Modification and multimerization of therapeutic oligonucleotides
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Platform technology in oligonucleotide therapeutics can be described according to a number of ‘dimensions’ (sets of parameters that are at least partly orthogonal to each other and work together to improve clinical performance). These include (i) a potent and relevant mechanism of action, such as RNAi, RNase H, or splice switching, (ii) robust nucleic acid chemistry, including modified sugars, phosphates and nucleobases, and (iii) the presence and nature of a ligand such as cholesterol or GalNAc, if applicable.

We suggest that the development of oligonucleotide clusters or “multimers” may be considered an additional dimension for optimization of therapeutic oligonucleotides. Several other groups have worked on multimeric species from two oligonucleotides to dozens or hundreds of oligonucleotides, resulting in improvements in tissue distribution and cellular uptake as well as, in some cases, changes in mechanism or increased potency. Our work focuses on the range from 2-8 oligonucleotides.

In one project, for example, we have been working in collaboration with Idera Pharmaceuticals to understand the mechanism of dimeric antisense oligonucleotides with their 3'-ends facing out (termed 3GA by Idera). These show improved potency relative to the analogous monomeric oligonucleotides, both in cells and animals, and we explore the mechanism for their increased potency.

In a separate project, we have tested series of higher clusters of oligonucleotides in terms of their effects on tissue distribution and potency of gene silencing. Our findings suggest that particular multimer designs may be optimal for targeting particular tissues. Our work on higher clusters has required the development of robust synthetic methods, and we also present a comparison of inline synthesis of multimeric species (using branching phosphoramidites) with post-synthesis assembly of individual oligonucleotides which revealed several synthetic surprises.

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Pyrrole-imidazole Polyamides: Artificial Genetic Switches for Therapeutic Gene Modulation

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We are interested in the chemical biology of nucleic acids. Using the tool of organic chemistry, molecular biology and physical chemistry, we explore the chemical principles underlining the recognition, reactivity and structure of nucleic acids. For example we have been undertaking original research on atom-specific reaction of DNA by antitumor antibiotics as well as sequence-specific recognition of DNA.\[1\] By reconstituting such knowledge, various functionalized sequence-specific DNA binding pyrrole-imidazole polyamides (PIPs) were synthesized as an artificial genetic switch, which can switch on and switch off the gene expression on demand. For example, recently developed alkylating PIP that could switches off cancer related KRAS gene,\[2\] and RUNX family controlling genes.\[3\] Especially, PIP targeting RUNX-binding sequences was highly effective against AML cells and against several poor prognosis solid tumors in a xenograft mouse model of AML without notable adverse events. Also designed and synthesized HDAC inhibitor\[4\] or HAT activator\[5\] PIP conjugates which effectively switch on specific gene networks. Therefore, strategies to expand our tunable PIPs could create an epoch-making approach to modulate the desired gene expressions. In this talk recent progress of regulation of the gene expression using designed PIPs will be discussed.

Session I: Nucleic Acid Chemistry
Jesper Wengel
Sunday, 24 September 2017
16.15 – 16.37

Lipophilic LNAs to improve oligonucleotide drug pharmacokinetics

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LNA (locked nucleic acid) and the corresponding 2’-amino-LNA derivative both mediate strong nucleic acid hybridization, and the latter furthermore the opportunity of attachment of a variety of functional groups along antisense and siRNA constructs. The focus of the presentation will be on Lipo-LNA, ie. on novel lipophilic 2’-amino-LNA-based antisense compounds. It will be presented how plasma half-life and biodistribution can be engineered, and gene knock-down data in cell culture and in vivo will be shown for both phosphorothioate and phosphorodiester derivatives. For a review on 2’-amino-LNA, see I. K. Astakhova and J. Wengel, Acc. Chem. Res. 2014, 47, 1768.

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Synthesis and Biological Activity of Thiomorpholino, Morpholino, and Imidoamidate DNAs

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Thiophosphoramidate morpholino (TMO) and their DNA chimeras (TMO/DNA) were prepared on DNA synthesizers using phosphoramidite chemistry. These new oligonucleotide analogues contain morpholino- and 2’-deoxyribonucleosides joined through thiophosphor/phosphor internucleotide linkages and were prepared from synthons having standard amide protection on the nucleoside bases. The TMO and TMO/DNA chimeras were observed to have higher melting temperatures when compared to natural DNA/DNA and DNA/RNA duplexes. Moreover the TMO/RNA duplexes exhibit the A-form structure and are RNase H1 active. Treatment of HeLa cells with fluorescently labeled TMO and TMO/DNA chimeras demonstrated that these analogues were efficiently taken up by cells in the absence of a lipid transfection reagent and stimulated biological activity in a dual luciferase assay. Because of the simplistic synthesis procedures, various TMO analogues and their DNA chimeras are now readily available and should therefore open new pathways for research into the antisense and diagnostic oligonucleotide fields.

2’-Deoxynucleosides prepared as acylamide, diisopropylaminophosphoramidite synthons have been used to synthesize a new DNA analogue having amino functionality at both nonlinking internucleotide phosphorus bonds. These imidoamidate derivatives form duplexes with complementary DNA, are positively charged, can be transfected into cells in the absence of lipid, are modestly active in a dual luciferase assay and are RNase H1 active.

Other chemistries have led to the synthesis of amino alcohol phosphotriester DNA, diboraneposphonate DNA, and 3’-5’ linked triazoylphosphonate DNA. The synthesis and biological activity of selected analogues will be presented. A summary of the structures for these various analogs is as follows:

Diboraneposphonate DNA  3’-5’ Triazoylphosphonate DNA  Aminoalcohol DNA  Thiomorpholino DNA  Imidoamidate DNA
Stereodefined phosphorthioate LNA oligonucleotides
Nanna Albaek, Troels Koch
Roche Innovation Center Copenhagen

Locked nucleic acid (LNA) is a proven platform for generating oligonucleotide drug candidates. One of the key learnings over the years has been the appreciation of the wide property variations for different LNA oligonucleotides when measuring e.g. compound efficacy/potency, toxicity, and cellular uptake. Prior to selecting a lead candidate these differentiated properties are thoroughly investigated to understand all drug parameters required for a specific indication.

The current drug discovery platform of LNA oligonucleotides utilizes in most cases a fully modified phosphorthioate (PS) backbone. Since each PS internucleoside linkage introduces a stereocenter which is not according to standard oligonucleotide synthesis procedures stereochemically controlled, these oligonucleotides are in fact a mixture of diastereoisomers and not just “one compound”. Hence, a 16-mer LNA oligonucleotide having 15 internucleoside PS linkages is in fact a mixture of $32 \times 2^{15}$ different diastereoisomers.

We have found that the property variations described above for diastereomeric mixtures can be further expanded when introducing stereodefined PS internucleoside linkages in LNA oligonucleotides. By the use of adapted chemistry procedures the PS stereochemistry in each internucleoside linkage can be controlled and allow for the synthesis of one single LNA isomer/compound. We have found wide property variations on e.g. potency for LNA’s with different fully controlled PS stereochemistries all having the same nucleobase and sugarmodification pattern. Moreover, we have found changes in drug properties by stereochemically controlling only a few internucleoside PS linkages and leaving the other PS’s still as a mixture of both possible configurations and thereby making a subset of diastereoisomers as the final product.

This presentation will focus on the diversity of properties for both diastereomeric mixtures and for stereodefined PS LNA oligonucleotides.
Triplex Forming Oligonucleotides (TFOs) LNA-containing: \textit{in silico} and \textit{in vitro} studies

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Oligonucleotides (ONs) that bind to the polypurine strand in the major groove of double strand DNA (dsDNA), by Hoogsteen (HG) (parallel orientation) or reverse HG (antiparallel) hydrogen bonds, thus forming a triplex structure, are called TFOs. In a parallel triplex, T\textbullet{}A\textendash{}T and C\textbullet{}G\textendash{}C base triads are formed. Sequence-specific DNA recognition by TFOs has been largely exploited to regulate gene expression at the transcriptional level. However, there are challenges facing TFO-targeting of dsDNA such as ON binding affinity and stability of the triplex structure in a genomic context. To overcome this we have used Locked Nucleic Acid (LNA), which is known to increase triplex stability. LNA is a synthetic nucleotide analogue characterized by a methylene bridging the 2'-oxygen and 4'-carbon of the ribose. Moreover, formation of cytosine-rich parallel triplexes is not favourable at physiological pH because it requires the protonation of cytosine. To overcome this we used ONs containing Pseudoisocytidine (ΨC), which can target the DNA duplex and form a parallel triplex at neutral pH. To further stabilize triplex formation we also examined the effect of conjugating TFOs with the triplex intercalating compound Twisted Intercalating Nucleic Acid (TINA), focusing on its position within the ON sequence. TINA is a flexible base-stacking monomer that has been shown to stabilize intermolecular triplex structures.

We performed Electrophoretic Mobility Shift Assays and characterized the structure of the formed triplex using molecular dynamics simulation. All hybridizations were performed in intra-nuclear salt conditions at pH 7.4, and in all cases a triplex-specific intercalating agent, Benzoquinquinaxalone (BQQ) was used in parallel experiments to analyse triplex formation. We found that DNA/LNA TFOs, were conformationally pre-organized for major groove binding, in contrast to non-modified DNA TFOs. Reducing the LNA content in the 3'-end impaired hybridization as compared to reduction in the 5'-end. Similarly, inserting TINA in TFOs potently stabilized triplex formation, with 3'-end TINA being more efficient than 5'-end inclusion and much more efficient than when TINA was inserted in the center. TFOs having three or more consecutive ΨCs, were unable to form triplexes under intranuclear salt conditions at pH 7.4, also when BQQ and TINA were included. Only with nonconsecutive ΨCs and in combination with alternating DNA/LNA residues, the C-rich TFO was able to form a triplex. Collectively, these findings should facilitate the design of potent anti-gene ONs.
Therapeutics targeting RNA offer great potential – not only can they be used to tackle the 80% of translated proteins that are difficult to modulate with either small molecule drugs or antibodies but can also be used to modulate the regulatory non-coding RNA. This opens up a great number of new targets for disease intervention and advances in RNA therapeutic chemistry to increase stability and potency are opening up a new wave of therapeutic molecules. The selective delivery of oligonucleotides to specific tissues has the exciting potential to open up even more cell types to potent modulation by these molecules and improve their therapeutic activity and safety profile. The specific targeted delivery to hepatocytes using GalNAc- oligonucleotide conjugates is well established and has been shown to lower the therapeutic dose needed for liver targets by approximately 10-fold [1,2]. To build on this early success with RNA targeting, AstraZeneca has in collaboration with IONIS Pharmaceuticals developed novel targeting approaches to enable specific delivery of oligonucleotide to cells other than hepatocytes and that are of interest in cardio-metabolic diseases. This talk will address some of the strategies and platforms developed to enable discovery and development of novel homing ligands that can be conjugated to antisense oligonucleotides (ASOs) for targeted delivery to pancreatic beta cells and that show efficient knock-down of genes in vitro and in vivo. In vitro, in GLP1R overexpressing HEK293 cells, GLP1-ASO treatment showed enhanced knock down of the target gene compared to the ASO, with a 40 fold increase in potency for MALAT1 ASO. In vivo, single subcutaneous injections of GLP1-ASOs dose dependently increased the ASO exposure and reduced gene expression in mouse islets while parent ASO was without effect.

Cellular dynamics visualized from molecules to organisms at increased spatio-temporal resolution

Tom Kirchhausen, *Harvard Medical School*

Frontier optical-imaging modalities exemplified by the lattice light-sheet microscope invented by Eric Betzig set new visualization standards for analyzing and understanding sub-cellular processes in the complex and dynamic three-dimensional environment of living-cells in isolation and within tissues of an organism. By using ultra-thin sheets of light to rapidly illuminate biological samples with extremely low photon doses, 3D experiments previously limited to seconds or minutes by photo-bleaching or by photo-toxicity, can now be done at diffraction limited resolution and high-temporal precision with unprecedented duration of minutes or hours. We believe this ability to image with minimal perturbations is ideally suited to support hypothesis-generating research geared towards new discoveries.

The talk will illustrate how we can ‘see’ in three dimensions the intracellular delivery of RNAi and antisense oligonucleotides in cells maintained in tissue culture conditions and also will describe our most recent efforts to link processes that mediate and regulate the movement of vesicular carriers throughout cells and the biogenesis of organelles in both, isolated cells maintained in tissue culture conditions and cells within tissues of a living zebrafish embryo.

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Unlocking the full potential of oligonucleotide therapeutics to address unmet medical needs requires development of strategies for efficient and functional delivery of oligonucleotides to target cells outside of the liver.

We established a scientific collaboration to explore extrahepatic delivery of chemically stabilized siRNAs as direct conjugates with Centyrins, a novel class of highly stable FN3 domain proteins. This presentation will discuss preparation, characterization, in vitro and in vivo gene silencing properties of Centyrin – siRNA conjugates.

Centyrins have been selected for conjugation on the basis of highly efficient internalization in target cells through receptor mediated endocytosis. Chemical and chemoenzymatic coupling of siRNA to Centyrins has yielded stable, well-behaved, fully homogeneous conjugates with good plasma stability. In vitro, these conjugates have demonstrated ability to knock down expression of mRNA and protein in cells expressing receptor target of the corresponding Centyrin, in the absence of any transfection reagent. In vivo, Centyrin-siRNA conjugates induced robust gene silencing in mouse tumor xenograft model with RNAi mechanism confirmation obtained through 5' RACE assay on tumor samples.

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Delivery of siRNA to the lung by lipid peptide nanoparticles as a therapy for cystic fibrosis

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Cystic fibrosis (CF) involves multiple organ systems but the main pathology is production of thick sticky mucus and chronic bacterial infection leading to loss of lung function. CF is caused by mutations in CFTR, a cAMP-regulated chloride channel in the apical membrane of the airway epithelium, but also involves upregulated activity of the epithelial sodium channel, ENaC, leading to defective ion and fluid transport in the lung epithelium which contribute to depletion of the airway surface liquid (ASL) and the viscous mucus, which impair mucociliary clearance enabling bacterial colonisation. We proposed that siRNA-mediated silencing of the αENaC major protein subunit, should reduce uptake of sodium and water, restoring the ASL depth restoring ciliary activity and so preventing bacterial infection. Short interfering RNAs have potential applications for CF and other respiratory diseases but have been limited by a lack of effective delivery formulations for the lung and so we have developed a novel nanoparticle for lung transfection, evaluated in air-liquid interface cultures of human CF epithelial cells and in normal mice.

Lipid-peptide nanoparticles of about 100 nm in size were produced from formulations of epithelial-targeted, cationic peptides for stable packaging of siRNA and receptor targeted endocytosis, and cationic and helper lipids that enhance transfection by enabling post-endocytic, endosomal escape. It is important not to silence ENaC activity completely as this would potentially lead to excess lung fluid but we found that αENaC mRNA silencing of 50% was sufficient to correct epithelial electrical ion transport properties, and to restore ASL depth, ciliary beating and fluid uptake measurements to normal levels in human ALI cell culture models of CF. These levels of siRNA-mediated silencing have not been achieved in ALI cultures with previous nanoparticle studies. In vivo, siRNA lipid-peptide nanoparticles were delivered to murine lungs by oropharyngeal instillation in doses of 16 µg with αENaC silencing levels of 30% achieved. Silencing persisted for at least one week while repeated administration of three doses at 48 h intervals enhanced silencing further to more than 50%, which is in the therapeutic window identified by ALI culture, with no evidence of toxicity.

In conclusion, we have identified a novel nanoparticle for lung siRNA delivery that silence ENaC to restore mucociliary parameters in CF ALI cultures and achieve therapeutic levels of ENaC silencing in vivo. Targeted, lipid-peptide nanoparticles thus offer a route to developing an ENaC siRNA therapy for CF and possibly other respiratory diseases.

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Cyclic peptides to improve delivery and bio-activity of antisense oligonucleotides in muscle tissue

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To be effective, antisense oligonucleotides (AONs) need to be delivered and effectively taken up by the target cells. From preclinical experiments it is known that a small amount of systemically administrated AON ends up in healthy skeletal and cardiac muscle tissue. Conjugation of tissue-homing peptides is an approach that aims to improve this. We performed phage display screens using a cyclic peptide library combined with next generation sequencing analysis to identify candidate muscle-homing peptides. Candidate peptides (with a fluorescent tag) were screened for improved uptake in differentiated myotubes and cardiomyocytes in vitro. Conjugation of the lead peptide to a 2'-O-methyl phosphorothioate AON designed to induce exon 23 skipping in the mdx mouse model, enabled a significant, two to three-fold increase in delivery and exon skipping in all analyzed skeletal and cardiac muscle, and appeared well tolerated. While selected as a muscle-homing peptide, uptake was increased in liver and kidney as well. Nonetheless, our results suggest that the identified peptide has the potential to facilitate delivery of AONs and perhaps other compounds to skeletal and cardiac muscle.

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Development of PPMO for the Treatment of DMD

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Duchenne muscular dystrophy (DMD) is a neuromuscular X-linked recessive disease most commonly caused by deletion mutations in the dystrophin gene resulting in disruption of the normal reading frame. One potential strategy to treat DMD is to use antisense oligonucleotides to alter the splicing pattern (i.e., exon skipping) so that an in-frame message is restored, resulting in the production of an internally truncated yet functional dystrophin protein. To this end, cell-penetrating peptides (CPPs) conjugated to phosphorodiamidate morpholino oligomers (PPMO) and unconjugated PMO compounds were injected into \textit{mdx} mice and cynomolgus monkeys to determine the levels of exon skipping and dystrophin in muscle. In the \textit{mdx} mouse studies, the PPMO compound is comprised of a CPP conjugated to the mouse oligonucleotide sequence designed to skip exon 23, whereas the PMO compound consists of the same sequence but without the CPP. PPMO treatment produced significant increases in exon skipping and dystrophin levels compared to PMO at all tested doses (10-120 mg/kg) and time points (7-90 days post-injection). Furthermore, PPMO treatment produced a widespread pattern of dystrophin expression on muscle tissue sections, whereas PMO treatment produced a limited and patchy expression pattern. Given that the sequences between the PPMO and PMO compounds are identical, the improved efficacy with PPMO is attributed to the CPP. To determine if similar improvements in efficacy occur in non-human primates, cynomolgus monkeys were treated with SRP-5051, a PPMO compound that consists of the same sequence conjugated to a human oligonucleotide sequence that promotes exon 51 skipping. Monkeys received 4 weekly injections of SRP-5051 at 20, 40 or 80 mg/kg before being sacrificed 48 hours after the last dose. The monkeys tolerated all the injections and none of the animals had to stop treatment due to in-life adverse events. Exon skipping was observed in all muscle tissues analyzed at the three dose levels with the exception of the heart at 20 mg/kg. The highest levels of efficacy occurred at 80 mg/kg, which produced 65.7-94.9%, 60.7%, and 43.9-66.6% exon skipping in skeletal (quadriceps, diaphragm, biceps, deltoid), cardiac (heart), and smooth (duodenum, esophagus, aorta) muscles, respectively. In summary, conjugation of the lead CPP to PMO improves muscle delivery \textit{in vivo}, and SRP-5051 targets and produces high levels of exon skipping in muscle tissues that are affected in DMD.

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Session III: Immune Effects and Safety of Nucleic Acids
Gunther Hartmann
Monday, 25 September 2017
11.00 – 11.25

Nucleic Acid Immunity
Gunther Hartmann, University of Bonn, Germany

Life has established sophisticated mechanisms to detect and eliminate foreign genetic material or to restrict its function and replication. Tremendous progress has been made in the understanding of these mechanisms which keep foreign or unwanted nucleic acids from viruses or phages in check. Mechanisms reach from restriction-modification systems and CRISPR/Cas in bacteria and archaebacteria to RNA interference and immune sensing of nucleic acids, altogether integral parts of a system which is now appreciated as nucleic acid immunity. With inherited receptors and acquired sequence information nucleic acid immunity comprises innate and adaptive components. Effector functions include diverse nuclease systems, intrinsic activities to directly restrict the function of foreign nucleic acids (e.g. PKR, ADAR1, IFIT1) and extrinsic pathways to alert the immune system and to elicit cytotoxic immune responses. These effects act in concert to restrict viral replication and to eliminate virus-infected cells. The principles of nucleic acid immunity are highly relevant for human disease. Besides its essential contribution to antiviral defense and restriction of endogenous retroelements, dysregulation of nucleic acid immunity can also lead to erroneous detection and response to self nucleic acids then causing sterile inflammation and autoimmunity. This review aims to provide an overview of the diverse mechanisms of nucleic acid immunity which mostly have been looked at separately in the past, and to integrate them under the framework nucleic acid immunity as a basic principle of life, the understanding of which has great potential to advance medicine, including the development of therapeutic immunostimulatory oligonucleotides for the treatment of viral infection and cancer.
Regulation of Inflammatory mRNAs via an Endoribonuclease Regnase-1
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Activation of innate immune cells by Toll-like receptors (TLRs) leads to the production of proinflammatory mediators of such as cytokines. The abundance of mRNAs encoding inflammation mediators is tightly controlled by a set of RNA binding proteins (RBPs) including Regnase-1 and Roquin. Roquin recognizes stem-loop structures present in mRNAs encoding inflammatory proteins and degrades them by recruiting a CCR4-NOT deadenylase complex to its target mRNAs. Roquin-mutant mice spontaneously develop autoimmunity by elevated expression of Icos on T cells and enhanced production of Tnf in innate immune cells. In contrast, Regnase-1 (also known as Zc3h12a) is an endonuclease essential for degradation of inflammation-related mRNAs such as Il6, Ptgs2 and Nfkbiz induced by TLR stimuli in innate immune cells. Regnase-1 and Roquin control early and late phase of inflammation, respectively. Regnase-1 is also critical for suppressing activation of T cells by degrading mRNAs including Rel, Icos and Il2 and maintains immune homeostasis in mice. Furthermore, Regnase-1 is important for the maintenance of iron homeostasis and prevention of anemia by controlling iron uptake via the degradation of Phd3 mRNA. Regnase-1 recognizes stem-loop structures present in mRNA 3’ untranslated regions, and mRNAs binding with Regnase-1 and Roquin are significantly overlapping. Regnase-1 specifically degrades translationally active mRNAs depending on UPF1, a helicase essential for the nonsense-mediated mRNA decay. Interestingly, Regnase-1 recognizes inflammatory mRNAs undergoing pioneer rounds of translation, which is triggered by the phosphorylation of UPF1 by a kinase SMG1. Taken together, our findings reveal that Regnase-1 is essential for the maintenance of immune homeostasis by acting as an endoribonuclease degrading inflammatory mRNAs in a unique mechanism.
Session III: Immune Effects and Safety of Nucleic Acids
Robert L. Coffman
Monday, 25 September 2017
11.50 – 12.15

Development of CpG Oligonucleotides for the Immunotherapy of Cancer
Robert L. Coffman
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CpG-oligonucleotides (CpG-ODN) target pattern the recognition receptor TLR9 and mimic the immunostimulatory activity of microbial and viral DNA. The ability of CpG-ODN to stimulate anti-tumor immunity has been demonstrated repeatedly in animal models; however, successful application to human cancers has been challenging. Dynavax is currently developing two C-class CpG-ODN for human cancers: SD-101 for intratumoral (IT) injection and DV281 for aerosol delivery in lung cancers. Both are proposed to act by stimulating antigen-presenting dendritic cells in the tumor and draining lymph nodes, potentially synergizing with immune checkpoint inhibitors to produce systemic immunity to both injected and un.injected tumors.

Preclinical studies in mouse tumor models demonstrated that SD-101 given IT in anti-PD-1 non-responders led to complete, durable rejection of essentially all injected tumors and a majority of un injected, distant-site tumors. The two agents synergize to enhance both the numbers and functionality of CD4+ and CD8+ T cells specific for tumor antigens. In humans, SD-101 has been studied in combination with the anti-PD-1 antibody, pembrolizumab, in a phase I dose escalation study in stage IIIc/IV metastatic melanoma patients. IT injection of SD-101 in combination with pembrolizumab was well tolerated and produced antitumor responses in both anti-PD-1 naïve and experienced melanoma patients. Importantly, responses were observed in both injected and non-injected tumor lesions.

Inhalation of aerosolized DV281 in patients with primary lung cancer or lung metastases of other cancer types represents an alternate strategy for the localized delivery of a TLR9 agonist to the tumor. In a mouse model of lung metastases, inhaled DV281 slows tumor progression and induces formation of tertiary lymphoid structures adjacent to tumors. In combination with anti-PD-1, lungs in most mice were completely cleared of tumor, and systemic anti-tumor immunity was generated leading to control of metastases in other organs.

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Investigation of Mortality Imbalance in Revusiran Phase 3 Study, ENDEAVOUR

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Alnylam Pharmaceuticals

Hereditary ATTR (hATTR) amyloidosis, a multisystem disease caused by transthyretin (TTR) amyloid deposits in nerves, heart and other tissues, manifests with a range of clinical presentations including neuropathy and cardiomyopathy. hATTR amyloidosis with cardiomyopathy carries high mortality, and is generally fatal within 2.5-5 years of diagnosis. The ENDEAVOUR study (NCT02319005) was a double-blind Phase 3 study of the investigational RNA interference therapeutic revusiran, a first generation GalNAc-siRNA conjugate targeting wild type and mutant TTR mRNA, in patients with hATTR amyloidosis with cardiomyopathy. Patients were randomized 2:1 to revusiran 500 mg or placebo (pbo) SC QD x 5 days and then weekly. Co-primary endpoints were 6-minute walk distance and reduction in serum TTR at 18 months. On 05 October 2016, dosing in all revusiran trials was discontinued, and further development was stopped, based on a mortality imbalance between treatment arms in this study. Subsequently, an investigation of the mortality imbalance on the ENDEAVOUR study was performed.

206 patients were randomized and treated (140 revusiran; 66 pbo). Median treatment duration was limited (6.7 months revusiran and 7.8 months pbo), precluding assessment of primary efficacy endpoints. The study population had baseline evidence of advanced heart failure (HF). Treatment arms were generally balanced for baseline characteristics, although a greater percentage of revusiran patients were age ≥ 75 years compared to pbo (31% vs. 18%). The mortality imbalance during the treatment period was 18 (13%) deaths on revusiran vs. 2 (3%) on pbo. Based on independent adjudication of events, deaths on both arms were primarily cardiovascular and due to HF, as expected in this population. There was no evidence of drug-related cardiotoxicity based on similar change over time in echocardiographic parameters and cardiac biomarkers, and similar time to first and recurrent CV and HF hospitalizations between treatment arms. There was no evidence that pharmacokinetics or TTR lowering contributed to mortality.

While a role for revusiran cannot be excluded, comparison to natural history and subgroup analysis of patients by age category suggest mortality in the pbo arm may have been lower than expected.

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**Immunologic reshaping and therapy of cancer by stimulation of innate nucleic acid sensor RIG-I**

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We describe a novel immunotherapy approach in which the viral defense system is harnessed to stimulate anti-tumor immunity. Cancer immunotherapy has revolutionized oncology in recent years, yet many tumors become resistant or do not respond to current treatments such as checkpoint inhibitors. Stimulation of the innate immune system opens a new therapeutic strategy that could be combined effectively with other immunotherapeutic regimens. The ubiquitously expressed cytosolic RNA receptor retinoic acid inducible gene I (RIG-I) recognizes double-stranded RNA bearing a 5′-triphosphate. Its activation induces apoptosis preferentially in tumor cells and simultaneously activates the innate immune system via type I interferon (IFN) signaling. We developed an optimized, fully synthetic oligonucleotide, designated RGT100, which is a RIG-I selective ligand. RGT100 activates the RIG-I pathway leading to the induction of Th1-dominated cytokines, including IFN-α and IFN-β. The treatment of tumor-bearing mice with RGT100 encapsulated in a delivery device demonstrated potent anti-tumor activity in a variety of tumor models. Histological and flow cytometric analysis of the tumors revealed infiltration and activation of immune cells after RGT100 treatment. Treatment of tumors by intratumoral injection led to efficacy of both the treated tumors as well as untreated contralateral tumors. Furthermore, systemic delivery of RGT100 was efficacious against both local subcutaneous B16 melanoma as well as its lung metastases, as well as a variety of other tumors. Data support both natural killer (NK) cell-mediated and T cell-mediated anti-tumor activities, and resistance to tumor re-challenge has been demonstrated. Immunologic characterization of the tumors and draining lymph nodes after therapy reveals expression of a type-I IFN signature and parameters of immunogenic cell death as well as markers of local activation and cytotoxic activity of NK- and T-cells. In summary, Rigontec’s RIG-I-selective ligand RGT100 shows strong anti-tumor activity in several clinically relevant mouse tumor models and routes, while bearing an advantageous safety profile. RGT100 has entered clinical evaluation in advanced cancer patients in Q1 2017, and will be clinically evaluated for single-agent activity as well as in combination with checkpoint inhibitors.
NF-κB signaling plays central role regulating cancer cell survival and self-renewal as well as immune cell activity. The microRNA-146a provides negative feedback inhibition of the NF-κB pathway and has been suggested a tumor suppressor in acute myeloid leukemia (AML). While it is a potential therapeutic target, the delivery of miRNA remains a challenge limiting clinical translation. We previously developed a strategy for targeted delivery of oligonucleotide therapeutics, such as siRNA, into human and mouse myeloid cells and B cells, using partly or completely phophorothioated (PS) single-stranded oligodeoxynucleotides containing CpG motif (CpG ODN). The CpG-conjugates undergo rapid scavenger receptor (SR)-mediated endocytosis followed by a Toll-like receptor-9 (TLR9)-facilitated cytoplasmic release. Here, we demonstrate that similar strategy based on type A CpG ODN (D19) can be employed for the delivery of functional miR146a mimics as well as anti-miR146a oligonucleotides (146AMO). Both CpG-miR146a mimic and CpG-146AMO conjugates were quickly internalized by target human and mouse non-malignant myeloid cells, as well as by AML cells. Unexpectedly, the CpG/TLR9 activation to accelerate endosomal escape, was not indispensable for the inhibitory effect of the CpG-146AMO. The GpC-146AMO or the conjugate using the PS-modified hydrocarbon chain only (CHPS-146AMO) were sufficient for the SR-mediated internalization and inhibition of the target miR-146a. In contrast, target myeloid cells did not internalize the 146AMO alone or when equipped with the unmodified hydrocarbon chain. Furthermore, we confirmed the functionality of CpG-miR146a mimic both in myeloid cells in vitro and in miR-146a knockout mice. The CpG-miR146a mimic reduced protein levels of downstream targets, such as Irak1 and Traf6, thereby inhibiting NF-κB activity. As expected, the CpG-146AMO had an opposite effect and strongly stimulated NF-κB signaling. Our initial in vivo studies in mouse Cbfb/Myh11/MPL AML detected antitumor activity of CpG-146AMO, with decreased numbers of leukemic cells in spleen and bone-marrow. These effects were likely caused by enhanced immune activity rather than direct leukemia cell cytotoxicity. Further studies will dissect the effect of miR-146a-dependent NF-κB modulation in AML cells and in leukemia-associated myeloid cells, aiming at limiting AML survival, while augmenting antitumor immune responses.
MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. Compared to miRNA biogenesis, pathways that mediate mature miRNA decay are not as well understood. We report that biologically functional miRNAs are degraded in human cells by the endonuclease Tudor-SN (TSN). In vitro, recombinant TSN initiates the decay of both protein-free and AGO2-loaded miRNAs via endonucleolytic cleavage at CA and UA dinucleotides, preferentially at scissile bonds located more than five nucleotides from miRNA ends. Consistent with this, cellular targets of TSN-mediated decay defined using miR-seq follow this rule. Inhibiting TSN-mediated miRNA decay by CRISPR-Cas9 knockout of TSN inhibits cell-cycle progression by upregulating a cohort of miRNAs that downregulates mRNAs encoding proteins that are critical for G1-to-S phase transition.


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tRNAs act as adaptors that play a fundamental role in translating the transcriptome into the proteome. While only 30-50 tRNAs (depending on species) are required for mRNA translation, the genome encodes significantly more tRNA genes (over 600 in the human genome). This genetic excess is evolutionary conserved, although the biological significance is poorly understood. After languishing in the background for many years, tRNA has reclaimed center stage with the discovery of its multiple non-canonical functions, which often involve subtle variations in the tRNA sequence of isoacceptors and isodecoders, or by base modifications. We and others showed that tRNA variants serve as an abundant reservoir to produce diverse, novel non-coding RNAs termed tRNA derived fragments (tRFs). We previously discovered that a variety of abiotic stresses activate the RNase angiogenin (ANG) to cleave the anticodon loop of tRNAs to producing tRFs known as tRNA-derived stress-induced RNAs (tiRNAs). Significantly, a subset of tiRNAs act as translation silencers to inhibit mRNA translation initiation. In contrast to other small non-coding RNAs (ncRNAs) that recognize their targets via base-pairing, tiRNA-mediated repression does not rely on a sequence complementarity, thus representing a novel mechanism of ncRNA-mediated target mRNA recognition/regulation. This tiRNA subset also promotes the assembly of Stress Granules, RNA granules with pro-survival and stress-adaptive functions, helping cells cope with adverse environmental conditions and enhance their survival. I will give an overview on the basic molecular mechanisms underlying functions of tiRNAs in cell physiology and proposed functions in various pathologies. I will also discuss a translational potential of tiRNAs as disease biomarkers and therapeutic targets for cancer and neurodegenerative diseases.
Epigenetic mechanisms underlie the orchestrated expression of the genome in different cell-types during development and are increasingly recognized as important in many disease states. Nuclear RNAs are key contributors to epigenetic regulation by impacting chromatin and nuclear structure. XIST RNA provided the precedent for a large non-coding RNA that regulates transcription by modifying chromatin architecture. RNA transcribed from the X-linked XIST gene spreads in cis across one female X chromosome and triggers numerous biochemical and structural changes to form silent heterochromatin, a stable epigenetic state perpetuated from cell to cell. Due to the multi-layered repressive changes, silencing is essentially irreversible. Although XIST evolved to silence the X-chromosome, by targeting the XIST gene into one chromosome 21 in trisomic Down Syndrome iPS cells, we showed that the RNA has a remarkable ability to comprehensively silence an autosome. Hence, XIST RNA operates on a genome-wide mechanism for inducing heterochromatin, and we are pursuing the implications of that for the problem of chromosomal abnormalities.

Understanding how this large (14 kb) XIST functions can also lead to development of tools for broader epigenetic control of therapeutic targets. Recent work indicates that XIST may act by silencing or displacing repeat-rich RNAs that are abundant across euchromatin, and which promote an open chromatin state. XIST and other RNAs directly or indirectly recruit histone modifying enzymes, such as polycomb proteins or HDACs, but our evidence indicates these RNAs also are essentially structural element of the nuclear chromosome. The interspersed repetitive sequences which comprise half the genome may prove key to underpinning the architecture of the epigenome. In other recent work, we discovered that a different type of repeat sequence is implicated in epigenetic instability common in cancers. The large pericentric blocks of human satellite II (HSATII) comprise one of the most prominent but poorly studied features of the human genome, long thought non-expressed “junk”, with no known function. We recently uncovered that HSATII is not only highly expressed in half of various human tumors, but that this exceptionally high-copy tiny repeat (24bp), at the level of DNA or RNA, can act as a molecular sponge and impact the distribution of two classes of master epigenetic regulators in the cancer cell genome, likely contributing to further epigenetic changes. The potential of chromosome associated RNAs and highly repetitive sequences merits exploration for development of epigenetic therapeutic strategies.

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A 3’ tRNA derived small RNA (tsRNA) affects ribosome biogenesis and translation in rapidly dividing cells and a target for hepatocellular carcinoma

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There are tens of thousands of different tRNA-derived small RNAs (tsRNAs) of 18-40 nucleotides in length in mammalian cells. In recent years there is accumulating evidence suggesting that these RNAs can play different yet important roles in gene regulations. Nevertheless, in most cases, the biological roles of these RNAs have yet to be defined. Using a variety of locked nucleic acid/antisense oligonucleotide (LNA/ASO)-mediated strategies, we found that inhibition of one specific 3’tsRNA, induces apoptosis in rapidly dividing cells. Inhibition of the tsRNA but not the mature tRNA reduced the translation of ribosomal protein S28 (RPS28) mRNA and led to a block in pre-18S ribosomal RNA processing, and ultimately a decrease in the number of 40S ribosomal subunits. Using a modified chromatin isolation by RNA purification method, we found that the tsRNA binds to ribosomal protein S28 mRNA and by genetic complementation analyses established two binding sites contained within the mRNA. Furthermore, we established that the binding of the tsRNA to these sites were required for optimal translation. The systemic delivery of a specific anti-tsRNA oligonucleotides into mice (the tsRNA sequence is the same in mouse and humans) did not cause liver injury in normal mice but induced apoptosis and a significant growth retardation of patient-derived orthotopic hepatocellular carcinomas surgically implanted into mouse livers. Our result establishes a newly defined post-transcriptional mechanism of gene regulation and provides a novel target for cancer therapeutics.

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Axiomer® technology: Therapeutic oligonucleotides for directing site-specific A-to-I editing by endogenous ADAR enzymes

Janne Turunen, Roxana Redis, Antti Aalto, Petra de Bruijn, Özcan Sahin, Hsin-Yi Huang-van Eekelen, Stephan Freriks, Lenka van Sint Fiet, Julien Boudet, Seda Yılmaz-Elis, Mierelle Schuijt, Gerard Platenburg, Bart Klein.

ProQR Therapeutics

Recruitment of endogenous RNA editing enzymes by oligonucleotides represents a significant therapeutic opportunity for a new type of drugs that can treat genetic disorders by reversing the underlying mutations. Deamination of adenosines into inosines (A-to-I editing) is the most common type of single-nucleotide posttranscriptional editing, with a predictable change in the base-pairing specificity: As inosine base-pairs with cytidine, the editing effectively results in an A-to-G conversion, which in turn can affect RNA processing (e.g. splicing or RNA stability) or the codon identity during translation. The reaction is catalysed by the ADAR enzymes (Adenosine deaminases acting on RNA), and takes place on different substrates, including (pre-)mRNAs, miRNAs and IncRNAs, and in a range of disease-relevant tissues. The specific challenge is to develop an approach where the endogenous ADAR can be recruited by using an oligonucleotide only, without the need for overexpression of ADAR (fusion) proteins. Furthermore, the oligonucleotide should be chemically modified to enable general drug-like properties, without interfering with ADAR binding and activity.

To employ ADARs for therapeutic applications, we have developed the Axiomer® platform, which takes advantage of Editing OligoNucleotides (EONs) containing specific patterns of chemical modifications. The modifications provide for stability and cellular uptake, and enable the EONs to recruit the endogenous ADARs and direct them to specifically edit one selected adenosine, while suppressing the editing of other, off-target adenosines. We provide proof of concept for the Axiomer® technology in a mouse model of the Hurler syndrome, a lysosomal storage disorder caused by inactivation of the alpha-L-iduronidase enzyme. The underlying G-to-A mutation is corrected by EON-directed A-to-I editing in the Idua transcript, resulting in restoration of protein translation and enzymatic activity. In vitro work with additional models indicates that the EONs are generally applicable for the correction of mRNA G-to-A mutations, over 20,000 of which are known to cause monogenetic disorders. We also discuss future applications, which include precise modifications of single amino acids to change protein functions, as well as ncRNA editing to modulate regulatory networks.

1Idua-W392X transgenic mouse licensed from The UAB Research Foundation

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Session IV: Emerging Topics in RNA Biology
Pedro M.D. Moreno
Monday, 25 September 2017
16.45 – 17.00

Hydrogel-assisted LNA gapmer delivery for in situ gene silencing in spinal cord injury
Pedro M.D. Moreno1,2, Ana R. Ferreira1,2, Daniela Salvador1,2, Ulf Tedebark4,5, Mónica M. Sousa1,3, Isabel F. Amaral1,2, Jesper Wengel6, Ana P. Pêgo1,2,7,8
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8 Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4050-313 Porto, Portugal

LNA-based antisense gapmer oligonucleotides (AONs) were designed against two possible targets involved in nerve regeneration inhibition, RhoA and GSK3β, and loaded in a fibrin hydrogel matrix as an integrated system for their local release in vivo in a spinal cord injury (SCI) setting.

Fibrin gels can be efficiently loaded with AONs which directly interact with the fibrin fiber components.

We demonstrate that gymnastic delivery of gapmers is efficient in fibrin embedded organotypic dorsal root ganglion culture system which could thus be used as an in vitro CNS model for AON delivery. An efficient reduction of target gene expression (>60% at RNA and protein level) was achieved when culturing DRG explants for 7 days in LNA-AON containing gels confirming that the AONs, besides being efficiently distributed throughout the DRG explant were also active inside the cells.

In vivo experiments were conducted in a rat model of spinal cord injury (hemisection). The lesion was filled with the AON-containing fibrin gel and covered with an additional gel patch. Five days post lesion, an extensive presence of the AON throughout the lesion site, but also traveling rostral and caudal to the lesion, was observed. Furthermore, when using functional LNA-AONs, we observed approximately 80% gene down-regulation in vivo.

Our results suggest that the delivery of free AONs from a fibrin gel matrix is a viable option for SCI application, potentially providing a combinatorial effect where the AONs are able to locally modulate cellular gene expression while fibrin hydrogel offers a permissive support matrix for cell infiltration and neuronal regeneration.

Acknowledgements
FCT (HMSP-ICT/0020/2010); SCML (MC-1068-2015); (PIEF-GA-2011-300485 to P.M.D.M.).

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Hydrogel-assisted LNA gapmer delivery for in situ gene silencing in spinal cord injury

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Targeting toxic microsatellite RNAs with CRISPR/Cas9.
Gene Yeo
UCSD, NUS

I will present my lab’s efforts in utilizing the CRISPR/Cas9 system for interrogating RNA in live cells and its recent application to eliminating toxic microsatellite RNAs such as those found in myotonic dystrophy type 1 and 2, Huntington’s disease and ALS.

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Robust In Vivo Gene Editing with Systemic Lipid Nanoparticle Delivery of CRISPR/Cas9 RNA Components

Amy Rhoden Smith, Jonathan D Finn, Mihir Patel, Lucinda Shaw, Maddy Younis, Jane van Heteren, Tanner Dirstine, Corey Ciullo, Reynald Lescarbeau, Jessica Seitzer, Ruchi Shah, Aalok Shah, Dandan Ling, Jacqueline Growe, Melissa Pink, Ellen Rhode, Kristy M Wood, Christian Dombrowski, Walter Strapps, Yong Chang, David V. Morrissey

Intellia Therapeutics

There is considerable interest in the therapeutic potential of CRISPR/Cas9-mediated gene editing to treat a wide variety of genetic diseases; however, clinically viable delivery of CRISPR/Cas9 components presents an obvious challenge. Here, we present development of a novel lipid nanoparticle (LNP)-mediated delivery system for Cas9 mRNA and chemically synthesized sgRNA capable of producing significant editing in both the mouse and rat transthyretin (Ttr) gene in the liver. In mouse, a single systemic dose of the LNP containing co-formulated Cas9 mRNA and sgRNA is able to achieve 70% editing of Ttr alleles in the liver to produce approximately 97% reduction in circulating levels of transthyretin protein, a response that is durable for at least 9 months. Similar dose-responsive results have been achieved in rat, with up to 66% editing at the target DNA site and up to 91% reduction in serum TTR protein levels. We have identified that RNA cargo optimization, including a novel modification pattern for sgRNA and optimization of the mRNA to give increased Cas9 protein expression, have been crucial for potent in vivo gene editing. Our work demonstrates that this LNP system can deliver clinically viable levels of in vivo editing and reduction of TTR serum protein, highlighting the potential of this method for effective therapeutic liver delivery.

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Session V: Developments in Gene and RNA Editing
Matthew Stanton
Tuesday, 26 September 2017
9.20 – 9.45

Messenger RNA as a Novel Therapeutic Approach
Matthew Stanton
Moderna Therapeutics

Messenger RNA represents a potentially attractive new therapeutic modality but requires solutions to RNA’s ability to activate innate immune sensors and to delivery. The introduction of chemical modifications can directly interfere with recognition by immune sentinels. Such modifications must remain compatible with the ribosome, a requirement that can severely limit the arsenal of available structural changes. Modifications to the uridine nucleobase have arisen as a class that meets these stringent criteria with pseudouridine (Y) serving as the prototypical example. Major groove modifications 1-methyl pseudouridine (m1Y) and 5-methoxy uridine (mo5U) also demonstrate attractive properties in this regard. The detailed immunostimulatory consequence of utilization of these modifications will be discussed including non-obvious influences on lipid nanoparticle delivery performance.

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Significant Improvements in CRISPR Specificity using Chemically Modified Single Guide RNAs

Although CRISPR systems have emerged as a transformative tool for performing alterations of genome sequence in living cells there remains keen interest in increasing their specificity. Multiple approaches have been explored to further increase the specificity of CRISPR-Cas9 systems including truncation of the guide RNA and engineering the Cas protein by mutating specific amino acids. We have explored an alternative approach employing site-specific chemical modifications in the 20 nucleotide DNA recognition sequence of guide RNAs by systematically evaluating the performance of a range of chemically modified single guide RNAs at on- and off-target sites using in vitro biochemical DNA cleavage assays and cell-based assays. Our results demonstrate that certain site-specific chemical modifications in guide RNAs can markedly reduce off-target cleavage while maintaining high on-target CRISPR-Cas9 efficiency for several clinically relevant targets, suggesting that this approach can significantly improve the performance of CRISPR systems for research and therapeutic applications.

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**Session V: Developments in Gene and RNA Editing**  
**Maria Montiel**  
Tuesday, 26 September 2017  
10.00 – 10.15

**Site-Directed RNA Editing for Correcting the Most Frequent Premature Termination Codon Mutations in CFTR**  
*Maria Montiel*, Isabel C. Vallecillo-Viejo, Joshua J. C. Rosenthal  
The Eugene Bell Center for Regenerative Biology and Tissue Engineering, The Marine Biological Laboratory, University of Chicago, Woods Hole, MA, Department of Pharmacology, University of Puerto Rico, Medical Sciences Campus, San Juan, PR.

RNA editing is a molecular process that catalyzes the conversion of specific adenosines (A) to inosines (I) within an RNA molecule. This process is initiated by the Adenosines Deaminases that Act on RNA (ADARs) family of enzymes. I is read as guanosine (G) by ribosomes and other biological processes. If this process occurs in a coding region, protein sequence can be modified and function can be altered. In our laboratory, we have engineered a recombinant ADAR that uses a guide RNA (gRNA) to target a specific A of our choosing. Using our system of site-directed RNA editing (SDRE) we have attempted to correct the five most common premature termination stop codons (PTCs) in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) (G542X, W1282X, R553X, R1162X and Y122X). As a first step, we tested SDRE’s efficiency in all five CFTR PTCs by transiently transfecting all the components in HEK293T cells; editing enzyme, gRNA, and CFTR with each PTC. Four days post-transfection, we measured correction at the level of RNA by RT-PCR and direct sequencing. We detected efficient editing (≥ 37 %) at the target adenosine in all CFTR PTCs tested: CFTR G542X (UGAG; 37%), W1282X (UGAA; 63%), R553X (UGAG; 46%), R1162X (UGAG; 86%), and Y122X (UAA; 83 and 75%). Our long-term goal is to deliver all the elements of our system for SDRE using viruses. Towards this end, as a first step we used a reporter system to monitor correction in mCherry-eGFP W58X PTC using an AAV virus expressing only our editing enzyme. We tested the ability of this virus to deliver the editing enzyme to cells when they were co-transfected with mCherry-eGFP W58X mutant and gRNA plasmids (both delivered by non-viral means). Preliminary results indicate ~20% editing of W58X, showing that viral delivery is a plausible means to deliver the enzyme. Future experiments will focus on delivering both the editing enzyme and the gRNA to assess correction in mCherry-eGFP and CFTR mutants. We will also determine functional correction of CFTR mutants corrected by SDRE in epithelial cell monolayers. We acknowledge funding from CFF Award ROSENT14XX0 and NINDS R01 NS087726.

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mRNA therapeutics must be efficiently translated into functional protein while simultaneously evading the innate immune system. Incorporation of chemically modified nucleotides can help exogenous mRNAs evade immune sensors that recognize foreign RNA, but the impact of these modified nucleotides on translation efficiency has not been fully explored. To determine how primary mRNA sequence and nucleotide modification combine to modulate functional protein expression, we performed extensive functional characterization on a set of synonymous mRNA sequences containing different nucleotide modifications. Here, we show that chemical modification impacts translation through gross alteration of mRNA secondary structure, independent of primary sequence. Contrary to expectation, we find that more structured ORFs lead to greater protein output. This increased protein output is primarily due to increased translation efficiency, not increased mRNA half-life. Thus, secondary structure (determined jointly by primary sequence and nucleotide modification) confers an advantage for translation, but not stability, of therapeutic mRNAs. Modified nucleotides provide a tool for modulating mRNA translation through changes in secondary structure without altering primary sequence or sequence properties such as codon usage. This finding facilitates the continued development of potent mRNA therapeutics.
Session VI: Preclinical Development
Frank Rigo
Tuesday, 26 September 2017
11.00-11.25

ATXN2 as a therapeutic target for SCA2 and ALS
Frank Rigo
Ionis Pharmaceuticals, Carlsbad, CA

The genetic cause for many neurodegenerative diseases is known, yet for most of them no therapies exist that directly exploit this information. The finding that single stranded antisense oligonucleotides (ASOs) distribute broadly in central nervous system (CNS) tissues following delivery into the cerebral spinal fluid has created the opportunity to treat several neurodegenerative diseases with ASOs. An attractive approach to reduce gene expression is via the use of ASOs that harness the RNase H mechanism, which have shown success in both preclinical models and in clinical trials for diseases of the CNS. We have shown that ASO-mediated reduction of ATXN2 ameliorates disease phenotypes in mouse models of spinocerebellar ataxia type 2 (SCA2) and amyotrophic lateral sclerosis (ALS). This supports the continued development of an ATXN2 ASO therapy for the treatment of SCA2 and ALS.

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Emerging microRNA targets for Brain Diseases
Anna M. Krichevsky
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MicroRNAs are the key regulatory molecules that control gene expression in health and disease. The work in our laboratory focuses on miRNAs that may underlie the pathogenesis of two devastating neurologic diseases. The first is the most common primary brain tumor glioblastoma (GBM), and the second is the major neurodegenerative disease of aging, Alzheimer’s disease (AD). In this talk, I will outline our strategy for identification and prioritization of miRNAs associated with these human pathologies, deciphering their targets and signaling in the pathologic context, and developing the miRNA targeting approaches toward new therapies. Both oligonucleotide-based and gene editing-based targeting will be discussed.

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Implementation of customized NGS technology for screening of LNA oligonucleotides

Lars Joenson, Lukasz Kielbinski, Troels Koch, Mads Aaboe Jensen
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Splice-switching oligonucleotides can be designed to elegantly manipulate alternative splicing such that it brings a therapeutic benefit in the context of disease. The strategy can be applied in many ways – both directly to target and restore aberrantly spliced RNA caused by mutations, indirectly by targeting adjacent RNA to restore open reading frames or to introduce de novo isoforms coding for novel proteins with particular activities. Recently, oligonucleotide drugs, based on the splice-switching approach, have been marketed for treatment of patients with severe rare genetic diseases.

Traditionally, most functional splice-switching oligonucleotides are identified through cell-based screens of a limited number of compounds. The typical readout is isoform-ratio changes produced by PCR-based methods, such as semi-quantitative hot RT-PCR or quantitative PCR. A common challenge for PCR-based methods is that not all alternative splicing events can be effectively captured due to primer-design limitations in the surrounding sequence or insufficient fragment size differences. Importantly, PCR-based methods mostly provide information about known isoforms.

Faced with this challenge, we decided to think about ways to develop a new method that would not only provide sufficient throughput for industry-scale discovery, whilst, at the same time could bring superior data quality and precision. Our solution to this problem is a sequence agnostic NGS-based process that can be easily customized for any particular gene-of-interest. Once it has been fully implemented, we believe our approach will provide a significant step forward for drug discovery of splice-switching oligonucleotides.

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Introduction: Antisense technology is a validated platform for drug development. Antisense oligonucleotide (ASO) treatments can downregulate their target mRNAs through an RNase H mechanism; alternatively, through steric blocking mechanisms, ASOs can upregulate target mRNAs leading to increased protein production. Nonsense mutations generate premature termination codons (PTCs) that can subject mRNA transcripts to rapid degradation through the nonsense mediated decay (NMD) pathway. The recessive genetic disease Cystic Fibrosis (CF) is caused by loss of function mutations in the CFTR gene. CFTR loss in the lung is the primary cause of mortality for CF patients. Of ~70,000 CF patients worldwide, approximately 10% have at least one nonsense mutated CFTR allele. Partly due to a limited set of nonsense mutated-CFTR models, there are no targeted therapies available for these patients. Therapeutics that generate relatively small increases in CFTR function could have a significant clinical impact.

Objective: To identify ASOs that upregulate CFTR with nonsense mutations by promoting PTC readthrough or through inhibition of the NMD pathway, with the eventual goal of developing this strategy for therapeutics.

Methods and Results: We have developed and validated an in vitro cell line model of the human CFTR W1282X mutation. To this model, we applied three ASO-mediated strategies to upregulate W1282X expression: 1) block the deposition of exon junction complexes downstream of PTCs, which serve as triggers for NMD; 2) block NMD-initiating mRNA cleavage by SMG6; and 3) downregulate NMD and translation termination factors through an RNase H mechanism. Screens are underway to determine the efficacy of the steric blocking ASOs. Data generated thus far show promising levels of sustained and dose-dependent mRNA upregulation by inhibiting multiple NMD pathway components with ASOs. Experiments are underway to determine the protein-level consequences of W1282X mRNA upregulation. After determining the most promising ASOs for W1282X upregulation, the ASOs will be tested in more human disease-relevant models.

Conclusion: Therapeutic strategies that promote PTC read-through or modulate the NMD pathway have the potential to generate a functional or partially functional CFTR protein, thereby providing benefit to CF patients.

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Acceleration of Diabetic Wound Healing by Local Delivery of PHD2-targeting sshRNAs

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In diabetes-associated chronic wounds, the normal response to hypoxia is impaired and many of the cellular processes involved in wound healing are hindered. HIF (Hypoxia Induced Factor)-1α activates multiple factors including VEGF and SDF-1 that enhance wound healing by promoting cellular motility and proliferation, new vessel formation, and re-epithelialization. PHD2 (prolyl hydroxylase domain-containing protein 2), a crucial cellular oxygen sensor, negatively regulates HIF-1α activity by targeting it for degradation. We have identified a highly potent sshRNA (short synthetic hairpin RNA) that inhibits expression of PHD2 in cell culture with IC50 = 15 pM. This sshRNA, which acts by a Dicer-independent RNAi mechanism, is chemically modified for biostability and to mitigate any potential immunostimulatory effects. Silencing PHD2 transcripts stabilized HIF-1α and led to increased proliferation and migration of keratinocytes in vitro. To assess activity and delivery in a mouse model of type II diabetes, PHD2-targeting sshRNAs were formulated for local delivery to wounds using layer-by-layer (LbL) technology. LbL nanofabrication was applied to incorporate sshRNA into a thin polymer coating on a Tegaderm™ mesh. This coating gradually degrades under physiological conditions, releasing sshRNA-containing nanoparticles for sustained cellular uptake. LbL-PHD2 sshRNA-formulated Tegaderm was applied directly to splinted 6-mm full-thickness excisional wounds 1 day post-wounding in db/db mice. Wound images were captured every other day to monitor progress of wound closure. Wounds treated with a single application of LbL-PHD2 sshRNA closed almost 4 days faster than control group wounds (n=6 per group; p=0.01). Cellular uptake was confirmed using fluorescent sshRNA. SDF-1 and VEGF levels were significantly increased in treated wounds on Day 2 post-wounding (p<0.05), and von Willebrand Factor staining showed a significant increase in neovascularization in wound area at Day 7 (p<0.05). These results suggest that silencing of PHD2 by localized delivery of sshRNAs is a promising approach for the treatment of chronic wounds, an urgent medical need for patients with diabetes.

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AAV5-miHTT gene therapy demonstrates broad vector distribution and strong mutant huntingtin lowering in a Huntington disease minipig model

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Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the HTT gene. The translated expanded polyglutamine repeat in the huntingtin protein is known to cause toxic gain-of-function, affecting numerous cellular processes. Our approach involves lowering huntingtin using gene transfer of a cassette encoding an engineered microRNA targeting human HTT, delivered via adeno-associated viral vector serotype 5 (AAV5-miHTT). We previously showed strong reduction in huntingtin and prevention of neuronal dysfunction in HD rodent models after single intracranial injection of AAV5-miHTT. Here, we investigated the feasibility, efficacy, and safety of AAV5-miHTT for the first time in HD animals with a larger brain size. AAV5-miHTT was administered bilaterally into the striatum and thalamus of transgenic HD (tgHD) minipigs using convection enhanced delivery. In total 24 animals were injected with 1E+13 and 3E+13 genome copies AAV5-miHTT, AAV5-GFP, or formulation buffer and followed for three months. Both surgical procedure and AAV5-miHTT treatment were well tolerated with no adverse events. Longitudinal cytokine profiles in cerebrospinal fluid demonstrated a consistent pattern with a transient mild increase in cytokine levels up to two weeks post AAV5-miHTT injection.

We detected a widespread dose-dependent distribution of the vector throughout the minipig brain that correlated strongly with the miHTT expression. Expression of mutant HTT mRNA was significantly reduced in all regions transduced by AAV5-miHTT. Next to HTT mRNA reduction, a clear dose-dependent reduction in soluble mutant huntingtin protein levels was observed by ultrasensitive single-molecule counting. The 3E+13 genome copies AAV5-miHTT resulted in a significant mutant huntingtin reduction of more than 50% in the striatum and thalamus. In accordance with the parenchymal huntingtin reduction, in the cerebrospinal fluid of tgHD minipigs mutant huntingtin was also reduced after AAV5-miHTT treatment.

The present study is the first demonstration of successful human mutant huntingtin lowering in a large animal model of HD. The combination of widespread vector distribution, extensive huntingtin lowering, long-term expression and tolerability observed with AAV5-miHTT support the continued preclinical development of HTT-lowering gene therapy for HD.

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Inhibiting the Androgen Receptor Interaction with the Long Non-Coding RNA SLNCR using 2’-FANA-Modified Oligonucleotides Decreases Melanoma Invasion and Proliferation
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Long non-coding RNAs (lncRNAs) are critical regulators of numerous physiological processes and diseases, especially cancers. The tissue- and disease-specific expression of lncRNAs, as well as their gene-regulatory functions, makes lncRNAs ideal therapeutic targets. However, development of lncRNA-based cancer therapies is limited because the mechanisms of many lncRNAs are obscure. We identified a novel lncRNA, SLNCR, whose expression is associated with worse overall melanoma survival. SLNCR contains a highly conserved sequence that binds to the androgen receptor (AR) and mediates increased melanoma invasion and proliferation in an androgen-independent manner. Thorough biochemical characterization of the AR-RNA interaction reveals that the N-terminal regulatory domain of AR binds to single-stranded RNA in a sequence-specific manner. To develop candidate novel therapeutics inhibiting the SLNCR- and AR-mediated invasion and proliferation, we designed short (21-28 nucleotide) oligos that are either (i) reverse complement to SLNCR’s AR binding sequence, which bind to SLNCR to generate double-stranded RNA incapable of AR binding, or (ii) mimics of the SLNCR AR binding sequence, which bind directly to AR to preclude SLNCR binding. Both SLNCR- and AR-binding oligos are capable of sterically blocking the AR-SLNCR association. Moreover, gymnotic delivery (i.e. delivery of naked oligos independent of any agents) of 2’-deoxy-2’-fluoro-D-arabinonucleic acid (2’-FANA) modified oligos to patient-derived melanoma cells significantly reduces melanoma invasion and proliferation. Thus, these SLNCR- and AR-binding oligos represent novel therapeutic approaches for inhibition of melanoma growth and metastasis. Moreover, these studies show that characterization of protein-lncRNA interactions is critical for the development of lncRNA-targeted therapeutics.

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A 'Guided' Tour of an Early Career in RNA and Nucleic Acid Therapeutics

Keith T. Gagnon

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Department of Chemistry & Biochemistry, Southern Illinois University

This talk will discuss some of the history and future of RNA-guided enzymes in research and therapeutics. It is from the vantage point of a young investigator who has worked on RNA-guided enzymes throughout his career. These opinions may offer different perspectives and spark new ideas for future nucleic acid therapeutics.

From RNAi to CRISPR-Cas9, and even RNase H-mediated ASOs, nucleic acid-guided enzymes have been an engine of inspiration and innovation. Nucleic acid-guided enzymes have translated into ground-breaking laboratory tools and a new generations of therapeutics. The more we understand nucleic acid-guided enzymes, the more innovations we can envision.

Before RNAi was discovered, complexes of small nucleolar noncoding RNAs and their associated proteins, known as snoRNPs, were the best known examples of RNA-guided enzymes. Investigations revealed important insight into the structure-function relationships between the RNA guide and the enzyme and established biochemical principles for activity. Their manipulation for therapeutics, however, has never been fully realized. Nonetheless, the knowledge gained and the tools developed facilitated the subsequent characterization of RNAi. The utility of RNAi was quickly recognized because it was reminiscent of ASOs, where only a small nucleic acid guide was needed. Chemical modification of the guide RNA has since been the focus of tremendous therapeutic development. More recently, CRISPR-Cas systems have been discovered and their potential to revolutionize gene therapy has become obvious. Knowledge and tools from previous RNA-guided research contributed to rapid progress. However, these systems require introduction of a large RNP. Thus, a logical question was whether such systems would fit into the world of nucleic acid therapeutics? But a little creativity and some nucleic acid chemistry are suggesting ways in which nucleic acid therapeutics and CRISPR can join together for new therapeutic approaches. With siRNAs destined for the clinic, the excitement of CRISPR, and the ability to engineer new RNA-guided systems, nucleic acid-guided enzymes are expected to continue shaping therapeutics for the foreseeable future.

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Session VII: Awards Presentations & Talks
Richard Finkel
Wednesday, 27 September 2017
9:20-9:40

Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study
Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, Norris DA, Xia S, Bennett CF, Bishop KM.

Spinal Muscular Atrophy (SMA) is debilitating pediatric neuromuscular disease with an incidence of ~1:11,000. SMA is a monogenic, autosomal recessive disorder due to deletions or mutations in the SMN1 gene. A small amount of normal SMN protein is produced by a “backup” paralogous gene, SMN2, which differs from SMN1 by a single nucleotide that affects splicing, largely excluding exon 7 from the transcript. The number of copies of the SMN2 gene is inversely related to the severity of the phenotype. Deficiency of SMN protein causes premature degeneration of motor neurons in the spinal cord and brainstem, leading to neurogenic atrophy of skeletal muscle. Progressive weakness of limb muscles results in lack of motor development, with a spectrum of phenotypes from weak babies who never sit (type 1) to young infants who can sit but not walk (type 2) and older children and adults who walk but later show gradual decline (types 3 and 4). Weakness of respiratory and oral-pharyngeal muscles in infants with type 1 leads to early respiratory failure and inability to eat, resulting in significant morbidity and early mortality, with the majority dying before 2 years of age. Until recently, the only therapy for these conditions was supportive management, which prolonged survival but without the prospect of improved motor function. Following discovery in 1995 of the SMN1 and SMN2 genes, numerous targeted treatment strategies have been investigated and some are now in the clinic.

Antisense oligonucleotides (ASO) have been developed for modulation of SMN2 pre-mRNA. Nusinersen (Ionis Pharmaceuticals, Biogen), a 2'-O-methoxyethyl phosphorothioate-modified ASO, targets ISS-N1, an intronic splice inhibitor site, to increase exon 7 inclusion and thereby increase SMN protein expression. Proof-of-concept was demonstrated first in an animal model of SMA and then in humans in a Phase 2 safety and proof-of-concept study (CS3A), and will be discussed here. This ASO is administered intrathecally via repeated lumbar punctures. Two Phase 3 studies (CS3B and CS4) subsequently demonstrated safety and efficacy of nusinersen, leading to regulatory approval by the FDA (2016) and EMA (2017). Nusinersen (Spinraza™) is the first approved drug for SMA. ASO therapy for neuromuscular diseases is now a reality. Nusinersen offers meaningful benefit in SMA, with improved survival and motor function.
A life’s work in oligonucleotides: from chemical synthesis to peptide-PMO for treatment of neuromuscular diseases

Michael J. Gait
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Synthetic oligonucleotides and their analogues have been the primary focus of my scientific career. During my postdoctoral studies in 1973-75 with Gobind Khorana in the USA, the synthesis of a 15- to 20-mer oligodeoxyribonucleotide towards gene synthesis by phosphodiester chemistry in solution required a whole year’s work. In the late 1970s in the UK and by use of solid phase methods on a polyamide support, we were able to extend a growing oligonucleotide chain by one nucleotide unit using diester chemistry in 2 days (1977-79), then using triester chemistry in 4 hours (1979-1982) and finally in 1 hour (1983-84), such that an oligonucleotide could be synthesized and purified within 2 days.

Subsequently the machine-aided phosphoramidite synthetic methods of Caruthers and others shortened the time further and reduced the labour needed considerably. Accordingly our lab moved on in the late 1980s to methods of end-labeling for mRNA detection as well as synthesis of RNA and their analogues for applications in studies of ribozyme function. Also at this time we learnt DNA cloning and Sanger shotgun DNA sequencing resulting in the cloning, expression and mutagenesis of T4 RNA ligase, previously thought to be unclonable.

During the 1980s and 1990s several companies were developing antisense phosphorothioate and 2’-O-funtionalized oligonucleotide analogues as therapeutic agents for targeting mRNA in cells and in vivo. However it became clear that antisense activity was severely limited by their poor ability to penetrate cells and reach their desired RNA targets. Following successful projects in the 1990s together with the Karn lab on the HIV-1 Tat and Rev proteins and their interactions with the respective HIV RNA targets, we utilized strongly binding 2’-OMe/LNA mixmers (with Wengel’s group in Odense) as antisense inhibitors of HIV Tat-dependent trans-activation and also developed (with the Lebleu lab in Montpellier) cell-penetrating peptides (CPP) as conjugates for oligonucleotide cell delivery. Particular success came with CPP delivery of charge-neutral PNA oligonucleotides. Moving to the convenient HeLa pLuc705 splice-switching assay of pre-mRNA, we developed a class of new Arg-rich CPPs called Pip. Teaming up in 2007 with the Wood group in Oxford, we applied such CPPs to delivery of similarly charge-neutral PMOs into muscle cells and into a mouse model of Duchenne muscular dystrophy. This exciting and fruitful collaboration has led us also into peptide-PMO conjugates for treatment of other neuromuscular diseases such as Spinal muscular atrophy and myotonic dystrophy. The culmination of this work is leading to ongoing plans for commercialization of this platform technology of PMO delivery by conjugated novel CPPs towards clinical trials, leading me to great optimism for the future.

I wish to pay tribute to my many LMB co-workers as well as several important collaborations throughout my career without which the culminating developments of therapeutic peptide-oligonucleotide conjugates would not have been possible.
Session VIII: Preclinical Topics in Nucleic Acid Therapeutics

Frank Slack
Wednesday, 27 September 2017
11.00 – 11.25

MicroRNA-based therapeutics in cancer
Frank J. Slack, Ph.D.
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MicroRNAs are small non-coding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. miR-21, miR-155, let-7 and miR-34 are microRNAs implicated in human cancer. Specifically, human let-7 and miR-34 are poorly expressed or deleted in lung cancer, and over-expression of let-7 or miR-34 in lung cancer cells inhibits their growth, demonstrating a role for these miRNAs as tumor suppressors in lung tissue. let-7 and miR-34 regulate the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for their involvement in cancer. We are focused on the role of these genes in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis. In contrast, miR-21 and miR-155 are oncomiRs and up-regulated in many cancer types. We are also developing effective strategies to target these miRNAs as a novel anti-cancer approach. Lastly we are examining the non-coding portions of the genome for mutations and variants that are likely to impact the cancer phenotype. We have successfully resequenced the 3’UTRome and microRNAome from cancer patients with a family history of cancer.

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RNA-based Infection Research
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Infections are exquisitely complex biological processes that involve a dynamic interplay between the pathogen and its host. Importantly, research on infectious diseases has traditionally focused on the role of proteins. However, RNA is now ascending as a promising class of biomolecules to characterize the state of cells, to be therapeutically targeted, and to be developed as a programmable drug.

This talk will give an overview of RNA-based infection research which includes the launch of a new federal institute dedicated to this topic. In addition, the promises and challenges for antisense oligonucleotides to be used for treatment of bacterial infections and interventions of microbiota-related diseases will be discussed.

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Exploiting the miRNA biogenesis pathway for effective oligonucleotide therapeutics

Richard I. Gregory

The Stem Cell Program at Boston Children's Hospital, Department of Biological Chemistry and Molecular Pharmacology, and Department of Pediatrics, Harvard Medical School (HMS), Co-Director, HMS Initiative for RNA Medicine, Harvard Stem Cell Institute

The classical microRNA (miRNA) biogenesis pathway involves the specific cleavage of stem-loop containing primary miRNAs (pri-miRNAs) by the Microprocessor, a complex containing DROSHA and DGCR8, to generate precursor miRNAs (pre-miRNAs). Pre-miRNAs are then processed to short duplexes by the ribonuclease DICER. The mature ~22 nt RNA serves as a guide to induce AGO2-mediated cleavage and/or repression of target mRNA. This endogenous pathway can be exploited for gene knockdown using either plasmids to express short hairpin RNAs (shRNAs) or by introducing small interfering (siRNA) oligonucleotides. Thus, RNA interference (RNAi) technology using shRNAs expressed via RNA polymerase (pol) III promoters has been widely exploited to modulate gene expression in a variety of mammalian cell types, and is of potential therapeutic utility. For certain applications however, such as lineage-specific knockdown, embedding targeting sequences into pol II-driven miRNA architecture is required. Therefore a complete understanding of the miRNA biogenesis pathway will aid the development of effective RNAi-based treatments for a variety of different diseases. Furthermore, the identification of new miRNA regulatory pathways can uncover possible ways to manipulate expression of endogenous miRNA(s) as an alternative oligonucleotide-based therapy.

Our most recent insights into understanding the mechanisms of miRNA biogenesis will be presented including the widespread relevance of a newly identified progenitor miRNA (pro-miRNA) biogenesis intermediate, a feedback mechanism regulating Microprocessor activity, and specific examples of where some of this knowledge is helping translate oligonucleotide-based therapies into the clinic will be featured including: 1) ongoing efforts aimed at RNAi-based targeting of the transcriptional repressor BCL11A as a strategy to activate expression of fetal goblin to alleviate sickle cell disease (SCD), and 2) targeting the LIN28-let-7 regulatory pathway with small molecules as a possible new therapeutic strategy to restore expression of the let-7 tumor suppressor miRNA in a variety of pediatric and adults cancers.

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Exploring chemical and structural diversity of lipophilic conjugates for extrahepatic siRNA delivery \textit{in vivo}.

Annabelle Biscans,$^{1,2}$ Andrew H. Coles,$^{1,2}$ Reka A. Haraszti,$^{1,2}$ Dimas Echeverria,$^{1,2}$ Anastasia Khvorova.$^{1,2}$

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A broad range of human diseases, including cancer, infection, and neurodegeneration, can be treated via the silencing of specific genes using small oligonucleotides as siRNAs. However, the delivery of siRNAs into affected cells and tissues remains a challenge.

One of the most promising strategy to improve global siRNA tissue distribution is the conjugation of the siRNA with a bioactive conjugate. Currently, the development of N-Acetylgalactosamine (GalNac)-conjugated siRNA which enables targeted delivery to hepatocytes has transformed the development of medicines for diseases with liver involvement. Although highly promising, the utility of this technology is limited to liver delivery only.

To expand siRNA distribution, our lab has explored the use of lipophilic conjugates.$^{1,2}$ We have synthesized a panel of 15 variants changing by their structure, length and degree of saturation to evaluate the impact of the lipophilic conjugate on siRNA tissue distribution \textit{in vivo}. All compounds have been generated from a functionalized solid support, purified by High-Performance Liquid Chromatography (HPLC), and characterized by Liquid Chromatography-Mass Spectrometry (LC-MS). The effect of the lipophilic conjugates on siRNA tissue distribution has been evaluated \textit{in vivo}, using a combination of three assays: Fluorescent microscopy (n-3), Peptide Nucleic Acid (PNA) hybridization assay (quantification of oligonucleotides in tissues, n-3) and QuantiGene® (n-8) to evaluate target gene silencing. We demonstrated that the chemical variety of the conjugate has a major impact on tissue distribution profiles, showing accumulation of functional oligonucleotides in several tissues beyond liver and kidney.

The distribution and efficacy data in several tissues including liver, kidney, spleen, intestine, heart, lung, adrenal glands, and skin will be presented. Enabling productive delivery to a range of tissues paves the path towards expanding RNAi clinical utility beyond liver.


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Small interfering RNAs, such as naturally occurring microRNAs (18- to 24-mer non coding double stranded RNA molecules), play roles in almost all aspects of biology and can be used for selectively silencing relevant gene expression. This class of agents represent innovative drug candidates with great potential to target a substantial part of the currently undruggable genes and gene products. Strong evidence supports a causal link between microRNA dysregulation and the development of many types of cancer. In addition, pattern of microRNA expression can be correlated with tumor type, stage, and other clinical variables. Consequently, these features and recent technical progresses have led to significant regained interest in academia and industry for this class of drugs as alternative approach for therapeutic intervention in Oncology. It has been postulated that microRNAs provide unique simultaneous regulation of multiple mRNA targets which safely and selectively improve long-term control of advanced tumors and reduce relapse rates.

InteRNA Technologies has developed agnostic lentiviral-based functional screening in tumor cell-based assays aimed to identify the best microRNA candidates. A wide range of microRNAs tackling major elements of the ‘hallmarks of cancer’ have been discovered, and selected for further development. Among them, a tumor suppressor microRNA targeting important and relevant cancer targets such as c-Kit, K-Ras, MCL1, Cyclin D1, CD73, CD39 and TIM-3, was identified. This microRNA candidate - inducing apoptosis, cell cycle arrest and anti-tumor immunity - has been used to design a chemically-modified synthetic 22-nucleotide double stranded microRNA mimic, coded INT-1B3. More recently, a novel lipid nanoparticle-based delivery vehicle ensuring effective distribution to cell(s) of interest, cellular uptake and endosomal escape, has been identified in experimental tumor-bearing mice.

The presentation will focus on pharmacokinetics/pharmacodynamics properties, as well as main promising pharmacology features of INT-1B3 in both tumor cell-based assays and experimental tumor-bearing mice which strongly support further preclinical development of the drug candidate for therapeutic intervention in Oncology.

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Targeting Long Non-Coding RNAs with Nanoparticle-mediated RNAi for Breast Cancer Therapy
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Long non-coding RNAs (lncRs) are biochemically versatile species that have emerged as promising therapeutic targets in oncology. LncRs exert multilevel gene regulation at the epigenetic, transcriptional, and translational levels in a tissue- and disease-specific manner. In heterogeneous and complex malignancies, such as triple-negative breast cancer (TNBC), numerous aberrantly expressed lncRs are known to significantly impact clinical outcomes by influencing global gene signatures. In this study, we demonstrate that the 855-nuc long DANCR (Differentiation Antagonizing Non-protein Coding RNA) acts as an oncogenic lncR in TNBC. DANCR is significantly overexpressed in tissues from TNBC patients and cell lines, compared to normal breast tissues and mammary epithelial cells, respectively.

Since DANCR cannot be targeted by conventional chemotherapy, we implemented the multifunctional cationic lipid carrier ECO to deliver therapeutic siRNA to silence DANCR in TNBC cell models. Targeted ECO/siRNA nanoparticles have been shown to efficiently deliver therapeutic siRNA in BC via systemic delivery, by means of effective cellular uptake and endosomal escape, resulting in efficient silencing of oncogenes. Stable self-assembly ECO/siDANCR nanoparticles were modified with PEG conjugated with the RGD peptide for improving biocompatibility and targeted delivery to cancer cells. ECO-PEG-RGD/NC nanoparticles with non-specific siRNA were used as negative control (NC).

Multiple TNBC cells, including MDA-MB-231, Hs578T, and BT549, were transfected with the therapeutic ECO-PEG-RGD/siDANCR and non-specific ECO-PEG-RGD/NC nanoparticles. The therapeutic formulation demonstrated efficient intracellular siRNA delivery and sustained target silencing, as evidenced by 80-90% knockdown in the expression of DANCR in all the cell lines for at least 7 days. Compared to the NC formulation, the ECO-PEG-RGD/siDANCR formulation demonstrated excellent therapeutic efficacy, reflected by the significant inhibition in the migratory and invasive abilities of the cells through Matrigel-coated Transwell inserts and scratch-wounds. The therapeutic formulation also resulted in significant decrease in cancer cell viability, proliferation, and tumor spheroid formation, as measured by MTT assay, BrdU incorporation ELISA, and 3D-Matrigel assays, respectively. At the molecular level, silencing of DANCR was found to mediate the functional effects by downregulating Epithelial-Mesenchymal transition (EMT)-associated proteins, such as ZEB1, N-cadherin, and BMI1, as well as the anti-apoptotic marker survivin.

This study demonstrates that DANCR acts as an onco-lncR by driving the invasion and proliferation of TNBC and targeted ECO/siRNA nanoparticle-mediated DANCR silencing is a promising approach for treating TNBC. The targeted ECO/siRNA nanoparticles can serve as a safe and effective delivery platform to target cancer-associated lncRs for the treatment of various types of cancers.

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Antisense Platform Technology: Recent Phase 3 Experience and Beyond
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The research and discovery activities at Ionis consistently provide 3 to 5 antisense drugs each year entering development across multiple therapeutic areas, and currently has resulted in a mature and growing development pipeline of more than 35 drugs. The efficiency of R&D technology has resulted in high rates of success in nonclinical and early stage clinical development moving more than 80% of drugs entering development into late stage clinical development. Three recent Phase 3 programs with multiple Phase 3 clinical studies have produced three antisense programs in various stages of registrations preparation, filing and ultimately approval globally. All three drugs are transformative in their respective targeted rare diseases. Following behind these three programs are more than 10 antisense programs in active early to late Phase 2 development positioned to move into Phase 3 development if successful. Phase 3 experience, challenges and opportunities will be discussed along with innovative progress of the overall pipeline.

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Emerging Therapeutic Profile of RNA Interference Against Hepatic Targets

Abstract not available at time of printing.
The Clinical Development of mRNA Drugs and Vaccines

Tal Zaks

*Moderna Therapeutics*

Messenger RNA offers unparalleled breadth and depth for the discovery and development of novel drugs and vaccines. The potential to direct specific tissue translation of both wild type and engineered intracellular, membrane-bound, and secreted proteins (and combinations thereof), coupled with rapid transition from preclinical to clinical development, has enabled Moderna and its partners to progress several development candidates into the clinic. This potential is being realized by the parallel development of different modalities (e.g. prophylactic and therapeutic vaccines, paracrine and systemic drugs) while ensuring that platform-wide preclinical, safety, and pharmacology attributes are carefully de-risked in a step-wise manner. This mRNA platform strategy will be reviewed in the context of the first wave of Moderna mRNA vaccines and therapeutics entering the clinic.

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Spinal muscular atrophy (SMA) is a progressive motor neuron disease with an incidence of ~1:11,000. SMA is a monogenic disorder that can present with a wide range of phenotypes: newborns with near quadriplegia and requiring ventilation support (type 0), young infants who never sit (type 1), older infants who can sit but not walk (type 2), and older children and adults who walk but later show gradual decline (types 3 and 4). The more severe type 0 and type 1 patients have markedly reduced survival. Supportive management can prolong survival in these infants, but without the prospect of improved motor function.

Nusinersen (Ionis Pharmaceuticals, Biogen), a 2'-O-methoxyethyl phosphorothioate-modified ASO, targets ISS-N1, an intronic splice inhibitor site, to increase exon 7 inclusion and thereby increase SMN protein expression. This ASO is administered intrathecally via repeated lumbar punctures. Proof-of-concept was demonstrated first in a murine model of SMA. Phase 1 and 2 safety studies began in humans in older children with SMA types 2 and 3, then in infants in a safety and efficacy Phase 2 study (CS3A). This was followed by two Phase 3 studies (CS3B in infants and CS4 in children), which demonstrated safety and efficacy of nusinersen, leading to approval by US (2016), European, Canadian and Japanese (2017) regulatory agencies. Nusinersen (Spinraza™) is the first approved drug for SMA. Intrathecal administration of nusinersen has been demonstrated in these studies to be safe, well-tolerated, and to have a significant disease modifying effect, with prolonged survival and improved motor function. Ongoing studies include an open-label extension study (SHINE) in symptomatic infants and children, and a small, open-label study (NURTURE) in pre-symptomatic infants. Preliminary data analysis indicates that treatment earlier in the course of SMA appears to provide the greatest benefit, with some pre-symptomatic infants demonstrating normal growth and development at 2 years of age. This observation supports the proposal to add SMA to the list of diseases endorsed for newborn screening, a request now under consideration.

Spinal muscular atrophy is a treatable disorder. Neurodegeneration can be halted and neuronal dysfunction can be reversed, at least in the short term, as observed by the course over 2 to 3 years in many of the clinical trial participants. Now, with regulatory approval of Spinraza, a wider range of patient phenotypes is being treated. Within the next few years we will learn what is the therapeutic window -- by age and severity of disease s – where a meaningful clinical response to treatment can be predicted, whether the benefit continues to accrue over time, and what other organ systems depend upon SMN protein.
Richard Finkel (2017) regulatory agencies. Nusinersen (SpinrazaTM) is the first approved drug for SMA. The efficacy of nusinersen, leading to approval by US (2016), European, Canadian and Japanese Phase 3 studies (CS3B in infants and CS4 in children), which demonstrated safety and efficacy in older children with SMA. Phase 1 and 2 safety studies began in humans in older children with SMA types 2 and 3, then in infants in a safety and efficacy Phase 2 study (CS3A). This was followed by two studies in severe type 0 and type 1 patients having markedly reduced survival. Supportive management is now under consideration.

Proof-of-concept was demonstrated first in a murine model of SMA. This ASO is administered intrathecally via repeated lumbar punctures. Over 2 to 3 years in many of the clinical trial participants. Now, with regulatory approval of Nusinersen Treatment of Spinal Muscular Atrophy, a wider range of patient phenotypes is being treated. Within the next few years we will learn what is the therapeutic window – by age and severity of disease – where a meaningful clinical response to treatment can be predicted, whether the benefit continues to accrue over time, and what other organ systems depend upon SMN protein.

In the BLM model, we demonstrated a dose-dependent and statistically significant reduction in lung weight, collagen deposition and histology and fibrosis scores following ND-L02-s0201 treatment. As a functional measure, statistically significant improvement in lung function determined by running endurance capacity was noted. Following cell enrichment, myofibroblasts contained the highest HSP47 mRNA expression. BLM led to a >5-fold increase in myofibroblasts and ND-L02-s0201 treatment reduced the myofibroblasts to sham levels. Comparable anti-fibrotic efficacy utilizing the endpoints was also observed in the silica model.

CONCLUSIONS: ND-L02-s0201 down-regulates HSP47 gene expression in pre-clinical animal lung and liver fibrosis models, which leads to significant inhibition of liver and lung fibrosis. These data suggest that HSP47 could be a clinically relevant target in liver and lung fibrosis.

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