Preconference Session: NextGen Early Career Scientist Meeting
Jonathan Watts

Sunday, 13 October
10.05-10.35

The First Few Bases: Career and Chemical Modification

Jonathan K. Watts
RNA Therapeutics Institute, UMass Medical School, Worcester, MA, USA

This plenary talk of the early career scientist symposium has been invited to be primarily about career trajectory and advice for trainee scientists. I will weave this information together with recent research results from my laboratory. In particular, this talk will describe some of our work on "tissue-specific medicinal chemistry" with a focus on optimization of antisense oligonucleotide chemistry for the lung and brain.

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Preconference Session: NextGen Early Career Scientist Meeting
Ysobel Baker

Sunday, 13 October
10.35-10.51

Backbone-modified locked nucleic acids for therapeutic antisense applications

Ysobel Baker, Jinfeng Chen, Cameron Thorpe, Pawan Kumar, Afaf El-Sagheer, Tom Brown
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Oligonucleotides with artificial backbones which mimic the natural phosphodiester linkage often show enhanced stability toward nucleases and have found applications in synthetic biology, nanotechnology, and gene synthesis. Whilst the incorporation of charge neutral analogues reduces the overall negative charge, these linkages typically reduce the thermal stability of the corresponding duplex, limiting their potential as therapeutics. In contrast, incorporation of conformationally restrained locked nucleic acid (LNA) into DNA improves the thermal stability of DNA:RNA duplexes. Therefore, we sought to combine the favourable properties of charge neutral linkages with those of LNA to create a new type of antisense oligonucleotide.

We have developed efficient strategies to prepare oligonucleotides containing multiple charge neutral linkages flanked by LNA on their 5′-side, 3′-side, or on both sides. The resulting oligonucleotides were found to bind their RNA targets with higher specificity and affinity that the corresponding unmodified DNA and have enhanced stability towards enzymatic degradation. We believe that these properties, along with the overall reduced anionic charge, make this class of oligonucleotides promising therapeutic agents and we are now carrying out cellular uptake and gene inhibition studies. I will present the synthesis, thermal and nuclease stability studies, along with crystal structures of the most promising candidates, and discuss preliminary data from exon skipping cell assays.

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Preconference Session: NextGen Early Career Scientist Meeting
Lonneke Verboon

Sunday, 13 October
10.51-11.07

Deciphering the interactive network of the *DLK1-DIO3* locus in hematopoiesis and pediatric leukemia

Verboon L[1], Schneider D[2], Xu J[3], Heckl D[1] and Klusmann JH[1]
[1]Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; [2]Hannover Medical School, Germany; [3]UT Southwestern, Dallas, USA.

Non-coding RNAs (ncRNAs) have emerged as central regulators of chromatin and gene expression. The expression patterns of ncRNAs, especially long noncoding RNAs (lncRNAs), are highly cell-type specific, posing a novel window for targeted therapies in pediatric acute megekaryoblastic leukemia (AMKL). Our group previously profiled mRNAs, microRNAs, and lncRNAs in the human normal and malignant hematopoiesis, with a special focus on pediatric acute myeloid leukemia (AML). We discovered that the *DLK1-DIO3* locus was highly expressed in human CD34+ hematopoietic stem and progenitor cells (HSPCs), megakaryocytes (MKs), and AMKL.

To gain insight into the role of the *DLK1-DIO3* locus in normal haematopoiesis and to explore the therapeutic potential of this locus in AMKL, we performed ChIP-Seq for human HSPCs, MKs and monocytes, which showed cell type specific activating H3K3me3 and repressing H3K27me3 histone marks. RNA-seq data confirmed cell type specific expression of the *DLK1-DIO3* locus. The ncRNAs from this locus are transcribed as one huge polycistrone of which the transcription starts at the *MEG3* gene. Using Bisulphite sequencing, a significant correlation between *MEG3* expression and the methylation status of a CpG island downstream of the first exon of *MEG3* was revealed. When deleting the transcription start site of *MEG3* with a CRISPR-Cas9 vector containing two sgRNAs, proliferation of AMKL cell lines was impaired. Single cell clones from K562 and HEL cells showed a more mature phenotype upon deletion of *MEG3*.
In addition, the *DLK1-DIO3* locus contains the largest microRNA cluster in the human genome with 54 microRNA. Lentiviral expression of several highly expressed miRNAs of the *DLK1-DIO3* locus in CD34+ HSPCs resulted in accelerated megakaryocytes maturation *in vitro*. Demonstrating the importance of several microRNAs in the formation of MKs.

Using different approaches, we propose the *DLK1-DIO3* locus is an important regulator of megakaryopoiesis with different members controlling this process. Our studies provide the foundation for further investigations towards targeted therapies in AMKL.

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Preconference Session: NextGen Early Career Scientist Meeting
Reka Haraszti

Sunday, 13 October
11.07-11.23

Artificial exosomes for oligonucleotide delivery to the brain

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Exosomes can serve as delivery vehicles for therapeutic oligonucleotides. However, the components necessary and sufficient to support exosomal delivery have not yet been established. Here, we connect biochemical composition and activity of natural exosomes to create effective artificial exosomes for siRNA delivery. We first compare natural exosomes from serum-deprived and control mesenchymal stem cells (frequently used exosomal source cells) and show that serum-deprived cells produce exosomes up to-twenty-two-fold more effective at delivering siRNAs to neurons. Based on the hypothesis that membrane composition defines trafficking properties we conducted extensive proteomic and lipidomic analyses of exosomes from serum-deprived and control cells. Correlating biochemical composition with biological activity we define six protein pathways and one lipid class, dilysocardiolipin, to potentially contribute to exosomal delivery of siRNAs. After testing each candidate as part of a proteoliposome, we identify an "artificial exosome" composition, in which of one lipid (dilysocardiolipin) and three proteins (Rab7, Desmoplakin, and AHSG) are incorporated into conventional neutral liposomes. These artificial exosomes mimic the properties of natural exosomes to deliver siRNAs to neurons *in vitro* and *in vivo*. Furthermore, artificial exosomes are on par with natural exosomes at delivering AAVs and CRISPR.

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Preconference Session: NextGen Early Career Scientist Meeting
Alejandro Garanto

Sunday, 13 October
11.23-11.39

Splice modulation therapy for a variety of *ABCA4* mutations underlying Stargardt disease

Alejandro Garanto[1,2], Mubeen Khan[1,2], Riccardo Sangermano[1,2], Tomasz Tomkiewicz[1,2], Sarah Naessens[3], Miriam Bauwens[3], Carel B. Hoyng[2,4], Frauke Coppieters[3], Silvia Albert[1,2], Michael E. Cheetham[5], Elfride De Baere[3], Frans P.M. Cremers[1,2], Rob W.J. Collin[1,2].
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Our proof-of-concept work on the correction of a recurrent splicing defect underlying *CEP290*-associated Leber congenital amaurosis (LCA) has led to the initiation of a clinical trial, with recently announced promising results, demonstrating safety and in some subjects efficacy of intra-ocular AON delivery in LCA patients. Here, we expand the use of antisense oligonucleotides (AONs) for the treatment of another subtype of inherited retinal disease caused by mutations in the *ABCA4* gene: Stargardt disease (STGD1).

We established a panel of *ABCA4* midigenes together encompassing the complete *ABCA4* gene, with which the effect of any *ABCA4* variant on pre-mRNA splicing can readily be assessed *in vitro*. For variants resulting in aberrant *ABCA4* splicing, AONs were designed, and co-transfected with the midigenes to assess their correction efficacy. For several variants, patient-derived fibroblasts and/or induced pluripotent stem cell (iPSC)-derived photoreceptor progenitor cells (PPCs) were treated with the AONs, and rescue efficacy was determined by RT-PCR analysis.

We identified more than a dozen different *ABCA4* variants that affect pre-mRNA splicing. The majority of variants resides in introns, leading to a pseudoexon (PE) insertion, in some cases in a retina-specific manner. Another, highly recurrent *ABCA4* variant (c.768G>T) affects the last nucleotide of exon 6, and leads to an exon extension of 35 nt, thereby disrupting the reading-frame. For all variants that resulted in PE insertion, this process could be corrected by one or more AONs targeting the corresponding PE. Intriguingly, also the 35-nt extension caused by the c.768G>T variant could be rescued completely by blocking the alternative splice donor site in intron 6, forcing the spliceosome to use the original splice donor site of exon 6.

AONs appear to be an effective and versatile tool to correct different types of splice defects that are caused by *ABCA4* mutations. Given the promising data obtained so far in a clinical trial for *CEP290*-associated LCA, AONs may serve as a more broadly applicable therapeutic strategy, not only for Stargardt disease but potentially also for other retinal diseases.

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Preconference Session: NextGen Early Career Scientist Meeting
Thomas Michler

Sunday, 13 October
11.39-11.55

RNAi-suppression of Hepatitis B Virus antigens enables therapeutic vaccination to induce curative CD8 T-cell responses in mice

Thomas Michler[1,2 *], Anna Kosinska[1*], Julia Festag[1], Till Bunse[1,2], Mathias Heikenwalder[1,3], Dirk Grimm[3], Stuart Milstein[4], Laura Sepp-Lorenzino[4], Percy Knolle[5] and Ulrike Protzer[1,2]

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250 million people world-wide are chronically infected with Hepatitis B Virus (HBV) causing 880.000 annual deaths. Cure is highly desired but rarely achieved with current therapies. HBV persistence was found to correlate with a dysfunctional T-cell response and high levels of HBV antigens were proposed as causative agent. We evaluated the capacity of stabilized, liver-targeted siRNAs to suppress HBV-antigen expression, and to allow recovery of HBV-specific B- and T cell responses either spontaneously or after therapeutic vaccination. High viremic HBV-transgenic mice were treated with standard of care nucleoside analogue entecavir, an shRNA-expressing Adeno-Associated Virus vector (AAV-shHBV) or N-Acetylgalactosamine (GalNAc)-conjugated siRNAs. B- and T-cell immunity were monitored following therapeutic vaccination with an HBV core and surface protein prime vaccination and a Modified Vaccinia Ankara virus - boost immunization. RNAi administered monthly at 3 mg/kg GalNAc-siRNA (s.c.) or $1x10^{11}$ geq AAV-shHBV (i.v.) efficiently suppressed HBsAg and HBV DNA by 2-3 log10 and HBeAg by >1 log10. Entecavir strongly reduced HBV DNA by 4 log10, but antigen levels remained unchanged. Therapeutic vaccination induced HBV-specific B-cell immunity and CD4 T cell responses independent of antigen levels, but HBV-specific CD8 T-cell responses were only seen in animals with reduced antigen levels after RNAi administration. Induction of CD8 T-cell responses coincided with suppression of HBV replication in the liver to levels undetectable by Southern blot or PCR. We then evaluated if the combinatorial RNAi and vaccination therapy could achieve cure of chronic hepatitis B. For this, high-titre, persistent HBV replication was induced in C57/Bl6 mice by transduction with $2x10^{11}$ geq of an AAV vector carrying a 1.2-overlength HBV genome. 3 monthly doses of GalNAc-siRNAs followed by therapeutic vaccination reduced HBsAg and HBeAg by 5 and 4 log10, respectively, to levels below the detection limit, and triggered seroconversion to anti-HBs and anti-HBe. Control of HBV replication was maintained for >5 months after the last siRNA dose. In conclusion, we developed a combinatorial RNAi and therapeutic vaccination therapy for hepatitis B that achieved long-term functional HBV cure in a preclinical mouse model, suggesting potential for clinical translation.

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Preconference Session: Oligonucleotide Therapeutics Education Workshop
Eduardo Paredes

Sunday, 13 October
13.35-14.05

Oligonucleotide process planning, development and scale-up: CMC strategy for sustainable manufacturing

Eduardo Paredes
Nitto Avecia

Oligonucleotide manufacturing technology has advanced significantly now allowing for the manufacture of synthetic oligonucleotides with ease. Still, advents in oligonucleotide design and decoration require diligent attention to manufacturing routes to ensure the highest quality of oligonucleotide APIs are manufactured. For this, several case studies are presented to highlight important considerations for specialty raw materials, manufacturing route vetting and scale-up consistency. A robust strategy in partnership with Avecia can ensure that manufactured processes are planned efficiently, developed adequately, and scaled-up reliably.

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Preconference Session: Oligonucleotide Therapeutics Education Workshop
Ekkehard Leberer

Sunday, 13 October
14.05-14.35

MiRNA Drug Development from Lead Selection to Phase 2

Ekkehard Leberer
Sanofi

The non-coding genome makes up 98.8% of the human genome. Most of this non-coding genome is transcribed into non-coding RNAs that may play an important role in cellular regulation in health and disease; these non-coding RNAs could be novel targets for future medicines.

MicroRNAs are short non-coding RNAs that regulate biochemical pathways and networks of pathways by the mechanism of RNA interference (RNAi). MicroRNA-21 has been implicated in multiple organs as a microRNA associated with fibrotic diseases and cancer (1).

The presentation will summarize the opportunities and challenges of developing microRNA-based drugs and will illustrate the successful generation of an anti-fibrotic microRNA-based therapeutic approach that we have developed in collaboration with our strategic partner Regulus (www.regulusrx.com) by targeting microRNA-21 with an antisense oligonucleotide (anti-miR-21) (1). This microRNA-based drug is now further developed by Sanofi in a phase 2 clinical trial for a fibrotic kidney disease called Alport Syndrome (2, 3).

I will use this therapeutic program as an example to describe the challenges of providing drug-like properties to oligonucleotides, and I will summarize the approaches that we have applied to overcome these challenges. These approaches include chemical modifications of the oligonucleotide backbone and side chains for chemical stabilization and for improving the intracellular delivery of the lead oligonucloetide. Moroever, I will use this example to summarize strategies for a translational path of moving an oligonucleotide drug candidate from animal pharmacology studies into phase 2 of clinical development.

(1) Chau B.N. et al.: MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. Sci. Transl. Med. 4, 121ra18 (2012)
(2) Gomez I.G. et al.: Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. J. Clin. Invest. 125, 141-156 (2015)
(3) Guo J.,…, **Leberer E.** et al.: Dysregulated expression of microRNA-21 and disease-related genes in human patients and in a mouse model of Alport Syndrome. Hum. Gene Ther. 30, 865-881 (2019)

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Preconference Session: Oligonucleotide Therapeutics Education Workshop
Anastasia Khvorova

Sunday, 13 October
14.35-15.05

Oligonucleotide Therapeutics: Technology Evolution for Clinically Relevant Tissue Delivery

Anastasia Khvorova
RNA Therapeutics Institute, University of Massachusetts Medical School

A decade of progress in oligonucleotide chemistry and formulation development has resulted in several compounds, both siRNAs and antisense, demonstrating robust clinical activity. Based on sequence, these types of drugs can be designed to modulate the expression of any disease-causing gene.

Clinical utility is defined by the ability to deliver to the tissue of interest. The most promising approach is modulation of oligonucleotide delivery through direct chemical modification. Full chemical stabilization, identification of optimal conjugates (GalNAc and others), and understanding the relationship between structure and pharmacokinetic/pharmacodynamic behavior are essential to enabling sustained (many month) efficacy following a single administration.

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Preconference Session: Oligonucleotide Therapeutics Education Workshop
Thomas Rupp

Sunday, 13 October
15.30-16.00

Current CMC and Regulatory Aspects for Oligonucleotide Therapeutics

Mr. Thomas Rupp
Thomas Rupp Consulting

21 years after the first market authorization of an antisense drug: Fomivirsen in 1998, the number of oligonucleotides in early to late stage clinical development has grown to approximately 550. After the conditional approval of Volanesorsen by the EMA in May 2019, which succeeded the market authorization of Patisiran and Inotersen by FDA and EMA in late 2018, a total of 9 (10) oligonucleotides (five antisense transcription inhibitors, two antisense splice modulators, one aptamer, one siRNA, and one immune stimulatory vaccine adjuvant) have so far been approved as therapeutics. Yet, as of today, there are no formal guidelines issued by local health authorities to regulate specifically the CMC strategy-development for therapeutic oligonucleotides, and as a result, agencies treat each IND individually on a case-by-case base, and drug sponsors interpret existing small molecule and/or biologicals guidelines. This presentation will provide an overview of the current status of key CMC considerations in early stage drug development.

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Preconference Session: Oligonucleotide Therapeutics Education Workshop
Brooke Rock

Sunday, 13 October
16.00-16.30

A Bioanalytical Toolkit to Support Oligonucleotide Therapeutics Throughout the Drug Development Pipeline

Brooke Rock, Amgen, Inc.

Oligonucleotides are a quickly involving modality with strong proof of concept now established in the clinic. Despite the clinical success of oligonucleotides, there is still limited understanding of ADME (absorption, disposition, metabolism and excretion) in comparison to other modalities (e.g. small molecules). In general, the physicochemical properties of oligonucleotides push this new modality outside of the small molecule classification; however, there exist opportunities to apply learnings from small molecules to underwrite the pharmacokinetics-pharmacodynamic (PK-PD) relationships, inform structural integrity, and understand biodistribution. The presentation will discuss the above three topics along with the strategy to effectively characterize oligonucleotides from early discovery into the clinical setting by discussing novel, robust, sensitivity bioanalytical tools.

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Session I: Nucleic Acid Chemistry
Eriks Rozners
Monday, 14 October
8.45-9.10

Sequence Selective Recognition of Double-Stranded RNA by Cationic Nucleobase and Backbone-Modified Peptide Nucleic Acids

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The important roles that non-coding double-stranded RNAs (dsRNA) play in biology and development of disease makes them attractive targets for molecular recognition. However, designing of small molecules that selectively recognize RNA has been a challenging and involved process because RNA helix presents little opportunity for shape-selective recognition. We discovered that cationic nucleobase- and backbone-modified peptide nucleic acids (PNA) bind with high sequence selectivity and low nanomolar affinity to dsRNA via major groove triple helix formation under physiological conditions. Most interestingly, the modified PNAs exhibited unique RNA selectivity and had up to two orders of magnitude higher affinity for the dsRNAs than for the same dsDNA sequences. Recent studies showed that the deep and narrow major groove of RNA presented a better steric fit and hydrogen bonding arrangements for the PNA ligands than the wider major groove of DNA. Conjugation of PNA with short lysine peptides further enhanced binding affinity and cellular uptake of PNA. PNAs carrying M and Lys modifications were efficiently taken up by cells, while the unmodified PNA showed little uptake.

Recently, we found that nucleobase- and backbone-modified PNAs recognized dsRNA sequences present in biologically relevant RNA, such as mRNAs and microRNAs, with high affinity under physiological conditions. In collaboration with Prof. Naoki Sugimoto's group at FIBER, Kobe, Japan, we demonstrated that triplex-forming PNA suppressed translation by forming a highly stable and sequence selective triple helix with a dsRNA region in the 5´UTR of long mRNA both in vitro and in cells. Triple helix formation was also able to inhibit maturation of pre-microRNA hairpins. Taken together our results suggest that the cationic modified PNAs may be promising compounds for modulating the function of biologically relevant dsRNA in live cells. Given the infancy of our understanding of the various roles that RNA plays in gene regulation, it is conceivable that triplex-forming PNAs may become valuable tools for studying biologically relevant dsRNA in live cells. The current presentation will discuss our most recent results on sequence selective recognition of complex biological dsRNA molecules and potential applications of this recognition in biomedical research and biotechnology.

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Session I: Nucleic Acid Chemistry
Hanadi Sleiman
Monday, 14 October
9.10-9.35

DNA nanostructures for cellular delivery of therapeutics

Hanadi Sleiman
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DNA nanotechnology has emerged as an exceptionally programmable method to organize materials. Most current strategies rely on assembling a complex DNA scaffold, often containing hundreds of different strands, and using it to position materials into the desired functional structure. Our research group has developed a different approach to build DNA nanostructures. Starting from a minimum number of DNA components, we create 3D-DNA host structures, such as cages, nanotubes and spherical nucleic acids, that are promising for targeted drug delivery. These can encapsulate and selectively release drugs, oligonucleotide therapeutics and materials, and accomplish anisotropic organization of targeting ligands.

We find that they resist nuclease degradation, silence gene expression to a significantly greater extent than their component oligonucleotides and have a favorable in vivo distribution profile. We designed a DNA cube that recognizes a cancer-specific gene product, unzips and releases drug cargo as a result, thus acting as a conditional drug delivery vehicle; as well as DNA structures that bind to plasma proteins with low nanomolar affinities, thus increasing stability in vivo. We will also describe a method to 'print' DNA patterns onto other materials, thus beginning to address the issue of scalability for DNA nanotechnology. Finally, we will discuss the ability of small molecules to reprogram the assembly of DNA, away from Watson-Crick base-pairing and into new motifs.

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Session I: Nucleic Acid Chemistry
Tom Brown
Monday, 14 October
9.35-10.00

Synthesis and properties of oligonucleotides with triazole, carbamate and amide backbones

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Oligonucleotide analogues that contain artificial backbone linkages in place of canonical sugar-phosphate groups are of potential value in therapeutic applications. The basic requirements of artificial linkages are that they do not inhibit duplex formation and also retard or prevent enzymatic degradation of the oligonucleotide *in vivo*. A more difficult objective is to design backbone analogues that enhance the uptake of oligonucleotides into human cells. We will describe the synthesis and properties of a series of DNA backbone analogues that we designed with the above properties in mind.[1-3] We will also discuss the effects of locked nucleic acid sugar moieties on the properties of such oligonucleotide analogues.

1. Thorpe, C., Epple, S., Woods, B., El-Sagheer, A.H. & Brown, T. Synthesis and biophysical properties of carbamate-locked nucleic acid (LNA) oligonucleotides with potential antisense applications. *Org. Biomol. Chem.* **17**, 5341-5348 (2019).
2. Kumar, P., Truong, L., Baker, Y.R., El-Sagheer, A.H. & Brown, T. Synthesis, Affinity for Complementary RNA and DNA, and Enzymatic Stability of Triazole-Linked Locked Nucleic Acids (t-LNAs). *ACS Omega* **3**, 6976-6987 (2018).
3. Kumar, P., El-Sagheer, A.H., Truong, L. & Brown, T. Locked nucleic acid (LNA) enhances binding affinity of triazole-linked DNA towards RNA. *Chem. Commun.* **53**, 8910-8913 (2017).

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Session I: Nucleic Acid Chemistry
John Chaput
Monday, 14 October
10.00-10.25

Generating Biologically Stable TNA Aptamers that Function with High Affinity and Thermal Stability

John C. Chaput[1,2,3]
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Aptamers generated from natural and modified nucleotides are often prone to nuclease digestion, which limits their utility in many biomedical applications, including therapeutics. Here we describe the in vitro selection of an unnatural nucleic acid polymer system based on α-L-threofuranosyl nucleic acid (TNA) that is completely refractory to nuclease digestion. The use of an engineered TNA polymerase permitted the isolation of functional TNA aptamers that bind to HIV reverse transcriptase (HIV RT) with K_D values of ~0.4 to 4.0 nM. The TNA aptamers remain active in the presence of strong nucleolytic enzymes, where DNA and FANA aptamers rapidly degrade, and exhibit markedly higher thermal stability than monoclonal antibodies (mAbs), which irreversibly unfold at elevated temperatures. The combined properties of biological stability, high target binding affinity, and thermal stability make TNA aptamers a powerful system for further development as future diagnostic and therapeutic agents.

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Session I: Nucleic Acid Chemistry
Marina Zenkova
Monday, 14 October
10.25-10.40

Novel mesyl phosphoramidate antisense oligonucleotides as an alternative to phosphorothioates for efficient silencing of oncogenic microRNA in vivo.

Marina A. Zenkova, Olga A. Patutina, Svetlana K.Miroshnichenko, Dmitrij A. Stetsenko, Catherine A. Burakova, Valentin V. Vlassov
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Forty years of studies proved that antisense oligonucleotides have great potential to target mRNAs of disease-associated genes and noncoding RNAs. Amongst vast number of oligonucleotide backbone modifications, the most widely used in research and clinic is phosphorothioate modification. However, along with merits, there are notable drawbacks of phosphorothioate oligonucleotides such as decreased binding affinity to RNA, reduced specificity and increased toxicity. We report herein on the synthesis and in vitro evaluation of a novel DNA analog, namely, mesyl (methanesulfonyl) phosphoramidate oligonucleotide (mesyl oligonucleotide). A novel type of DNA analogs which substitute mesyl group for the natural phosphodiester group in every internucleotidic position show significant advantages over often used DNA phosphorothioates in their RNA binding affinity, nuclease stability and specificity of their antisense action, which involves activation of cellular RNase H enzyme for hybridization-directed RNA cleavage.
In vitro. Biological activity of the mesyl oligonucleotides was demonstrated with respect to pro-oncogenic miR-21, miR-155 and miR-17. A 22 – 24 nt anti-miR mesyl oligodeoxynucleotides specifically decreased levels of corresponding microRNA in melanoma B16 cells, induced apoptosis, reduced proliferation and impeded migration of tumor cells, showing superiority over isosequential phosphorothioate oligodeoxynucleotide in the specificity of its biological effect. Reduced overall toxicity compared to phosphorothioate and efficient activation of RNase H are the key advantages of mesyl phosphoramidate oligonucleotides, which may represent a new promising type of antisense therapeutics[1].
In vivo. The basic features of mesyl oligonucleotides applied as antitumor therapeutics were investigated. Evaluation of kinetics of tumor growth suppression, systemic toxicity, biodistribution and specificity of action as well as primary analysis of pharmacokinetic parameters of new analogs were performed in comparison with phosphorothioate oligonucleotides. Using drug-resistant model of human epidermoid carcinoma KB-8-5/SCID mice it was shown that peritumoral administration of anti-miR-21 mesyl oligonucleotide pre-complexed with folate-containing liposomes provides effective accumulation of therapeutic oligonucleotide in tumor tissue leading to 8-fold reduction in primary tumor growth. It was accompanied by significant increase in the expression of direct protein targets of miRNA-21 such as PTEN and PDCD4 in tumor tissue caused by manifold reduction in miRNA-21 level. No effects on the expression of other miRNAs were observed. Morphometric investigation of liver and kidneys as well as biochemical blood analysis demonstrated that mesyl oligonucleotides are devoid of pronounced toxicity in respect to liver and kidneys. The data obtained give evidence that mesyl-oligonucleotides represent novel vigorous implement of antisense technology.

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Session I: Nucleic Acid Chemistry
Ken Yamada
Monday, 14 October
10.40-10.55

Structurally Constraining of Inter-nucleotide Linkage Impacts on siRNA Potency and Allele Specificity

Ken Yamada[1, 2], Loic Roux[1, 2], Sarah Davis[1,2], Faith Conroy[1, 3], Julia Alterman[1, 3], Dimas Echeverria[1, 2], Edith Pfister[1, 3], Neil Aronin[1, 3], Anastasia Khvorova[1, 2]

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Phosphate backbone stabilization has a large impact on metabolic stability of oligonucleotides. In the RNA therapeutics field today, chemically stabilized synthetic RNAs are a requirement for prolonged therapeutic effects. To date, various phosphate analogues have been reported, however, phosphorothioate (PS) is the only backbone modification used extensively in the field, proving its high compatibility with RNAi, RNaseH, and CRISPR-Cas systems.[1,2] While PS is robust and has proven to be beneficial, multiple PS modifications are potentially toxic and can be positionally limited depending on the gene regulating species (antisense, siRNA, CRISPR, etc.). To further stabilize siRNAs, several RNAi therapeutics research groups, including our lab, have been investigating "*in vivo* potent" siRNA with 5′-(*E*)-vinylphosphonate, 5′-(*E*)-VP. This modification enables higher siRNA potency *in vivo*, which attributed to its high phosphatase resistance and its shape-fitting in 5′-phosphate binding site in Argonaute 2.[3] Motivated to develop alternative phosphate backbones beyond phosphorothioate and with the benefit of 5′-(*E*)-VP on siRNA in mind, we began investigating the potential benefits of the (*E*)-VP structure as an "inter-nucleotide" backbone modification for metabolic stabilization and/or other novel effects on siRNA arising from VP's local stereo-constraint of the siRNA backbone. To survey the effect of the inter-nucleotide (*E*)-VP modification on RISC function a panel of siRNAs was synthesized using synthetic (*E*)-VP- nucleotide dimer phosphoramidites, in which every inter-nucleotide position of the guide strand was (*E*)-VP modified individually. To our surprise, (*E*)-VP showed significant position-specific modulation of RISC activity, both positively and negatively. In addition, we found that structural constraint of the guide strand drastically enhanced Single Nucleotide Polymorphism (SNP)-based target mRNA discrimination. The data presented here is the first description of the structurally "locked" inter-nucleotide phosphate analogue, (*E*)-VP, as a new paradigm of backbone modification for enhancement of stability, efficacy, and specificity of therapeutic siRNAs.

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Keynote Session:
Thomas Carell
Monday, 14 October
11.25-12.15

Epigenetic base modification and the potential for chemical intervention

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DNA stores genetic information in the form of the sequence of the four canonical nucleosides dA, dC, dG and dT. DNA contains in addition epigenetic information, which is encoded by the four modified cytidine nucleosides 5-methylcytidine (mdC), 5-hydroxymethylcytidine (hmdC), 5-formylcytidine (fdC) and 5-carboxycytidine (cadC) (Fig. 1, left).[1] Additionally, 5-hydroxymethyluridine (hmdU) may play a role as well. The positions and the kind of modified dC-base present at a specific position in the genome establishes an unknown 2nd code. Setting and erasing of these epigenetic bases controls the complete development process starting from an omnipotent stem cells and ending with an adult specialized cell (Fig. 1, right). I am going to discuss results about the function and the distribution of the new epigenetic bases hmdC, fdC and hmdU in the genome[2] and I am showing how metabolic states influence the chemistry involved with setting and erasing these modified nucleosides in the genome. Synthetic routes to the new nucleosides will be discussed that enable the preparation of oligonucleotides containing the 2nd code. Particularly, results from isotope dilution and isotope tracing mass spectrometry will be discussed. They allowed to decipher the pathways of active demethylation.[3] Interesting is the fact that the base excision repair system plays a central role during erasure of the bases. Again mass spectrometry helped us to quantify the repair processes involved in epigenetics.[4] Finally, I am discussing potential präbiotic origins of modified bases[5] and chemicals that are able to modulate the epigenetic information layer.

Figure 1. Illustration of the 2nd code present in DNA (left) and proposal of how the epigenetic bases are interconverted to establish dynamic changes.

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Session II: Nucleic Acid Functional Interference Techniques
Thorsten Stafforst

Monday, 14 October
13.30-13.55

Site-directed RNA Editing by Harnessing ADARs

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The manipulation of genetic information at the RNA transcript is a promising alternative to gene therapies including gene replacement and gene editing as it may overcome some of the major limitations of the latter. These include technical and ethical hurdles resulting from permanent off-target edits, insertional mutagenesis, lack of efficiency in many tissues, and the delivery of several components. A particularly attractive strategy is the harnessing of endogenous ADARs for site-directed RNA editing. We recently described guideRNA designs that enable the recruitment of natural ADARs. These can either be genetically encoded or administered as chemically stabilized antisense oligonucleotides. Interestingly, the ASO chemistry dramatically influences the efficacy and precision of the transcript manipulation. We will give insights into the concept of site-directed RNA editing, which may represent a novel platform for drug discovery.

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Session II: Nucleic Acid Functional Interference Techniques
Martin Egli

Monday, 14 October
13.55-14.20

Structure-Activity Correlations of Novel Sugar and Backbone-Modified siRNA Analogs

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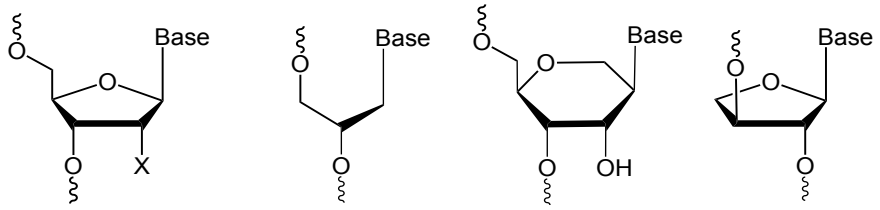
First-generation nucleic acid analogs such as 2′-deoxy-2′-fluoro-RNA (2′-F RNA), 2′-*O*-methyl RNA (2′-*O*Me RNA), and the phosphorothioates (PS-DNA/RNA) have remained staples of oligonucleotide modification strategies in the discovery and optimization of potential RNAi therapeutics.[1,2] The first FDA-approved siRNA drug, patisiran (Onpattro) contains eleven 2′-*O*Me ribonucleotides in the sense and antisense strands formulated in a lipid nanoparticle (LNP) delivery system.[3] Despite their overall appeal in terms of enhanced nuclease resistance, increased pairing stability (2′-F/*O*Me RNAs) and safety, newer analogs are needed to further improve the therapeutic potential of unformulated siRNAs like GalNAc conjugates. Considering the complexity of protein-RNA interactions in RNA interference, it is imperative to expand the process of discovery and optimization of modified siRNAs to new analogs. We have explored the potential benefits for efficacy of incorporating into siRNAs sugar modifications like *N*-methylacetamide (NMA), 5′-*E*-vinylphosphonate (5′-*E*-VP) and, more recently, xeno-nucleic acid (XNA)[5] residues such as glycol nucleic acid (GNA),[6] altritol nucleic acid (ANA),[7] and threofuranosyl nucleic acid (TNA).[8] The presentation will summarize results from structural and modelling studies and correlations with *in vitro* and *in vivo* activities of siRNAs with incorporated novel nucleotides.



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Session II: Nucleic Acid Functional Interference Techniques
Annabelle Biscans

Monday, 14 October
14.20-14.45

siRNA chemical engineering for extra-hepatic delivery

Annabelle Biscans[1], Andrew Coles[1,2], Maire Osborn[1,3] and Anastasia Khvorova[1]
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Achieving robust and sustained extrahepatic delivery is a crucial next step in RNA therapeutics development. Lipophilic conjugation of fully chemical stabilized small-interfering RNA (siRNA) supports significant and effective delivery throughout the body. Therefore, chemically engineering lipid conjugates may be a strategy to improve siRNA delivery to extrahepatic tissues.

In this talk, I will describe recent progress in understanding the relationship between conjugate chemical structure and siRNA pharmacokinetic/pharmacodynamic behavior. We systematically evaluated tissue accumulation and efficacy *in vivo* of a panel of siRNAs conjugated to different classes of lipid moieties—saturated and non-saturated fatty acids, steroids, and vitamins—with or without a phosphocholine polar head group, and a panel of siRNAs conjugated to mono-, di-, and tri-meric fatty acids. Although overall clearance (from 0 to 90%), retention, and the liver/kidney distribution ratio are predominantly defined by conjugate hydrophobicity, the chemical nature of the conjugate significantly impacts efficacy, toxicity, and intra-organ distribution. Slight chemical alterations may have a significant effect on degree of immune cell accumulation and toxicity.

We also systematically evaluated the impact of oligonucleotide structural configuration and conjugate linker chemistry. We demonstrated that both siRNA configuration and linker chemistry alter accumulation, which does not necessarily correlate with efficacy.

Overall, the chemical evolution of engineered conjugate supports functional delivery to a range of tissues, including muscle. We confirmed that a single subcutaneous injection of hydrophobic siRNA targeting myostatin promotes significant muscle growth, validating the potential of conjugated siRNAs to achieve clinically-relevant phenotypes. Therefore, the rational engineering of lipid conjugates is a promising platform for extrahepatic delivery of siRNA, opening up a multitude of tissues for therapeutic intervention.

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Session II: Nucleic Acid Functional Interference Techniques
Frank Rigo
Monday, 14 October
14.45-15.00

Cleavage of pre-mRNA in the nucleus by RNase H1-dependent antisense oligonucleotides causes RNA polymerase II transcription termination

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An attractive approach to reduce gene expression is via the use of antisense oligonucleotides (ASOs) that harness the RNase H1 mechanism. RNase H-dependent ASOs have been used successful as therapeutic agents in numerous animal models of disease and patients with a wide range of disease. ASOs have been shown to target RNA both in the nucleus and cytoplasm. However, much remains to be learnt about the activity of ASOs in the nucleus. Here we show that RNase H ASOs targeted to introns or exons robustly reduce the levels of mRNA associated with chromatin. Surprisingly, only intron targeted ASOs robustly reduce the levels of pre-mRNA associated with chromatin. This indicates that exon targeted ASOs remain inactive until pre-mRNA has undergone splicing, but before the mRNA is released from chromatin. After ASOs guide RNase H1 to cleave RNA, the cleaved fragments are known to be degraded in the nucleus. However, the consequences of ASO-mediated RNA cleavage on transcription have never been documented. Here we show that intron targeted ASOs, but not exon targeted ASOs, cause RNA polymerase II transcription termination in cultured cells and mice. Furthermore, ASO-mediated transcription termination is mediated by the nuclear exonuclease XRN2, as has been shown for transcription termination mediated by cleavage at polyadenylation signals. Our work has significant implications for using ASOs to mechanistically dissect the function of ncRNAs since both RNA reduction and transcription termination can occur simultaneously. We show that judicious design of ASOs allows one to reduce the expression of RNA without affecting transcription, which is essential for demonstrating that the function of a non-coding gene is mediated by RNA.

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Session II: Nucleic Acid Functional Interference Techniques
Pieter Mestdagh

Monday, 14 October
15.00-15.15

HTPathwaySeq, a novel application for high-throughput RNAi off-target gene identification

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RNAi-based library screens provide a powerful approach to identify novel therapeutic targets. These libraries target hundreds of candidate genes using multiple siRNAs (or shRNAs) per gene. Genes for which all (or the majority of) available siRNAs induce a phenotype of interest are considered as hits while individual siRNAs that induce the phenotype are considered to do so through off-target effects. Dozens of such individual siRNAs can be identified from a single screen. While they are typically ignored, they harbour an enormous untapped potential to identify novel candidate therapeutic targets. To reveal these off-target genes, we have applied HTPathwaySeq, a novel cost-effective and high-throughput RNA-seq based platform to quantify gene expression directly from crude cell lysates. HTPathwaySeq reproducibly detects 5,000-10,000 genes per sample at shallow sequencing depth and enables robust differential gene expression analysis between conditions (siRNA versus control treated cells). To demonstrate the potential of HTPathwaySeq for siRNA off-target gene identification, we selected 90 siRNAs that induced the phenotype of interest through presumed off-target effects from an siRNA library screen experiment. Cells were treated with each of these siRNAs in quadruplicate flowed by cell lysis and shallow 3' end mRNA sequencing. Genes that were significantly downregulated between siRNA and control treated cells were enriched for siRNA seed sequences in their 3' UTR, confirming off-target behavior. Aggregating results from these 90 siRNAs revealed multiple off-target genes that were identified across 10 or more conditions. Several of these recurrent off-target genes were either known to induce the phenotype or were interaction partners of other genes known to induce the phenotype. Taken together, HTPathwaySeq can be applied to repurpose off-target siRNA hits from library screens to reveal novel candidate therapeutic targets for drug development.

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Session II: Nucleic Acid Functional Interference Techniques
Sabrina Leslie

Monday, 14 October
15.15-15.30

Deconstructing ASO-RNA hybridization with single-molecule resolution

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McGill University[1], ScopeSys, Inc.[2], Ionis Pharmaceuticals[3]

Oligonucleotide drugs are dependent on a myriad of molecular interactions ranging from how Watson-Crick duplexes are formed at target sites to how various protein interactions impact cellular trafficking, stability and molecular scanning. Most existing tools to measure drug interactions make "ensemble" measurements and report a single result, typically averaged over millions of molecules or more. The problem is that these ensemble measurements cannot provide a clear and complete picture of how individual molecules interact with each other: they can miss rare events; they average out the natural variations or sub populations within biological samples; and they obscure insights into multi-step and multi-state reactions. These limitations can hinder our ability to understand complex drug mechanisms.

In this work, we present a single-molecule microscopy technique called Convex Lens-induced Confinement (CLiC) and apply it to deconstruct the interactions between antisense oligonucleotides and RNA targets. With CLiC, freely diffusing, individual molecules are trapped in arrays of micro- or nano-sized features so that many single, isolated molecules can be observed, and their reactions can be followed in time. Here, we investigate how mismatch structures and other variables affect ASO/RNA hybridization. By labelling ASO and RNA species with different fluorophores, we demonstrate high-throughput tracking of many individual ASO-RNA interactions over time to construct accurate distributions of rates and affinities. This approach and detailed analysis enable new insights into how structural variations such as higher-order RNA structure, and how modifications such as mismatches, affect drug performance and can furthermore be used to inform drug design.

Figure. CLiC single-molecule imaging of nucleic acid molecules. (A) Flow cell is compressed to trap molecules in embedded glass "nano wells". (B) Fluorescence image of singly-labeled oligonucleotides diffusing in wells.

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Session III: Nucleic Acid Damage and Repair
Nathan Luedtke
Monday, 14 October
16.00-16.25

RNA or DNA: Unexpected Mechanism of a Classic Nucleoside Drug

Nathan W. Luedtke[1], Alessandra Messikommer[1], Katja Seipel[2], Stephen Byrne[1], Peter J. M. Valk[3], Alexandre Theocharides[4], Thomas Pabst[2]
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Bioorthogonal functional group chemistry, such as azide-alkyne “click” and tetrazine-alkene cylcloaddition reactions, can be used to address long-standing mechanistic questions in the clinic. For example, we developed a traceable mimic of the common anti-cancer drug cytarabine (ara-C) by converting a single hydroxyl group to azide, giving “AzC”. This compound exhibited the same biological profile as ara-C in cell cultures and in vivo. Using azide-alkyne "click" reactions and super-resolution imaging, we uncovered an apparent contradiction: drug-resistant cells incorporated relatively large quantities of AzC into DNA and entered S-phase arrest, whereas drug-sensitive cells incorporated only small quantities of AzC. Upon removing AzC from the media, resumption of DNA synthesis and completion of the cell cycle occurred prior to complete removal of AzC from genomes in vitro and in vivo. These results revealed a new type of resistance mechanism, where efficient incorporation of the drug into DNA gave rise to highly stable, stalled replication forks that limited incorporation of the drug, yet allowed for the resumption of DNA synthesis following treatment. In this talk, the molecular targets of AzC in primary white blood cells from acute myeloid leukemia patients will be presented. These results demonstrate that the introduction of a bioorthogonal functional group into known drugs can provide candidates for new “theranostic” agents that are used in both therapeutic and diagnostic modalities.

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Session III: Nucleic Acid Damage and Repair
Matthias Altmeyer
Monday, 14 October
16.25-16.50

The DNA Damage Response – Friend or Foe?

Matthias Altmeyer
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The DNA Damage Response (DDR) comprises a sophisticated molecular signaling network that protects and maintains genome integrity. Defects in the DDR can lead to an accumulation of mutations, and DDR genes are frequently found mutated in different cancers. While a functional DDR acts as natural barrier against carcinogenesis, cancer-specific defects in DDR genes often entail cellular vulnerabilities that can be exploited therapeutically. Moreover, current CRISPR-mediated genome engineering approaches rely on the cellular DNA repair machinery for precise gene editing. I will discuss how we use multi-dimensional high-throughput single cell microscopy to delineate DNA damage responses in a cell cycle resolved manner and estimate implications for therapeutic interventions, both in the context of targeted interference with DDR functions for improved cancer therapy and in the context of CRISPR-mediated gene editing.

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Session III: Nucleic Acid Damage and Repair
Dong Hyun Jo
Monday, 14 October
16.50-17.05

Application of Genome Editing for Treatment of Retinal Diseases

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Genome editing with clustered regularly interspaced short palindromic repeats (CRISPR) system can be used as a tool to correct pathological mutations or modulate gene expression levels associated with the pathogenesis of human diseases. Owing to well-established local administration methods including intravitreal and subretinal injection, it is relatively easy to administer therapeutic genome editing machinery to ocular tissues for treating retinal diseases. In this context, we have investigated the potential of *in vivo* genome engineering as a therapeutic approach in the form of ribonucleoprotein or viral vectors. Major issues in the therapeutic application of genome editing include specificity and efficacy according to types of the CRISPR system. CRISPR-associated protein (Cas)9 from *Streptococcus pyogenes* and *Campylobacter jejuni* or Cas12a effectively disrupted the expression of genes in ocular tissues after the induction of non-homologous end joining after double-strand breaks. In addition, therapeutic genome editing including homology-directed repair was feasible to treat a nonsense mutation in the *RPE65* gene which is related to inherited retinal degeneration. It was also notable that there were no significant *in vivo* off-target effects of genome editing machinery targeting the well-selected target for more than 14 months. In this context, it is logical to utilize genome editing technology in addition to conventional treatment options to treat patients with retinal diseases. Further translational research on genome editing will help us realize its therapeutic application in clinical settings.

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Session III: Nucleic Acid Damage and Repair
Christopher Barkau
Monday, 14 October
17.05-17.20

Small Nucleic Acid-Based Inhibitors of CRISPR-Cas9

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Safety is a primary concern for development of CRISPR-based medicines. CRISPR inhibitors that are straightforward to design, manufacture, and implement would help control the timing and persistence of editing, potentially reduce off-target editing, or provide a fail-safe kill switch. We developed small nucleic acid-based inhibitors (SNuBs) that can efficiently inhibit Cas9 editing (*1*). CRISPR SNuBs comprise a chemically modified antisense oligonucleotide-like module and an aptamer-like module that act together to bind Cas9 with very low nanomolar affinity independent of the guide sequence. We have explored designs and chemistry for each module and the linker connecting the modules. We will present optimized CRISPR SNuBs and progress toward their application for inhibiting multiple types of Cas9 enzymes, controlling off-target effects, and inhibition of Cas9 in diverse model systems. This approach has the potential to open the door for enhanced safety of clinical CRISPR-Cas9 gene-editing applications (*2*).

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Session III: Nucleic Acid Damage and Repair
Laura Croft
Monday, 14 October
17.20-17.35

Targeting DNA repair pathways in cancer using oligonucleotides

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Cancer is the second leading cause of death globally, with numbers expected to rise by about 70% over the next two decades. There currently exists an unmet need for new, less toxic and more efficient therapeutics to improve treatment outcomes in cancer patients. Genome stability pathways offer a promising area of therapeutic intervention in cancer. Specific inhibitors of key DNA repair proteins have shown promising results as combination therapies and as single agents. We use single stranded oligonucleotides to target DNA repair proteins in cancer and explore this technology as a potential cancer therapeutic. We show that specific oligonucleotides inhibit DNA repair, induce DNA damage and apoptosis in cancer cell lines and suppress tumour growth in mouse xenograft models of prostate and lung cancer. We also explore their efficacy as combination treatment with first-line therapy in breast, ovarian and lung cancer cell line panels. Our lead molecules may have the potential to act as broad-spectrum therapeutics in cancer and may offer novel, effective and less toxic treatment options for cancer patients.

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Session IV: Nucleic Acid Immunity
Shizuo Akira
Tuesday, 15 October
8.30-8.55

Endoribonuclease Regnase-1 controls inflammation and immune responses

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Immune responses are accompanied by dynamic changes in gene expression. Gene expression is controlled at multiple points, including signal transduction, transcription and mRNA stability. So far, transcriptional regulation has been extensively studied. Many transcription factors including NF-kB and AP-1 are involved in induction of genes involved in inflammatory and immune responses. However, recent studies have revealed that control of gene expression at the mRNA level is as important as transcriptional control in the immune response. We have shown that Regnase-1 encoded by the Zc3h12a gene is an endoribonuclease involved in destabilization of a variety of mRNAs including IL-6, IL-12, and Regnase-1 itself mRNAs via the stem loop structure present in the 3' UTR of these genes
Although originally identified as LPS-inducible gene, Regnase-1 protein is present in unstimulated cells, and disappears in response to Toll-like receptor ligands via an IKK-dependent proteasome degradation pathway or in response to T cell receptor stimulation through the cleavage by Malt-1. Thus, Regnase-1 acts as a brake in unstimulated cells as well as a negative feedback regulator after cellular activation. Recently we found that IL-17 signal also inhibits the function of Regnase-1, triggering IL-17 response. I would like to discuss the role of Regnase-1 in the inflammation and immune responses.

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Session IV: Nucleic Acid Immunity
Veit Hornung
Tuesday, 15 October
8.55-9.20

Nucleic Acid Sensing in Human Myeloid Cells

Veit Hornung
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A central function of our innate immune system is to sense microbial pathogens by the presence of their nucleic acid genomes or their transcriptional or replicative activity. In vertebrates, a receptor-based system is largely responsible for the detection of such "non-self" nucleic acids. Tremendous progress has been made in the past years to identify host constituents that are required for this important task. To this end, nucleic acid sensing pattern recognition receptors such as the TLRs, RIG-I like receptors, AIM2 and the cGAS-STING axis have been identified. While many sensing modalities are conserved between mouse and man - the former being the classic model organism to study these pathways - certain species-specific differences exist that need to be considered when extrapolating from the murine to the human system.
In the past years, we have established cell culture models and genome engineering technologies to study the role of nucleic acid sensing pathways directly in human myeloid cells. Doing so, we have uncovered important differences between the human and the murine system with regards to nucleic acid recognition and response patterns. In this talk an update is given on our latest progress on nucleic acid sensing by human myeloid cells.

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Session IV: Nucleic Acid Immunity
Grace Chen
Tuesday, 15 October
9.20-9.45

Circular RNA Immunity

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Circular RNAs (circRNAs) are prevalent in eukaryotic cells and viral genomes. Mammalian cells possess innate immunity to detect foreign circRNAs, but the molecular basis of self vs. foreign identity in circRNA immunity is unknown. We show that N6-methyladenosine (m^6A) RNA modification on human circRNAs inhibits innate immunity. Foreign circRNAs are potent adjuvants to induce antigen-specific T cell activation, antibody production, and anti-tumor immunity in vivo, and m^6A modification abrogates immune gene activation and adjuvant activity. m^6A reader YTHDF2 sequesters m^6A-circRNA and is essential for suppression of innate immunity. Unmodified circRNA, but not m^6A-modified circRNA, directly activates RNA pattern recognition receptor RIG-I in the presence of lysine-63-linked polyubiquitin chain to cause filamentation of the adaptor protein MAVS and activation of the downstream transcription factor IRF3. CircRNA immunity has considerable parallel to prokaryotic DNA restriction modification system that transforms nucleic acid chemical modification into organismal innate immunity.

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Session IV: Nucleic Acid Immunity
Eva Bartok
Tuesday, 15 October
9.45-10.05

Immune sensing by TLR8 requires processing by endosomal RNases

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In humans, TLR8 is an essential sensor of bacterial RNA for the induction of proinflammatory and Th1 cytokines. Crystal analysis revealed that TLR8 binds uridine and short single-stranded(ss) RNA in two binding pockets, but the RNases responsible for processing RNA are still unknown. Herein, we demonstrate that endosomal endoribonucleases contribute to RNA processing for TLR8 recognition. Bacterial RNA, plasmodium RNA and a number of synthetic ORN TLR8 ligands required endosomal RNase expression for TLR8 activation. Supplementation of uridine restored recognition of ssRNA, but not structured RNA, demonstrating that these RNases act both to release uridine and to release ssRNA from complex ligands for TLR8 binding. Strikingly, peripheral blood mononuclear cells from RNase T2 hypomorphic patients lacked a response to bacterial RNA while the response to small molecule TLR8 agonists was intact. Our data provide a novel perspective for understanding TLR8 activity and differences between cell types, individuals and species.

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Session IV: Nucleic Acid Immunity
Guizhi (Julian) Zhu
Tuesday, 15 October
10.05-10.20

Responsive STING-activating nanovaccines to treat tumors refractory to immune checkpoint blockade

Guizhi (Julian) Zhu, Yu Zhang, Ting Su
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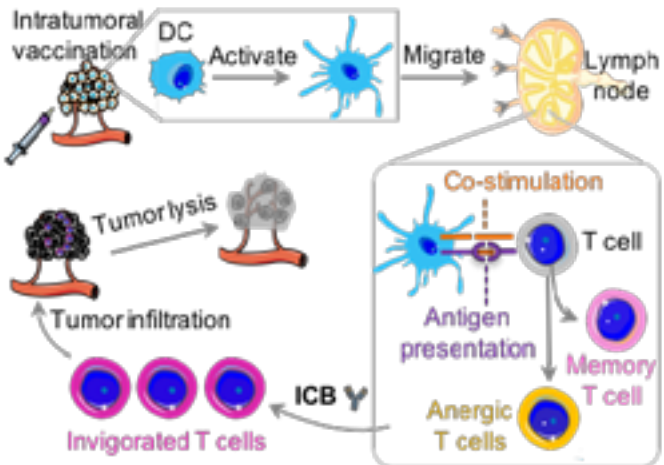
Despite the recent breakthrough of immune checkpoint blockade (ICB) in cancer immunotherapy, the objective response rates (ORRs) to single ICB agents remain low (<25%), due to sparse preexisting target immune cells for ICB, heterogeneous immune checkpoint levels, and multi-tier immunosuppression. Combining multiple ICB agents can increase ORRs but also aggravate immune-related adverse events (irAEs). Rational combination of therapeutic vaccines and ICB can enhance ORRs while minimizing irAEs. Stimulator of interferon genes (STING) agonists are promising immunostimulators for cancer immunotherapy. STING is an intracellular receptor that senses cytosolic cyclic dinucleotides (CDNs) to induce the production of type I interferons (IFNs) and proinflammatory cytokines that activate immune cells such as $CD8^+$ T cells and natural killer cells. However, the clinical translation of CDNs has been challenged by their susceptibility to enzymatic degradation or hydrolysis, poor pharmacokinetics, negative charges that hamper efficient cell uptake, and potential irAEs associated with systemic CDN dissemination. To address these challenges, we designed novel nanovaccines that deliver, protect, and conditionally release CDNs in target cells (see *Scheme*). In one study, we synthesized nanovaccines using FDA-approved biodegradable polymers as nanoparticulate cores, and modified nanoparticle surfaces with designer DNA that binds to CDNs under physiological pH but undergoes a conformational configuration to release CDNs in the acidic endosome. The nanoparticles protected CDNs from degradation. In DCs, this nanovaccine elicited three integral signals for antigen presentation and T cell priming: upregulation of MHC-II (Signal 1), upregulation of co-stimulatory factors (Signal 2), and secretion of type I IFNs and proinflammatory cytokines (Signal 3), all of which outperformed that of natural CDNs and chemically-stabilized CDNs. In mouse models of anti-PD-1-refractory melanoma and colorectal cancer, intratumoral vaccination using these nanovaccines remodelled the immune milieu and synergized with anti-PD-1 for tumor immunotherapy. In another study, we synthesized nanovaccines that were loaded with CDNs or STING-activating oligonucleotides *via* cationic pH-responsive co-polymers that had increased positive charge and hydrophilicity in the acidic endosome of target immune cells, leading to nanoparticle dissociation and the release of CDNs/DNA and facilitating their endosome escape for the immunotherapy of multiple types of murine cancer.



Scheme: Responsive STING-activating nanovaccines for combination immunotherapy of ICB-refractory tumors.

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Session IV: Nucleic Acid Immunity
Robert MacLeod

Tuesday, 15 October
10.20-10.35

Development and characterization of AZD8701, a high affinity antisense oligonucleotide targeting FOXP3 to relieve immunosuppression in cancer.

Revenko AS[1], Sinclair C[2], , Peter A[2], Taylor M[2], Johnson RB[2], Hettrick LA[1], Klein S[2], Solanki A[2], Chapman M[3], Yates J[4], Angell HK[5], Watt A[1], Monia BP[1], Barry ST[2], Lyne P[6], Edbrooke M[2] ,Goldberg, F[2], MacLeod AR[1]

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Regulatory T cells (Treg) critically maintain immuno-suppression in the tumor microenvironment, representing an attractive immuno-oncology target. The Treg lineage is defined by expression of the FOXP3 transcription factor, which controls immune-suppressive functions. We have developed the clinical candidate AZD8701, a next-generation antisense oligonucleotide inhibitor of FOXP3 (utilizing the Ionis Gen 2.5 cEt-modified ASO platform).

AZD8701 treatment knocked down FOXP3 in primary human Tregs via free uptake (IC_{50} 65nM), which was also associated with modulation of known FOXP3 target genes including 25-50% reduction in CTLA4, ICOS, CCR8 and GITR. Tregs treated with AZD8701 further exhibited reduced suppressive functions in *in vitro* suppression assays, which confirmed the functional effects of FOXP3 modulation. Finally, AZD8701 promoted dose-dependent knockdown of FOXP3 in humanized mice, including >50% FOXP3 knockdown at doses that can be feasibly achieved with the Gen 2.5 ASO platform in the clinic.

To support the importance of FOXP3 in immuno-oncological settings, we characterized murine surrogate FOXP3 ASOs in the context of syngeneic tumour bearing mice. Murine FOXP3 ASOs similarly promoted >50% FOXP3 knockdown in mice and were well tolerated with no overt toxicological findings at high doses over more than six weeks of treatment. Murine FOXP3 ASOs significantly attenuated tumour growth in A20 and ID8-VEGF syngeneic models, which was associated with some complete tumour regressions. Moreover, we found that mouse surrogate FOXP3 ASOs promoted additive/enhanced therapeutic effects when combined with immune checkpoint blockade.

Collectively, FOXP3 ASOs represent a first-in-class strategy to target Tregs in cancer in a highly selective manner. The clinical application of AZD8701 may provide therapeutic benefit to patients either as a monotherapy or in combination with immune checkpoint blocking agents.

A. Robert MacLeod
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Session V: Dr. Alan M. Gewirtz Memorial Scholarship Award
Bruno M.D.C. Godinho

Tuesday, 15 October
11.05-11.25

Divalent siRNA Scaffold for Robust Gene Modulation in the Central Nervous System

Bruno M.D.C. Godinho[1], Julia F. Alterman[1], Matthew R. Hassler[1], Chantal Ferguson[1], Andrew H. Coles[1], Dimas Echeverria[1], Ellen Sapp[2], Reka A. Haraszti[1], Richard Moser[3], Miguel Sena-Esteves[4], Heather Grey-Edwards[5], Marian DiFiglia[2], Neil Aronin[1,6], Anastasia Khvorova[1,7]

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RNA interference (RNAi)-based gene silencing holds great promise as a therapeutic strategy for incurable, genetically-defined neurological diseases, such as Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS). However, non-toxic and efficient delivery of synthetic oligonucleotides to the central nervous system (CNS) remains a primary challenge that hinders fast progression of this technology for the treatment of CNS disorders.

Here we describe a neuroactive divalent siRNA scaffold (Di-siRNA) that allows broad and long-lasting gene silencing (up to 6 months) in the mouse brain after a single intracerebroventricular (ICV) injection. In these studies, we found a strong correlation between guide strand tissue accumulation and level of silencing with 2 ng siRNA/mg tissue sufficient to produce more than 80% target gene downregulation. This novel fully chemically modified scaffold also demonstrated widespread distribution in the brains and spinal cords of Dorset sheep and Cynomolgus macaques after a single injection (ICV or intrathecal catheter). Furthermore, Di-siRNAs exhibited similar subcellular perinuclear localization, both in neurons and glia, to that observed in previous rodent studies. Potent silencing of the Huntingtin (Htt) mRNA target and protein was achieved in various regions of the non-human primate (NHP) brain, including cortex, hippocampus and striatum (caudate), but also in the spinal cord 1 month after injection. Preliminary toxicity assessments revealed no detectable pathology (bleeding, edema, etc.) and no major inflammatory response in the NHP brain. In addition, no significant changes were observed in complete blood counts and in a panel of biochemical markers, including liver enzymes and electrolytes, suggesting minimal systemic impact.

Together these data validate the utility of Di-siRNAs for potent and sustained modulation of gene expression in larger mammalian brains, and greatly contribute to the advancement of RNAi-based therapeutics for neurogenetic disorders.

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Session V: Mary Ann Liebert publishers Inc Young Investigator Award Holly Kordasiewicz

Tuesday, 15 October
11.25-11.45

Antisense oligonucleotides for the treatment of neurodegenerative diseases

Holly Kordasiewicz; Dan Norris; Anne Smith; Roger Lane; Frank Bennett; Eric Swayze
Ionis Pharmaceuticals

Antisense oligonucleotides (ASOs) are emerging as a viable therapeutic approach to alter production of proteins implicated in currently untreatable neurodegenerative diseases. ASOs are single stranded nucleotides, typically 20 bases in length, that bind complementary target RNA through Watson and Crick hybridization. Depending on ASO design, hybridization can lead to selective degradation of the target RNA, alteration of RNA splicing or another highly specific post-transcriptional modification. Ionis has achieved transition from the bench to the clinic for certain neurodegenerative diseases, as evidenced by regulatory approval of an ASO for the treatment of spinal muscular atrophy and initiation of clinical trials for ASOs in Huntington's disease, amyotrophic lateral sclerosis and other neurological conditions. Tools that guide drug development and mitigate risk include pharmacology studies in rodents to demonstrate proof-of-concept phenotypic benefit of ASOs and biodistribution studies in larger species. Additionally, modeling can be built using preclinical data to predict ASO effects in humans. Here, I describe our experiences in ASO development, including proof-of-concept and biodistribution animal studies, pharmacokinetics and pharmacodynamics (PKPD) model-building and clinical trial design and interpretation. These experiences demonstrate the possibility of advancement of ASO therapies to treat neurodegenerative disease.

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Session V: Paper of the Year Award
Xue-hai Liang
Tuesday, 15 October
11.45-12.05

Chemical modification of PS-ASO therapeutics reduces cellular protein-binding and improves the therapeutic index

Wen Shen, Cheryl L. De Hoyos, Michael T. Migawa, Timothy A. Vickers, Hong Sun, Audrey Low, Meghdad Rahdar, Melanie Bell, Stan Riney, Susan F. Murray, Sarah Greenlee, Xue-hai Liang; Punit P. Seth, and Stanley T. Crooke
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The molecular mechanisms of toxicity of chemically modified phosphorothioate antisense oligonucleotides (PS-ASOs) are not fully understood. Here, we report that toxic gapmer PS-ASOs containing modifications such as constrained ethyl (cEt), locked nucleic acid (LNA) and 2′-*O*-methoxyethyl (2′-MOE) bind many cellular proteins with high avidity, altering their function, localization and stability. We show that RNase H1–dependent delocalization of paraspeckle proteins to nucleoli is an early event in PS-ASO toxicity, followed by nucleolar stress, p53 activation and apoptotic cell death. Introduction of a single 2′-*O*-methyl (2′-OMe) modification at gap position 2 reduced protein-binding, substantially decreasing hepatotoxicity and improving the therapeutic index with minimal impairment of antisense activity. We validated the ability of this modification to generally mitigate PS-ASO toxicity with more than 300 sequences. Our findings will guide the design of PS-ASOs with optimal therapeutic profiles.

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Session V: Lifetime Achievement Award
Muthiah Manoharan
Tuesday, 15 October
12.05-13.00

Living in the World of Oligonucleotide Therapeutics

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As the 2019 Lifetime Achievement Awardee of the Oligonucleotide Therapeutics Society, I would like to start by thanking the society. Thinking about my path through the world of oligonucleotide therapeutics, I find it amazing that molecules that we once thought of as totally lacking in drug-like properties, due to poor biodistribution, tissue delivery, and cell permeation capabilities, are now used to treat patients with diseases once considered "undruggable" and function as lifesaving drugs. I am honored and humbled to have been part of the process and have this recognition.

I was born in Madurai, Tamil Nadu, India and received my B.Sc. and M.Sc. degrees in chemistry at the American College, located in the same town. After my education, I served as a member of faculty of the College for four years before I was persuaded by my professors A. R. Venkitaraman, Divakar Masilamani, and Solomon Pappiah to study in United States. I began Ph.D. studies in chemistry with Professor Ernest L. Eliel at the University of North Carolina, Chapel Hill in 1978. I carried out synthesis and conformational analysis of heterocyclic compounds including substituted tetrahydropyrans – the skeletal compound of pyranose sugars present in GalNAc. I also attempted to correlate the pharmacological effects of molecules like phencyclidine with conformation: For example, I asked why the phenyl group prefers to have an axial orientation when positioned geminally to the methyl group in phencyclidine. Following my graduate work, attracted by the publications of Professor John A. Gerlt related to stereochemistry, ^{17}O NMR, and enzymology of nucleic acids, I worked with him in the field of oligonucleotides at Yale University and then at University of Maryland as a Leukemia Society post-doctoral research fellow. We delineated how DNA repair enzymes uracil-DNA glycosylase and UV endonuclease V function to generate and repair abasic sites in DNA using ^{13}C-labeled oligonucleotides synthesized using phosphotriester chemistry.

I started my industrial career as an oligonucleotide chemist at Lifecodes Corporation in Valhalla, NY in 1988 in the then-nascent field of therapeutic oligonucleotides. I worked under Alan F. Cook (a superb nucleoside chemist from Roche who studied earlier with John Moffatt at Syntex) who suggested that I research the area of conjugation chemistry of oligonucleotides. When Lifecodes changed its focus from therapeutics to diagnostics in 1990, I moved to Isis Pharmaceuticals to work with P. Dan Cook and Stan Crooke. At Isis, I contributed to the team in the areas of sugar (2'-*O*-MOE, 2'-*O*-NMA, 2'-*O*-DMAEOE, and their cousins), linkage, and base modifications, lipidic nucleic acids, targeting ligands, conjugation chemistry, and a successful universal solid support. I also learned much about pharmacokinetics, pharmacodynamics, safety, and structural biology of oligonucleotides. In the 12 and a half years I spent at Isis, the field matured and multiple modes of antisense oligonucleotide action were identified: RNase H mechanisms, translation arrest, pseudo half-knots, immunity-based applications, and splice modulation.

In 2003, I was the first chemist hired at Alnylam by John Maraganore. There I was exposed fully to the fascinating world of Argonautes and RNA interference (RNAi). We built a chemistry team with the broad mandate to study chemical modifications and motifs,

conjugation chemistry with multiple ligands, and delivery platforms (lipid nanoparticles, polymer conjugates, and complex-forming strategies), RNA synthetic and analytical methods for siRNAs. Our team pioneered the discovery and development of the chemical modifications and delivery that make RNAi-based human therapeutics possible. Our work led to ONPATTRO (patisiran), the first RNAi therapeutic approved by the FDA; FDA approval came in August 2018, and it is now approved for use in Europe, Canada, and Japan.

Our research group demonstrated for the first time the human therapeutic applications of hepatocyte-targeting GalNAc-conjugated oligonucleotides, a platform that has revolutionized the nucleic acid-based therapeutics field with several compounds in the advanced clinical trials (givosiran, inclisiran, fitusiran, virtrusiran, and lumasiran, to name a few). We expect that many of these compounds will be approved and available for the needy patients in the near future. With our academic collaborators, we developed antagomirs – the antagonists of miRNAs (a direct example of John Maraganore's *kainotomia* (καινοτομία, i.e., innovation). and continue to study structural biology of RNAi; we enabled immunostimulatory siRNAs and RIG-I modulating siRNAs, nuclear RNAi, RNA activation, and selective modulation of biotherapeutics using RNAi. We also maintained productive relationships with pharmaceutical partners including Merck, Novartis, Metronix, Roche, Immunogen, Takeda-Japan, Genzyme-Sanofi and Regeneron. I continue to serve the American Chemical Society, Oligonucleotide Therapeutics Society, International Society of Nucleosides, Nucleotides, and Nucleic Acids and Gordon Research Conferences and am proud to function as an ambassador for oligonucleotide therapeutics around the globe. ("Yaadhum oore yaavarum kelir-□□□□□□ □□□ □□□□□□□ □□□□□□-": i.e., We all hail from same village, and we are all kins!)

In closing I would like to share some questions that I still ponder regarding the future of the oligonucleotide therapeutic world: ("I'm still turning over stones, hoping to find something new!").

1. What are the "undruggable" diseases that oligonucleotide therapies will make treatable in the near future?
2. What other applications are there for GalNAc conjugates platform that will expand the "GalNAc universe" beyond reversirs and bis-conjugates?
3. What novel oligonucleotide chemical modification will next revolutionize the field?
4. Are there alternatives to solid-phase synthesis that will make oligonucleotide synthesis more efficient and scalable?
5. What we have done in the area of delivery is just the "end of the beginning". How do we achieve efficient delivery of oligonucleotides to all remaining tissues?
6. How do we achieve endosomal release of oligonucleotides?

Finally, thank you again for this recognition as the Lifetime Achievement Awardee of the Oligonucleotide Therapeutics Society for the year 2019 for "commitment to the field of oligonucleotide therapeutics through outstanding contributions to education, research, and therapeutics application". I am grateful to my parents, mentors, heroes, colleagues, collaborators, consultants, post-docs, students, interns, friends, and family for their help, support, and understanding that made my journey in the world of Oligonucleotide Therapeutics so rewarding and memorable.

This presentation is dedicated to all of them.

Session VI: Oligonucleotide Delivery and Targeting
Bruce Sullenger
Tuesday, 15 October
14.30-14.55

Aptamers as Reversible Ligands for Therapeutic, Imaging and Clean Cell Purification Applications

Bruce Sullenger[1], Bethany Gray[1], George Pitoc[1], Martin Requena[1], Mike Nichols[1], Kady-Ann Steen-Burrell[1], Debra Wheeler[2], Shahid Nimjee[2], Linsley Kelly[1], Juliana Layzer[1] and Rachel Rempel[1]

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We have been exploring the concept of utilizing aptamer-antidote oligonucleotide pairs as reversible ligands now for over a decade. Our studies demonstrate that aptamer- binding can be rapidly reversed *in vitro*, *in vivo* and in patients using short antidote oligonucleotides. We will describe three different recent applications of this powerful approach to generated switchable ligands. First our results evaluating this approach to control a therapeutic antiplatelet aptamer in the setting of ischemic stroke will be described. As stroke treatment is limited by serious bleeding, we believe that the development of a rapidly reversible antiplatelet agent for this clinical setting will significantly improve the treatment options of the millions of patients that experience stroke around the world each year. Next we will describe how a rapidly reversible aptamer can be utilized to image an active thrombus *in vivo* in real time. As current methods of detecting thrombi are indirect, we believe that the development of this approach will greatly facilitate rapid and definitive clinical diagnoses and more accurately direct clinical care of patients experiencing acute thrombotic events. Finally we discuss our studies demonstrating that aptamer-antidote pairs can be utilized to purify cells in their native states, which will greatly improve their function following isolation. As antibodies are difficult to remove from cells and aptamers are not through the use of our antidote-reversal approach, we believe that aptamer-antidote based cell purification methods represent a novel and more useful strategy to isolate cells in their "native state" for both research and clinical applications. In summary our discovery that aptamer-antidote pairs represent rapidly reversible ligands has many potential applications particularly in settings where rapid, active control of binding is important and valuable.

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Session VI: Oligonucleotide Delivery and Targeting
Peter Lutwyche
Tuesday, 15 October
14.55-15.20

mRNA Therapy for Rare Diseases: Optimization of Payload and Delivery System

Peter Lutwyche
Genevant Sciences Corp.

mRNA therapeutics have great potential in the treatment of rare diseases caused by a missing or faulty protein by utilizing cells' natural translation apparatus to produce the desired protein from an introduced mRNA template. To do this the mRNA must be efficiently delivered to the target cells, intact and without activating immune defences. Lipid nanoparticles (LNPs) have been used in research to deliver a variety of nucleic acid (NA) payloads, protecting the NA from degradation and facilitating uptake into target cells and subsequent endosomal release. LNPs have also enabled the only approved siRNA therapeutic, patisiran, for the treatment of polyneuropathy caused by a type of hereditary amyloidosis. When developing mRNA-LNP products for potential therapeutic use, both the mRNA payload and the LNP must be thoughtfully designed. For mRNA payload, strategies such as base modification, codon and UTR optimization and modulation of polyA tail length can be used to reduce immune-stimulatory potential and to increase message stability and translation of protein. For LNPs, among the four customary lipid components, the choice of cationic and PEG lipids in particular have critical influence on potency and tolerability. This presentation describes the optimization of mRNA and LNP components of a product candidate in development to treat a severe rare liver disease. Using relevant rodent and primate animal models, we demonstrate that mRNA-LNP can be designed to provide significant protein production in multiple dose regimens with an acceptable safety profile, suggesting the feasibility of this therapeutic approach.

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Session VI: Oligonucleotide Delivery and Targeting
Karyn O'Neil
Tuesday, 15 October
15.20-15.45

Centyrin Mediated Delivery of siRNA to Extra-hepatic Cell Types

Karyn T. O'Neil, Rob Kolakowski, Russ Addis, Steve Nadler
Aro Biotherapeutics

Aro Biotherapeutics is a preclinical stage biotechnology company focused on discovery and development of Centyrins, a new class of small, structurally simple, ultra-stable and highly soluble proteins engineered to specifically bind antigens with high affinity. Aro has created therapeutic lead candidates combining Centyrins specific for receptors on selected tumor types with siRNAs that address intracellular targets involved in tumor growth and immune regulation. Centyrin-drug conjugates provide a means to deliver macromolecules to intracellular targets that have been considered "undruggable" and enable access to disease targets beyond hepatocytes where GalNAc targeted delivery has been validated.

We have shown that Centyrins enable extended exposure in early endosomes with intracellular trafficking patterns that are differentiated from antibodies targeting the same receptor. Using Centyrins targeted to cell surface receptors on tumor cells, we have also demonstrated efficient internalization and trafficking of Centyrins to the cytosol via protein complementation assays. These studies confirm our hypothesis that the pH stability and chemical properties of Centyrins enable slow release from endosomes, and ultimate delivery of the conjugated siRNA moiety to cytosolic RISC.

We are developing Centyrin-KRAS siRNA conjugates that are designed to inhibit tumors driven by KRAS mutations across a variety of solid tumor types. Using a novel screen for internalizing Centyrins, we have identified Centyrins that bind and are internalized via several different highly expressed receptors on epithelial tumors. In addition, we have optimized KRAS siRNA payloads for Centyrin deliver and have identified highly potent and specific leads. Conjugates prepared from Centyrins targeting epithelial receptors and optimized siRNAs demonstrate highly potent and specific knockdown activity in epithelial tumor cell lines *in vitro*. The time course for maximal knockdown is consistent with a model whereby Centyrin-siRNA conjugate retained in early endosomes is released over an extended time period. These data highlight the broad utility of this platform.

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Session VI: Oligonucleotide Delivery and Targeting
Ivan Chernikov
Tuesday, 15 October
15.45-16.00

Investigation of the mechanism of internalization of fluorescently labeled cholesterol-modified siRNA into cultured tumor cells

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Conjugation of small interfering RNA (siRNA) with lipophilic molecules of natural origin which has natural mechanisms for cell internalization is a promising approach of delivering siRNA to cells for biomedical purposes *in vitro* and *in vivo*. Earlier, we showed that the attachment of cholesterol to the 5'-end of the sense strand of nuclease-resistant siRNA (Ch-siRNA) through an optimized linker allows it to penetrate into the cells and suppress the expression of the target gene. It has also been demonstrated that suppression of target gene expression in *in vivo* tumors occurs following a single intravenous, intraperitoneal, or peritumoral injection of Ch-siRNA.
Hematopoietic cells are considered to be one of the most difficult for delivery siRNA to them, while the development of siRNA delivery systems for these cells can significant increase in the effectiveness of antiretroviral therapy and the effectiveness of treatment of tumors of the hematopoietic origin. However, it was shown that despite the high efficiency of accumulation of Ch-siRNA in hematopoietic cells, Ch-siRNA did not possess silencing activity in them. In this work we investigated the mechanisms of accumulation of fluorescently-labeled Ch-siRNA into cancer cells KB-3-1 and leukemic cells K562 using inhibitors of different types of endocytosis. We showed that fluorescently-labeled Ch-siRNA penetrate into KB-3-1 and K562 cells by several mechanisms whose contribution differs depending on cell type and serum availability. In a serum-free medium, it was found that macropinocytosis and clathrin-dependent endocytosis contribute to the accumulation of the conjugate in KB-3-1 cells, while clathrin-dependent endocytosis makes the main contribution to K562 cells. Interesting that inhibitors of different types of endocytosis do not reduce the silencing activity of Ch-siRNA without fluorescent label.
It can be assumed, that Ch-siRNA penetrates to cells through several mechanisms and that most of the Ch-siRNA accumulates in cells "unproductively" and does not participate in RNA-interference.
This work was supported by the Russian Foundation for Basic Research № 17-04-01100.

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Session VI: Oligonucleotide Delivery and Targeting
Julia Alterman

Tuesday, 15 October
16.00-16.15

A comprehensive RNAseq analysis shows that siRNA based modulation of huntingtin in the central nervous system of non-human primates is highly specific

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siRNAs are a powerful tool that support potent, sequence specific targeting of a particular gene. However, as with other classes of drugs there are concerns about the potential for a number of off-target effects. First, by potently silencing the expression of a particular protein, any interacting molecules could be dis-regulated. Second, siRNAs, as a result of their interaction with Argonaut, could, by partial complementarity, exhibit miRNA like off-target effects. Finally, miRNA post-transcriptional regulation of gene expression is a fundamental biological process that if disrupted could cause widespread negative impact on mRNA expression levels. Therefore, it is critical to ensure that the introduction of large amounts of chemically modified siRNAs to the system does not disrupt the natural miRNA population.

We have developed a novel chemical scaffold that shows significant silencing of the huntingtin transcript throughout the non-human primate (NHP) brain, exhibiting as much as 90% silencing in the cortex at one month post injection. In order to answer questions about potential off-target effects we performed RNAseq on samples from the non-human primate cortex (n=12 samples per group) and looked for changes in overall gene expression, the presence of potential miRNA like off target effects, and the degree of RISC occupancy with chemically modified siRNAs. Our results show that (1) one month long potent modulation of HTT expression has no major impact on the overall transcriptome and (2) fully chemically modified siRNAs induce limited off-targeting effects in the CNS of NHPs. This study represents the first report evaluating the specificity of oligonucleotide therapeutic molecules in NHPs and the CNS in general.

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Session VI: Oligonucleotide Delivery and Targeting
Anders Wittrup

Tuesday, 15 October
16.15-16.30

Unravelling the Mysteries of Endosomal Escape

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With current nucleic acid delivery strategies, only a small fraction of administered nucleic acids are believed to reach the cytosol, while most is trapped within the endosomal system. A key issue, complicating efforts to enhance the delivery efficiency of various delivery strategies, has been a lack of tools to detect and characterize successful endosomal escape and cytosolic delivery of the nucleic acid cargo.

Here, we present a number of methods that enables detailed characterization of the endosomal escape process of most nucleic acid delivery strategies. These methods are all fluorescence microscopy based and make it possible to monitor endosomal escape events in real-time in live cells. The methods can be grouped into three distinct, highly optimized, imaging strategies, each providing information relating to different aspects of the endosomal escape process. The first strategy is direct imaging of fluorescently labeled cargo in the cytosol. Recent efforts in our lab has enabled confident detection and imaging down to ~500 cytosolic siRNA molecules. The second strategy is to use fluorescent protein tagged galectins to identify releasing vesicles. Galectins are endogenous membrane damage sensors that rapidly (within seconds) are recruited to damaged endosomal structures at the moment of endosomal escape. The third strategy is to monitor the intensity of fluorescent nucleic acid containing endosomal structures. This strategy is, on its own, very challenging to use to detect endosomal escape as mobile endosomal structures fluctuate in fluorescence intensity but can be combined with the other strategies to enable measurements of release fractions and dynamics. By such an approach, we have for the first time visualized endosomal escape of a ligand-conjugated siRNA facilitated by a small molecule release enhancer.

We believe these methods can be widely applied by the scientific community to address multiple aspects of endosomal escape for most delivery systems. Key questions of interest that now can be addressed include determination of release dynamics and frequency, compartment of release, fraction of release, absolute delivery amount and more. Advancing our understanding of the endosomal escape process of various delivery strategies will aid substantially in bringing more nucleic acid therapeutics to the clinic.

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Session VII: Oligonucleotide Preclinical Stage Discovery

Luca Cartegni

Wednesday, 16 October
8.35-9.00

Abstract not available at the time of printing

Session VII: Oligonucleotide Preclinical Stage Discovery
Thomas Thum

Wednesday, 16 October
9.00-9.25

Clinical transition of microRNA-based therapeutic concepts in cardiovascular disease

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Heart failure is a growing global pandemic with increasing burden. Treatments that improve the disease by reversing heart failure at the cardiomyocyte level are lacking. MicroRNAs (miRNA) are transcriptional regulators of gene expression, acting through complex biological networks, and playing thereby essential roles in disease progression. Adverse structural remodelling of the left ventricle due to myocardial infarction (MI) is a common pathological feature leading to heart failure (HF). We previously demonstrated increased cardiomyocyte expression of a miRNA family during pathological cardiac conditions. Transgenic mice overexpressing the miRNA family develop pathological cardiac remodelling and die prematurely from progressive HF. Using both knockout and antisense strategies, we have shown this noncoding RNA to be both necessary and sufficient to drive the pathological growth of cardiomyocytes in a murine model of left ventricular pressure overload. Based on the findings, we proposed this miRNA to serve as a therapeutic target in heart failure therapy. In a large animal model of ischemic/reperfusion injury in pigs (*n*=135) we successfully demonstrated dose-dependent efficacy on diverse cardiovascular functional parameters, strong PK/PD relationships, and overall high clinical potential of a novel lead-optimized synthetic locked nucleic acid phosphorothioate backbone antisense oligonucleotide inhibitor as a next-generation heart failure therapeutic. After successful GLP-compliant toxicology in two species the new drug is currently tested in a clinical phase Ib study in heart failure patients (NCT04045405).

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Session VII: Oligonucleotide Preclinical Stage Discovery
Rusty Montgomery
Wednesday, 16 October
9.25-9.50

Development of MRG-110, an LNA-AntimiR Targeting miR-92a for Use in Tissue Repair and Revascularization

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miRNAs are small, non-coding RNAs that act as negative regulators of gene expression. miR-92a has previously been shown to inhibit angiogenesis. Conversely, inhibitors of miR-92a accelerate angiogenesis and tissue repair, improving function following myocardial infarction, limb ischemia, vascular injury, bone fracture, and wound healing in rodents. Due to their pro-angiogenic and tissue repair effects, miR-92a inhibitors offer potential therapeutics to accelerate the tissue repair process in humans.

The effect of miR-92a inhibitors on tissue repair was investigated in a GLP pig wound healing study. A Locked Nucleic Acid (LNA)-modified inhibitor of miR-92a, MRG-110, was administered by intradermal injection around the periphery of a full thickness excisional wound at multiple dose levels randomized across wound sites. MRG-110 significantly ($p<0.05$) increased vascularization within the dermal portion of the wound bed which was associated with significantly ($p<0.01$) increased granulation tissue. Laser Doppler assessment on demonstrated significant ($p<0.05$) increases in perfusion in drug treated wounds compared to controls. These effects accelerated wound healing, leading to an approximately 5-day improvement in achieving 50% wound closure in healthy farm pigs compared to standard of care. The lowest dose tested had maximal benefit indicating even lower doses may be effective.

MRG-110 was then investigated in two Phase I clinical studies assessing the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single and multiple local administrations of MRG-110 in excisional wounds as well as a systemic dosing study assessing safety and tolerability in healthy volunteers. MRG-110 was safe and generally well-tolerated when given as a single intravenous dose up to 1.5 mg/kg or as 3 weekly intradermal doses up to 4.5 mg/dose (0.08 mg/kg per dose in a 60 kg person). Despite the variability in wound healing in the healthy volunteers, MRG-110 treatment demonstrated an apparent dose-dependent increase in angiogenesis, as demonstrated by increased perfusion and histological markers of neoangiogenesis, as well as reduced αSMA expression. These data support moving forward in cutaneous wounds of varying etiology where increased perfusion may result in improved wound closure and reduced a-SMA expression may reduce scar formation and contracture. This mechanistic proof of concept study combined with the favorable safety and tolerability of MRG-110 via systemic administration also support additional clinical studies to evaluate the ability of the product candidate to enhance vascularization and function in the setting of heart failure.

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Session VII: Oligonucleotide Preclinical Stage Discovery
Kotaro Yoshioka

Wednesday, 16 October
9.50-10.05

Heteroduplex-oligonucleotides for highly efficient silencing of microRNA through a novel intracellular mechanism.

Kotaro Yoshioka[1,], Taiki Kunieda[1], Yutaro Asami[1], Huijia Guo[1], Haruka Miyata[1], Kie Yoshida-Tanaka[1], Yumiko Sujino[1], Wenying Piao[1], Hiroya Kuwahara[1], Kazutaka Nishina[1], Rintaro Iwata Hara[1, 2], Tetsuya Nagata[1], Satoshi Obika[3], Takeshi Wada[2] and Takanori Yokota[1]

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AntimiR is an antisense oligonucleotide that silences microRNA (miRNA) for the treatment of intractable diseases. Since high affinity chemistries have been developed to enable efficient miRNA-binding, chemical modified antimiRs have been tested in clinical trials. However, no antimiRs now has been approved for clinical use and enhancement of its *in vivo* efficacy and improvement of its toxicity remain challenging.

We here design heteroduplex oligonucleotide (HDO)-antimiR as a new technology comprising an antimiR and its complementary RNA. HDO-antimiR binds targeted miRNA *in vivo* more efficiently by 12-fold than the parent single-stranded antimiR. HDO-antimiR also produced enhanced phenotypic effects in mice with upregulated expression of miRNA-targeting messenger RNAs. Surprisingly, the enhanced potency of HDO-antimiR was not explained by its bio-stability or delivery to the targeted cell, but reflected an improved intracellular potency. Finally, we demonstrated that HDO-antimiR has a unique intracellular mechanism for silencing targeted miRNA in comparison with the conventional single-stranded antimiR.

Our findings provide new insights into biology of miRNA silencing by double-stranded oligonucleotides and support the *in vivo* potential of this technology based on a new class of intracellular mechanism for the treatment of miRNA-related diseases.

1) Yoshioka K, Kunieda T, Asami Y et al. Highly efficient silencing of microRNA by heteroduplex oligonucleotides. *Nucleic Acids Res.* 2019.

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Session VII: Oligonucleotide Preclinical Stage Discovery
Ulrich Lächelt
Wednesday, 16 October
10.05-10.20

Oligo(ethylenamino)-Lipopeptide PMO Conjugates: Impact of Unsaturation and Particle Formation on Splice-Switching Activity

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A therapeutic approach based on morpholino oligonucleotides (PMO) is the restoration of functional gene expression by modification of pre-mRNA splicing. In a recent library screen, potent artificial lipopeptides were identified for the synthesis of conjugates with splice-switching PMO via copper-free click chemistry. The compounds generated by solid-phase synthesis are based on natural α-amino acids, artificial oligo(ethylenamino) acids and hydrophobic modifications. In a systematic variation of the lead structure by substitution of contained fatty acids with 0 to 6 double bonds, the degree of unsaturation turned out to have critical impact on the extent of splice-switching activity in pLuc/705 reporter cells. A compound containing linolenic acid with 3 double bonds exhibited highest activity and significantly increased functional protein expression in the HeLa pLuc/705 model *in vitro* and *in vivo* after intratumoral injection. Two key parameters of high splice-switching activity were identified: the lipopeptide-PMO conjugates associate to nanocomplexes in a concentration dependent manner and the contained fatty acids determine the pH-dependent lytic activity and endosomal release of the PMO formulations. The potent PMO conjugates mediate significant splice-switching in H2K-mdx52 dystrophic skeletal myotubes at low nanomolar concentrations and are considered a promising platform for the generation of PMO-therapeutics with favourable activity/toxicity profile.

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Session VII: Oligonucleotide Preclinical Stage Discovery
Patrick Carroll

Wednesday, 16 October
10.20-10.35

A novel ASO based approach to treat peripheral neuropathic pain

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Peripheral neuropathic pain affects about 8% of the population and is a major public health concern. There is an urgent need for novel therapeutic approaches since current treatments (opioids, repurposed anti-anxiolytics and anti-epileptics) cause serious side-effects and are only moderately effective in a minority of patients. We identified the NaK-ATPase modulator protein Fxyd2, expressed in specific subsets of somatosensory neurons of the dorsal root ganglia in rodents and humans. Analysis of null mutant *Fxyd2* mice in a rodent model of neuropathic pain showed that *Fxyd2* expression is necessary to maintain pain behavior.

Here, we tested the capacity of antisense oligonucleotides (ASOs) directed against *Fxyd2* mRNA to attenuate pain symptoms in different experimental models. Having identified a 20-mer ASO-Fxyd2, with strong inhibitory efficiency in a human embryonic kidney cell line, we then used this sequence to inhibit Fxyd2 expression in rat DRG neurons *in vivo*. Daily intrathecal injections of an ASO-Fxyd2, chemically modified for direct transfection without transfection reagent, in rat models of neuropathic and also chronic inflammatory pain, led to a complete attenuation of mechanical hypersensitivity. Injection of equivalent quantities of a control scrambled-ASO had no effect. Our results point to Fxyd2 inhibition by antisense oligonucleotides as a novel therapeutic approach to treating pain conditions.

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Session VIII: Oligonucleotide Preclinical Stage Development
Daniel Curtis

Wednesday, 16 October
11.00-11.25

Antisense Oligonucleotide Therapies for Rare Neurodegenerative Disorders

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Genetic diseases provide essential insights into disease mechanisms and, in defining disease causing mutations, suggest therapeutic targets for specific disorders. Antisense oligonucleotide approaches expand the potential for therapeutic intervention in the cases where small molecules or other approaches are problematic. One interesting mechanism which can be applied is the modulation of pre-mRNA splicing to include or exclude exons to counteract the detrimental effect of disease mutations. The Dutch mutation in the Amyloid Precursor Protein affects the Amyloid Beta peptide sequence, leading to amyloid accumulation in the cerebral vasculature and early onset hemorrhagic stroke at approximately age 50. This autosomal dominant condition, called D-CAA (Dutch Cerebral Amyloid Angiopathy), appears to be a pure amyloidopathy, and is an accelerated version of sporadic CAA which affects elderly patients with normal APP sequences. The Dutch mutation is confined to three large pedigrees from Katwijk and nearby villages in The Netherlands, as well as a related population near Perth Australia. We have developed candidate therapeutic ASOs which modulate the splicing of APP by exclusion of exon 17. Exon 17 contains the central region of Amyloid Beta including the Dutch mutation. By eliminating the potential to produce Amyloid Beta, the ASOs reduce amyloid accumulation and should delay or prevent the onset of stroke in D-CAA. We will present data showing in vitro and in vivo activity of candidate ASOs. We explore data from natural history studies suggesting biomarker-based approaches for clinical studies.

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Session VIII: Oligonucleotide Preclinical Stage Development
Daniel Lindén

Wednesday, 16 October
11.25-11.50

PNPLA3 antisense oligonucleotide, a novel precision medicine approach to treat non-alcoholic steatohepatitis

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Objective: Non-alcoholic fatty liver disease (NAFLD) is becoming a leading cause of advanced chronic liver disease. The progression of NAFLD, including the most aggressive variant, non-alcoholic steatohepatitis (NASH), has a strong genetic component, and the most robust contributor is the patatin-like phospholipase domain-containing 3 (*PNPLA3*) rs738409 encoding the 148M protein sequence variant. We hypothesized that suppressing the expression of the PNPLA3 148M mutant protein would exert a beneficial effect on the entire spectrum of NAFLD.

Methods: We examined the effect of *PNPLA3* silencing using antisense oligonucleotide (ASO) and small interfering RNA (siRNA) in human HepG2 hepatoma cells homozygous for the *PNPLA3* rs738409 sequence variant encoding for the 148M protein. We also explored the effects of liver-targeted GalNAc-conjugated ASO-mediated silencing of *Pnpla3* in a knock-in mouse model in which we introduced the human *PNPLA3* I148M mutation.

Results: *PNPLA3* suppression decreased intracellular lipids in HepG2 cells. ASO-mediated silencing of *Pnpla3* reduced liver steatosis (p=0.038) in homozygous *Pnpla3* 148M/M knock-in mutant mice but not in wild-type littermates fed a steatogenic high-sucrose diet. In mice fed a NASH-inducing diet, ASO-mediated silencing of *Pnpla3* reduced liver steatosis score and NAFLD activity score independent of the *Pnpla3* genotype. Furthermore, the ratio between cholesteryl palmitoleate and cholesteryl palmitate (CE 16:1/16:0) was reduced in both plasma and liver after *Pnpla3* silencing in both genotypes. However, reductions in liver inflammation score (p=0.018) and fibrosis stage (p=0.031) were observed only in the *Pnpla3* knock-in 148M/M mutant mice and not in wild-type littermates. These responses were accompanied by reduced liver levels of Mcp1 (p=0.026) and Timp2 (p=0.007) specifically in the mutant knock-in mice. This may reduce levels of chemokine attracting inflammatory cells and increase the collagenolytic activity during tissue regeneration.

Conclusion: This study provides the first evidence that a Pnpla3 ASO therapy can improve all features of NAFLD including liver fibrosis and suppressing the expression of a strong innate genetic risk factor, *PNPLA3* I148M, may open up for the first precision medicine approach in NASH.

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Session VIII: Oligonucleotide Preclinical Stage Development
Elizabeth Vroom

Wednesday, 16 October
11.50-12.15

The Role of Patient Organisations in Drug Development: The example of Duchenne Muscular Dystrophy

Elizabeth Vroom
World Duchenne Organization

Duchenne parents decided to shoulder responsibility and become an active partner in the development of viable treatments or a cure for this disease. Duchenne parents worldwide have played a significant role not only as funders of research or the initiative for start-up companies, but also in raising awareness, improving care, setting up standards of care and the dissemination of this information. Furthermore they took the initiative to collect data on patient and caregiver preferences, to develop outcome measures, including Patient Reported Outcome Measures, and bridge the so-called 'Valley of Death' in drug development. They focus on optimal (re)use of data and optimal trials design. They are involved in regulatory and HTA discussions and European policies.

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Session VIII: Oligonucleotide Preclinical Stage Development
Marc Thibonnier

Wednesday, 16 October
12.15-12.30

Metabolic and Energetic Benefits of microRNA-22 Inhibition

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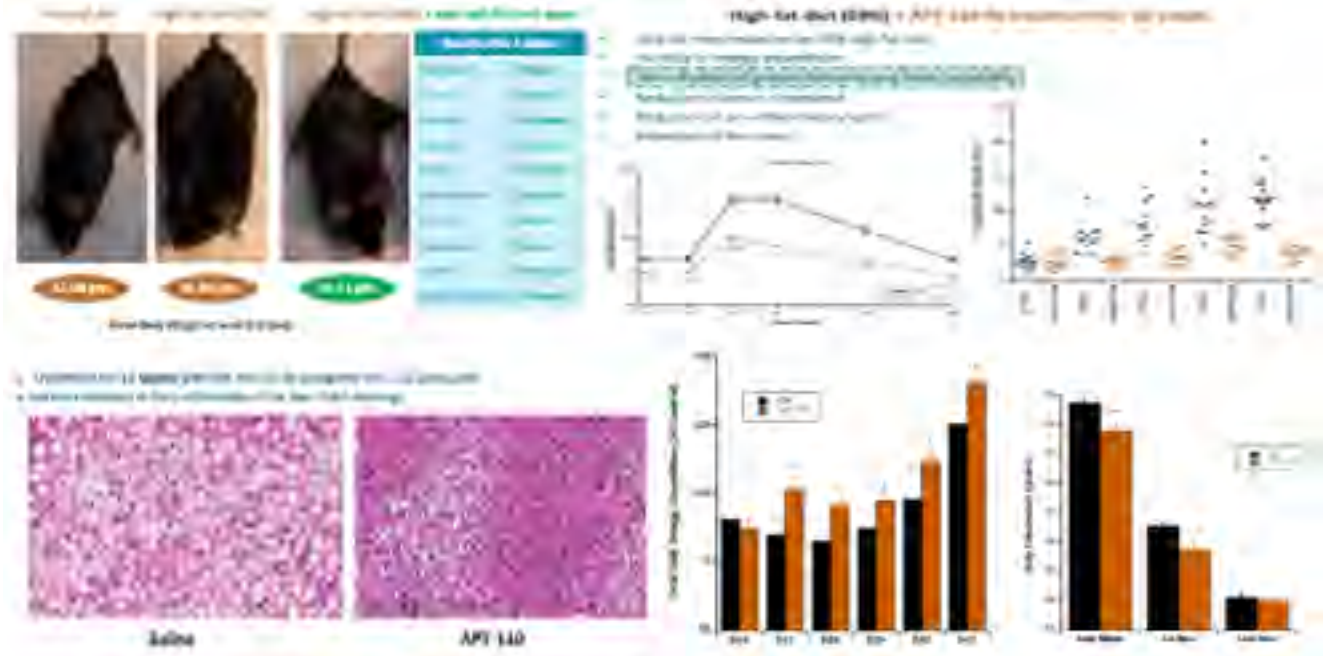
Diabesity and Non Alcoholic Fatty Liver disease (NAFLD) are growing pandemics with substantial health and financial consequences. We are developing microRNA-based drug candidates that increase lipid oxidation and energy expenditure to tackle metabolic diseases.

Phenotypic screening of miRNA antagomirs and agomirs in primary cultures of human subcutaneous adipocytes revealed that inhibition of microRNA-22-3p resulted in increased lipid oxidation, mitochondrial activity and energy expenditure. These effects may be mediated through activation of target genes like *KDM3A*, *KDM6B, PPARA, PPARGC1B and SIRT1* involved in lipid catabolism, thermogenesis and glucose homeostasis.

We investigated whether these in vitro findings could be replicated in vivo in diet-induced obese (DIO) mice of various ages. In DIO male C57Bl/6 mice fed a 60% fat diet, weekly s.c. injections of different miR22-3p antagomirs for 8 weeks produced a significant fat mass reduction, but no change of appetite nor body temperature. Insulin sensitivity, circulating glucose and cholesterol were also improved. A follow-up study exploring the metabolic and energetic effects of our first miR-22-3p antagomir drug candidate (APT-110) in DIO mice showed that weekly s.c. injections of APT-110 for 12 weeks produced a sustained increase of energy expenditure as early as Day 11 of treatment, a significant fat mass reduction, but no change of appetite, physical activity nor body temperature. Insulin sensitivity, glucose, cholesterol and leptin were also improved. Oral glucose tolerance tests were normalized. There was a dramatic reduction of liver steatosis at completion of the study after 3 months of active treatment. RNA sequencing by NGS revealed an activation of lipid metabolism pathways.

These original findings suggest that microRNA-22-3p inhibition could be an effective treatment of fat accumulation and related complex metabolic disorders like obesity and type 2 diabetes mellitus (the so-called diabesity) and NAFLD.



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Session VIII: Oligonucleotide Preclinical Stage Development
Yi-Wen Chen
Wednesday, 16 October
12.30-12.45

Gapmers targeting DUX4 as a therapeutic strategy for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common inherited muscular dystrophies with an incidence of 1:8,000 to 1:20,000. Studies showed that FSHD is caused by aberrant expression of double homeobox 4 (DUX4) due to epigenetic changes of the D4Z4 macrosatellite repeat region at chromosome 4q35. The aberrant expression of DUX4 misregulates downstream genes and pathways, which leads to muscle pathologies and weakness. To date, there is no effective treatment for FSHD. The goal of this study is to evaluate antisense oligonucleotide strategies to reduce the pathogenic DUX4 mRNA in affected muscles and improve muscle pathology and function using an FSHD mouse model. LNA and 2'-MOE gapmers were delivered by either intramuscular injections (i.m.) or subcutaneous injections (s.c.) to the DUX4-expressing FLExDUX4 mouse model. Our results showed that i.m. injections (20ug) of gapmers into the tibialis anterior muscles significantly reduced DUX4 transcripts in the muscles of the FLExDUX4 mice. Short-term treatment by s.c. injections (20mg/kg) of the gapmers reduced DUX4 expression in the muscles. In a long-term 10-week trial, s.c. injections (20mg/kg) twice a week significantly reduced DUX4 expression. In addition, muscle function measured by grip strength testing showed functional recovery after the treatment. Muscle fibrosis was also reduced by the treatments. Our findings showed that the gapmers targeting DUX4 significantly reduced DUX4 transcripts. The reduction of DUX4 was accompanied by recovery of muscle functional deficits and improvement of muscle pathology in the FLExDUX4 mice. The data support the use of gapmers as a viable therapeutic approach for FSHD.

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Session VIII: Oligonucleotide Preclinical Stage Development
Atze Bergsma
Wednesday, 16 October
12.45-13.00

Development and optimization of antisense therapy for Pompe disease

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Pompe disease, a lysosomal storage disease that affects 1 in 40.000 newborns, is an autosomal recessive genetic disorder which causes the gradual loss of muscle function leading to loss of ambulance and respiratory insufficiency. Since 2006, enzyme replacement therapy (ERT) has been approved for the treatment of Pompe disease. Our center currently treats approximately 120 patients and is the single reference center for Pompe disease in The Netherlands. However, ERT has its drawbacks including variable treatment efficacy, antibody response, long and frequent infusions, and high costs.

Over 90% of adults and 60% of children with Pompe disease in the Caucasian population carry the c.-32-13T>G splicing variant that causes partial skipping of *GAA* exon 2, leading to a loss of functional acid α-glucosidase (GAA) protein activity. We recently identified a pseudoexon located upstream of exon 2. In the presence of c.-32-13T>G , this pseudoexon is included in the mRNA transcript at the cost of *GAA* exon 2 inclusion, leading to loss of the transcription start site and subsequent degradation *GAA* mRNA. We developed antisense oligonucleotides (AONs) targeting this pseudoexon that are able to restore canonical splicing of *GAA* exon 2 in patient iPSC-derived myotubes, leading to a GAA enzymatic activity well above the disease threshold.

Improvements in RNA uptake in skeletal muscle cells is required for the clinical development of splice-switching RNA-based therapy as an alternative treatment option for Pompe disease. In an effort to test improved versions of identified AONs, we have developed a 3D Muscle-on-Chip system in which human iPSC-derived myogenic progenitors are used to generate cultured 3D muscle bundles. This system allows evaluation of AONs on uptake and splicing correction in a personalized manner using contractile force as functional read out. It will not only be useful for testing improvement of AONs for Pompe disease, but also for the testing of novel therapies for muscle disorders in general.

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Session IX: Oligonucleotide Clinical Studies
Tal Zaks
Wednesday, 16 October
14.30-14.55

Bringing mRNA medicines to patients

Tal Zaks, MD, PhD
Moderna, Inc.

Using mRNA to guide the translation of proteins in-vivo has the potential to lead to a wide range of medicinal applications, spanning from infectious disease and cancer vaccines to localized tissue-specific therapies and ultimately systemically active therapeutics. However, given LNP-encoded mRNAs have a biological similarity to RNA viruses, harnessing the therapeutic potential requires avoiding non-specific immune activation, whether by the delivery vehicle or the mRNA itself.
By combining our understanding of the underlying mRNA science and the invention of new delivery technologies, we have been discovering and developing mRNA medicines a range of specific therapeutic modalities. Guided by preclinical models and their translatability to humans, we have now advanced multiple mRNA medicines in clinical trials across therapeutic areas spanning infectious diseases, oncology, and rare genetic diseases. This talk will review these applications, with a focus on systemically administered mRNA therapeutics.

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Session IX: Oligonucleotide Clinical Studies
Julian Gillmore
Wednesday, 16 October
14.55-15.20

Abstract not available at the time of printing

Session IX: Oligonucleotide Clinical Studies
Toby Ferguson
Wednesday, 16 October
15.20-15.45

Safety, PK, PD, and exploratory efficacy in single and multiple dose study of a SOD1 antisense oligonucleotide (BIIB067) in participants with ALS

Timothy Miller (1), Merit Cudkowicz (2), Pamela Shaw (3), Danielle Graham (4), Stephanie Fradette (4), Hani Houshyar (4), Frank Bennett (5), Roger Lane (5), Ivan Nestorov (4), Laura Fanning (4), Ih Chang (4), Toby Ferguson (4)* *presenting author*
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Objective: To evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of an antisense oligonucleotide (BIIB067) designed to reduce superoxide dismutase (SOD1) mRNA in people with amyotrophic lateral sclerosis (ALS) with SOD1 gene mutation (SOD1-ALS).
Background: ALS is a fatal, neurodegenerative disease characterized by loss or dysfunction of upper and lower motor neurons. Approximately 2% of ALS cases are linked to SOD1 mutations. Over 200 SOD1 mutations have been identified with substantial variation in rate of disease progression. Toxicity of mutant SOD1 is secondary to gain of function, not loss of SOD activity, suggesting SOD1 reduction may be therapeutic. BIIB067 is under development for treatment of SOD1-ALS.
Methods: This randomized, placebo-controlled, single- and multiple-ascending dose (SAD/MAD) study enrolled participants with ALS. In the MAD portion of the study, 50 participants with confirmed SOD1 mutation were randomized (3:1 BIIB067:placebo) to receive BIIB067 (20, 40, 60, or 100 mg) or placebo for 12 weeks. Safety (primary), PK/PD (secondary), and efficacy (exploratory) were assessed.
Results: The majority of adverse events (AEs) were mild or moderate in severity. Dose-dependent increases in BIIB067 concentrations in plasma and CSF were observed. A statistically significant reduction of CSF SOD1 was observed in the 100-mg cohort (n=10) versus placebo (n=12) (p=0.002) and suggested substantial reduction of CNS tissue SOD1. Lowering of CSF phosphorylated neurofilament heavy and slowing of functional decline as measured by ALS Functional Rating Scale Revised scores, slow vital capacity, and muscle strength were observed in the 100 mg cohort versus placebo. In participants with SOD1 mutations known to be rapidly progressive, a greater difference between the 100-mg and placebo groups was observed across these measures compared to those with other mutations.
Conclusions: This first report of BIIB067 in SOD1-ALS demonstrates reduction of SOD1 in CSF and strongly supports further investigation of BIIB067 efficacy in people with SOD1-ALS.
Study supported by: Biogen.

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Session IX: Oligonucleotide Clinical Studies
Timothy Yu
Wednesday, 16 October
15.45-16.10

Individualized oligos, orphan diseases, & new opportunities in genomic medicine

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Over 30 million patients in the United States live with a rare disease. Genome sequencing is revolutionizing their diagnosis, but 95% still lack effective therapy; given the diversity of diseases to tackle, creative frameworks will be necessary to address this gap. We will review our experience with the accelerated development of the patient-specific drug milasen as rescue therapy for a young girl suffering from vision loss, seizures, and neurologic regression. We will describe how arriving at a precise molecular diagnosis prompted a collaborative effort to design, test, and deploy a novel and allele-specific splice-switching oligonucleotide to treat her. Implications of this work for other patients, and prospects for further development of individualized genomic medicine will be discussed.

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Session IX: Oligonucleotide Clinical Studies
David Giljohann
Wednesday, 16 October
16.10-16.25

Clinical Results from XCUR17, a Topically Applied Antisense Spherical Nucleic Acid in Patients with Psoriasis

David Giljohann, *Exicure Inc.*

Exicure is developing topical antisense gene regulating agents, including XCUR17. XCUR17 is designed to locally inhibit interleukin 17 receptor alpha mRNA for the treatment of chronic plaque psoriasis. Drug discovery and development efforts by Exicure revolve around the use of spherical nucleic acid (SNA) constructs, which are 3-dimensional arrangements of oligonucleotides where the nucleic acids are densely packed and radially oriented. When arranging oligonucleotides in this way, properties arise that are distinct from the "linear" nucleic acids (i.e., nucleic acids not arranged in the SNA format). Most importantly, oligonucleotides arranged in the SNA geometry exhibit skin penetration properties and increased cellular uptake when compared to linear oligonucleotides. A Phase 1 clinical trial of XCUR-17 indicated that the drug was safe, well tolerated and produced a dose dependent clinical response in the psoriatic skin. The presentation will describe the preclinical results of the compounds, and the clinical and biomarker results from the first in patient study of XCUR-17.

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Session IX: Oligonucleotide Clinical Studies – Late Breaking
Nagy Habib

Wednesday, 16 October
16.25-16.40

Activation of CEBPA in myeloid cells by saRNA in HCC patients: The emergence of an immunomodulatory switch for anti-cancer therapy

Vikash Reebye[1], Sheba Jarvis[2], Jenni Vasara[3], Nina Raulf,[3] Pinelopi Andrikakou[1], Robert Habib[3], David Blakey[3], John Rossi[4], **Nagy Habib[1]**.

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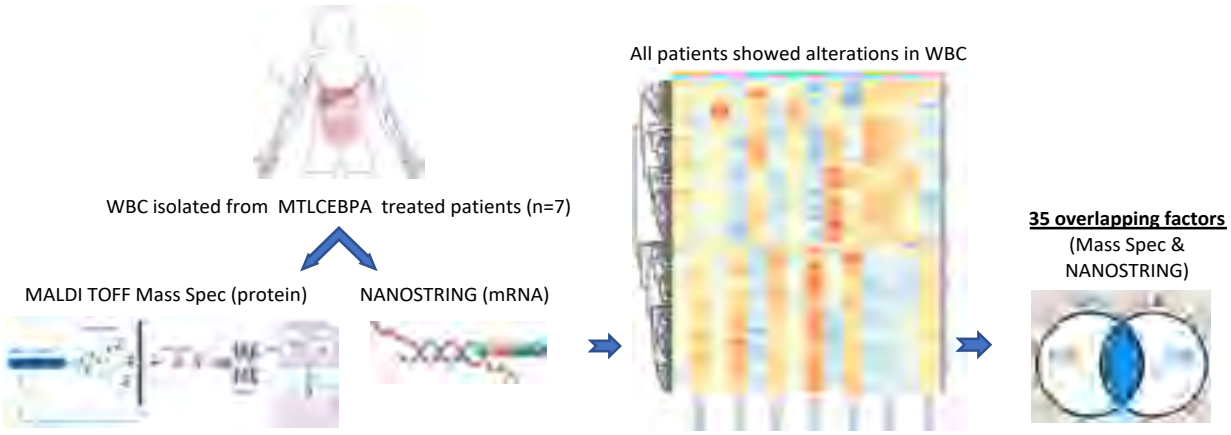
Background: MTL-CEBPA is a first-in-class small activating RNA (saRNA) oligonucleotide which specifically up-regulates the myeloid cell master regulator, C/EBP-α (CCAAT/enhancer-binding protein alpha). Here we present for the first time the quantitative changes observed in the WBC of patients administered with CEBPA-saRNA.

Method: MTL-CEBPA is currently in a Phase I, dose escalation and dose expansion trial in adults with HCC or secondary liver cancer. Patients receive intravenous MTL-CEBPA at 28-160 mg/m2 for 3 weeks either QW, BIW at d1 and d2, BIW at d1 and d3, or TIW at d1, d2, and d3 followed by a rest period of 1 week. Adverse events, serum PK, anti-tumour activity were assessed. Circulating WBC were captured using Ambion's LeukoLOCK isolation columns before treatment for baseline measurements and post-treatment at Day 2 , 8 and 15 for quantitative protein expression (MALDI-TOF Mass spectroscopy) and quantitative gene expression (NANOSTRING).

Results: MTL-CEBPA demonstrated a good safety profile with several durable complete responders and partial responders observed. Treatment altered the expression of 522 protein and 656 genes in circulating WBC. An overlapping analysis revealed 35 key targets were affected in all of the patients. MTL-CEBPA treatment enhanced *CEBPA* expression affecting macrophage biology through regulation of *BAX, CD14, CEBPB, ELANE, MAPK*; and the gate keeper of the immune system, *NFkB1*. These are well defined immunomodulatory factors known to polarise macrophages towards an anti-tumour phenotype.



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Session IX: Oligonucleotide Clinical Studies – Late Breaking
Ilja Kubisch

Wednesday, 16 October
16.40-16.55

ENVISION, a Phase 3 Study of Safety and Efficacy of Givosiran, an Investigational RNAi Therapeutic, in Acute Hepatic Porphyria Patients

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Objectives:
Acute Hepatic Porphyria (AHP) is a family of rare genetic diseases due to enzyme defects in heme synthesis that most commonly includes acute intermittent porphyria (AIP), but can also include hereditary coproporphyria (HCP) and variegate porphyria (VP). Induction of 5-aminolevulate acid synthase 1 can lead to accumulation of neurotoxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG), resulting in neurovisceral attacks and chronic manifestations. Givosiran, an investigational RNAi therapeutic, targets liver ALAS1 to reduce ALA/PBG and is being evaluated for its ability to reduce attacks and disease manifestations.
Methods:
ENVISION (NCT03338816), a Phase 3 global, multicenter, randomized, double-blind, placebo-controlled trial with an open label extension (OLE), evaluated the efficacy and safety of subcutaneous givosiran in AHP. Endpoints were evaluated in genetically confirmed AIP patients unless noted otherwise. The primary endpoint was composite annualized attack rate requiring hospitalizations, urgent care, or hemin administration at home over six months. Secondary endpoints at six months included ALA/PBG levels, hemin use, composite attack rate in all AHP patients, daily pain, fatigue, nausea, and quality of life.
Results:
Ninety-four AHP patients were enrolled in the study (89 AIP, 2 VP, 1 HCP, 2 AIP without an identified genetic mutation). Givosiran treatment led to significantly fewer composite attacks in AIP patients relative to placebo ($p=6.04 \times 10^{-9}$), as well as reductions in ALA/PBG, hemin use, and composite attacks in all AHP patients ($p < 0.0001$). Worst daily pain was also significantly reduced in AIP patients ($p=0.0455$). Adverse events were reported for most patients (placebo=80.4%; givosiran=89.6%), and serious adverse events were reported in 20.8% of givosiran and 8.7% of placebo patients. There was one discontinuation in a patient on givosiran due to a transaminase elevation, and 93/94 patients continued in the OLE phase.
Conclusions:
In a Phase 3 study, givosiran treatment led to clinically meaningful efficacy with an acceptable tolerability profile.
Presenter: Dr. Ilja Kubisch, Klinikum Chemnitz, Flemmingstraße 2, 09116 Chemnitz / i.kubisch@skc.de

Session IX: Oligonucleotide Clinical Studies – Late Breaking
The Medicines Company

Wednesday, 16 October
16.55-17.10

Further progress with siRNA for atherosclerotic cardiovascular disease: Update on the ORION program including results from recently completed ORION-11 and ongoing ORION-1/3

Presenter: TBA, *The Medicines Company*

Inclisiran is a GalNAc conjugated siRNA designed to inhibit PCSK9, reduce low-density lipoprotein (LDL-C) cholesterol levels, and potentially be the first oligonucleotide drug to benefit a large patient population.

We presented the background, rationale, situation and plans for the development of inclisiran (an investigational agent not yet approved in any country) in patients with ASCVD and risk equivalents to the OTS on 1st October 2018. Since then progress towards fulfilling the promise of oligonucleotide therapeutics for large population chronic diseases has been promising. By the end of 2019, inclisiran will have completed its Phase III pivotal studies in 3660 patients utilizing 300mg doses of inclisiran administered on day-1, day-90 and then every 6 months for 18 months. The first of the three confirmatory Phase III trials (ORION-11), representing 1617 patients, was reported as a late-breaker at the European Society of Cardiology on 2nd September 2019.

ORION -11 met its co-primary efficacy endpoints – inclisiran treatment resulted in a 54% reduction in LDL-C compared to placebo at day 510 ($p<0.00001$) and a 50% time-averaged reduction (day 90-540) in LDL-C compared to placebo ($p<0.00001$). Safety measurements, including treatment-emergent adverse events, were similar in the two populations with the sole exception of adverse events at the injection site (4.19% inclisiran compared with placebo).

In addition, during the last year, 3-years of follow-up treatment for Phase II patients from the ORION-1/3 study showed persistent LDL-C lowering (average 51% for all patients treated and 56% for patients given the dose selected for Phase III), highly consistent with pharmacodynamic models built previously. The long-term observations in Phase II patients continues to provide promising data, with no reports of hepatic, renal or immune-related toxicity. Meantime, other Phase I data have confirmed no need for dose adjustment in patients with renal impairment.

Detailed clinical trial results for ORION -11 and ORION 1/3 will be presented at the 2019 OTS meeting and will further clarify the potential for inclisiran to be the first broadly used oligonucleotide drug.

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