1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

DATS

Abstracts and Progam

Radboudumc

OTS OLIGONUCLEOTIDE THERAPEUTICS SOCIETY

Local Delivery

Universitair Medisch Centrum Groningen

ENCORE

Eurogentec

LUMC Leids Universitair Medisch Centrum

DCRT Dutch Center for RNA Therapeutics

Erasmus MC University Medical Center Rotterdam

1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

Keynote speakers:

Prof. Annemieke Aartsma-Rus
A brief history of exon skipping therapy development: Developments in the current millennium

Dr. Bruno Godinho
Delivery of Stabilized siRNA scaffolds to the Central Nervous System

Invited speakers:

Prof. Roland Brock - *Peptide-based delivery strategies for antisense oligonucleotides*

Dr. Julie Rutten - *Skipping NOTCH3 exons to prevent protein aggregation in CADASIL patient arteries*

Dr. Peter van den Akker - *Exon skipping therapy for dystrophic epidermolysis bullosa*

Dr. Marjon Pasmooij - *Antisense to medicine: a regulator's perspective*

Elizabeth Vroom - *The role of patient organisations in antisense oligonucleotide drug development*

Dr. Marlen Lauffer - *The Dutch Center for RNA Therapeutics*

For early career scientists:

10 minute talks for the best abstracts
Selected abstracts to present elevator pitches to introduce their poster
Prize for best poster and talk

Abstract submission deadline: May 6th 2022

Registration / Abstract submission:
Registration on website:
http://www.DutchAntisense.org
Registration deadline: May 20th 2022
Registration fee: €20,-
Mail abstracts to:
DutchAntisense@gmail.com

Location:
Meeting & Event Center
Fletcher Wellness-Hotel
LEVEL building 5th Floor
Next to Leiden Centraal
Bargelaan 180, Leiden

Organizing Committee:
Dr. Alex Garanto, Radboudumc
Dr. Ronald Buijsen, LUMC
Dr. Jeroen Bremer, UMCG
Dr. Atze Bergsma, Erasmus MC

Radboudumc

OTS Oligonucleotide Therapeutics Society
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Universitair Medisch Centrum Groningen

ENCORE

Eurogentec

DCRT Dutch Center for RNA Therapeutics

Leids Universitair Medisch Centrum

Erasmus MC University Medical Center Rotterdam

1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

9:30 **Arrival, Registration and Coffee**

9:55 **Welcome**

10:00 **National keynote lecture:**
Annemieke Aartsma-Rus (LUMC) - *A brief history of ASO therapy development: Developments in the current millennium.*

SESSION 1

10:45 **Roland Brock (Radboudumc)** - *Peptide-based delivery strategies for antisense oligonucleotides*

11:15 **Peter van den Akker (UMCG)** - *Exon-skipping therapy for dystrophic epidermolysis bullosa*

11:45 **Julie Rutten (LUMC)** - *Skipping NOTCH3 exons to prevent protein aggregation in CADASIL patient arteries*

12:15 **Marlen Lauffer** - Dutch Center for RNA Therapeutics

12:30 **LUNCH**

SESSION 2

13:15 **Early Career Investigator Presentations**
13:15 - **Yvonne K. Jongejan (LUMC)** - *Small interfering RNAs for allele-selective silencing of murine von Willebrand factor*
13:30 - **Jurriën Prins (LUMC)** - *A novel antisense strategy to inhibit BK polyomavirus replication*
13:45 - **Irene Vázquez-Domínguez (Radboudumc)** - *Deciphering the efficacy and safety of different antisense oligonucleotide chemical modifications in a retinal context*

14:00 **International Keynote Lecture:**
Bruno Godinho (Atalanta Therapeutics) - *Delivery of stabilized siRNA scaffolds to the central nervous system*

14:45 **Marjon Pasmooij (Medicines Evaluation Board)** - *Antisense to medicine: a regulator's perspective*

15:15 **Poster Pitches**

15:30 **POSTER SESSION with Coffee and Cookies**

SESSION 3

16:45 **Early Career Investigator Presentations**
16:45 - **Claudia Milazzo (Erasmus MC)** - *ASO treatment rescues UBE3A expression and multiple phenotypes of an Angelman Syndrome mouse model*
17:00 - **Tom Metz (LUMC)** - *Biodistribution of radioactively labelled AONs after ICV and IT injection in mice*
17:15 - **Janine Reurink (Radboudumc)** - *WGS identifies deep-intronic variants in USH2A that are amenable for AON-based splice correction therapy*

17:30 **Elizabeth Vroom (Duchenne Parent Project Netherlands)** - *The role of patient organizations in antisense oligonucleotide drug development*

18:00 **Awards, closing remarks**

18:10 **Drinks and Networking**

19:30 **Dinner (not included, pre-registration required)**

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Keynote biosketches

(in chronological order)

Annemieke Aartsma-Rus

Prof. Dr. Annemieke Aartsma-Rus is a professor of Translational Genetics at the Department of Human Genetics of the Leiden University Medical Center. She played an important role in the development of antisense mediated exon skipping for Duchenne muscular dystrophy during her PhD research (2000-2004) at the Leiden University Medical Center (the Netherlands). As of December 2007 she became leader of the "DMD exon skip group". Since 2013 she has a visiting professorship at the Institute of Genetic Medicine of Newcastle University (UK). In 2020 she co-founded the Dutch Center for RNA Therapeutics (DCRT), a non-for-profit academic collaboration aiming to develop clinical treatment with exon skipping therapies for eligible patients with unique mutations.

Her work currently focuses on developing antisense-mediated exon skipping as a therapy for Duchenne muscular dystrophy and other rare diseases. This involves work in cell and animal models to improve efficiency of exon skipping, studies in muscle pathology, the identification of biomarkers, studying the basics of pre-mRNA splicing and transcript processing and the generation and detailed analysis of mouse models. Finally, she aims to bridge the gap between stakeholders (patients, academics, regulators and industry) involved in drug development for rare diseases and to develop exon skipping therapies for patients with unique mutations.

She has published over 220 peer-reviewed papers, 11 book chapters and 15 patents. She has given many invited lectures at scientific conferences and patient organization meetings, where she is known for her ability to present science in a clear and understandable way. She created and maintains an overview of different therapeutic approaches for Duchenne on the TREAT-NMD website (https://treat-nmd.org/research-overview/dmd-research-overview/).

In 2011 she received the Duchenne Award from the Dutch Duchenne Parent Project in recognition of her dedication to the Duchenne field. In 2020 she received the Black Pearl Science Award from Eurordis for her work in educating patients. In 2021 she received the Ammodo Science Award for her contribution to developing exon skipping therapies for Duchenne and the outstanding achievement award from the Dutch Society of Gene and Cell Therapy for her work. She has been selected as most influential scientist in Duchenne muscular dystrophy in the past 10 years by Expertscape based on contributions to the understanding and treatment of Duchenne muscular dystrophy annually since 2015.

She is chair of the TREAT-NMD Advisory Committee for Therapeutics (TACT), Chair of the Dutch Center for RNA Therapeutics, vice-chair of COST Action CA17103 (Delivery of antisense RNA therapies), was President of the Oligonucleotide Therapeutics Society (2019-2021), and was Chair of the TREAT-NMD executive committee (2013-2016 and 2019-2020). She was a junior member of the Dutch Royal Academy of Sciences (DJA), consisting of the top 50 scientists in the Netherlands under 45, from 2014-2019. She is part of the core group of the N-of-1 collaborative and 1 mutation 1 medicine (1M1M) network.

Bruno Godinho

Bruno Godinho received his foundational pharmacy training at the Lisbon School of Health Technology (ESTESL, Portugal) where he obtained his Bachelor's degree. During his time in college he undertook research internships at Utrecht University (Utrecht, The Netherlands) and Universidade NOVA de Lisboa (Caparica, Portugal), which were key shaping his interest in pursuing a career in scientific research. His Master degree in Clinical Pharmacology obtained at the University of Glasgow (Scotland, UK) fostered, for the first time, his interest in gene therapy and gene silencing. He later received his Doctorate degree from University College Cork (Cork, Ireland) where he studied formulation-based approaches for the delivery of therapeutic oligonucleotides to the central nervous system (CNS). He then joined Prof. Khvorova's Lab at the RNA Therapeutics institute (UMass Medical School, USA) as a postdoctoral fellow to learn and gain experience in the design and delivery of fully-modified conjugated therapeutic oligonucleotides. During his training he made significant contributions towards the identification of neuroactive siRNA scaffolds that enable potent and sustained gene silencing in the CNS, without the need for synthetic formulation. In 2016, he received the Milton-Safenowitz Post-Doctoral Fellowship Award from the Amyotrophic Lateral Sclerosis Association (ALSA) to support his research. Bruno is also the recipient of other career awards, including the Dr. Alan M. Gewirtz Memorial Scholarship Award (Oligonucleotide Therapeutics Society), the STAT 2019 Wunderkind Award (STAT News) and a Silver Medal from the Polytechnic Institute in Lisbon. Bruno has also held several teaching positions throughout his career: Invited Lecturer in Biopharmaceutics at University College Cork (Ireland); Adjunct Professor at the Lisbon School of Health Technology (ESTESL, Portugal) and Guest Lecturer at the Instituto de Engenharia de Lisboa (ISEL, Portugal), teaching pharmacy-related subjects and advanced therapeutics.

At Atalanta Therapeutics, a CNS-focused RNAi therapeutics company, Bruno leads platform development and innovation efforts, and is the lead in vivo pharmacologist for several internal and partnership programs.

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National keynote lecture by Annemieke Aartsma-Rus 10:00-10:45

A brief history of ASO therapy development: Developments in the current millennium.

Annemieke Aartsma-Rus

Leiden University Medical Center, Leiden, The Netherlands

Antisense oligonucleotides are a unique therapeutic modality that have unprecedented specificity due to their ability to sequence-specifically hybridize to target transcripts. They can be used to reduce production of toxic proteins or to restore protein production. Currently 16 oligonucleotide treatments and vaccines are approved by the FDA and/or the EMA. During the presentation, I will outline what is required to turn oligonucleotides into drugs. I will also introduce the different therapeutic modalities giving examples of approved drugs or oligonucleotides in clinical development.

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ABSTRACTS
Session 1
10:45-12:30
(chronological order)

Peptide-mediated delivery of antisense oligonucleotides

Najoua El Boujnouni[1,2], Rik Oude Egberink[1], Alexander H. van Asbeck[1,4], Derick G. Wansink[2], Roland Brock[1,3]

[1]Dept. of Biochemistry and [2]Dept. of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, The Netherlands
[3]Dept. of Medical Biochemistry, College of Medicine, Arabian Gulf University, Kingdom of Bahrain
[4]present address: RiboPro, Oss, The Netherlands

Peptides have been broadly explored as delivery vectors for antisense oligonucleotides (AONs). In general, two methods for formulation can be distinguished. On one hand, peptides can be conjugated covalently to the AON. This approach is the method of choice for uncharged AON analogs such as morpholino oligomers and for conjugation of uncharged ligand (peptides) to negatively charged AONs. On the other hand, positively charged peptides and negatively charged AON can form nanoparticles through charge-driven complexation. Peptides employed for charge-driven nanoparticle formation belong to the class of cell-penetrating peptides. Whereas many peptides yield uniform nanoparticles, only few peptides yield efficient cytosolic delivery and the structural and functional principles that guide this process are only poorly understood. Here, I will summarize our understanding of structural principles decisive for cytosolic delivery and methodological approaches to understand structure-activity relationships of oligonucleotide delivery. Very clearly, a delicate balance of positive charge, stability of nanoparticles and an amphipathic character is needed to hit a sweet spot between efficient uptake and membrane-toxicity. Since peptides can be considered short polyamide oligomers, these structural insights are also relevant for the broader field of polymer-based oligonucleotide delivery.

Exon-skipping for dystrophic epidermolysis bullosa

Aileen Sandilands,[1] Jeroen Bremer,[2,3] Michael Conneely,[1] F. Cisse Vermeer,[2] Bnar Abdul Kader,[1] W.H. Irwin McLean,[1] Robyn P. Hickerson[1], Peter C. van den Akker[1,2,3]

[1]Division for Biological Chemistry and Drug Development, School of Life Sciences, University of Dundee, Dundee, Scotland, UK.
Departments of [2]Genetics and [3]Dermatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

Recessive dystrophic epidermolysis bullosa (RDEB) is a devastating genetic disorder that results in severe blistering of the skin and mucosal membranes after only minor friction. Patients usually die before the age of 40 years due to aggressive skin cancer. It is caused by biallelic null variants in the *COL7A1* gene that encodes type VII collagen (C7), an extracellular protein that forms anchoring fibrils that secure epidermal attachment to the underlying dermis. No curative therapies exist for RDEB, and there is much room for disease-modifying therapies. In recent years, we have demonstrated proof-of-concept for antisense oligonucleotide (ASO)-mediated exon skipping in *in vitro* and *in vivo* human skin models. *In vitro*, ASO treatment induced exon skipping and C7 production in keratinocytes and fibroblasts from a patient with RDEB. In human skin grafts, generated on the backs of nude mice from the same patient's skin cells, exon skipping and C7 production were also observed after systemic ASO treatment of the mice, highlighting the potential of systemic ASO treatment which is highly preferable for RDEB patients. In another study we then demonstrated that the C7 protein lacking the skipped exon was functional, and a literature study confirmed that exon skipping should be expected to ameliorate the severe RDEB phenotype. Recently, we have shown for the first time that our ASOs are able to induce exon skipping in intact human control skin using a new *ex vivo* skin model, which is an important prerequisite for further considering any systemic ASO delivery approach. The current focus of our research is on improving the exon skipping efficiency in intact human skin and addressing the outstanding questions before we can move towards patients.

Skipping NOTCH3 exons to prevent protein aggregation in CADASIL patient arteries

Julie Rutten MD. PhD
Department of Clinical Genetics, LUMC

CADASIL is a disabling hereditary vascular dementia and stroke syndrome, for which no treatment is available. The disease is caused by stereotyped mutations in *NOTCH3*, which alter the number of cysteines in one of the epidermal growth factor-like repeat (EGFr) domains of the NOTCH3 protein. This causes toxic NOTCH3 aggregation and accumulation in the (cerebro)vasculature, leading to vascular smooth muscle cell degeneration and a reduced cerebral blood flow. We have used antisense-mediated exon skipping to restore the canonical number of 6 cysteines within EGFr, while maintaining normal NOTCH3 signaling function. Data from patients with naturally occurring exon skipping provide in-human evidence that cysteine corrective NOTCH3 exon skipping is associated with less NOTCH3 aggregation and an attenuated phenotype.

The Dutch Center for RNA Therapeutics: a center to develop antisense oligonucleotide therapies for patients with nano-rare mutations

Marlen C. Lauffer[1], Annemieke Aartsma-Rus[1], Rob W.J. Collin[2], Ype Elgersma[3], Willeke van Roon-Mom[1]

[1]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Human Genetics and the Donders Institute for Brain, Cognition and Behavior , Radboud University Medical Center, Nijmegen, the Netherlands

[3]Department of Clinical Genetics and The ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus University Medical Center Rotterdam, the Netherlands

Antisense oligonucleotides (AONs) offer the potential to treat patients with genetic diseases. Notably, for tissues allowing local injection, such as the brain and eye, high local exposure can be achieved with 3-4 infusions of low amounts of AONs annually. Proof-of-concept for this treatment strategy has been shown for example in spinal muscular atrophy and Leber congenital amaurosis. This approach can also benefit patients with private mutations, as was recently evidenced by the development of the custom-made AON milasen for a patient with Batten's disease. Such advancements underline the potential of AONs as personalized medicines, specifically for patients with unique mutations that are associated with brain or eye phenotypes. However, pharmaceutical companies are usually not interested in the development of such approaches, due to the extreme rarity of these variants and high costs of drug development.

The Dutch Center of RNA Therapeutics (DCRT) is a collaboration of three Dutch academic medical centers with a track record in AON development that aims to develop therapies for patients with nano-rare variants and to offer these therapies in a not-for-profit manner. The DCRT works in alignment with the N-of-1 collaborative (N1C, global) and the 1mutation1medicine consortium (1M1M, European). In the first two years, we have identified several patients with mutations that are suitable for splice modulation by AONs. Here, we outline our pre-clinical development pipeline of AON-based splice correction therapies and explain patient selection criteria. We describe the Dutch roadmap towards clinical implementation, highlighting also the efforts to align developments internationally.

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ABSTRACTS
Session 2
13:15-15:30
(chronological order)

Identification of small interfering RNAs for allele-selective silencing of murine von Willebrand factor

Yvonne K. Jongejan[1], Richard J. Dirven[1], Kalina Paunovska[2], Kim D. van der Gouw[1], Elisa Schrader[2], Annika de Jong[1], Noa A. Linthorst[1], James E. Dahlman[2], Bart J.M. van Vlijmen[1], Jeroen C.J. Eikenboom[1]

[1] *Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands.*
[2] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia, United States.*

High von Willebrand factor (VWF) plasma levels are associated with (arterial) thrombosis. Antiplatelet therapy increases bleeding risk and often fails to prevent arterial thrombosis. Lowering of VWF through allele-selective silencing of *VWF* with small interfering RNAs (siRNAs) would be a personalized approach which averts complete *VWF* knockdown and thus minimizes bleeding risk. We aimed to investigate the feasibility of strain-selective siRNA-mediated VWF inhibition *in vivo* in mice. 15 siRNAs targeting murine *Vwf* were studied on activity and allele-selectivity *in vitro* in transient co-transfected HEK293 cells expressing C57BL/6J (B6) and 129S1/SvImJ (129S) *Vwf*. Of those siRNAs three lead candidates were chosen. These candidates, a non-selective si*Vwf*, and two strain-selective siRNAs (si*Vwf.B6* and si*Vwf.129S*), together with corresponding scrambled controls were encapsulated in 7C1 polymeric nanoparticles for endothelial targeting *in vivo*. Male and female B6 and 129S mice were intravenously injected with the siRNA-encapsulated nanoparticles. 72 hours post-injection, citrated blood and lung tissue were collected for measuring VWF plasma protein and *Vwf* mRNA expression, respectively. For both male and female mice, all three lead candidate siRNAs dose-dependently inhibited *Vwf* expression on mRNA and plasma protein levels in the corresponding mouse strains. The highest inhibitory effect was shown at the dose of 1.5 mg siRNA/kg body weight. The median inhibition was 70% [63-95] on lung mRNA and 75% [66-82] on plasma levels for si*Vwf*-treated mice, 87% [85-90] and 83% [80-86] for si*Vwf.B6*-treated mice, and 70% [54-79] and 62% [51-72] for si*Vwf.129S*-treated mice. The strain-selective siRNAs did not inhibit *Vwf* in their non-corresponding strains. To conclude, we have shown efficient *in vivo* endothelial targeting of strain-selective siRNAs with up to 90% inhibition of *Vwf* in corresponding mouse strains with no inhibition in non-corresponding strains.

This study was financially supported by the Dutch Thrombosis Foundation (grant #2018-1).

A novel antisense strategy to inhibit BK polyomavirus replication

Jurriën Prins[1], Janneke Kouwenberg[1], Anouk Spruit[2], Lizanne Daleman[1], Anton Jan van Zonneveld[1] and Eric van der Veer[3].

[1]Leiden University Medical Center (Nephrology), [2]Leiden University Medical Center (Human Genetics), [3]Hybridize Therapeutics.

Post-kidney transplantation infection of BK virus (BKV) leads to a loss of kidney graft function and increases the risk of rejection. Since no treatment option exists for preventing BKV reactivation in patients, reducing immunosuppression is standard care but at the risk of allograft rejection. The early cellular stage of BKV infection is critically dependent on correct splicing of the BKV-derived key regulator Large T antigen (T-Ag). Our aim was therefore to develop an RNA-based therapeutic compound, in the form of an antisense oligonucleotide (ASO), that intrinsically homes to the kidney and abrogates BKV infection through interference with T-Ag splicing.

Bioinformatics analysis of publicly available whole genomic sequences was performed to study T-Ag splice site conservation. Multiple 20-mer 2'OMe-PS ASOs were designed to target the T-Ag donor and acceptor splice sites and tested in BKV-infected human HK-2 and primary tubular epithelial cells (PTEC). RNA, protein and supernatant was harvested to study ASO activity on BKV replication. Activity was validated in a separate 3D urothelial cell model. In addition, findings were validated in a 3D urothelial cell model. Last, 8-week-old male C57BL6 mice were administered i.v. with 40 mg/kg ASO to study biodistribution and retention using immunofluorescent staining and hybridization-ELISA.

Bioinformatics analysis of BKV genomic sequences indicated a high splice site sequence conservation between BKV subtypes, allowing for a one-size-fits-all approach. Screening of ASOs in BKV-infected HK-2 revealed log10-fold reductions in mRNA expression of both T-Ag and the late-coding viral packaging protein VP1. This resulted in the downstream suppression of VP1 protein expression and release of virus particles. Validation of three lead candidate ASOs in BKV-infected PTEC resulted in 91-97% suppression of VP1 protein expression and 86-94% reductions in virus particle production. Activity was found to be dose-dependent, reliant on 2'OMe and phosphorothioate chemistry, and unaffected by BKV strain origin. Preliminary findings from a 3D urothelial model indicated activity under both lipofectamine-assisted and gymnotic conditions. The most effective candidate (HYB_03) was selected to proceed with in vivo testing. Intravenous administration of 40 mg/kg HYB_03 in mice was well tolerated and resulted in efficient uptake and retention in the kidney up to 90 days after a single administration.

We have developed a novel therapeutic in the form of a 20-mer 2'OMe-PS ASO that directly interferes with BKV by targeting the donor splice site of Large T antigen. This therapeutic shows a high activity in vitro and is efficiently taken up by the kidney.

Deciphering the efficacy and safety of different antisense oligonucleotide chemical modifications in a retinal context.

Authors: Irene Vázquez-Domínguez[1,2], Alejandro Allo-Anido[1], Lonneke Duijkers[1], Tamara Hoppenbrouwers[1,2], Anita Hoogendoorn[1], Rob WJ. Collin[1,2], Alejandro Garanto[1,2,3].

[1]Department of Human Genetics, Radboud university medical center (Radboudumc); [2]Donders Institute for Brain, Cognition and Behavior, (Radboudumc); [3]Department of Pediatrics, Amalia Children's hospital and Radboud Institute of Molecular Life Sciences (RIMLS), Radboudumc (Nijmegen).

Inherited retinal diseases (IRDs) are a group of heterogenous neurodegenerative diseases which lead to visual impairment. In IRDs, a 15% of associated genetic defects affect pre-mRNA splicing, resulting in aberrant transcripts which hamper normal cell function. In the last years, antisense oligonucleotide (AON)-based therapies have widely explored for IRDs. However, little is known about how the AON chemical modifications can affect uptake, biodistribution and toxicity in the retina. Here, we present to our knowledge the first comparison of chemical modifications in a retinal context.

Different genes exclusively expressed in specific retinal cells affected in IRDs (rods, cones, bipolar and retinal pigment epithelium cells) were selected. Subsequently, splicing modulation capacity of AONs harboring three different commonly used chemical modifications [2'-O-methyl-phosphorothioate (2-OMe), 2'-O-methoxyethyl-phosphoriate (2-MOE), and phosphorodiamidite morpholino oligomers (PMO)] was studied first, *in vitro* by employing splice reporter vectors, and *in vivo* by direct delivery to the mouse retina.

Our *in vitro* results indicated that 2-OMe and 2-MOE AONs were able to redirect splicing similarly, but 2-MOE AONs showed a slightly higher efficacy in most of the cases. PMO transfection *in vitro* requires a different delivery method, but resulted in the lowest efficacy for all selected genes. These observations were confirmed *in vivo*, in which 2-MOE and 2-OMe modified AONs presented the highest splicing modulation efficacies. Furthermore, PMO oligonucleotides injection resulted in a clear phenotype present in 88.5% of the injected mice. The ensuing isolated retinas were whiter and smaller than the retinas obtained from controls, suggesting a PMO-mediated toxic effect. Histological studies are ongoing to assess how the retina is affected upon PMO delivery.

Overall, our results constitute the first comparison of different chemical modifications of AONs in a retinal context, demonstrating different properties amongst them. Our data show that 2-MOE is the most efficacious to modulate splicing. In contrast, PMO modification not only led to a poor splicing modulation but also to potential toxic effects, at least in the mouse retina.

1st Dutch Antisense Therapeutics Symposium

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International keynote lecture by Bruno Godinho 14:00-14:45

Delivery of Stabilized siRNA scaffolds to the Central Nervous System

Bruno M.D.C. Godinho, PhD

Atalanta Therapeutics, Boston, MA.

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

Transvascular delivery of therapeutic oligonucleotides is one of the most desired paradigms since it would exclude the need for invasive procedures, like neurosurgery, to treat the brain. Nonetheless, the low permeability of the blood-brain barrier (BBB) and the rapid clearance kinetics of these drugs from the blood precludes meaningful distribution to this target organ. Several research groups have attempted transcytosis-based strategies with little success. Here we show an alternative approach that uses transient osmotic disruption of the BBB to enable transvascular delivery of a lipid-modified siRNA to the rat brain. This approach allowed for potent gene silencing throughout the brain without major neurotoxicity/neuroinflammation. Although further studies are required to evaluate the long-term effects of repetitive BBB disruptions on brain homeostasis, this study provides valuable proof-of-concept for the utility of mannitol-based BBB disruption strategies for productive brain delivery of therapeutic oligonucleotides.

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

Antisense to medicine: a regulator's perspective

A.M.G. (Marjon) Pasmooij, PhD

Marjon is Science Programme Manager at the Medicines Evaluation Board (MEB) and since 2022, co-chair of the Regulatory Science Network Netherlands Steering Committee. She has an outstanding background in cell biology, dermatology, and molecular genetics. Marjon has been employed by the MEB in various roles since 2007 and has always combined her work at the MEB with research at the University Medical Centre of Groningen. As co-promotor of Jeroen Bremer, and together with Peter van den Akker, Marjon laid the foundation for antisense oligonucleotide-mediated exon skipping as potential therapeutic approach for dystrophic epidermolysis bullosa.

Currently, her focus within the MEB is on the development of the new science policy 2020-2024. She is also part of the Big Data Steering Group that is working on the 10 priority recommendations regarding Big Data, including having skills to perform analyses on Big Data within the network.

At the 1st Dutch Antisense Therapeutics Symposium, Marjon will provide a regulator's perspective on antisense RNA therapeutics.

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Poster Pitches
15:15-15:30

#5 Edward Geeurickx, Biogazelle
#9 Dyah Karjosukarso, Radboudumc
#11 Elsa Kuijper, LUMC
#14 Hilde Smeenk, Erasmus MC
#16 Nuria Suárez Herrera, Radboudumc
#20 Alper Yavas, LUMC

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Poster Session

(with coffee and cookies)

15:30-16:45

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ABSTRACTS
Session 3
16:45-17:00
(chronological order)

Antisense oligonucleotide treatment rescues UBE3A expression and multiple phenotypes of an Angelman Syndrome mouse model

Claudia Milazzo,[1,2] Edwin J. Mientjes,[1,2] Ilse Wallaard,[1,2] Søren Vestergaard Rasmussen,[3] Kamille Dumong Erichsen,[3] Tejaswini Kakunuri,[1,2] A.S. Elise van der Sman,[1,2] Thomas Kremer,[4] Meghan.T. Miller,[4] Marius Hoener,[4] Ype Elgersma[1,2*].

1. Department of Neuroscience, Erasmus MC, Rotterdam, The Netherlands. 2. The ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus MC, Rotterdam, The Netherlands. 3. Therapeutic Modalities, Roche innovation Center Copenhagen, F. Hoffmann-La Roche Ltd. 4. Neuroscience and Rare Diseases Discovery & Translational Area, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd.

Angelman syndrome (AS) is a severe neurodevelopmental disorder for which only symptomatic treatment with limited benefits is available. AS is caused by mutations affecting the maternally inherited *UBE3A* gene. Previous studies showed that the silenced paternal *Ube3a* gene can be activated by targeting the antisense *Ube3a-ATS* transcript. Here, we investigated antisense oligonucleotide (ASO) induced *Ube3a-ATS* degradation, and its ability to induce UBE3A reinstatement and rescue of AS phenotypes in an established *Ube3a* mouse model. We found that a single intracerebroventricular injection (ICV) of gapmer ASOs in newborn (P1) or juvenile (P21) AS mice results in potent UBE3A reinstatement in the brain and a full rescue of sensitivity to audiogenic seizures. AS mice treated with ASO at P1 also showed full rescue of previously established AS phenotypes such as anxiety and depressive like behaviors, a significant improvement of motor coordination and a notable improvement of hippocampal plasticity. No rescue was observed for repetitive and innate behaviours. Taken together, our findings highlight the promise of ASO mediated reactivation of *UBE3A* as a disease modifying treatment for AS. The Angelman Syndrome clinic of the ENCORE expertise center for Neurodevelopmental disorders at Sophia/ Erasmus MC is currently involved in a clinical trial to translate these findings to patients (NCT03768063, sponsored by Hoffmann-La Roche).

Biodistribution of radioactively labelled antisense oligonucleotides after intracerebroventricular and intrathecal injection in mice measured with SPECT

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Antisense oligonucleotides (AONs) are promising candidates to treat a wide range of diseases. Neurological diseases are an interesting target for AON treatment, because AONs are efficiently taken up by brain cells. Direct injection into the cerebrospinal fluid surpasses the blood brain barrier. Intracerebroventricular (ICV) injection is the predominantly used route of administration of AONs in the mouse brain. Injecting AONs ICV gives diffusion throughout the whole brain, and AONs are stable for at least three months. In regard to future clinical applications, it is important to know AON distribution of different routes of administration. In this study we investigated the distribution of AONs injected ICV or intrathecal (IT) in a mouse.

We analyzed the biodistribution of a radioactively labeled, splice modulating AON in the first hours and days after IT and ICV injection in mice using SPECT imaging. Wild-type mice were injected either ICV or IT with an AON targeting exon 17 of amyloid precursor protein (APP) pre-mRNA[1,2] labeled with radioactive indium-111 and scanned in a SPECT scanner at 0, 4, 24, 48, 72 and 96 hours after injection. Organs and tissues were collected for radioactivity counting. Paraformaldehyde-fixed brain tissue was collected for immunofluorescent staining. Fresh-frozen tissue was collected for RNA analysis.

Imaging with SPECT shows that the signal diffuses throughout the central nervous system and kidneys in the first hours after injection. The radioactive signal in the central nervous system persists over the course of five days, while signal in the kidneys rapidly decreases. Intracerebroventricular injection gives a better spread throughout the whole brain and spinal cord. Post mortem counting in the different organs and tissues shows that ICV injection gives a higher signal in various brain regions, even though peripheral organ counting shows a similar pattern of distribution of signal in both ICV as IT injection.

These results show that, although most of the signal disappears from the body in the first hours and days after injection, a relatively high signal is observed within the central nervous system compared to the rest of the body. Both IT and ICV injection spread throughout the central nervous system, but ICV injection seems to give a higher signal in the brain. Currently, we are performing tissue PCR analysis to confirm exon skipped APP RNA present in the different brain regions.

[1] Amylon Therapeutics,

[2] Daoutsali et al., Nucleic Acid Ther. 2021

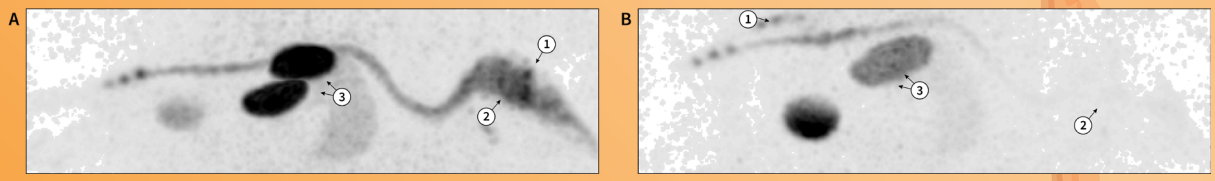

Figure 1: Sagittal summary of SPECT signal four hours after a) intracerebroventricular and b) intrathecal injection with indium-111 labelled APP AON. Both injections show a spread throughout the body away from the injection-site (1). Intracerebroventricular injection shows a higher signal in the brain (2). The majority of the signal is in the kidneys (3) at this time-point.

Whole genome sequencing identifies several deep-intronic variants in *USH2A* that are amenable for antisense oligonucleotide-based splice correction therapy

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A significant portion of Usher syndrome type 2 (USH2) and autosomal recessive retinitis pigmentosa (arRP) cases remain genetically unexplained because only one, or no causative variants are detected in the *USH2A* exons. We assessed USH2 and arRP cases with a mono-allelic *USH2A* variant using whole genome sequencing (WGS) and developed an antisense oligonucleotide (AON)-based therapeutic strategy for a subset of the identified variants.

One-hundred cases were screened using WGS to detect missed *USH2A* exonic, deep-intronic, structural, and regulatory variants, and variants in other genes associated with arRP and USH2. Variants with a predicted effect on pre-mRNA splicing were assessed using minigene splice assays. For variants that were confirmed to cause inclusion of a pseudoexon, we designed target-specific AONs and evaluated their splice-correcting potential using minigene splice assays and patient-derived photoreceptor-precursor cells.

Fourty-nine cases were (likely) solved as we identified novel splice variants, structural variants or bi-allelic variants in arRP or USH2-associated genes. Thirteen *USH2A* variants showed a deleterious effect in the minigene splice assays. Four of these variants resulted in the inclusion of a pseudoexon, which was reversed upon delivery of AONs.

Through our study, the genetic diagnosis was (likely) completed for 49/100 cases. This highlights that WGS is a powerful approach to genetically explain cases that remain mono-allelic after pre-screening of the *USH2A* exons and flanking intronic sequences. We identified four novel deep-intronic variants and we confirmed that AONs are a valuable tool to correct the associated splice defects.

The role of patient organizations in antisense oligonucleotide drug development

Elizabeth Vroom
Duchenne Parent Project Netherlands

Duchenne patient/parent organisations have played, and are still playing, an important role in the development of anti-sense oligonucleotide. Initially the preclinical phase as sponsors of fundamental research,in fact they provided the seed money to start this line of research. In the translational and clinical research they also contributed to research projects, to the development of outcome measures including for non-ambulant patients, standard operating procedures (SOP) for studies in animal models, trial design and data collection. They also initiated patient preference studies. The World Duchenne Organization set up an international Community Advisory Board (CAB) to advise industry. Patient organizations played a vital role in multistakeholder meetings with regulators and as experts at the European Medicines Agency.

1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

Awards and closing remarks
18:00-18-10

Drinks and networking
18:10-19:30

1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

ABSTRACTS
Posters

Poster # 1

ANALYSIS OF OLIGONUCLEOTIDE IMPURITIES ON AN UHPLC-TOF MS SYSTEM WITH A MODIFIED SURFACE TECHNOLOGY

Perry Derwig[1], Catalin Doneanu[2] Christopher Knowles[3], Jonathan Fox[3], Emma Harry[3], Ying Qing Yu[2], Joseph Fredette[2] and Weibin Chen[2]

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2 Waters Corporation Milford MA, USA;
3. Waters Corporation, WImslow, UK

Oligonucleotide therapeutics have emerged in recent years as a powerful alternative to small molecule and protein therapeutics. Manufacturing and quality control of oligonucleotide therapeutics requires highly selective and sensitive LC-MS method for impurity identification and quantification.
Oligonucleotides contain a negatively charged phosphate backbone known to interact with metal surfaces typically found in the fluidic path of conventional stainless steel LC systems. These interactions are often responsible for all oligonucleotide losses, poor chromatographic peak shapes or poor data reproducibility. To address these challenges, Waters has developed a family of new technologies containing a more inert surface specifically designed to address difficult to analyze analytes – MaxPeak™ High Performance Surfaces (HPS). Here we investigated the capabilities of this type of metal surfaces in a UPLC system and column technology, for the intact mass confirmation of oligonucleotides and their associated impurities.
Critical advantages were observed in impurity measurement of oligonucleotides, including lower detection limits and reproducibility with excellent mass accuracy.

Poster # 2

Advancing antisense therapy against DM1 in a patient-directed manner

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Myotonic dystrophy type 1 (DM1) is caused by an unstable $(CTG)_n$ repeat expansion in the *DMPK* gene for which, despite much effort, no curative therapy is yet available. Antisense oligonucleotides (AONs) are promising therapeutics as these can specifically target the source of the disease, i.e. the toxic *DMPK* transcripts. In DM1, repeat instability and patient variability with respect to repeat length and clinical manifestation result in a highly heterogeneous patient population. To provide an effective treatment against DM1, these factors must be addressed.

We, and others, have identified several AONs able to sterically block the aberrant binding of RNA-binding proteins to expanded *DMPK* transcripts or induce RNase H-mediated degradation thereof, thereby diminishing downstream effects and restoring cellular function. To decide which AON would be the most promising candidate for further (pre-)clinical development, we compared the activity of AONs that differ in mechanism of action (blocking- versus RNase H-recruiting) and/or target sequence (the repeat or a unique sequence) in DM11 myoblasts. AON efficacy was determined by measuring *DMPK* expression, nuclear foci and disease-associated mis-splicing, along with RNA sequencing to investigate on- and off-target effects. We also addressed the impact of DM1 heterogeneity on AON activity using primary cultures originating from DM1 patients with varying repeat lengths.

In DM11 myoblasts, the repeat blocking AON and both gapmers led to *DMPK* knock-down and were equally potent in correcting aberrant splicing. However, the repeat blocking AON was more effective in MBNL1 protein displacement and had the fewest off-target effects, indicating it to be superior over other candidates. Determination of AON efficacy in our primary DM1 cell panel was less straight-forward, as these cultures exhibited a milder disease phenotype regardless of repeat length, limiting the corrective potential of the tested AONs. The complexities observed for primary DM1 cells highlight the importance of testing drug candidates against a broad panel of cell lines to capture patient heterogeneity (especially in diseases like DM1).

Lastly, the use of patient material prompted us to search for an alternative read-out, as a sensitive, robust and clinically relevant parameter by which therapeutic potency can be measured already at the *in vitro* stage. Since increasing evidence for a link between DM1 and mitochondrial (dys)function is emerging, we are currently investigating whether mitochondrial morphology and function in isogenic cell lines is altered by the presence of the repeat expansion.

Poster # 3

Efficient exon 53 skipping of the human dystrophin transcript in a mouse model for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the DMD gene encoding for dystrophin. The absence of dystrophin results in continuous contraction-induced damage in skeletal muscle. One way of restoring dystrophin expression is by using antisense oligonucleotides (AONs) to reframe the disrupted open reading frame of the transcript. AONs bind to the target exon in the pre-mRNA dystrophin transcript, thereby hiding it from the splicing machinery, which results in skipping of the target exon. This leads to production of a shorter but semi-functional dystrophin protein. Although some AONs are already conditionally approved for DMD, this is based on restoration of very low levels of dystrophin, because delivery of AONs to muscle and efficiency of exon skipping is still a challenge.

In an effort to optimize AON efficiency, we assessed exon 53 skipping in the DMD gene with AONs of the following chemical modifications, all with a phosphorothioate (PS) backbone: FANA, FRNA, LNA-2'OMe, LNA-FRNA and αLNA-FRNA. First, we determined exon 53 skipping in immortalized human control myoblast cultures. Here we found efficient exon 53 skipping was induced whether the AONs were delivered via transfection or gymnosis. The FRNA and LNA-FRNA modifications were most efficient. Pronounced exon 53 skipping levels were also observed in the skeletal muscles and heart of hDMDdel52/*mdx* mice receiving weekly subcutaneous injections of 50 mg/kg for six weeks starting at 4 weeks of age. In the gastrocnemius of these mice, the LNA-2'OMe and LNA-FRNA modified AONs were most efficient (70.9 ±10.7% and 86.5 ± 7.3% skip, respectively) with comparable levels in triceps, diaphragm and heart. All AONs were well tolerated based on plasma markers for liver and kidney function. We are currently evaluating dystrophin protein levels in skeletal muscles and heart.

Poster # 4

Exploring dystrophin stability and processing under oxidative stress

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Mutations in the *DMD* gene encoding the dystrophin protein are causative for Duchenne muscular dystrophy (DMD), a severe X-linked muscle-wasting disorder. Potential treatment for DMD consists of restoring the *DMD* reading frame by treatment with antisense oligonucleotides (AONs). Four AONs are currently FDA approved for DMD treatment. However, further development of these therapies is necessary as only a low level of dystrophin is restored in the skeletal muscle of treated patients. A more thorough understanding of the processing and degradation of dystrophin could help to improve the restoration of dystrophin by AON therapies.

Previously, it has been shown that muscles of DMD animal models and patients exhibit elevated levels of reactive oxygen species (ROS). ROS lead to increased oxidative stress, which damages muscle tissues through various mechanisms. We hypothesize that oxidative stress might directly influence the degradation mechanism and stability of both wild-type and AON-restored dystrophin. To explore the effect of oxidative stress and antioxidants on dystrophin processing, immortalized human myotubes were exposed to oxidative stress-inducing agents after which levels of dystrophin mRNA and protein, as well as dystrophin localization were analyzed.

Preliminary data indicate that oxidative stress may cause accelerated degradation of dystrophin. Significant decreases of DMD mRNA and protein can be observed in immortalized human myotubes in response to ROS exposure. Future experiments will look into whether co-treatment of DMD patient myotubes with AONs and antioxidants can further improve AONs efficacy and contribute to stabilization of dystrophin levels. Moreover, we will attempt to elucidate the dystrophin processing pathways which are involved in DMD degradation (e.g. proteasomal degradation) in response to oxidative stress in order to extend our understanding of how AON therapies for DMD patients could be enhanced.

Poster # 5

Identification of potent and specific antisense oligonucleotides for target gene knockdown or splice modulation using a high-throughput RT-qPCR based cellular screening platform

Geeurickx E, Dewaele S, Van Peer G, Mestdagh P, Reinartz A, Van Cauwenberghe R, Hellemans J, Vandesompele J

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While antisense oligonucleotides (ASOs) are in principle capable of efficiently silencing gene expression or modulating splicing, *in silico* prediction of the potency of ASO sequences is challenging. Identifying potent ASOs often requires an *in vitro* evaluation of many candidates that typically tile the target RNA (or splice site) of interest. To enable such analyses, we have developed a comprehensive and high-throughput platform to screen hundreds of ASOs for on-target knock-down or splice modulation efficiency. We established a panel of 20 model cell lines that have high free-uptake capacity and developed a dedicated pipeline to design expression-modulating or splice-modulating ASOs. Candidate ASOs are delivered to 96-well culture plates in a single dose and target gene expression or splicing is quantified by RT-qPCR or fragment analysis on crude cell lysates using various independent assays and multi-gene normalization. For ASOs modulating target gene expression, these data typically reveal one or multiple hotspot regions in the target gene that are highly accessible for ASO binding and target knock down. Based on these regions, additional ASOs can be designed for secondary screens in order to further expand the set of potent ASOs. Hits identified from these screens are further validated in dose response experiments to determine the most potent candidates based on IC-50 values. Candidate ASOs are subsequently annotated based on their off-target gene potential through a proprietary *in silico* prediction pipeline considering the number of mismatches and their position in the ASO sequence. Off-target predictions are complemented with gene expression data of ASO treated cells using our high-throughput RNA-sequencing platform HTTargetSeq. HTTargetSeq enables library preparation directly from cell lysates for hundreds of samples in a single experiment. At shallow sequencing depth, robust gene expression data for over 10,000 genes is generated, enabling the identification of primary off-target genes. As a result, potent and specific ASOs can be identified for any target gene of interest. To demonstrate the robustness and potential applications of our workflow, we have screened over 10,000 ASOs with different chemistries targeting 250 genes in multiple cell lines. Our data reveal differences in the potency of ASO chemistries, varying targetability across genes, and differences in target gene knock-down among qPCR assays that need to be considered when screening for potent ASOs.

Poster # 6

Investigating the effect of DMD non-sequential splicing on exon skipping strategies

Remko Goossens 1, Nisha Verwey 1, Leslie CL Wu 2, Fred Schnell 2, Annemieke Aartsma-Rus 1
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2: Sarepta Therapeutics, Inc., Cambridge, Massachusetts, USA

Duchenne muscular dystrophy is one of the most common myopathies, with an incidence of 1:5000 in newborn boys. The root cause of the disease are mutations in the dystrophin (*DMD*) gene which disrupt the reading frame. The use of antisense oligonucleotides (AON) to induce exon skipping is a therapy aimed at restoring the dystrophin reading frame to allow for production of an internally truncated but functional dystrophin protein. Four AONs have been approved by the Food and Drug Administration (FDA, USA).

DMD is the longest gene in the human genome, spanning over 2 megabases on chromosome X. The large DMD muscle transcript (Dp427m) precursor is composed of 79 exons separated by 78 introns, which length can be up to 250 kilobases each. Introns are removed from the transcript by splicing, and exons ultimately join together to generate a messenger RNA for protein translation.

Previous work from our lab has determined that of the 78 *DMD* introns, almost half are not spliced sequentially. This means that certain introns are only removed from the transcript after a downstream intron has already been spliced out. Moreover, exons can be flanked by introns which are spliced out rapidly ("fast") or by those retained in the transcript longer ("slow"). This has implications for the skippability of dystrophin exons, where exons flanked by slowly spliced introns may be easier to skip than those flanked by quickly spliced introns.

To test this hypothesis, we have selected a series of *DMD* exons preceded and/or followed by either slow- or fast-splicing introns. We used a large set of unique phosphorodiamidate morpholino oligomers (PMOs) to assess whether there is a correlation between the speed of intron removal and the efficiency of exon skipping in *in vitro* cultured myocytes. Data obtained from this approach furthers our understanding of the dynamics of the *DMD* transcript, and can prove valuable for determining appropriate exons to be targeted by AON therapeutics for DMD.

Poster # 7

Development of a gapmer ASO screening workflow using high-throughput RT-qPCR and automated data analysis

Niki van der Steenstraten, Maria Blanca Torroba, Raymond de Wit, Roxana Redis, and Ilse Haisma
Charles River Laboratories, Leiden, The Netherlands

Gapmer antisense oligonucleotides (ASO) are a powerful tool for knocking down disease-relevant targets but identifying efficacious ASOs in a qPCR assay is typically costly and time consuming due to low-throughput protocols. In this project, we developed a high-throughput qPCR assay and automated analysis templates for screening 4,000 gapmer ASOs for their ability to knockdown a large disease-relevant target in immortalized patient-derived epithelial cells. Cells in 96-well microplates were transfected with ASOs, stained with Hoechst and imaged after 48 hours to quantify nuclei count as an indicator of cytotoxicity, and subsequently lysed using the Invitrogen™ Cells-to-CT™ 1-Step TaqMan™ Kit. Lysate was added to 384-well qPCR plates containing mastermix with primer-probe sets against the gene of interest and housekeeping gene, and plates were frozen down for staggered multiplexed RT-qPCR analysis. Due to lack of a known positive control against the target, an ASO targeting a long non-coding RNA was taken along on each plate and knockdown was analyzed with appropriate primer-probe sets to confirm successful ASO delivery and assess intra- and inter-plate variability. Critical liquid handling steps were performed semi- or fully automated to ensure high throughput. A data analysis pipeline was set up using our data management and visualization software packages to analyze assay performance and target knockdown in an automated and thus reliable manner. Molecular screens as described here are part of Charles River's lead candidate selection pipeline which also includes ASO immunotoxicity assays, functional assays, off-target analyses, in vivo pharmacokinetics, safety and efficacy studies, and further IND-enabling studies.

Abstracts need to be submitted as word files to DutchAntisense@gmail.com

Poster # 8

ExonCheck: a tool to prioritize ultra-rare disease patients for personalized antisense oligonucleotide therapies

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Around 80% of all rare diseases are of genetic origin, with each rare disease affecting fewer than 1 in 2,000 people worldwide. However, up to 7,000 rare diseases have been described to date, which strikingly accounts for 1 in 17 people. For most of these disorders, no therapeutic approaches are yet available and innovative treatment strategies are urgently needed. In recent years, antisense oligonucleotides (ASOs) have arisen as one option to tackle rare diseases, and ASO therapies have been approved for disorders like spinal muscular atrophy and Duchenne muscular dystrophy.

Besides treating whole patient groups, the first successful personalized RNA therapies have been reported, whereby ASOs are designed to specifically target single mutations of one patient at a time. With this proof-of-feasibility to generate the ultimate personalized therapies, there is a need to quickly and efficiently evaluate which patients in general and mutations in specific are eligible for this treatment approach. Currently, this type of variant evaluation of rare-disease patients is done manually.

Here, we introduce ExonCheck, a new tool that we are building to automate variant stratification. For each mutation, the tool allows input of the protein sequence, genomic localization or RNA localization and provides a prediction if the variant is suitable for so-called ASO based exon-skipping therapy. For these individualized therapies, the employed ASOs will modulate pre-mRNA splicing, thereby changing and restoring the reading frame of the mRNA.

ExonCheck needs to complete several steps in order to predict the outcome for each variant. The tool will consider whether the variant is present in transcripts expressed in brain and eye, the tissues currently being targeted with individualized ASO therapies. Further, ExonCheck will determine whether the variant is located in an in-frame or out-of-frame exon and if there are important functional domains located within the exon. Taking the different domains into consideration will help in determining whether a partial protein function can be expected after skipping this exon. The size of the exon to be skipped is taken into account, as well as the change in codons (amino acids) on the exon-exon junction. Eventually, ExonCheck will facilitate assessing whether a patient's rare variant is suitable for ASO therapy.

Poster # 9

Towards clinical application of antisense oligonucleotides for the treatment of Stargardt disease

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Stargardt disease (STGD1) is a progressive retinal disorder that initially affects central vision, and often leads to complete blindness. It is associated with bi-allelic mutations in the *ABCA4* gene, which encodes an ABC-transporter that is exclusively expressed in the retina. Dysfunction of ABCA4 leads to the accumulation of toxic by-products of the visual cycle in the retina, leading to retinal cell death. To date, more than 1200 different *ABCA4* variants have been described, a significant proportion of which affects pre-mRNA splicing. Based on the prevalence and previous proof-of-concept data, we carefully selected three prime candidate variants for which we aim to further develop antisense oligonucleotides (ASO) for the treatment of STGD1, up to the point of approval for therapeutic interventions in humans. As a first step, we have performed an extensive ASO screen for each of these candidates in patient-derived photoreceptor precursor cells, which allowed us to narrow down the list of candidate ASOs. This will be followed up by further testing of the top most promising ASO candidates in patient-derived retinal organoids, to asses both of the potency (i.e. correction at RNA, protein and toxic by-product level) as well as safety.

Poster # 10

RNA therapy development for SCA1 using RNA degrading- and splice-modulating antisense oligonucleotides

L.M.C.Kerkhof[1,2], R.A.M. Buijsen[1], M.C. Lauffer[1,2], A.M. Aartsma-Rus[1,2] and W.M.C. van Roon-Mom[1,2]
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Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by a CAG repeat expansion in exon 8 of the *ATXN1* gene (ATXN1-201). SCA1 is characterized by disturbances in balance and gait and has a fast disease progression. Eventually, the disease can result in respiratory failure, which is the main cause of death in patients. At the moment, no curative treatment is available for SCA1. We here aim to develop RNA targeting therapies for SCA1 using antisense oligonucleotides (AONs) to achieve modulation of pre-mRNA splicing and RNase H mediated knockdown. The RNA splice AONs are fully modified and aim to remove the extended toxic CAG repeat in exon 8 of the *ATXN1* gene, resulting in less mutant protein. Furthermore, splice-modulators targeting other exons of the *ATXN1* gene in the 5' untranslated region will be investigated for their ability to modulate ataxin-1 protein levels. For RNase H-mediated knockdown, gapmers will be designed to downregulate levels of mutant ataxin-1 mRNA.
Initial selection of these AONs will be performed in a neuroblastoma cell line using PCR and Western blot analysis to assess efficacy on both the RNA and protein level. Subsequently, the most efficient AONs will be tested in a dose-response curve prior to further screening in a standardized neuronal human induced pluripotent stem cell (hiPSC) platform. AONs will be tested in 2D human iPSC-derived neuronal platform by studying network development using multi-electrode arrays, metabolic dysfunction by assessing mitochondrial function and disease mechanism using single-cell RNA sequencing data. Ultimately, the most promising AONs will be screened in a SCA1 3D hindbrain organoid model that will be developed.

Poster # 11

THERAPEUTIC EFFECT OF ANTISENSE OLIGONUCLEOTIDE TREATMENT IN YAC128 HUNTINGTON MICE

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In Huntington's Disease (HD), cellular toxicity is particularly caused by protein fragments of the mutant huntingtin (Htt) protein generated by proteolytic enzymes. Lowering the levels of these toxic Htt protein fragments is hypothesized to ameliorate the consequences of an expanded CAG-repeat in the Htt gene. To that end, an antisense oligonucleotide (AON) molecule has been developed in our group that targets Htt RNA and induces skipping of exon 12 in Htt mRNA. As exon 12 contained the particular proteolytic cleavage sites, the resulting HttΔ12 protein can no longer be cleaved into its toxic fragments. In this study, we aimed to determine whether skipping of exon 12 in Htt mRNA can rescue the phenotype of YAC128 mice, a model of HD that contains the full-length human Htt gene including 128 CAG-repeats.

In total, 1500 µg AON was administered via three intracerebroventricular (ICV) injections starting at 6 months of age. Subsequently, motor behaviour was assessed monthly by a rotarod test and (hypo)activity was studied using PhenoTyper cages (Noldus). At 12 months of age, MRI was performed to assess cerebral volume, blood flow and connectivity, whereupon mice were sacrificed and blood and brain were collected.

Around 40% skip efficiency was detected in RNA from cortex of YAC128 mice. Although AON treatment did not reverse the YAC128 phenotype observed on the rotarod, it normalized the increased body weight levels of YAC128 mice to wild-type levels. Furthermore, untreated YAC128 mice showed lower activity levels compared to wild-type during the dark phase which was normalized by AON treatment.

Next, we will quantify skip efficiency on RNA level in other brain regions and assess protein modification. We will assess neuropathology using immunohistochemistry and MRI data. Furthermore, we will study dysregulation of gene expression in brain.

Poster # 12

Identification of heart and skeletal muscle homing peptides to improve the uptake of phosphorodiamidate morpholino oligomers (PMOs) for Duchenne Muscular Dystrophy therapy

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Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disorder caused by reading frame disrupting mutations in the DMD gene leading to the loss of dystrophin protein. This protein is involved in stabilizing and protecting muscle cells during contraction and relaxation. Without dystrophin, muscle cells get damaged over time and therefore patients eventually lose motor functions. Exon skipping therapy uses antisense oligonucleotides (AONs) to restore the mRNA reading frame which results in production of an internally truncated dystrophin protein. Hence, this therapy could help to slow down disease progression. While uptake by muscles is achieved after systemic delivery of AONs, including the class of phosphorodiamidate morpholino oligomers (PMOs), there is a need for further improvement. Currently, four exon skipping PMOs have been approved as treatment for DMD patients. However, we believe that with improved uptake of PMOs by muscle, an improved therapeutic effect can be achieved. This project focusses on identifying heart and skeletal muscle homing peptides, aiming to improve PMO uptake in skeletal muscles and heart by conjugating identified homing peptides to PMOs. To do so, in vivo biopanning with a phage display library consisting of millions of peptides was carried out in mdx, D2.mdx and WT mice to select candidate peptides. Next generation sequencing was used to identify peptide sequences enriched in skeletal muscle and heart but not in off-target organs, such as kidney and liver. Identified muscle-homing peptides will then first be fluorescently labeled to evaluate their uptake in an in vitro set-up whereafter leading candidates will be conjugated to PMOs to test for improved delivery to skeletal muscles and heart in DMD mouse models.

Poster # 13

Title: Modelling genetic disorders of lysine metabolism in a dish.

Authors: **Imke M.E. Schuurmans**[1,2,3,4], Clara D.M. van Karnebeek[4,5], Katrin Linda[7,8], Sara van Katwijk[2,7], Karlien L.M. Coene[2,6], Udo Engelke[2,6], Tessa Peters[2,8], Nael Nadif Kasri[7,8] and Alejandro Garanto[1,2,3,7].

Affiliations: [1]Department of Pediatrics, [2] Radboud Institute for Molecular Life Sciences, [3]Amalia Children's Hospital, [4]Department of Pediatrics Emma Children's Hospital Amsterdam University Medical Center Amsterdam (The Netherlands), [5]United for Metabolic Diseases (The Netherlands), [6]Translational Metabolic Laboratory, Department of Laboratory Medicine, [7]Department of Human Genetics and [8]Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen (The Netherlands).

Abstract:

Background: Pyridoxine-dependent epilepsy (PDE-ALDH7A1) and glutaric aciduria type-1 (GA1) are two rare neurometabolic disorders of lysine metabolism, caused by pathogenic variants in *ALDH7A1* and *GCDH,* respectively. Deficiency of the encoded enzymes results in accumulation of neurotoxic metabolites causing debilitating neurological sequelae in patients. We aim to develop human cellular models to investigate the disease mechanisms and test potential therapeutic strategies.

Materials & Methods: Knock-out induced pluripotent stem cells (iPSC) have been generated using CRISPR/Cas9 technology and PDE patient-derived fibroblasts have been reprogrammed into iPSCs. Subsequently, they have been differentiated towards neurons and astrocytes for further morphological and functional characterization using multi-electrode arrays (MEAs). Medium from PDE patient-derived fibroblasts was also collected for metabolomic analysis.

Results: ALDH7A1 and *GCDH* knock-out iPSC lines have been generated and subsequently characterized at electrophysiological level. Preliminary MEA data show a higher (network) burst rate and shorter (network) burst duration compared to control neurons. MEA analysis of PDE patient-derived neurons is ongoing. Furthermore, PDE patient-derived fibroblasts have shown to reproduce the altered metabolomic profile described in patients.

Conclusions: In-depth characterization of fibroblasts and neural co-cultures will allow us to define novel robust cellular PDE-ALDH7A1 and GA1 models that will serve to shed further light on disease mechanisms and treatment development, including antisense technology.

Poster # 14

USING ANTISENSE OLIGONUCLEOTIDES TO REACTIVATE PATERNAL *UBE3A* IN ANGELMAN SYNDROME PATIENT-DERIVED NEURONS

Abstracts need to be submitted as word files to DutchAntisense@gmail.com

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Neurodevelopmental disorder treatment discovery remains complicated by phenotype discordances between patients and mouse models. Among the well-established issues are the differences in the genome between humans and non-human model systems, an issue that has been widely discussed for the therapeutic modality of antisense oligonucleotides (AON). Angelman Syndrome (AS) is a severe neurodevelopmental syndrome characterized by developmental delay, behavioral abnormalities, and seizures. For AS therapeutic discovery, non-homology between the human and non-human model system genomes is a major problem for genetic therapies such as AONs, particularly for the widely used AS mouse model. AS is caused by loss-of-function mutations of the maternal *UBE3A* gene, which exhibits parent-of-origin imprinting in mature neurons. The paternal copy of *UBE3A* is silenced through expression of the *UBE3A* Antisense Transcript (*UBE3A-ATS*). Therefore, *UBE3A-ATS* is an interesting potential target for AON therapy, as targeted degradation of the *UBE3A-ATS* could reverse paternal imprinting, thereby reintroducing *UBE3A* expression in mature neurons. However, due to lack of sequence homology between mice and humans, the AS mouse model is not suitable for preclinical testing of AONs designed for targeting the human *UBE3A-ATS*. In an effort to address this fundamental problem, we have generated human neuronal cultures from induced pluripotent stem cells (iPSCs) of two siblings carrying a nonsense mutation (W577*) in the *UBE3A* gene, in addition to an unrelated reference control line (WTC). Neurons were generated through overexpression of neurogenin-2, which rapidly induces neuronal differentiation in iPSCs. These neurons form networks within three weeks and recapitulate *UBE3A* gene imprinting, resulting in the loss of UBE3A expression in neurons derived from AS patients. Here, we show that treatment with an AON against the *UBE3A-ATS* reactivates *UBE3A* expression from the paternal *UBE3A* allele. After a week of treatment with *UBE3A-ATS* targeting AONs through addition to the cell culture medium, we observe a robust increase in UBE3A protein in AS patient neurons, compared to a non-targeting AON. Future studies will be focused on investigating whether *UBE3A-ATS* targeting AONs can also rescue functional deficits in AS patient neurons using multi-electrode arrays as an assay of clinical efficacy.

Poster # 15

Personalized antisense-oligonucleotide therapy for a patient with beta-propeller protein-associated neurodegeneration

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Beta-propeller protein-associated neurodegeneration (BPAN) is an X-linked progressive disorder caused by mutations in the *WDR45* gene. The resulting neurological disorder is characterized by early-onset seizures, developmental delay, and intellectual disability, including a behavioral phenotype comparable to autism spectrum disorder. While seizure occurrence fades with age, patients later present with a cognitive decline and a progressive movement disorder. An important hallmark of the disease is the increasing iron accumulation in the brain, especially in the substantia nigra and globus pallidus. To date, there is no available therapy and patient care solely relies on symptom management.

Personalized RNA therapies have recently emerged as a possibility to provide treatment for individual patients with unique mutations. Especially deep-intronic, splice-altering variants, for which antisense-oligonucleotides (ASOs) can restore normal splicing, can be tackled with a tailor-made approach. Here, we report the case of a young adult female who was diagnosed with a likely pathogenic deep intronic mutation in *WDR45* (c.235+159C>G) that is eligible for ASO therapy. The mutation leads to skipping of the canonical exon 4 and inclusion of part of intron 4 into the mRNA, causing a frameshift.
To this end, we designed multiple ASOs targeting the deep intronic mutation to correct splicing and restore the canonical mRNA. The different ASOs were tested on patient-derived fibroblasts and we identified one ASO that was able to efficiently reduce inclusion of the part of intron 4. To evaluate to what extent the restoration of the reading frame also leads to an amelioration of the cellular phenotype, we made use of the role of WIPI4 (the protein encoded by *WDR45*) in autophagy and measured p62 and LC3B levels in patient cells. We employed a high-throughput cellomics platform to evaluate the occurrence of p62 and LC3B aggregates in control, patient non-treated, and treated cells simultaneously.

Poster # 16

Combined AON-U7snRNA therapy decreases aberrant splicing caused by multiple deep-intronic ABCA4 variants *in vitro*

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Introduction

Stargardt disease is a progressive inherited retinal disease caused by *ABCA4* mutations. Over the years, an increasing number of pathogenic intronic *ABCA4* variants has been reported, usually causing splicing alterations at the pre-mRNA level. Antisense oligonucleotides (AONs) are an attractive therapeutic strategy to rescue these splicing defects, yet they are designed to target individual variants. In this study, we experimentally analyze the potential of combined AON-U7snRNA splicing modulation therapy to target multiple intronic *ABCA4* variants simultaneously.

Methods

To test the U7snRNA splicing modulation tool, midigene-based splice assays were performed *in vitro*. Wild-type or mutant *ABCA4* midigenes were co-transfected in HEK293T cells with single or multiple AON-U7snRNA cassette vectors, including the corresponding positive (naked AONs previously reported as efficient) or negative (single or multiple empty U7snRNA cassette vectors) controls. Splicing correction was analyzed by RT-PCR, and semi-quantified based on the recovery of the correct *ABCA4* transcript.

Results

The multiple AON-U7snRNA cassette vector could efficiently correct splicing defects caused by the targeted deep-intronic variants. Overall, the U7snRNA system reached similar rescue levels compared to the respective positive controls. No relevant effect was observed after delivering empty controls or single-cassette vectors targeting a different region. To corroborate this, delivery of the U7snRNA system through adeno-associated viruses (AAVs) in patient-derived photoreceptor cells and retinal organoids is currently ongoing.

Conclusion

Our research is supporting the development of AON-containing U7snRNA system to target multiple deep-intronic *ABCA4* variants with a single AAV. Ultimately, this novel combined therapy might contribute to increase the eligible patient population for treatment.

Poster # 17

Antisense oligonucleotide-based correction of the splicing defect caused by the c.769-784C>T variant in *ABCA4*

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Stargardt disease is an inherited retinal disease caused by mutations in the photoreceptor-specific gene ATP-binding cassette transporter type A (*ABCA4*). Interestingly *ABCA4* has a high occurrence of splicing-affecting mutations. Here, we screened 9 antisense oligonucleotides (AONs) designed to correct pseudoexon (PE) inclusion caused by the c.769-784C>T recurrent deep-intronic variant in *ABCA4*. AON efficacy to induce PE exclusion was assessed in three cell models. First, AONs were in HEK293T cells transfected with an *ABCA4* midigene (splice reporter vector) carrying the deep-intronic variant. Next, AONs were tested in compound heterozygous patient-derived fibroblasts. Based on the splicing-correction efficacy of each individual AON, the three most efficacious AONs at 0.5 μM were selected for a final assessment in photoreceptor-progenitor cells (PPCs). Selected AONs were delivered at two final concentrations (0.5 μM and 1 μM). One-way ANOVA test with subsequent Bonferroni correction was used for statistical analysis. The AON screening using the HEK293T-midigene model indicated high efficacy of all AONs. Given these results, the same pool of AONs was evaluated in the patient-derived fibroblasts. Based on PE correction AON2, AON5 and AON7 were selected for further assessment in the patient-derived PPC model. AON2-mediated splicing correction was the most efficacious at the lower concentration (0.5 μM, $p<0.001$), followed by AON7 ($p<0.001$). AON5 was least efficacious reaching statistical significance at final concentration 1 μM only ($p=0.03$). PE exclusion is a thoroughly researched therapeutic approach that has been shown to rescue protein function. It can be achieved using AONs, however it is crucial to select the most efficacious therapeutic molecule for further testing by employing different models. Taking this into account, AON2, AON5 and AON7 showed a high degree of PE skipping in PPCs. Further research of the 3 selected AONs includes protein rescue studies and safety profile assessment in advanced retina models such retinal organoids to mimic the cellular environment in patients.

Poster # 18

Deciphering the efficacy and safety of different antisense oligonucleotide chemical modifications in a retinal context.

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Inherited retinal diseases (IRDs) are a group of heterogenous neurodegenerative diseases which lead to visual impairment. In IRDs, a 15% of associated genetic defects affect pre-mRNA splicing, resulting in aberrant transcripts which hamper normal cell function. In the last years, antisense oligonucleotide (AON)-based therapies have widely explored for IRDs. However, little is known about how the AON chemical modifications can affect uptake, biodistribution and toxicity in the retina. Here, we present to our knowledge the first comparison of chemical modifications in a retinal context.

Different genes exclusively expressed in specific retinal cells affected in IRDs (rods, cones, bipolar and retinal pigment epithelium cells) were selected. Subsequently, splicing modulation capacity of AONs harboring three different commonly used chemical modifications [2'-O-methyl-phosphorothioate (2-OMe), 2'-O-methoxyethyl-phosphoriate (2-MOE), and phosphorodiamidite morpholino oligomers (PMO)] was studied first, *in vitro* by employing splice reporter vectors, and *in vivo* by direct delivery to the mouse retina.

Our *in vitro* results indicated that 2-OMe and 2-MOE AONs were able to redirect splicing similarly, but 2-MOE AONs showed a slightly higher efficacy in most of the cases. PMO transfection *in vitro* requires a different delivery method, but resulted in the lowest efficacy for all selected genes. These observations were confirmed *in vivo*, in which 2-MOE and 2-OMe modified AONs presented the highest splicing modulation efficacies. Furthermore, PMO oligonucleotides injection resulted in a clear phenotype present in 88.5% of the injected mice. The ensuing isolated retinas were whiter and smaller than the retinas obtained from controls, suggesting a PMO-mediated toxic effect. Histological studies are ongoing to assess how the retina is affected upon PMO delivery.

Overall, our results constitute the first comparison of different chemical modifications of AONs in a retinal context, demonstrating different properties amongst them. Our data show that 2-MOE is the most efficacious to modulate splicing. In contrast, PMO modification not only led to a poor splicing modulation but also to potential toxic effects, at least in the mouse retina.

Poster # 19

Prioritization of the development of exon skipping therapy for epidermolysis bullosa

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Abstract: Epidermolysis bullosa is a group of genetic skin conditions characterized by abnormal skin (and mucosal) fragility caused by pathogenic variants in various genes. The disease severity ranges from early childhood mortality in the most severe types to occasional; acral blistering in the mildest types. The subtype and severity of EB is linked to the gene involved and the specific variants in that gene; which also determine its mode of inheritance. Given the current level of understanding; the broad spectrum of genes and variants underlying EB make it impossible to develop a single treatment strategy for all patients. It is likely that many different variant-specific treatment strategies will be needed to ultimately treat all patients. Antisense-oligonucleotide (ASO)-mediated exon skipping aims to counteract pathogenic sequence variants by restoring the open reading frame through removal of the mutant exon from the pre-messenger RNA. This should lead to restored production of the protein absent in affected skin and consequently; improvement of the phenotype. Several pre-clinical studies have demonstrated that exon skipping can restore protein production in vitro; in skin equivalents; and in skin grafts; derived from EB-patient skin cells; indicating that ASO-mediated exon skipping could be a viable strategy as a topical or systemic treatment. A prioritization strategy for the development of exon skipping based on genomic information of all EB-involved genes can assist in the identification of variants most amenable to treatment. Here, we present one such strategy based on the amount of pathogenic variants reported in skippable exons.

Poster # 20

Development of an allele-specific antisense oligonucleotide targeting a heterozygous *ACTL6B* variant

Max Voslamber[1], Marlen C. Lauffer[1], Anouk Spruit[1], Pleuntje J. van der Sluijs[2], Gijs W. E. Santen[2], Willeke van Roon-Mom[1], Annemieke Aartsma-Rus[1]

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Neurodevelopmental disorders are a heterogenous group of diseases with the common hallmark of defective nervous system development, often caused by germline mutations. Patients present with a wide range of phenotypes, from mild intellectual disability to severe cognitive impairments and refractory seizures. Mutations in the actin-like protein 6B (*ACTL6B*) gene were recently described as being causative for an autosomal-recessive as well as an autosomal-dominant neurodevelopmental disorder, leading to global developmental delay, epileptic encephalopathy, and cerebral atrophy. *ACTL6B* is exclusively expressed in neurons and encodes for a protein that makes up an important component of the neuronal BRG1/brm-associated factor (nBAF) complex, which is required for chromatin remodelling in postmitotic neurons.

For this project, we aim to develop an allele-specific splice modulating antisense-oligonucleotide (AON) for the treatment of the heterozygous c.1027G>A (p.Gly343Arg) variant that is inherited in an autosomal dominant manner. The AON shall cause skipping of the exon containing the variant of only the mutated allele. We hypothesize that inactivating the mutated allele will transform the patient's phenotype to that of an unaffected heterozygous carrier.

To this end, we are using two different sets of *ACTL6B*-expressing cells for the study of the allele-specific AONs. First, we transdifferentiate patient fibroblasts into neurons by lentiviral-mediated overexpression of *NGN2* and *ASCL1*. Secondly, a midigene assay will facilitate the partial expression of *ACTL6B* in HEK cells. Site-directed mutagenesis is performed to introduce the c.1027G>A (p.Gly343Arg) mutation. The AONs will be tested using both systems and the efficacy of the AONs in general and their ability to target the mutated allele in specific will be measured using quantitative PCR methods.

Poster # 21

MODULATION OF *IGF1* SPLICING TOWARDS MECHANO GROWTH FACTOR ISOFORM BY ANTISENSE OLIGONUCLEOTIDE-MEDIATED EXON INCLUSION

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Insulin-like growth factor-1 (IGF-1) is a peptide hormone that is involved in normal body growth, and regulation of many other biological processes like cell proliferation, differentiation and migration. Together with other growth factors, IGF-1 plays a significant role in skeletal muscle maintenance making it a promising target for the treatment of secondary defects in muscle wasting diseases. The general consensus is that IGF-1 signaling is mostly mediated by mature IGF-1 but multiple transcript isoforms are produced by the *IGF-1* gene. The full-length IGF-1 precursor (prepro-IGF-1) contains a signal peptide, mature IGF-1 and E-peptide. After translation, signal peptide is cleaved and IGF-1 is found as mature IGF-1 or pro-IGF-1 (with E-peptide). The *IGF1* gene consists of six exons in which exons 5-6 are alternatively spliced to form different E-peptides isoforms namely IGF-1Ea (4-6), IGF-1Eb (4-5) and Mechano growth factor (MGF)/(IGF-1Ec) (4-5-6) (Figure 1). Because the variation of E-peptides is the only difference between IGF-1 isoforms on protein level , it has been suggested that E-peptides may have distinct functions within the pro-peptides or alone. MGF isoform is produced when the internal splice site on exon 5 is used and due to the out of frame insertion, coding region of exon 6 is shortened and unique Ec peptide is produced. Expression profile of MGF is associated with mechanical load or muscle damage. Furthermore, MGF has been shown to increase the proliferation of myoblasts via ERK pathway and is implicated to play a role in satellite cell activation. Therefore increasing MGF levels could be a strategy to induce muscle regeneration. In this study, we designed five different splice modulating antisense oligonucleotides (ASOs) (of the 2'OMePS chemistry) to induce the inclusion of exon 5 by targeting splicing silencer motifs around the internal splice site. Lipofectamine-mediated transfection experiments were performed on mIMCD3 kidney cells using different ASO concentrations. After 48h, total RNA and proteins were harvested. Assessment of exon inclusion by qPCR showed that only one ASO was functional which led to 30% increase in exon 5 inclusion. Using Western blot, we confirmed by that the ASO treatment resulted in approximately three-fold increase on ERK phosphorylation, as expected with MGF increases. Our findings, as proof of principle, show that ASO-mediated exon inclusion approach can be used to increase MGF levels.

Poster # 22

Personalised Antisense oligonucleotide therapies for rare neurodevelopmental disorders

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Neurodevelopmental disorders (NDDs) are a group of diseases characterised by disturbances in brain development and function. They are highly heterogeneous for etiology and clinical presentation, and they include autism spectrum disorder, intellectual disability, and epilepsy.
Next-generation sequencing has significantly improved the ability to identify the causative mutations underlying monogenic NDDs, leading to a rapid increase in the number of known conditions and diagnosed cases. However, given the rarity of these - often private - mutations, disease-specific therapies remain currently limited.
Antisense oligonucleotides (ASOs) offer the potential to treat patients with ultra-rare disorders, as they represent an opportunity to design a disease-modifying treatment that can be customised to the patient's specific variant.

This study will address various approaches to the use of ASOs in the context of different NDDs.
Firstly, we aim to identify developmental and epileptic encephalopathies (DEEs) patients carrying mutations eligible for ASO therapies. In particular, customised splice-modulating ASOs will be designed to target defined rare pathogenic variants. Furthermore, we aim to develop a standardised 2D neuronal human induced pluripotent stem cell (hiPSC)-derived platform to enable robust screening of patient-tailored ASO therapies. This platform will be used to quantify therapeutic efficacy at a cellular level and investigate further the pathomechanism associated with DEE genes. Our goal is to deliver these personalised therapies to patients within the course of this project.
Secondly, in a proof-of-concept study, we aim to assess to what extent ASOs can interact with secondary RNA structures. Two variants, located on opposite sides of a loop within the proteolipid protein 1 (*PLP1)* pre-mRNA, were previously identified in patients showing hypomyelination of early myelinating structures. ASOs targeting these deep intronic variants will be studied in patient-derived fibroblasts to evaluate the possibility of disrupting the pre-mRNA secondary structure and impeding the use of the cryptic splice site. Additionally, we will investigate *PLP1/DM20* alternative splicing to characterise the temporal expression of these two transcripts and allow the assessment of a therapeutic window for ASO treatment.

1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

List of attendees

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1st Dutch Antisense Therapeutics Symposium

Friday June 3rd 2022

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DATS

Thank you for your attendance, we hope to see you next time!

Also, big thanks to the following sponsors that made this meeting possible:

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