter Level
Animal Care Systems
ARCTURUS Therapeutics
SomaLogic
Applied Instruments