NVGCT Nederlandse Vereniging voor Gen- & Celtherapie

ANNUAL SYMPOSIUM

June 15th and 16th 2022

Congrescentrum De Werelt

Westhofflaan 2, 6741 KH Lunteren

WEDNESDAY JUNE 15th 2022

9.00 **Registration and Coffee**

9.30 **Welcome and Opening Announcements**

Victor van Beusechem, President of the NVGCT

9.45 **OPENING KEYNOTE LECTURE**

KLEOPAS KLEOPA, The Cyprus Institute of Neurology & Genetics, Nicosia
Gene replacement or gene silencing strategies to treat demyelinating CMT neuropathies

10.30 – 10.40 **EXHIBITOR PITCHES**

Chair: Jeroen de Vrij

10.40 - 12.45 **SESSION I** **Cancer Therapy**

Chair: Sabine van der Sanden

10.40 **Jürgen Kuball, University Medical Center Utrecht**
Translating gdT cells and their receptors to the clinic: 15 years later… go or no-go?

11.10 *Coffee break*

11.30 **Marco Huberts, Erasmus Medical Centre, Rotterdam**
Three-dimensional models for pancreatic ductal adenocarcinoma for evaluation of oncolytic virus efficacy

11.45 **Tanja de Gruijl, Amsterdam University Medical Center, Amsterdam**
Constitutive activation of GSK3beta in the melanoma microenvironment to overcome immune suppression

12.15 **Jella van de Laak, Maastricht University**
In vitro and *in vivo* validation models for bacteria-mediated cancer therapies

12.30 **Inge Jedema, The Netherlands Cancer Institute, Amsterdam**
DARE-NL: establishment of a Dutch infrastructure for cancer-specific ATMP Research of cell and gene therapy

12.45 - 13.15 **Greiner Award for Best Thesis 2020 & 2021**

Chair: Rob Collin

13.15 - 14.00 **LUNCH**

14.00 – 15.30 **PARALLEL PROGRAM**

14.00 - 15.30 **SESSION II-A**

NVGCT - ZonMW YOUNG INVESTIGATOR SPEED DATE CHALLENGE

Chair: Jan Theys

14.00 Introduction / explanation Speed Date Challenge

14.10 Speed-date sessions & start drafting projects

14.00 - 15.30 **SESSION II-B**

NVGCT MEETS PATIENT REPRESENTATIVES (in Dutch)

Co-organized by VSOP and Spierziekten Nederland

Chair: Gerard Wagemaker

14:00 Opening

14:05 **Rob Collin, Radboud University Medical Centre, Nijmegen**
The Dutch Center for RNA therapeutics

14.35 **Jan Benedictus, Patientenfederatie Nederland**
Introductie patiënten platform gen- en celtherapie

14:55 **Ria de Haas, Spierziekten Nederland**
De weg naar de eerste gentherapie voor een spierziekte

15.30 *Coffee break*

16.00 **JOINT KEYNOTE LECTURE NVGCT, VSOP & SPIERZIEKTEN NEDERLAND**

Chair: **Enrico Mastrobattista**

SABINE FUCHS, University Medical Center Utrecht
PRIME TIME

17.00 **NVGCT General Assembly**

17.30 **Leisure time / draft projects Speed Date**
Proposals need to be handed in at 22.30 hrs ultimately

19.00 **Dinner**

20.30 **Famous NVGCT Pubquiz**

Quizmaster: **Jan Theys**

THURSDAY JUNE 16th 2022

9.00 Announcement finalists Speed Date Challenge to be prepared for a pitch!

9.05 - 9.35 SESSION III IMMUNOTHERAPY

Chair: Max Medina

9.05 **Fréderique de Graaf, Immunetune**
Synthetic linear DNA as a novel platform for the development of personalized neoantigen vaccines

9.20 **Tereza Brachtlova, Amsterdam University Medical Center, Amsterdam**
RNAi library screening to identify target genes for more effective oncolytic immunotherapy of melanoma

9.35 - 10.05 SESSION IV-A GENE DELIVERY

Jerome Cabeau, Thermo Fisher
Designing gene delivery tools to enable cell and gene therapy

10.05 *Coffee break*

10.30 - 11.35 SESSION IV-B

GENE THERAPY PRODUCT DEVELOPMENT WITHIN INDUSTRY (sponsored by GENSCRIPT)

Chair: Rob Collin

10.30 **Jeroen de Vrij, ExoVectory**
ExoVectory (spin-out Erasmus MC): Harnessing the natural power of exosomes for regenerative medicine and delivery of (gene) therapies

10.45 **Di Yu, Elicera**
The journey of Elicera Therapeutics

11.10 **Jeroen van Heijst, Neogene**
Development of the T cell engineering platform for a phase 1 clinical trial of fully-individualized neoantigen-specific TCR therapy to treat advanced solid tumours

11.40 - 12.20 **NVGCT - ZonMw Young Investigator Speed Date Challenge**

Chair: Jan Theys

11.40 **Jan Theys** - Introduction to Speed Date Challenge

11.45 **Irene Vázquez Domínguez & Marieke Roefs** (last year's winners)
Project progress and future plans

12.00 **Pitches three finalists** (5 minutes pitch, 5 minutes questions)

12.20 - 13.15 **Lunch**

13.15 - 13.45

13.15 **Simona Guidi, ProPharma**
Turning a promising ATMP candidate into a medicinal product

13.45 - 15.30 **SESSION V** **DNA & RNA EDITING**

Chair: Jeroen de Vrij

13.45 **Irene Vázquez Domínguez, Radboud University Medical Center**
The importance of having a relevant model system to investigate splicing modulation: the unforeseen case of the c.11+5G>A variant in *RPE65*

14.00 **Mert Öktem, Utrecht University**
Amphipathic peptide mediated CRISPR-Cas9 ribonucleoprotein delivery for gene knockout and correction in various cell types

14.15 **Rick Wansink, Radboud University Medical Center**
Therapeutic approaches against RNA toxicity caused by an expanded microsatellite repeat

14.45 **Elena Sendino Garvi, Utrecht University**
GENE SURGERY: a potential CRISPR/Cas9-based treatment option for nephropathic cystinosis *in vitro*

15.00 **Dyah Karjosukarso, Radboud University Medical Center**
Towards clinical application of antisense oligonucleotides for the treatment of Stargardt disease

15.15 *Coffee break*

15.45 - 16.00 CLOSING CEREMONY

Chair: Victor van Beusechem

15.45 *Announcement winner ESGCT Best Abstract Award*
Announcement winners ZonMW Speed Date

16.00 CLOSING KEYNOTE LECTURE

JOHN VAN DER OOST, Wageningen University, Wageningen
CRISPR-Cas - from bacterial defense to gene & cell therapy

16.45 Meeting Closure
Victor van Beusechem, President of the NVGCT

NVGCT is grateful to its partners

ABSTRACTS

KLEOPAS KLEOPA

Gene replacement or gene silencing strategies to treat demyelinating CMT neuropathies

K. A. Kleopa[1,2]

[1]Department of Neuroscience and [2]Center for Neuromuscular Disorders, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Charcot-Marie-Tooth (CMT) inherited neuropathies result from variable molecular-genetic alterations in neurons and their axons, or in Schwann cells and the myelin sheath they form, leading to either loss or toxic gain of function cellular mechanisms. In order to treat the cause of the most common demyelinating CMT neuropathies resulting from either cell-autonomous loss of function or toxic mechanisms in myelinating Schwann cells, we have developed cell-targeted gene replacement or gene silencing approaches. Using clinically translatable intrathecal injection of adeno-associated viral (AAV) vectors we have demonstrated widespread biodistribution and expression throughout the peripheral nervous system (PNS) including lumbar spinal roots and distal nerves. Delivery of the *GJB1* gene associated with X-linked CMT1X neuropathy resulted in restoration of Cx32 expression in Schwann cells. Pre- and post-onset gene replacement therapy provided therapeutic benefit including improved motor function, nerve conduction velocities, and nerve pathology in different knockout as well as transgenic mouse models of the disease. Likewise, replacement of the *SH3TC2* gene associated with autosomal recessive CMT4C neuropathy resulted in functional and morphological improvement in the *Sh3tc2*-/- model of the disease. To treat CMT1A, the commonest CMT type, caused by *PMP22* gene duplication, we developed a microRNA-based gene silencing approach. Delivery of microRNA by AAV9 both at early as well as at late stages of the neuropathy efficiently silenced *PMP22* expression in PNS tissues, leading to phenotypic functional and morphological improvement in a CMT1A model overexpressing the human *PMP22* gene. Clinically relevant treatment-responsive blood biomarkers, including neurofilament light (NF-L), neural cell adhesion molecule 1 (NCAM1), and growth differentiation factor 15 (Gdf15) have also been validated in these neuropathy models at baseline and following treatment. Our studies provide a proof of concept for the therapeutic potential of gene replacement or gene silencing therapies to treat patients suffering from inherited demyelinating neuropathies.

SABINE FUCHS

PRIME TIME

S.A. Fuchs.[1]

[1]*Department of Metabolic Diseases, UMC-Utrecht Wilhelmina Childrens' Hospital, Utrecht. The Netherlands*

Metabolic diseases are genetic diseases. Although individually rare, as a group they affect many patients. Generally, they are severe and therapeutic options insufficient.
To improve care for these undeserved patients, I try to approach these individually rare disorders with a general approach, which can then be tailored to each disease and/or patient. Because the liver is the main metabolic organ where almost all metabolic processes are expressed at least to some extent, I focus on the liver. From liver tissue from patients, we culture liver organoids as unique personalized *in vitro* models to develop and test therapies and do drug screens for metabolic diseases.
Liver organoids also provide an interesting source of liver cells that may be infused through the portal vein as an alternative for liver transplantation to treat patients with metabolic diseases. By transplanting healthy donor liver organoids or a patients' own organoids after gene correction, many patients can be cured already when the function of as little as 5% of the liver cells is restored.
But in my opinion, it is now prime time for patients with metabolic disease because current gene editing technologies - and specifically prime editing – have the potential to be used for in vivo gene editing. Gene-correction of the root cause of genetic diseases would revolutionize care for the many patients with metabolic or other genetic diseases.

RICK WANSINK

Therapeutic approaches against RNA toxicity caused by an expanded microsatellite repeat

Derick G. Wansink

Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands

Myotonic dystrophy type 1 (DM1) is one of the most common inherited forms of muscular dystrophy. Patients suffer from skeletal muscle wasting and myotonia, but also organs like the brain and heart can be affected. The underlying cause is a dominant, unstable, non-coding (CTG)n repeat in *DMPK*. Transcripts with an expanded (CUG)n repeat abnormally bind important splicing factors leading to mis-splicing of several genes *in trans*. Therapeutic options for DM1 are currently limited to symptomatic treatment.
To evaluate the use of somatic gene editing as a promising approach for the correction of the expansion mutation in DM1, we used CRISPR/Cas9 in myoblasts from patients and DM1 mouse models. We managed to precisely and efficiently excise the repeat from expanded *DMPK* using a dual cleavage at either side of the (CTG)n sequence. Downstream molecular and cellular disease features turned out to be reversible, which is important information for the future development of gene therapy for DM1.
To investigate the dose-toxicity relationship of expanded (CUG)n RNA, we developed myoblast models in which we customized the expression of expanded (CUG)n RNA using catalytically inactive Cas9 (dCas9) fused to transcription activators and repressors, also known as CRISPR activation and interference (CRISPRa/i). We were successful in upregulating *DMPK* expression up to ten-fold in a transient manner, which was confirmed by RT-qPCR and single molecule RNA FISH. Mis-splicing as a result of (CUG)n RNA expression looked more severe in these myoblasts. In the opposite direction, we managed to downregulate *DMPK* expression >80% using CRISPRi. Downstream analysis confirmed a partial rescue of mis-splicing and could clarify the dose-dependent pathogenic mechanisms underlying DM1. Our new cell models thus mimic the broad spectrum of disease severity in tissues and patients, and provide a solid foundation for therapeutic strategies.

JEROME CABEAU

Designing Gene Delivery Tools to Enable Cell and Gene Therapy

J. Cabeau

Thermo Fisher Scientific, Cell & Gene Therapy, Paris, France

Cell and gene therapies are steadily delivering on their promise of providing significant and durable health and quality-of-life improvements for patients in a growing number of indications. Therapeutic successes today have frequently resulted from close academic-industrial collaborations. Scientific ideas developed within academia frequently require the support of industrial partners to bring their therapy to the clinic through the provision of platform technologies, qualified ancillary materials, process development and manufacturing capabilities, understanding of cGMP and quality systems, analytical expertise, financial support, clinical trials support and eventually scale-up for commercialization. Close cooperation between academia and industry will accelerate the successful development of novel therapeutics. Our success will be measured by our ability to deliver effective treatments to the broader patient population at an affordable cost.

Today, Thermo Fisher Scientific, one of the world's largest life science companies, has a corporate mission to enable its customers to make the world healthier, cleaner, and safer and is playing a major role in driving novel cell and gene therapies into the clinic. In this presentation, I will cover two main topics: Firstly, I will provide a brief introduction to Thermo Fisher's broader capabilities and experience in developing, partnering, and industrializing cell and gene therapies. I will then focus on the output of three major internal research programs which have resulted in the development of clinically compliant gene delivery capabilities suited to various clinical applications including a novel electroporation unit, clinically compliant viral vector production platforms for LV and AAV, and finally more recent research work in the exciting field of Lipid Nanoparticles (LNPs).

Three-Dimensional Models for Pancreatic Ductal Adenocarcinoma for Evaluation of Oncolytic Virus Efficacy

M. Huberts[1], E.A. Farshadi[2], D. Mustafa[2], C. van Eijck[2], R.A.M. Fouchier[1], B.G. van den Hoogen[1]

[1]Department of Viroscience, Erasmus Medical Centre, Rotterdam, The Netherlands; [2]Department of Pulmonary Surgery, Erasmus Medical Centre, Rotterdam, The Netherlands.

Oncolytic viruses (OVs) offer a promising, safe therapeutic option in the treatment of haematological malignancies and solid tumours like Pancreatic Ductal Adenocarcinoma (PDAC). Published and preliminary data demonstrate that multiple OVs can efficiently exert direct and indirect oncolytic effects in preclinical *in vivo* models. Unfortunately some of the clinical trials with OVs have shown suboptimal responses. It is well known that this is based on the heterogeneity within tumor subtypes. The observed heterogeneous anti-tumour responses to OVs emphasize the clinical need for better stratification of cancer patients for viro-immunotherapy by selecting the most promising candidate OV for treatment of PDAC in future clinical trials. Therefore, the information on susceptibility to different viruses needs to be correlated to genetic profiles of patients to define oncolytic predictor markers. To this end, we need robust PDAC 3D models with predictive value that fill the translational gap.
For translation from 2D to 3D cultures, we developed spheroids (3D) derived from human pancreatic cancer (HPAC) cells grown in 2D, and in parallel we developed a 3D model based on patient-derived pancreatic cancer organoids.
HPAC-spheroids were less susceptible to oncolytic Newcastle Disease Virus (NDV)-induced cell killing than the monolayers (2D), while repeated inoculations of spheroids with oncolytic NDV eradicated the different layers of a spheroid. Expertise obtained with the 3D spheroids was translated to study the efficacy of oncolytic virus therapy in patient-derived PDAC organoids (3D). Subsequently, the sensitivity of several organoids to virus induced cell killing was assessed upon inoculation with various OVs including NDV, Adenovirus, Reovirus and Measles Virus with the aim to correlate the sensitivity to genomic markers.
Here we show intra-organoid and inter-organoid heterogeneity in sensitivity to the different viruses. These data, in combination with genetic profiling, will allow the identification of predictive markers for sensitivity to specific OVs.

Keywords: *oncolytic viruses, pancreatic ductal adenocarcinoma, personalized medicine, Newcastle Disease Virus, Reovirus, Adenovirus, Measles Virus*

***In vitro* and *in vivo* validation models for bacteria-mediated cancer therapies**

G.M. van de Laak[1], A.M. Mowday[1,2], L. Dubois[1], J. Theys[1]

[1]Precision Medicine, Maastricht University, GROW Research institute for Oncology and reproduction, Maastricht, the Netherlands; [2]Auckland Cancer Society Research Centre, School of Medical Sciences, University of Auckland, New Zealand.

Most solid cancers contain regions of necrotic tissue and the extent of necrosis is associated with poor survival. Despite the high frequency of tumour necrosis, particularly in advanced disease, no therapeutic modalities exist to explicitly address this clinical issue. In our alternative anti-cancer approach, we use the harmless anaerobic bacteria *Clostridium sporogenes*, which upon injection as spores, will germinate and thrive in these necrotic regions, providing cancer-specific colonisation, a totally natural exquisitely specific phenomenon. We have developed innovative genetic tools that enable us to "arm" *C. sporogenes* with therapeutic genes. As such, our *Clostridium* vector functions as a delivery vehicle that can specifically transfer its cargo to the tumor. We have shown that systemic administration of various genetically engineered therapeutic strains results in significant treatment efficacy. In order to systematically investigate effects of newly developed *C. sporogenes* strains, we are currently determining the potential of a variety of *in vitro* and *in vivo* necrosis models. Several *in vitro* 3D cellular models were developed and results showed that the majority developed necrosis. Preliminary data indicate these models can be infected with *C. sporogenes,* leading to colonization. These promising results show the high potential of 3D *in vitro* models that can potentially serve as important intermediate step before embarking *in vivo* studies. Parallel to the *in vitro* experiments, necrotic fractions have been quantified in eight syngeneic mouse models. Four models that display necrotic fractions will be taken forward into next experiments involving quantification of colonization. The discrepancies in necrosis between the models will allow us to see a possible correlation between necrosis and colonization. Taken together these models will allow us to vigorously test our current and future strains for their efficiency, safety, and therapeutic properties, and to select those strains with the highest potential to be taken forward to clinical trial.

DARE-NL: establishment of a Dutch infrastructure for cancer-specific ATMP Research of cell and gene therapy.

I. Jedema[1], H. Dolstra[2] , E. Bremer[3], T. Straetemans[4]

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While Advanced Therapy Medicinal Products (ATMPs) represent a therapeutic breakthrough in cancer care, academic ATMPs only sporadically reach clinical practice and market authorization. Thus, there is an urgent need to stream-line ATMP research at all stages. Key bottlenecks that hamper ATMP development in the Netherlands include regulatory challenges, uncertain perspectives of the registration pathway, scattered knowledge, challenges in supply of GMP-grade ingredients such as vectors, lack of highly skilled staff, limited IP and regulatory affairs expertise and limited insight into development and manufacturing costs and reimbursement options. All Dutch ATMP research centres have joined forces to establish DARE-NL to solve these bottlenecks with the aim to accelerate translation of ATMPs into clinical practice.
DARE-NL will comprise an “ATMP knowledge framework”, “ATMP biologics & technology hub” and an “ATMP implementation and patients outreach platform”. Within the ATMP knowledge framework, efforts will be directed at establishment of a sustainable data, training, and valorisation platform; harmonizing GMP-production processes; and harmonizing and validating QC and characterization assays. Within the ATMP biologics & technologies hub, efforts will be directed at establishment of an academic viral vector platform and quality control assays; identifying requirements of new technologies for GMP production and clinical application; and artificial intelligence in production devices. An interactive “ATMP implementation and patient outreach platform” will be established, with efforts directed at assessment of regulatory, HTA and reimbursement evidence requirements in the ATMP development cycle; creating an (informal) communication platform to allow consultation with regulators, policy makers and patients; expansion of patients’ access by actively involving patient representatives; and setting up HE and clinical trial registries.
The proposed infrastructure centrally tackles bottlenecks and will accelerate safe and effective therapies and improve early and sustainable access to novel academic ATMPs in the Netherlands.

Synthetic linear DNA as a novel platform for the development of personalized neoantigen vaccines

Fréderique de Graaf[2], Tsolere Arakelian[1], Koen Oosterhuis[2], Kedar Moharana[2], Bram Teunisse[2], Iris Zoutendijk[3], Marcel Camps[1], Ramon Arens[1], Ferry Ossendorp[1*], Gerben Zondag[2,3,*], Jeroen van Bergen[1,2,*]

[1]Department of Immunology, Leiden University Medical Center, Leiden, The Netherlands; [2]Immunetune BV, Leiden, The Netherlands; [3]Synvolux Therapeutics BV, Leiden, The Netherlands

**Corresponding authors, shared senior authors*

The field of cancer immunotherapy is progressing towards the development of personalized medicine. The use of synthetic linear DNA offers a platform for the quick and cost-effective production of personalized neoantigen vaccines. A GMP-compatible method (AmpliVax) was developed that allows the simultaneous production of milligram amounts of multiple DNA vaccines in single vessel reactions within two days. This method relies on a primer-free, isothermal, rolling-circle amplification using high fidelity DNA polymerase and RNA polymerase to amplify circular DNA templates into linear double-stranded concatemers. Concatemers are digested into single linear expression cassettes which are subsequently protected by nuclease-resistant caps. In contrast to plasmid DNA, this method is cell-free, which simplifies the production process and hence decreases the costs. To test the expression, immunogenicity and anti-tumor efficacy of this synthetic linear DNA vaccine platform, polyepitope vaccines were generated encoding luciferase or multiple (>20) neoantigens and their efficacy was compared to that of plasmid DNA vaccines. Luciferase encoded by linear DNA was as effectively expressed as luciferase encoded by plasmid DNA upon intradermal injection with equimolar doses in mice. In addition, vaccination with linear DNA induced antigen specific CD8 and CD4 T-cell responses to similar levels as vaccination with plasmid DNA. As a result, tumor protection was observed between mice vaccinated with either plasmid DNA or the equivalent linear DNA polyepitope vaccine in a prophylactic melanoma challenge. What's more, improved overall survival were observed in vaccinated melanoma tumor-bearing mice when linear DNA vaccination was combined with checkpoint inhibitors compared to treatment with only checkpoint inhibitors. In conclusion, we have created a novel synthetic DNA vaccine platform suitable for the production of effective personalized cancer vaccines.

RNAi library screening to identify target genes for more effective oncolytic immunotherapy of melanoma

Tereza Brachtlova[1,2], Maxime Blijlevens[1], Renée X. de Menezes[3], Femke Sluiter[1], Rieneke van de Ven[4], Ida H. van Meulen-Muileman[1], Wenliang Dong[2], Tanja D. de Gruijl[1], Victor W. van Beusechem[1]

Amsterdam UMC, Vrije Universiteit Amsterdam, Depts. [1]Medical Oncology, [3]Epidemiology and Biostatistics, Netherlands Bioinformatics Center and [4]Otolaryngology, Head and Neck Surgery, Tumor Biology section, Cancer Center Amsterdam, 1081 HV Amsterdam, Netherlands; [2] ORCA Therapeutics B.V., 1081 HV Amsterdam, The Netherlands.

Oncolytic adenoviruses provide effective means for antitumor immune responses and are developed as new immunotherapeutic anti-cancer agents. However, suppression of myeloid differentiation and activation in the tumor microenvironment (TME) limits immunotherapy. To allow design of oncolytic adenoviruses silencing (so far unknown) immunosuppressing genes in melanoma (as we showed previously), we identified targets using siRNA library screening.
Important determinants of immune responses in the TME are immunosuppressive immature dendritic cells (DC) and M2-like macrophages; and immunostimulatory mature DC and M1-like macrophages. In the screens, melanoma cells were transfected with a custom siRNA library comprising potential targets involved in TME immunosuppression. Next, transfected cells were co-cultivated with primary human monocytes stimulated to develop into antigen-presenting DC or M1 macrophages and analyzed using flow cytometry. Three discovery screens were done using CD14+ monocytes from different donors. Control cultures of monocytes without melanoma cells showed monocyte-to-DC differentiation and presence of M1-like macrophages. In contrast, co-cultures transfected with irrelevant siRNA inhibited formation of mature DC or M1-like macrophages, showing that melanoma cells produced immunosuppressive factors that could be mimicked *in vitro*.
Upon silencing of (previously unidentified) immunosuppressive factors produced by melanoma cells, we observed improved differentiation of monocytes into CD45+/CD1a+ DCs and skewing of M2-like (CD163+/CD80-) macrophages towards an immunostimulatory M1-like (CD14-/CD80+) phenotype.
Nine candidate hit genes were validated on two melanoma cell lines with monocytes from three additional donors. Silencing these genes in melanoma cells induced loss of suppression-related CD14/CD163 (M2-like macrophages) or CD14 only (immature monocytes) markers, for two genes with a concomitant increase of the activation marker CD1a (CD1a+ DCs). Further validation using shRNA-expressing lentiviral vectors and stable shRNA-expressing melanoma cell lines is ongoing. Ultimately, selected RNAi molecules will be expressed from oncolytic adenovirus in pri-miRNA format, improving the efficiency of the novel cancer immunotherapy even more.

The importance of having a relevant model system to investigate splicing modulation: the unforeseen case of the c.11+5G>A variant in *RPE65*.

I. Vázquez-Dominguez[1,2], L. Duijkers[1,2], Z. Fadaie[1], E. Alaerds[1], F.P.M Cremers[1,2], S. Roosing[1,2], R.W.J Collin[1,2] and A. Garanto[1,3]

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Inherited retinal diseases (IRDs) are genetically heterogeneous disorders which lead to visual impairment. *RPE65* is one of the more than 270 genes involved in IRDs, and it is specifically associated with early-onset blindness. RPE65 is an enzyme involved in the visual cycle that is expressed in the retinal pigment epithelium (RPE). According to *in silico* predictions, the recurrent *RPE65* pathogenic variant c.11+5G>A leads to a 124 nucleotides (nt) elongation of exon 1, disrupting the reading frame. Splicing defects can be corrected by antisense oligonucleotides (AONs), which are short RNA molecules that complementarily interact with their target pre-mRNA. Here, we explored whether this variant can be a model to further study AON-mediated splicing modulation *in vitro* and *in vivo*.
First, the splicing defect was assessed in midigenes transfected into different cell lines, demonstrating, as predicted, that this variant generated a 124-nt exon elongation. We designed four AONs to correct splicing back to normal. Our results showed that all AONs were able to successfully redirect the splicing. Subsequently, blood cells from a patient carrying this variant in homozygosis were reprogramed into induced pluripotent stem cells (iPSCs). These iPSCs were differentiated to RPE for AON testing. Unexpectedly, the 124-nt exon elongation was barely detected. In addition, the overall *RPE65* expression was severely reduced in comparison with control RPE, even upon blocking nonsense-mediated decay. Furthermore, the absence of protein was confirmed by immunocytochemistry and western-blot. To further investigate this, luciferase assays were performed showing an RPE cell-specific defect on *RPE65* expression.
Overall, the *RPE65* c.11+5G>A variant was initially believed to cause a splicing defect, however studies in more relevant models, like iPSC-derived RPE, have shown that this variant affects *RPE65* expression, discouraging the use of AONs to investigate splicing modulation therapies.

Amphipathic Peptide Mediated CRISPR-Cas9 Ribonucleoprotein Delivery for Gene Knockout and Correction in Various Cell Types

Mert Öktem[1], Danny Wilbie[1], Olivier Gerrit de Jong[1], Enrico Mastrobattista[1]

[1]*Department of Pharmaceutics, Utrecht Institute of Pharmaceutical Sciences (UIPS), Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands*

Introduction: The CRISPR-Cas9 system has recently emerged as one of the most versatile gene editing tools for a broad range of gene editing applications. CRISPR-Cas9 mediated gene editing requires efficient delivery of the CRISPR components into the target cells.
Aims: We investigated if a *de novo* designed amphipathic peptide LAH5 is suitable for functional delivery of Cas9 RNPs into a variety of cells in culture for both gene knockout and gene correction applications.
Methods: Peptide mediated nanocomplexes were formed by incubating ribonucleoproteins (RNP) and HDR templates with LAH5 peptides at various ratios. Electrostatically-driven nanocomplexation was tested by a gel retardation assay. To assess efficiency of delivery at the cellular level, peptide nanocomplexes were tested in two different reporter cell lines: HEK293T NHEJ and HEK293T HDR Stoplight cells. These cell lines constitutively express mCherry, but start to co-express eGFP upon introduction of a +1 or +2 frameshift (NHEJ) or upon template-directed correction of a stop codon (HDR). In this way, CRISPR-Cas mediated gene knockout (NHEJ) and gene correction (HDR) can be easily quantified. Additionally, the delivery efficiency was also tested in various cell types (HEK293T, HEK293-F suspension cells, HeLa, HepG2 and primary fibroblasts) using CCR5 as a target for knockout.
Results / Conclusions: Nanocomplexes of LAH5 peptide and CRISPR-Cas9 components were approximately 200nm in size with a polydispersity index of 0,4. These nanocomplexes were taken up by the cells leading to efficient functional delivery with ~ 70% knockout efficiency and ~ 20% HDR efficiency.
These results show that peptide-based delivery of CRISPR-Cas9 RNP to cells *in vitro* is efficient, safe, and only requires minute amounts of Cas9 RNP and therefore forms a good alternative to other commercial transfection agents. Future research should demonstrate potential therapeutic use of these peptides for *ex vivo* or local *in vivo* applications.

GENE SURGERY: a potential CRISPR/Cas9-based treatment option for nephropathic cystinosis *in vitro*

E. Sendino Garví[1], M. Oktem[2], R. Masereeuw[1], P. Harrison[3], E. Mastrobattista[2], M.J. Janssen[1]

[1]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University. Universiteitsweg 99, 3584 CG Utrecht, The Netherlands; [2]Division of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University. Universiteitsweg 99, 3584 CG Utrecht, The Netherlands; [3]Department of Physiology, Biosciences Research Institute, University College Cork, College Road, Cork, Ireland. Contact: e.sendinogarvi@uu.nl (E.S.G.); m.j.janssen1@uu.nl (M.J.)

Background: Nephropathic cystinosis is a rare monogenetic kidney disease caused by mutations in the *CTNS* gene. In this project, we aim to develop a novel gene repair strategy for the most predominant 57kb deletion of *CTNS* using CRISPR-Cas9-HITI technology. This approach allows for the delivery of a large dsDNA linear *CTNS* repair template into a specific location in the genome.
Methods: For this study, we used two conditionally immortalized proximal tubule epithelial cells (ciPTEC). ciPTEC *CTNS*$^{-/-}$ cells were generated using CRISPR/Cas9 and ciPTEC *CTNS*^{Patient} were obtained from a cystinosis patient. For the delivery of the repair complex, we used a novel non-viral peptide-mediated delivery system. The repair construct for *CTNS* (3.2 kb) contains the *CTNS* promotor and the first 10 exons of the *CTNS* gene, as well as a fluorescent reporter gene (mCherry). Additionally, a second repair construct was designed (1,7Kb), excluding the mCherry gene, to study a more therapeutic-like repair construct.
Results: After transfection of the repair construct, we achieved a ~5% mCherry positive cell population, indicating these cells had successfully taken up and inserted the repair template into their genomic DNA. Further analysis of individual clonal cells showed restoration of lysosomal cystine levels in ~60% of the clones transfected with the big template (3.2Kb) and in >70% of the clones transfected with the therapeutic-like template (1.7kb), indicating that in most of the cells the *CTNS* function was indeed restored.
Discussion/Conclusion: In conclusion, these data show that the *CTNS* repair template can be precisely inserted into the genome, leading to the translation of a functional cystinosin transporter, which consequently restores the lysosomal cystine accumulation. Eventually, this gene repair system may offer a potential curative therapy for cystinosis, as well as a system for the *in vitro* restoration of several other genes involved in monogenic diseases.

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 mainly 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 mutations for which we aim to further develop antisense oligonucleotides (AONs) for the future treatment of STGD1. As a first step, we have performed an extensive AON screen for each of these candidates in patient-derived photoreceptor precursor cells, which allowed us to narrow down the list of candidate AONs. This is currently being followed up by testing of the most promising AON candidates in patient-derived retinal organoids, to assess potency aspects (i.e. correction at RNA, protein and toxic by-product level) as well as off-targets, with the incentive to select lead AONs for therapeutic intervention in humans.