



11th Annual Meeting of the Oligonucleotide Therapeutics Society

The Oligo Meeting

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11th Annual Meeting of the Oligonucleotide Therapeutics Society Meeting Report and Session Summaries

Preconference Session: Oligonucleotide Therapeutics Education Workshop *Making Drugs Out of Oligonucleotides*

Chair: Marc LeMaitre, PhD, *M_L Consult*

For the first time the OTS annual meeting offered an elective Oligonucleotide Therapeutics Education Workshop entitled "Making Drugs Out of Oligonucleotides". The goal of this workshop was to provide young and new researchers in the field with a top-level review of important aspects of the discovery and early development steps of therapeutic oligonucleotides.

Muthiah (Mano) Manoharan (Alynlam) spoke about Chemistry of Therapeutic Oligonucleotides. His presentation covered different classes of oligonucleotides and their respective mechanism of action. Important chemical modifications at the sugar level or at the base level were described as well as morpholino/PMO and gapmer oligonucleotides containing some of the mentioned modifications. Finally delivery systems were described [Cholesterol, PEG, GalNac DPC, LNPs].

Brett Monia (Isis pharmaceuticals) spoke about administration and *in vivo* delivery. Oligonucleotides can be taken up by cells via different pathways and can end in sinks instead of producing productive delivery into the cytoplasm or nucleus. As oligonucleotides accumulate differently in different organs and in different cells it is very important to consider the cell type(s) within a given organ that needs to take oligo up for pharmaceutical effect. Local delivery was also reviewed and the influence of medicinal chemistry on the optimization of pharmacodynamics relationship in animals was discussed.

Art Krieg (Checkmate) presented on toxicity and, ADME (absorption, distribution, metabolism and excretion) [presentation prepared with **Art Levin** (Avidity NanoMedicines)]. Safety parameters and the mechanism of action of some toxic effects, immunological side effects were reviewed, as well as the effect of chemical modifications on these matters. ADME is quite similar for all oligonucleotides of a given chemistry. Mostly, primate data are reasonably predictive for human trials.

Seokjoo Yoon (KIT) spoke about RNAseq as a new approach for safety evaluation of oligo therapeutics. RNAseq has the advantages of a broader dynamic range and low background noise compared to the DNA microarray platform. Comparing samples from treated and control animals shows the direct impact of treatment on target mRNA expression and off-target effects. This could be an approach for safety evaluation of oligonucleotides in early stages of development.

Finally a very active panel discussion allowed the audience to ask questions to the speakers, concluding very nicely this first [and successful, according to many testimonies] pre-meeting workshop.

Keynote Presentation: Tandem Repeats, RNA Diseases and Treatment

Charles A. Thornton, MD, *University of Rochester Medical Center*

Charles Thornton (University of Rochester) introduced tandem repeat diseases, such as myotonic dystrophy (DM), which are caused by a toxic gain of function of RNA. DM patients

carry an expanded CUG repeat in the *DMPK* gene or a CCTG repeat in the *ZNF9* gene. Upon transcription, repeats form stable secondary structures (loops) in the RNA. These loops sequester muscleblind proteins to the nucleus, which leads to a misregulation of alternative splicing of numerous genes resulting in the multisystemic pathology that characterizes DM. Using an RNase H dependent MOE-gapmer ASO targeting a CUG repeat containing transgene in mouse, very good muscle knockdown was achieved, leading to a correction of splicing defects and an improved phenotype. An ASO targeting endogenous *DMPK* was also able to achieve good knockdown in the muscles of wild type mice and monkeys. This ASO is currently being tested in a phase 1/2a dose escalation safety study in DM1 patients by ISIS pharmaceuticals.

Session I: Medicinal Chemistry and Novel Mechanisms of Action (non splicing)

Co-chairs: Mike Gait, PhD, *MRC Laboratory*

Jonathan Watts, PhD, *RNA Therapeutics Institute, UMass Medical School*

The chairs began this session by noting the relationship between the two themes, since novel chemical approaches and the ability to exploit novel mechanisms often go hand in hand.

Sudhir Agrawal (Idera) demonstrated that chemically modified oligonucleotides can serve as true antagonists of Toll-like receptors. These compounds can thus be used to interfere with inappropriate immune feedback loops. Examples were shown in autoimmune disease, B-cell lymphoma and Duchenne muscular dystrophy (in the latter case, loss of dystrophin stresses cells and leads to inflammation that causes some of the pathology). **Claes Wahlestedt** (U. Miami) described progress in gene upregulation by inhibition of natural antisense transcripts (NATs). Nine validated targets have now been activated in vivo. This talk focused on activation of BDNF (via gapmer ASO) for various neurological diseases, and of SCN1A for childhood epilepsy. **Maire Osborn** (RTI, UMass Medical School) then described a fully metabolically stabilized hydrophobic siRNA for gene silencing in the CNS (application to Huntington's disease). When constructed as a cholesterol conjugate, uptake was good but limited to areas of the brain near the infusion site. Changing to a DHA conjugate led to broader uptake and lower toxicity.

Anton McCaffrey (TriLink) described novel modifications to synthetic (in vitro transcribed) mRNA. 5-MeO-uridine and 1-methylpseudouridine, in particular, were promising novel modifications, but their activity depended on the nature of the mRNA. Both immune stimulation and stress granule formation were (independently) negatively correlated with translational activity from the synthetic mRNA. **Thorsten Stafforst** (Tübingen) has tethered ADAR enzymes via SNAP tag to a 'guide' oligonucleotide, and this construct induces site-specific A-to-I editing in arbitrary target transcripts. Mismatches and chemical modifications can be used to improve the specificity when there are multiple adenosines near the target site (editing preferentially occurs at A-C mismatches, and does not occur opposite 2'-OMe-RNA).

The final two talks of the session concerned aspects of CRISPR technology. First, **Sheena Saayman** (Scripps) demonstrated that a fusion protein of catalytically inactive dCas9 with VP64, with appropriate guide RNAs, could be used to reactivate latent HIV as part of a 'shock and kill' strategy. The response was more consistent than that of chemical stimuli. Finally, **Mark Behlke** (IDT) showed that chemically modified guide RNAs could be used to for CRISPR/Cas9 based DNA cleavage. While some positions are sensitive to chemical modification, active crRNAs and tracrRNAs have been made with up to 78% and 70% of 2'-OMe-RNA, respectively.

Session II: Exon Skipping

Co-chairs: Annemieke Aartsma-Rus, PhD, *Leiden University Medical Center*

Frank Rigo, PhD, *Isis Pharmaceuticals*

Annemieke Aartsma-Rus introduced the session and explained how antisense oligonucleotides (ASOs) can be utilized in several way to manipulate splicing for therapeutic purposes. This approach is developed furthest for Duchenne muscular dystrophy (DMD), where the aim is to skip an exon during pre-mRNA splicing to restore the dystrophin reading

frame and allow production of a partially functional protein rather than a non-functional one. ASOs of two different chemistries have been submitted to FDA for accelerated approval: Drisapersen (BioMarin) and Eteplirsen (Sarepta, presented by **Ryszard Kole** (Sarepta)). **Alberto Malerba** (Royal Holloway London) presented on combining dystrophin reading frame restoring ASOs with reading frame disrupting ASOs targeting the muscle growth inhibiting protein myostatin in an effort to increase muscle mass in the *mdx* mouse model.

In an adaptation of this approach Julie Rutten (LUMC) and Jeroen Bremer (University Medical Center Groningen) presented on skipping mutated exons without disrupting the reading frame, allowing the production of partially functional Notch3 proteins (mutated in CADASIL) and Collagen Type VIIa (mutated in dystrophic epidermolysis bullosa).

ASO-mediated inclusion of exon 7 of the *SMN2* gene is being explored for the treatment of spinal muscular atrophy. **Frank Bennett** (Isis pharmaceuticals) presented data on the widespread distribution of ASOs in the central nervous system (CSN) of rodents and monkey after CNS ASO delivery. He also provided an update on ongoing clinical trials.

Splicing modulation can also be used to restore splicing that is disrupted due to a cryptic splicing mutation. **Batsheva Kerem** (Alexander Silverman Institute) and **Alejandro Garanto** (Radboud University Medical Center) presented on restoring cryptic splicing for *CFTR* (cystic fibrosis) and *CEP290* genes (Leber's congenital amaurosis), respectively.

Tomoki Nomaguchi (Cold Spring Harbor) presented on the use of ASOs to target exon junction complex formation to prevent nonsense mediated decay and facilitate therapies aiming at readthrough of premature stop codons. **Benjamin Blencowe** (University of Toronto) presented on very small exons (microexons) that are alternatively included in genes expressed in neurons. Microexon inclusion is decreased in brains of autism spectrum disorder patients, and a mouse model where microexon inclusion was impaired showed phenotypic signs that resemble autism.

Session III: Emerging Concepts in RNA Biology, co-hosted by the RNA Society

Chair: Tracy Johnson, PhD, *University of California, Los Angeles*

Our understanding of the functions of RNAs and their regulation has evolved immensely over the last several decades. The session chair, **Tracy Johnson** (UCLA), highlighted some of the emerging concepts in RNA biology and introduced how these new insights are leading to important translational breakthroughs, such in RNA-based therapeutics.

Anna Marie Pyle (Yale University/HHMI) presented studies describing the structural basis for pathogen-specific dsRNA binding and ATPase activation of RIG-I like receptor proteins (RLRs), a family of proteins central to the innate immune response. She described new insights into the requirements for dsRNA activation and the differential roles for ATP binding vs. hydrolysis in RIG-I signaling by viral RNAs. **Jon Staley** (U. of Chicago) focused on the DEAH family of RNA-binding ATPases that direct the RNA-rearrangements during pre-mRNA splicing. Single molecule and biochemical studies revealed how these proteins promote the rejection of suboptimal splice sites and the selection of optimal sites, with important implications for the regulation of splicing and alternative splicing. **Kathleen Schoch** (Washington University, St. Louis) presented studies that elucidated the neuropathogenic consequences of two isoforms of microtubule-associated protein tau (MAPT) generated by alternative splicing. Using ASOs that modulate MAPT splicing, she showed that the four (4R) repeat isoform is associated with tau-mediated neurodegeneration. Treatment of mice that express the human mutant MAPT (with elevated 4R-tau) with these ASOs reduced tau accumulation and improved an impaired movement phenotype, suggesting therapeutic applications of ASOs to treat 4R-tauopathies. **Brian Adams** (Harvard University) used profiling studies to reveal a severe loss of miR-34a in triple-negative breast cancer (TNBC), leading to misregulation of c-Src. These insights provide powerful prognostic value and opportunities to develop targeted anti-tumor therapies, as reintroduction of miR-34a into TNBC cell lines inhibited proliferation and promoted sensitivity to the drug Dasatinib, and introduction of miR-34a into tumors in mice dramatically reduced tumor growth. Finally, while the canonical, textbook view of the translation initiation pathway has been well-characterized, little is known about in vivo functions and specific mRNA targets of non-canonical translation factors. **Kent Duncan** (U. Medical Center Hamburg-Eppendorf) described the first in vivo analysis in animals of the non-canonical translation factor, Ligatin/eIF2D, which has revealed a surprisingly specific role in synaptic function in the *Drosophila* nervous system.

Session IV: Delivery

Co-Chairs: David Blakey, PhD, *MiNA Therapeutics*

Rudy Juliano, PhD, *University of North Carolina*

David Blakey opened the session by describing the challenges of systemic oligonucleotide distribution including avoiding nucleases and rapid renal filtration, evading uptake by the mononuclear phagocyte (MPS), sequestration by the extracellular matrix and once the target cell is engaged, uptake and escape typically from an endosomal compartment to the cell cytosol; the latter can be extremely inefficient representing less than 1% of the internalised oligonucleotide. With appropriate chemical modification single strand oligonucleotides can distribute broadly (brain is an exception). Nevertheless, targeting approaches would have two major benefits – achieving more drug at the target site and limiting distribution to non-target cells. GalNAc ligand targeting has been used to improve uptake by hepatocytes. Dr. Blakey further described studies that indicate antibodies can be used to deliver oligonucleotides. A range of lipid based nanoparticles have also been used to delivery oligonucleotides.

Steve Dowdy (UCSD School of Medicine, La Jolla) described his recent work on Ribonucleic acid Neutrals (siRNAs) that aid delivery to cells. He highlighted that achieving effective endosomal escape was critical for these molecules and described a high throughput fluorescent screen based on split GFP proteins to identify peptide sequences that could aid their endosomal escape.

Johannes Winkler (University of Vienna) described the use of Darpins (designed ankyrin repeat proteins) to deliver siRNA to tumour cells. Using fluorescently labeled siRNA binding of the Darpin-siRNA to EPCAM positive cells was confirmed and active cellular uptake was demonstrated into an endosomal compartment. Unfortunately, with the conjugate alone little gene silencing in cells was seen although using an endosomal disrupting agent the effects were enhanced indicating that, as with other targeting approaches, endosomal escape is critical and will probably need to be built into the conjugate for successful translation of the approach in vivo.

Bethany Powell Gray (Duke University Medical Centre) described the use of oligonucleotide aptamers as delivery agents for toxins or potent small molecules. She described their lead aptamer E3 (a 36mer) that binds to a range of cancer but not normal tissues.

Brett Schrand (University of Miami) also discussed aptamer targeting but in his talk he described bispecific aptamers in which one aptamer arm targeted the tumour stroma while the other aptamer arm targeted 4-1BB(CD137) a member of the tumour necrosis receptor family that can act as a co-stimulatory molecule for activated T-cells and so is of great interest in the cancer immunotherapy field.

Rudy Juliano (University of North Carolina) described recent work on small molecules that enhance the pharmacological effects of antisense and siRNA oligonucleotides. The small molecule enhancers act by selectively releasing oligonucleotides from the late endosome compartment thus allowing increased access to the cytosol and nucleus.

Zheng-Rong Lu (Case Western Reserve University) described siRNA delivery using lipid nanoparticles that were pH sensitive and stabilized by disulphide bridges (ECO/siRNA).

Anders Høgset (PCI Biotech) described photochemical internalization (PCI) as an approach to the problem of endosomal entrapment of oligonucleotides. In PCI a photosensitizing compound designed to localize in endosome membranes is used. Illumination triggers photochemical reactions that lead to permeabilization and release of entrapped molecules to the cytosol.

Katharina Meijboom (University of Oxford) presented on splice switching antisense oligonucleotides as a potential therapy for spinal muscular atrophy (SMA) in animal models and in clinical trials, focusing on cell penetrating peptide conjugates to allow crossing of the blood brain barrier.

Session V: Oligonucleotide Safety: Issues, Mechanisms, Mitigations

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

Sebastien Burel (ISIS Pharmaceuticals), spoke about the potential for hepatotoxicity due to bridged nucleic acid (BNA) antisense oligonucleotides (ASOs). Previously, there had been controversy over a conclusion from the Isis pharmaceuticals team that locked nucleic acid (LNA) oligonucleotides were more prone to toxicity than methoxyethyl oligonucleotides that contain a less high affinity substitution. In his talk, Burel noted that the tendency for some ASOs to be toxic was a property of other high affinity substitutions, including Isis' bridged cET chemistry. The mechanism of hepatotoxicity involves hybridization to off-target transcripts and is RNase H-dependent. The mechanistic understanding providing by this study can guide the design process and increase the likelihood that development candidates will avoid hepatotoxicity.

Hiroki Kato (Kyoto University) examined the link between the RNA sensor MDA5 and autoimmune disorders. MDA5 is a viral sensor that binds long duplex RNA. Mice with mutant MDA5 develop Lupus-like nephritis. He described an ASO that induced sequence-specific toxicity through an MDA5-dependent mechanism. **Ann Durbin** (Harvard University and MIT) examined how the introduction of modified nucleotides would affect RIG-I activation. She concluded that naturally occurring modifications have the potential to suppress the RIG-I response. **Peter Jarver** (Stockholm University) investigated the structural requirements for oligonucleotide TLR3 antagonists. He concluded that single-stranded RNA-based oligonucleotides can bind TLR3 with high affinity. The final talk of the session was from **Ivan Zlatev** (Alnylam). He suggested that it might be useful to possess single-stranded compounds that could be administered to patients and reverse the effect of previously administered duplex RNAs. These compounds were designed to compete for binding to the guide strand RNA of the initially administered duplex. He showed that these compounds, termed "reversers" were able to block the activity of duplex RNAs within a few days of administration.

Session VI: Early Drug Discovery

Co-chairs: Marc Abrams, PhD, *Dicerna Pharmaceuticals*

Willeke van Roon-Mom, PhD, *Leiden University Medical Center*

Punit Seth (Isis Pharmaceuticals) illustrated the development of an allele selective suppression of mutant huntingtin by SNP targeting antisense oligonucleotides. Incorporation of modified nucleotides in the Gap region of a Gapmer ASO yielded ASOs with 100x selectivity for the mutant allele. This works by preventing RNase H-mediated cleavage of the mismatched transcript. Elegant modelling and biochemical assays confirmed this. Interestingly, the lead SNP-targeting ASOs also were matched perfectly to another unrelated target (BMPRA1). Modification screening and target counter screening were used to reduce off-target activity. In an transgenic model, a single bolus injection of 75ug achieved maximum efficacy. **Thomas Thum** (Hannover Medical School) presented on short and long non coding RNA therapeutics for cardiac remodelling. Both lncRNAs and miRNAs are implicated in cardiovascular disease. MicroRNA-24 is dysregulated in myocardial infarction and an LNA antagonist reduced heart size in a pig model of myocardial infarction. Secondly, microRNA-132 knock out mice showed cardiomyocyte hypertrophy and blockage of these miRNAs prevented stress-induced cardiac hypertrophy, fibrosis and heart failure. Finally, a lentiviral shRNA library targeting lncRNAs was used in a cardiomyocyte-based functional screen and identified the lncRNA CHAST. In vivo overexpression of CHAST in cardiac myocytes resulted in increased heart size while silencing with an LNA gapmer targeting CHAST in a mouse model of aortic constriction was efficacious. **Nicole Datson** (BioMarin) presented on the therapeutic benefit of a huntingtin-lowering antisense oligonucleotide targeting the CAG-repeat in the R6/2 Huntington's disease mouse model. Ninety percent knock down was observed throughout the brain after six weekly intracerebroventricular, leading to a reduction in huntingtin protein and improved performance in motor skill endpoints. The mice showed an increase in brain volume and a shift in the striatal metabolite profile was also observed. This ASO is applicable to other CAG repeat diseases. **David S. Greenberg** (The Hebrew

University of Jerusalem) presented about an anti-MicroRNA-132 oligonucleotide as a potent treatment for non-alcoholic fatty liver disease. Non-alcoholic fatty liver diseases are probably due to high fat diet, affects 25% of population and there are few therapeutic options. Systemic administration of an antagomiR targeting miRNA-132 (either LNA or 2'-O-Me chemistry) reduced steatosis and related phenotypes. **Alexander McCampbell** (Biogen) presented on antisense oligonucleotides for SOD1 to improve function and extend life of SOD1-G93A mice. Intracerebroventricular injection significantly improved survival and neuronal function without an effect on central nervous system inflammatory markers. It delayed onset, increased survival, animals preserved mobility throughout, CMAP assay of neuromuscular function and muscle fibre diameter assay improved.

Session VII: Late Drug Discovery

Co-chairs: Rachel Meyers, PhD, *Alnylam Pharmaceuticals*
Veit Hornung, MD, *University Hospital Bonn*

Gunther Hartmann (University Hospital Bonn) presented an overview of PRRs with an emphasis on RIG-I activation as a strategy to treat melanoma and a variety of other cancers. Having synthesized a small specific RIG-I activator (ImO1100), he showed efficacy in multiple tumor models using reduction of tumor growth type I IFN levels as a measure of RIG-I activity. The antitumor response resulted in long term memory as rechallenged mice were protected. In addition, combination strategies with RIG-I activators + PD1 inhibitors or irradiation showed an enhanced effect. **Andy Turnball** (AstraZeneca) shared work done in collaboration with Regulus Therapeutics to develop antagomir to miR-103/107 for the treatment of diabetes and NASH. Though miR-103/107 is ubiquitously expressed, it is upregulated in liver so they generated a GalNAc targeting Gen 2.5 antagomir (RG-125/AZD4076) for optimal specificity. This antagomir induced positive results on many glucose measures in an animal model of diabetes. **Bruce Wentworth** (Sarepta) introduced the phosphorodiamidate morpholino oligomer platform for treating rare and infectious diseases. A new peptide conjugated morpholino is currently being developed to target New Delhi Metallo-beta-lactamase-1 (NDM-1), an enzyme that renders bacteria insensitive to a broad range of antibiotics of the beta-lactam family (so called "superbugs"). **Rachel Meyers** (Alnylam) provided an update on the ESC-GalNAc technology. New data on TTRsc02, the latest GalNAc conjugate targeting TTR for the treatment of TTR-Amyloidosis, was presented. Changing gears, **Pavlina Konstantinova** (uniQure) presented data on the development of AAV5-miHTT a miRNA mimic delivered via AAV vector for the treatment of Huntington's disease.

Session VIII: Awards Presentations & Talks

Chair: Masad Damha, PhD, *McGill University*

Dr. Alan Gewirtz Memorial Scholarship. Established in 2011 as a tribute to the life and work of Alan Gewirtz, this award is given to outstanding new scientists and recognizes their contributions in the field of oligonucleotide therapeutics. This year's recipient, **Jana McCaskill** (University of Edinburgh) presented on the use of host-targeted miRNA mimics to suppress respiratory viral infection, and showed that four candidates caused broad-spectrum antiviral effects against all tested strains of influenza and respiratory syncytial virus. Preliminary insights were also given into the potential mechanism of action, with miRNAs causing the down-regulation of host factors previously shown to be important for influenza virus infection.

2015 Mary Ann Liebert Publisher Young Investigator Award. This award recognizes the outstanding achievements and contributions by a young scientist in the field of oligonucleotide therapeutics who has recently received his or her advanced professional degree. This year's recipient, **Frank Rigo** (Isis Pharmaceuticals) presented his progress on developing an ASO for the treatment of C9orf72 caused ALS/FTD. This disease is caused by a hexanucleotide repeat expansion (G₄C₂) in the first intron of C9orf72, a gene with unknown function. Work in rodents, though generation of KO mice and BAC Tg mice, suggests a toxic-

gain-of function mechanism of disease. An RNase H ASO administered to the CNS of BAC Tg mice was able to reduce pathological hallmarks of the disease, such as RNA foci and dipeptide repeat proteins.

Lifetime Achievement Award (LAA). This award recognizes an individual who has demonstrated a lasting commitment to the field of oligonucleotide therapeutics through fundamental and sustained contributions to education, research, and therapeutic application. **Fritz Eckstein** (Max-Planck-Institute for Experimental Medicine), the inaugural recipient of this award, delivered a wonderful lecture entitled “**50 Years of Phosphorothioate Nucleic Acids**”. The key experiment, in 1966, was the observation that adenosine 5'-phosphorothioate was resistant to alkaline phosphatase, a property later shown to apply to all phosphorothioate monoesters. This surprising result stimulated the synthesis of phosphorothioate diesters which were isolated as pairs of diastereomers. The Rp-diastereomer, whose configuration had been determined by X-ray structural analysis, permitted to determine the stereochemistry of cleavage by RNase A. This was the first example of approximately 50 enzymes involved in phosphate or nucleotidyl transfer to be characterised by the phosphorothioate approach, clarifying an in-line mechanism for most. Eckstein's observations that these building blocks are resistant to enzymatic hydrolysis became the basis for the antisense-methodology for the inhibition of gene expression with phosphorothioate oligonucleotides, two of which have been clinically approved, and many others are in clinical trials. These results were instrumental in identifying phosphorothioates in bacterial DNA where they are present in isomerically (Rp) pure form.

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Session IX: Clinical Studies

Co-chairs: Brett Monia, PhD, *Isis Pharmaceuticals*

Art Krieg, MD, *Checkmate Pharmaceuticals*

Akshay Vaishnav (Alnylam Pharmaceuticals) presented an overview of Alnylam's liver-targeting siRNA therapeutic strategy. This strategy is focused on genetically-defined targets utilizing an evolving GalNAc-conjugation strategy that improves both potency and durability in the liver. The first clinical program utilizing this strategy is Revusiran, a GalNAc-siRNA targeting transthyretin for TTR-related cardiomyopathy, currently in Phase 3 development. In addition to an overview of the Revusiran program, a new program utilizing an improved GalNAc design that targets antithrombin 3 for the prevention of bleeding events in patients with hemophilia was highlighted. Encouraging early Phase I data in hemophilia patients has shown evidence for reduced annualized bleeding events with good tolerability. This program is projected to advance rapidly to pivotal Phase 3 studies.

Mark Edbrooke (AstraZeneca) presented Phase 1B results of AZD9150 (ISIS481464), a Generation 2.5 (constrained ethyl phosphorothioate) antisense oligonucleotide targeted to the transcription factor STAT3. Emphasis was placed on preclinical results demonstrating superior activity for STAT3 knockdown in preclinical animal tumor models with Generation 2.5 ASOs over earlier generation antisense chemistries. Furthermore, Phase I data was presented demonstrating durable clinical responses in patients with refractory diffuse large B-Cell Lymphoma and in hepatocellular carcinoma with evidence of a tumor microenvironment mechanism of action. AZD9150 has now progressed to Phase 2 development in cancer. In addition, AZD5312 (ISIS 560131), a Generation 2.5 antisense oligonucleotide targeting the androgen receptor for prostate cancer was touched on and evidence of clinical efficacy was presented.

Joe Witztum (University of California, San Diego) presented an overview of triglyceride metabolism and the role of apoCIII as a central player in diseases related to hypertriglyceridemia including familial chylomicronemia (FCS), severe non-FCS hypertriglyceridemia, and diabetes. Following this introduction, the clinical program for Volanesorsen (ISIS 304801), a second generation 2'-methoxyethyl phosphorothioate antisense oligonucleotide targeted to the liver expressed lipoprotein apoCIII, was reviewed. Volanesorsen has successfully completed Phase I and Phase II studies with good tolerability. Moreover, substantial reductions in apoCIII and triglyceride levels have been demonstrated in multiple patient populations in Phase II, including FCS patients, severe non-FCS hypertriglyceride patients and type II diabetics. In addition, improved insulin sensitivity was

demonstrated in type II diabetics. Volanesorsen is currently under Phase III evaluation in FCS patients.

Bruce Given (Arrowhead Research Corporation) presented an overview of the development of 3 different approaches for RNAi delivery including i) unconjugated dynamic polyconjugates (DPC) for hepatic delivery, in development for HBV therapy with early positive results; ii) conjugated DPC for extra-hepatic applications; and iii) a subcutaneous delivery platform for hepatic delivery using GalNac, in development for targeting apa(a).

Jerry Horan (Celgene) provided an overview of the oral antisense program using first generation phosphorothioate ASO designed to target Smad7, called Mongerson, which is now moving into phase 3 clinical development based on positive phase 2 results. The ASO is formulated in modified release tablets with a pH sensitive coating designed to release the drug in the distal portion of the gut.

Paul Lammers (Mirna Therapeutics) described the clinical development of the first miR mimic, of the tumor-suppressor miR-34. The unmodified RNA is delivered IV using a lipid nanoparticle that avoids TLR activation. Several responses have been seen in the dose escalation studies using 3 different dose regimens, and biomarker studies on WBC have shown the expected changes in expression of miR-34 target genes.

The final talk of the session was presented by **Andreas Kuhn** (BioNTech RNA Pharmaceutical GmbH), who presented the evolution of their work on mRNA vaccines for cancer immunotherapy. The initial approach that went into clinical trials in 2012 used mRNA encoding conserved tumor antigens, and did not reach an MTD in 28 patients. Since 2013 they have changed strategy to vaccinate against patient-specific neoantigens by sequencing tumors, using bioinformatics for epitope prediction, and then cloning 5 predicted class I and II epitopes into a single mRNA. Several patients have shown neoantigen-specific T cell responses.