NVGCT Nederlandse Vereniging voor Gen- & Celtherapie

ANNUAL SYMPOSIUM
June 3rd and 4th 2024

Congrescentrum De Werelt

Westhofflaan 2, 6741 KH Lunteren

MONDAY JUNE 3rd 2024

9.00 **Registration and Coffee**

9.40 **Welcome and Opening Announcements**

Victor van Beusechem, President of the NVGCT

9.45 - 11.00 **SESSION I CANCER THERAPY** **SPONSORED BY M-FILTER**
Chair: Vera Kemp

9.45 **INGE JEDEMA, NKI**
Generation of personalized neoantigen-specific TCR-T-cell products using CRISPR/Cas-9 gene editing

10.15 **JACEK LUBELSKI, NanoCell Therapeutics**
tLNPs can effectively deliver DNA to T-cells and generate long-acting CAR-T cells in vivo

10.30 **JELLA VAN DE LAAK, Maastricht University**
Genetically modified bacteria as anti-cancer Trojan horse; intratumoral delivery of immunotherapy by Clostridium sporogenes

10.45 **TEREZA BRACHTLOVA, Amsterdam UMC & ORCA Therapeutics B.V.**
ORCA-010 oncolytic adenovirus activates the tumor microenvironment and induces systemic tumor-specific T-cell responses in prostate cancer: results from a Phase I/IIa clinical trial

11.00 **EXHIBITOR PITCHES**
Chair: Fréderique de Graaf

ChemoMetec, Eurofins, Thermo Fisher Scientific, ACROBiosystems, PlasmidFactory GmbH, Promega, VectorBuilder, Twist Bioscience

11.15 Coffee break

11.45 **ZonMw Young Investigator SpeedDate Challenge part 1**
Chair: Jan Theys

Opening: *Presentation by 2023 winners **Merve Yildiz** (Maastricht University) & **Julia Minnee** (Radboudumc)*

12.45 Lunch

13.30 **SPONSOR TALK**
Chair: Fréderique de Graaf

NICK BOELEN, M-Filter
Closed System Manufacturing

14.00 - 15.30 SESSION II FROM CLINICAL RESEARCH to CLINICAL PRACTICE
Chairs: Niek van Til and Nathalie Jansen

14.00 **LAURA CAMPBELL, Orchard Therapeutics**
Pharmaceutical industry perspective: study design and approval - the example of Libmeldy

14.12 **ANDREAS SCHUIL, Orchard Therapeutics**
Pharmaceutical industry perspective: market access

14.24 **CHRIS VAN LIESHOUT, UMC Utrecht**
Early assessment of CGT products

14.36 **RUDY DUPREE, National Health Care Institute**
CGT products: Assessment and reimbursement dossier evaluation

14.48 **ARJAN LANKESTER, LUMC**
Access to gene therapies for rare diseases (AGORA)

15.00 **CEES SMIT**
The patient perspective

15.12 **ROUNDTABLE DISCUSSION**

15.30 Coffee break

16.00 NVGCT OUTSTANDING ACHIEVEMENT AWARD CEREMONY
Chair: Victor van Beusechem

CHIARA BONINI, San Raffaele Hospital, Milan, Italy
Genome editing of T cells for cancer immunotherapy

17.00 NVGCT General Assembly

17.30 POSTER SESSION AND DRINKS

P1 YUNIATHI DINH-SUTARDO, UniQure Biopharma B.V.
P2 DAAN PERIC-HUPKES, Annogen B.V.
P3 CHRISTINE VAN HATTEM, Utrecht University
P4 NIEK VAN DER ZEE, Amsterdam UMC
P5 IDA VAN DER MEULEN - MUILEMAN, Amsterdam UMC
P6 ATHINA MAVROPOULO, University of Amsterdam
P7 DANIEL KOGAN, Utrecht University

19.00 Dinner

20.30 FAMOUS NVGCT PUBQUIZ
Quizmasters: Jan Theys & Victor van Beusechem

TUESDAY JUNE 4th 2024

9.00 **Announcement Speed Date Finalists**

9.05 - 10.20 **SESSION III RNA THERAPIES** **SPONSORED BY NECSTGEN**
Chair: Enrico Mastrobattista

9.05 **PIETER VADER, UMC Utrecht**
Extracellular vesicle-mediated RNA delivery: from mechanistic insights towards therapeutic applications

9.35 **ERIC VAN DER VEER, Hybridize Therapeutics**
AIC468 - a first-in class antiviral antisense oligonucleotide for the treatment of BK virus infection in kidney transplant recipients

10.05 **CISSE VERMEER, UMC Groningen**
ASO-mediated exon skipping in intact human skin pointing towards a treatment for RDEB

10.20 **SPONSOR TALK**
Chair: Fréderique de Graaf

MELISSA VAN PEL, NecstGen
Translational considerations for your future cell therapy production process

10.50 Coffee break*

11.15 - 12.00 **Greiner Award for Best Thesis 2023**
Chair: Massimiliano Caiazzo

12.15 - 12.45 **ZonMw Young Investigator SpeedDate Challenge part 2**
Chair: Jan Theys

Pitches three finalists (5 minutes pitch, 5 minutes questions each)

12.45 Lunch*

13.30 - 15.00 **SESSION IV PSIDER: PLURIPOTENT STEM CELLS, HEREDITARY DISEASES AND ETHICAL ISSUES**
Chair: Karien de Rooij

13.30 **NAEL NADIF KASRI, Radboudumc**
BRAINMODEL: standardized, IPSC-based medicine for immediate application in monogenic neurodevelopmental disorders

14.00 **LOUKIA YIANGOU, LUMC**
Developing hiPSC-cardiomyocyte models for interpreting the pathogenesis of variants associated with inherited cardiac disorders

14.20 **MIKE BROEDERS, Erasmus MC**
Pushing the boundaries of in vitro disease modelling: single cell analysis of a "population on a dish' to decipher monogenetic diseases

14.40 **JEROEN WIEGERTJES, NEMO Kennislink**
Festivaltour embryolike structures: dialogue with society

15.00 Coffee break*

15.30 **CLOSING KEYNOTE LECTURE**
Chair: Rob Collin

LUIGI NALDINI, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy
Genetic engineering of human hematopoiesis: state of the art and future perspective

16.15 **Award session & Closing remarks**
Victor van Beusechem, President of the NVGCT

16.30 END

* During the coffee breaks and lunch, symposium attendees can actively participate in NEMO Kennislink 'Holland's Next Embryo Model'.

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Awards

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ABSTRACTS

NVGCT Outstanding Achievement Award Winner

CHIARA BONINI

Genome editing of T cells for cancer immunotherapy

C. Bonini[1,2]

[1]University Vita-Salute San Raffaele and [2]Ospedale San Raffaele Scientific Institute, Milan, Italy.

Adoptive cellular therapy with engineered lymphocytes is an emerging therapeutic pillar that is rapidly revolutionizing cancer research and therapy. This therapeutic approach stems from the pioneering work, based on naturally occurring T cells, that demonstrated the ability of T cells to mediate anti-tumor clinical responses. In the autologous setting, expanded tumor-infiltrating lymphocytes obtained from resected solid tumors, such as metastatic melanoma, proved capable of recognizing tumor-associated antigens and to induce complete remissions once reinfused to patients. In the allogeneic setting, cellular therapies against relapsed hematologic malignancies used donor lymphocyte infusions after allogeneic hematopoietic stem cell transplantation, to exploit the alloreactive graft versus leukemia effect of donor lymphocytes, although at the price of a major complication, named graft versus host disease. By using viral vectors, T cells have been genetically engineered for enhanced persistence, improved safety, and, more recently, to express an artificial receptor that provides tumor-specific antigen recognition. By enabling the transgenic creation of antigen-specific T cells, genetic engineering technologies have widely broadened the use of adoptive T cell therapy. In recent years, the U.S. Food and Drug Administration and European Medicines Agency have approved six engineered T-cell therapeutic products, all chimeric antigen receptor (CAR) engineered T cells directed against B cell malignancies. In parallel, T-cell receptor (TCR) gene therapy is emerging as a promising approach for the treatment of several tumors. Despite encouraging clinical results, engineered T cell therapy is still constrained by several challenges, including the paucity of tumor specific CARs and TCRs, the variable fitness of the therapeutic cellular products and the sensitivity of engineered T cells to the immunosuppressive tumor microenvironment. Several of these challenges can be theoretically addressed by the genome editing technologies, that allow not only to add a transgene to T cells, but also to permanently delete multiple gene and/or substitute endogenous genes with genes of interest. Our lab is exploiting genome editing tools to: 1) Fully redirect T cell specificity through TCR gene editing; 2) improve the ability of therapeutic cells to resist immunosuppressive signals active in the tumor microenvironment. Overall, these innovative approaches should widen the safe and effective use of ACT to larger numbers of patients affected by cancer.

Keynote Lecture

LUIGI NALDINI

Genetic engineering of human hematopoiesis: state of the art and future perspective

L. Naldini[1,2]

[1]San Raffaele Telethon Institute for Gene Therapy and [2]San Raffaele University, Milan, Italy.

Genetic engineering of hematopoietic stem cells (HSC) with lentiviral vectors has been providing substantial benefit to growing numbers of patients affected by primary immunodeficiencies, hemoglobinopathies and storage disorders. Long-term follow up shows stable hematopoietic reconstitution by high numbers of corrected HSC without signs of clonal expansion or exhaustion. Precise engineering by gene editing may further improve the reach and safety of HSC gene therapy by achieving in situ gene correction or targeted transgene integration. Homology-driven editing, however, remains limiting in long-term HSC and the genetic outcome at target sites heterogenous and, for some by-products, potentially genotoxic. Template delivery by Integrase-defective lentiviral vectors rather than AAV6 and the use of lipid nanoparticles instead of electroporation may increase safety and efficiency of the procedure. Coupling selection for the intended edit and purging adverse outcomes may provide a preferred path towards clinical application of this currently unique modality enabling long-range edits. On the other hand, the emergence of base and prime editors that bypass the requirement for DNA double-strand breaks (DSB) allows editing single/few mutant nucleotides with limited activation of DNA damage response. We have shown, however, that DSBs are significantly lowered but not abrogated. Moreover, the expression of constitutive deaminase domains within the editors may impact the mutagenic load of treated cells. While these potentially genotoxic outcomes can be mitigated by optimizing expression and culture conditions, they should be better investigated and monitored in emerging clinical applications. Overall, our work should advance HSC gene therapy by a combination of transformative approaches leveraging on precision genetic engineering while alleviating the morbidity of the procedure, broadening application to several diseases and patients worldwide.

INVITED SPEAKERS

INGE JEDEMA

Generation of personalized neoantigen-specific TCR-T-cell products using CRISPR/Cas-9 gene editing

I. Jedema[1], W. Scheper[1], B. Raud[1], S. Scheij[2], A. de Vries-Egan[1], R. Voogd[1], J. Hagg[2], I. Pardieck[2], R. van Amerongen[2], C. Nijenhuis[2], J.B.A.G. Haanen[1,3]

[1]Division of Molecular Oncology and Immunology, Netherlands Cancer Institute, Amsterdam, The Netherlands ,[2] Biotherapeutics Unit, Hospital Pharmacy, Netherlands Cancer Institute, Amsterdam, The Netherlands, [3]Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands.

Cancer immunotherapy with immune checkpoint inhibitors can induce robust clinical responses in patients with solid cancers. However, many patients do not experience durable benefit, for instance because their tumors are only poorly infiltrated by T cells or because the pre-existing tumor-reactive T cells are too exhausted to be reinvigorated. T-cell receptor (TCR) gene therapy is a form of cellular immunotherapy that involves the genetic transfer of TCRs with desired tumor-reactivity to peripheral blood T cells of the patient, thereby circumventing the dependence on the fitness of the pre-existing intratumoral T cells.
In our project we developed a high-throughput platform for the identification and specificity testing of patient tumor-derived TCRs. For this, the mutational landscape of the autologous tumor is determined using whole exome and RNA sequencing, followed by introduction of these tumor-specific mutations (e.g. neo-antigens) as antigen libraries into immortalized B cells. Tumor infiltrating lymphocytes are isolated from the autologous tumor and their TCRs are identified using a single cell sequencing platform. A library of these TCRs is introduced into peripheral blood T cells and tested for their capacity to recognize neoantigens in functional screens.
Next, we developed a gene editing strategy using the CRISPR/Cas9 technology that allows efficient replacement of endogenous TCR expression with a selection of 5 neoantigen-specific TCRs in autologous patient-derived T cells. To achieve this, the expression of the alpha and beta chains of the endogenous TCR must be interrupted by targeting the first exon of the constant regions of both the TCR-alpha and TCR-beta loci (encoded by the genes TRAC and TRBC, respectively).
To allow clinical application of these CRISPR/Cas-9 gene edited TCR-T products, we performed upscaling of our production workflow using large-scale systems allowing T-cell isolation and activation, electroporation and subsequent culture in a fully closed GMP-compliant system using the CliniMACS Prodigy® platform.

The long and winding road of developing and reaching sustainable access to atidarsagene autotemcel

A.A.J. Schuil[1], L. Campbell[2]

[1]Orchard Benelux and partner markets EMEA, Orchard Therapeutics (Netherlands) B.V., Amsterdam, The Netherlands; [2]Orchard Therapeutics (Europe) Limited, London, United Kingdom.

There are few ATMPs which have successfully reached the commercialization phase of development. Furthermore, some ATMPs have faced challenges in reaching or maintaining sustainable market access. Atidarsagene autotemcel (arsa-cel) is a cryopreserved autologous *ex-vivo* HSC gene therapy to treat early-onset metachromatic leukodystrophy (MLD). MLD is an ultra-rare inherited lysosomal storage disease. Patients suffer from progressive neurological deterioration and, if untreated, it leads to premature death. Arsa-cel clinical trials in early-onset MLD commenced in 2010 and to date 39 patients have been treated in the clinical development program, including 9 patients treated via expanded access programs who also contribute to the evidence generation. Data was collected for 49 untreated patients to capture natural disease progression and act as a comparator. Arsa-cel was approved by the EMA in December 2020 and by the FDA in March 2024. Since registration in Europe several reimbursement agreements have been reached, qualified treatment centers (QTCs) have been activated and multiple patients have been treated. Through development to the present day, many hurdles have been overcome, trial design, acceptance of a comparator, moving to a cryopreserved formulation for access, funding the development, reimbursement, pricing challenges and cross-border access. Throughout development it is important to think about the implications for sustainable access following registration. The experience gained with arsa-cel can benefit the development of other ATMPs as many of the challenges remain in the current environment and will be important to take into account by all stakeholders.

CHRIS van LIESHOUT

Early Health Economics and Health Technology Assessment of CGT Products

C. van Lieshout[1]

[1]Department of Epidemiology and Health Economics, Julius Center for Primary Care and Health Sciences, UMC Utrecht, Utrecht University, Utrecht, The Netherlands.

During the development of any innovation, thinking ahead about health economic factors can improve the efficiency of clinical research and implementation efforts. There are several ways in which elements and methods of economic evaluations can be used in early stages of product development. For example, to determine in which patient population the most impact can be made or to inform the design of clinical research. When new treatments are developed, there may still be a lot of uncertainty about the scope of the innovation. In which patient group can the most impact be expected? In which treatment line can the innovation be best implemented? These are questions where conducting an early health economic analysis can be of added value. Another value of conducting early health economic analyses may be to inform clinical trials. In clinical research we see that there is often an idea about what the most important factors for positive patient and health economic outcomes are. However, practice teaches us that there may be factors that have more influence on the impact of the innovation than previously expected. By modeling the innovation, these factors can be identified at an early stage. It is often thought that the investment for such an early analysis is very high for a one-off analysis. However, the models created can be updated with recent data during the further development process and clinical evaluation phase. Ultimately, the model can be further developed for use in reimbursement decisions. During my presentation on the panel, I will provide further explanation about what economic evaluations exactly are, how they are carried out, what pitfalls there are and how these can best be navigated so that the chance the new therapy is used in the right patient population is as large as possible.

RUDY DUPREE

Reimbursement of ATMPs: challenges and opportunities

R. Dupree[1]

[1]Department of Development, Science and International Affairs, National Health Care Institute (ZIN), Diemen, The Netherlands.

In order to be used in daily practice, ATMP's need to be reimbursed by the healthcare insurers. Because everyone pays premiums and taxes, the government must ensure that the basic package of the health care insurance includes care that is necessary and that works. At the same time the government must ensure that healthcare is, and remains, accessible and affordable. The National Health Care Institute advises the Minister of VWS on whether to include medicines, including ATMP's, in the basic package. In order to be included in the basic package, care must, above all, be sufficiently proven effective. Cost-effectiveness can also play a role in reimbursement decisions.
As with all inpatient drugs, assessments of ATMP's are done by the National Healthcare Institute (Zorginstituut Nederland; ZIN) in case of high expected budget impact, and else by health care insurers.

The assessment of ATMP's often poses multiple challenges in comparison with other drugs:

1. Single-arm trials, lack of natural history data
2. Small and sometimes heterogeneous patient populations
3. Short follow-up on outcomes in trials while long-term effectiveness is claimed
4. High upfront costs and large uncertainty about cost-effectiveness

These challenges need to be balanced against the often high unmet need of the patients with diseases treated with ATMP's.

Several conditions for reimbursement of ATMP's can be put in place to mitigate those challenges:

1. Additional research, especially on longer follow-up
2. Orphan drug arrangement, with agreed start-stop criteria and indication committee
3. Financial agreements with Minister of Health or with health insurers or, potentially, other forms of risk-sharing agreements to achieve a cost-effective price
4. Reimbursement in study setting only (conditional approval) in case of promising but insufficiently proven effectiveness

The above highlights the need to take reimbursement decisions in early developmental stages and study design, and the need for actions following market access to achieve appropriate, cost-effective use in daily practice.

ARJAN LANKESTER

Access to Gene Therapies for Rare Disease: the AGORA initiative

A.C. Lankester[1]

[1]Willem-Alexander Children's Hospital, Department of Pediatrics, Division of Stem Cell transplantation, Leiden University Medical Center, Leiden, The Netherlands.

In the last two decades pioneering research, conducted predominantly in academic institutions, has led to development of stem cell-based gene therapies for a number of rare Inborn Errors of Immunity (IEI) and Metabolism that were shown to be effective and safe in clinical trials, and were demonstrated to be transformative for patients. The final step, translation to market authorization was hampered in a number of cases which resulted in curative, life-saving therapies not reaching patients in need for non-medical reasons, illustrating that the traditional commercialisation model appeared to be not fit for purpose.
For this reason a group of European multidisciplinary experts has taken the initiative to address this access challenge and work on solutions. For this purpose a foundation, named AGORA (Access to Gene Therapies for Rare Diseases) has been set up with the mission to facilitate sustainable access to effective and affordable gene therapies for treatment of patients with rare diseases, focusing on IEI and IEM. The AGORA foundation is an independent, not-for-profit entity that has already organized several meetings with international stakeholders including academic developers, pharma, regulators, funders and patient organizations to explore the needs and identify common, individual product transcending issues that require specific solutions. Dedicated working groups will focus on these topics and work on solutions.
During the panel discussion AGORA's goals and strategy will be further discussed.

CEES SMIT

The patient's perspective on gene and cell therapy: 'from dream to reality'

C. Smit[1], A. van Eekelen[1]

[1]Authors of the book 'Gen- en celtherapie, van droom tot praktijk', Eburon Academic Publishers, 2024.

In 2023, we have spoken with relevant stakeholders in the field of gene and cell therapy. Stakeholders from the world of researchers and physicians, industry, insurers and HTA economists, policy institutions and patient groups. The result of our conversations has been published in Dutch in the book titled: 'Gen- en celtherapie, van droom tot praktijk' in 2024.

The dreams and promises on gene therapy, that already have been described in a report by the Dutch Health Council (Gezondheidsraad) in 1997, have become reality. A grant program from the Ministry of Health (VWS) for ZonMw and its Translational Gene Therapy Research Programme (TGO) has contributed to the possibilities to gain and to build knowledge on gene and cell therapy in most academic hospitals and universities. Great results have been reached in the field of stem cell transplantations for sickle cell disease and gene therapy for hemophilia. Especially, the gene therapy for hemophilia B (and before for LPL-deficiency) has been the result of the Amsterdam based company UniQure (before AMT).

Many patient groups are involved with the education of their members in order to present them with a realistic view on the possibilities and impossibilities of gene and cell therapy. Researchers and industry groups are enthusiastic about the funding of the Italian Telethon Group and their research facilities for gene therapy and clinical trials.

The main problem in The Netherland lies in the fact that legislation and reimbursement models for gene and cell therapy lag behind scientific developments.

For more information: info@smitvisch.nl and www.smitvisch.nl

PIETER VADER

Extracellular vesicle-mediated RNA delivery: from mechanistic insights towards therapeutic applications

P. Vader[1,2]

[1]CDL Research, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Laboratory for Experimental Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands.

Extracellular vesicles (EVs) play a pivotal role in intercellular communication through functional transfer of bioactive cargo, including nucleic acids. Despite increasing interest in EV-mediated nucleic acid transfer, understanding of the pathways and mechanisms regulating EV-mediated nucleic acid delivery is limited. Here, we show a CRISPR/Cas9-based reporter system that allows the study of EV-mediated RNA transfer at single-cell resolution. We employed this system to compare the delivery efficiency of EVs to clinically approved state-of-the-art lipid nanoparticles and found that EVs delivered RNA several orders of magnitude more efficiently than these synthetic systems. To overcome challenges related to the difficulty of RNA loading into EVs, we prepared EV-liposome hybrid nanoparticles and evaluated them as siRNA delivery systems in terms of cellular uptake, toxicity, and gene-silencing efficacy. We show that hybrids combine benefits of both synthetic and biological drug delivery systems and might serve as future therapeutic carriers of siRNA.

NAEL NADIF-KASRI

Leveraging human neuronal networks on micro-electrode arrays to study disease-specific genotype-phenotype correlations

S. Puvogel[1], U. Ciptasari[1], E. van Hugte[1], N. Doorn[2], M. Frega[2], N. Nadif Kasri[1]

[1]*Human genetics department, Radboudumc, Nijmegen, the Netherlands;* [2]*Clinical Neurphysiology, UTwente, Twente, The Netherlands.*

Advancements in human genetics have enabled the identification of numerous genes linked to neurodevelopmental disorders (NDDs), including epilepsy and autism spectrum disorders (ASD). Despite progress in understanding the genetic underpinnings of NDDs, a significant disparity remains between genetic discoveries and understanding the underlying pathophysiology.
Induced pluripotent stem-cell (IPSC)-based model-systems provide new avenues to understand human disease and develop personalized treatments. In BRAINMODEL we aim to implement IPSC-technology to characterize disease mechanisms and inform individualized treatment decisions for two classes of monogenic neurodevelopmental disorders (mNDDs). iPSC-based strategies are particularly urgent for mNDDs given the severity, low therapy success, limited access to tissue, and heterogeneity. However, iPSC-technology also poses ethical questions. Within BRAINMODEL we have established standardized, scale-able assays, based on patient's own tissue, to test mNDD-relevant disease concepts, such as disturbances in excitation/inhibition (E/I) balance. In particular, by integrating RNA-sequencing and micro-electrode array (MEA) recordings in iPSC-derived neurons with NDD-related mutations, we established a platform to bridge the gap between molecular and functional aspects of neurodevelopment, with potential implications for NDD treatment development.

LOUKIA YANGOU

Developing hiPSC-cardiomyocyte models for interpreting the pathogenesis of variants associated with inherited cardiac disorders

L. Yiangou[1], N. Popović[1], A. Blanch Asensio[1], T. Visser[1], C.L. Mummery[1,2], R.P. Davis[1,2]

[1]Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands; [2]The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, Leiden University Medical Center, Leiden, The Netherlands.

Long QT Syndromes (LQTS), are a group of cardiac channelopathies, estimated to affect 1 in 2000 people. Type 2 LQTS (LQT2) is responsible for about one-third of LQTS cases and is caused by mutations in the gene KCNH2, which encodes the α-subunit of the potassium ion channel. Such mutations result in action potential duration prolongation in cardiomyocytes and thus life-threatening arrhythmias. However, they are characterised by reduced penetrance and variable disease severity, meaning that not all patients carrying the same mutation will have severe disease. Genetic variants play a paramount role in the variability of LQT2 severity and prognosis among patients. Thus, there is a great need for identifying benign or pathogenic variants, which will allow for patient risk stratification and precision medicine. Human induced pluripotent stem cells (hiPSCs) can differentiate to any cell type of the human body, thereby allowing disease investigation in the cell type affected, providing superior models to animals and heterologous expression systems. hiPSC-derived cardiomyocytes (hiPSC-CMs) have been used to successfully model cardiac arrhythmias, including LQT2. Here, we generated a hiPSC platform for high throughput assessment of the pathogenicity of various genetic KCNH2 variants in order to classify them as severe or beginning, as well as assess the contribution of variants of unknown significance (VUS) to disease. Recently, we developed a genetic modification method called STRAIGHT-IN. Using this method, we successfully generated a panel of hiPSC lines harbouring variants either known to cause LQT2 or variants of unknown significance (VUS). We have validated the pathogenicity of known KCNH2 variants and also identified a VUS to cause field potential duration prolongation, indicating that it is a pathogenic variant causing LQT2. In summary, we now have a platform that we can use to classify variants and also perform future drug screening studies to rescue the disease phenotype.

MIKE BROEDERS

Pushing the boundaries of in vitro disease modelling: single cell analysis of a "population on a dish' to decipher monogenetic diseases.

M.Broeders[1], I.J. Bakker[2], E.M. Bindels[3], M.A. Sanders[3], H.F.E. Gleitz[2], R Narcisi[1]

[1]Department of Orthopaedics and Sports Medicine, Erasmus MC, Rotterdam, The Netherlands; [2]Department of Developmental Biology, Erasmus MC, Rotterdam, The Netherlands; [3]Department of Hematology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands.

The identification and characterization of mutations in monogenic disorders is an important aspect of diagnosis, genetic counselling, prediction of disease severity, and the development of novel therapies. Therefore, unraveling the study disease mechanisms and genotype-phenotype correlation for monogenic disorders is crucial and can have a big clinical impact. A well-recognized strategy involves creating hiPSC based disease models to generate a "patient on a dish" to study the pathology *in vitro*. However, these rely on patient material and/or the generation of isogenic hiPSCs pairs. Creating isogenic pairs is labour intensive and time-consuming and access to patient material is often limited in rare diseases. In this project, we are developing an adaptable approach which will take in vitro disease modeling from a "patient on a dish" to a "population on a dish" approach. We use Single-cell RNA expression analysis to get a deeper insight into the disease mechanisms and genotype-phenotype correlation of monogenetic diseases. This will eliminate the need of patient material and the generation, expansion, differentiation, and analyses of every multiple individual cell lines. As a proof of concept, we applied this method to Aneurysms-osteoarthritis syndrome (AOS), a rare monogenic disorder caused by mutations in the SMAD3 gene.

JEROEN WIEGERTJES

Hollands Next Embryo Model, public dialogue on embryo models for festival settings

J.M. Wiegertjes[1], J.E. Kluvers[1], E. Grob[1]

[1]Department of Science Communication, NEMO Science Museum, Amsterdam The Netherlands.

Embryo-like structures made in the lab are increasingly looking like real embryos. For example, it is already possible for a pregnancy test to test positive in a petri dish. What do the Dutch find ethically sensitive in research into embryo models and what values and arguments play a role in this? NEMO Kennislink and Rathenau Institute are working together with support from ZonMw to raise values and ideas surrounding research into and with embryo-like structures (ELS) from the Dutch public in an accessible way. The result is a traveling mini-catwalk where festival visitors sit as jury members, can give their opinion and discuss the research into embryo models. In our presentation we will describe the 'why, how and what' of this experience. We will share the first results of the week at the Libelle Zomerweek, the big outdoor lifestyle fair of the women's magazine Libelle.

If you would like to experience Hollands Next Embryo Model for yourself, you can today. During the break we will be present with the installation in the central room. Dutch language only, though.

SPONSOR TALKS

NICK BOELEN

Closed system manufacturing

N. Boelen[1]

[1]*M-Filter B.V., Culemborg, The Netherlands.*

Cell therapy is an innovative and rapidly advancing field in medical science that holds and delivers great promise for revolutionizing the treatment of various diseases. At its core, cell therapy involves the use of living cells to restore, repair, or replace damaged tissues or organs within the body. This groundbreaking approach represents a paradigm shift from traditional pharmaceutical interventions, as it harnesses the regenerative potential of cells to address the root causes of diseases. Despite the countless scientific and industrial breakthroughs, there are still challenges to overcome. During this presentation I would like to inform you about a solution that addresses a set of these challenges. The addressed challenges are not confined to R&D, clinical trial manufacturing or commercial manufacturing, but apply to all three of them, and also involves the transfer from R&D to various manufacturing stages. The solution we will talk about is Closed system manufacturing. Closed system manufacturing is not just a single product or single method. Closed system manufacturing is a way of thinking, a way of designing, a way of working. Before we move into details about closed system manufacturing, we need to address some of the current issues in developing and manufacturing cell therapies: - The need for a large workforce of highly trained operator which can perform aseptic actions - high-level cleanrooms - Large area of cleanroom space - High risk of contamination - High risk of extractable accumulation - Lack of quality-by-design - Long process times - Large batch to batch variation - Scale-out is hard - Scale-up is hard - R&D to clinical-trial manufacturing is hard Closed system manufacturing makes sure that nowhere in the manufacturing process the drug product is exposed to the environment or transferred by complex human handling. This can be achieved by smart designed disposable technologies. In figure 1 we see the typical cell therapy manufacturing steps. By smart design and choices for the right disposable single-use technologies, we can make sure that the needles in a patient's arm are the only transfer steps left where human aseptic handling is required. It's important to note that this approached should be integrated early in the R&D phase. This will enable you to transfer not only a drug product, but a full process that's easy to replicate. This will make scale-out and scale-up easy. Because the system is fully closed, the need for A-grade cleanrooms becomes redundant which will translate to lower CAPEX and maintenance costs. In non-closed systems, transfer steps often contain actions like sterile tube welding or aseptic handling under LAF-conditions. These actions are time consuming and notorious for introducing plastic extractables in the process. By replacing these steps for sterile connectors, we can both cut on process time and extractable accumulation. Figure 1 Typical cell therapy manufacturing steps Closed system manufacturing enables research group and companies to deliver a robust process that outputs a drug product, instead of just a drug product. Therefore scale-out and scale-up will be simplified. Risk of contamination, processing time and extractable accumulation will be drastically reduced. The need for skilled operators will be decreased as well as the CAPEX for additional cleanrooms.

MELISSA van PEL

Translational considerations for your future Cell Therapy production process

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Cell and Gene Therapies have the potential to provide a cure for thus far uncurable diseases. Although very promising, the manufacturing of living cells as a therapy brings its specific challenges including cost and related patient access. As the field of Cell and Gene Therapy is currently still young, manufacture methods often involve open processes requiring grade B clean rooms although a trend towards the use of closed processes and bioreactors are emerging. Each cell type has its own requirements with respect to culture conditions, medium additives, and cell densities.
Process development aims to translate a research-grade process into a GMP compliant manufacture method, which is robust and reproducible despite the donor-to-donor variations. During process development it is important to identify the critical process steps and the critical quality attributes, and to develop adequate analytical assays and potency tests. However, at this stage key decisions should be made, and activities can be undertaken to challenge the cost of goods of a therapy.
Manufacture of Cell Therapies, such as Car T cells, Mesenchymal Stromal Cells and products derived from induced Pluripotent Stem Cells (iPSC), includes selection of starting population, cell activation, transduction, expansion and fill and finish of the product. During process development, many decisions have to be made on raw material usage, transduction method, cell expansion methods, use of bioreactors and quality control testing. It is key to understand the product and the process in the greatest detail possible.
Here we aim to share our knowledge on process development and testing strategies for Cell Therapies by providing an overview of the pros and cons of the different process choices.

JACEK LUBELSKI

tLNPs can effectively deliver DNA to T-cells and generate long-acting CAR-T cells *in vivo*

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Ex-vivo modification of immune cells to express Chimeric Antigen Receptor (CAR) has shown tremendous clinical and commercial success as a cancer treatment. Although widely adopted, *ex-vivo* CAR-T approaches are not without their challenges. Soaring production expenses, extended timelines, inherent toxicity risks and the operational intricacies of today's conventional cell therapy treatment models calls for a next wave of improvements.

Here we present a novel cell-targeted lipid nanoparticle (tLNP) that can deliver both DNA and RNA to T cells. This new tLNP based vector consists of an LNP formulation containing transposon DNA (encoding for CAR), mRNA (encoding for transposase) and activating and targeting protein moieties. We will discuss the ability of this vector to activate resting primary T cells, thereby allowing their permanent modification with a DNA encoded CAR construct *in vitro* in the absence of exogenous activation resulting in the generation of fully functional CAR-T cells. In addition, the capacity of this non-viral vehicle to drive the generation of functional T-cells *in vivo* will be presented. We will demonstrate the generation of persistent CAR-T cells, tumour control and extended survival after tLNP treatment in a human PBMC engrafted NSG mouse model injected with a human B-cell leukaemia cell line.

We believe that the CAR-T cells generated *in vivo* have a variety of advantages compared to currently employed ex-vivo manufacturing technologies.

JELLA van de LAAK

Genetically modified bacteria as anti-cancer Trojan horse; intratumoral delivery of immunotherapy by *Clostridium sporogenes*

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In recent years, cancer immunotherapy has been revolutionized as there is an ongoing growth in novel immunotherapeutics to treat various cancer types. Although remarkable successes, ineffective treatment or adverse effects in a subset of patients, due to systemic exposure, remain major challenges. Therefore, there is a need for development of tumor-specific delivery methods. Strikingly, the necrotic environment present in most solid tumors provides such an opportunity: upon injection as spores, the non-pathogenic anaerobic *Clostridium sporogenes* selectively penetrates necrotic tumor areas and germinates into vegetative bacteria. This bacterium can function as a vehicle transporting biotherapeutics specifically to the tumor microenvironment, a Trojan horse strategy. We recently developed novel genetic tools to "arm" *Clostridium sporogenes* with therapeutic genes. Here, we present the development of immunotherapeutic *Clostridium sporogenes* and the *in vitro* potential as an anti-cancer delivery system.

Clostridium sporogenes was genetically modified using CRISPR/Cas9 techniques, inserting the immunotherapeutic genes murine interleukin(mIL)-2, murine granulocyte-macrophage colony-stimulating factor (mGM-CSF), and anti-programmed death-ligand 1 (aPD-L1) stably into the chromosome. Secretion and biological activity of the various therapeutics were assessed using ELISA and cellular assays.

Sequencing results confirmed insertion of therapeutic genes into the chromosome. We verified that these insertions did not affect sporulation or growth abilities. Furthermore, we optimized a necrotic 3D spheroid model and demonstrated that the bacteria germinate and colonize herein. ELISA results showed successful secretion of mIL-2 and mGM-CSF up to 42,1 and 186,5 pg/mL (respectively). ELISA mimicking PD-1/PD-L1 interaction to test clostridial production of aPD-L1 has been optimized and validation will be performed soon.

Our results show that we can develop mGM-CSF and mIL-2 producing *Clostridium* strains with potential to secrete immunotherapy specifically in necrotic tumor area. The next step is to take these strains towards *in vivo* studies.

TEREZA BRACHTLOVA

ORCA-010 oncolytic adenovirus activates the tumor microenvironment and induces systemic tumor-specific T-cell responses in prostate cancer: results from a Phase I/IIa clinical trial

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Immunotherapies using oncolytic adenoviruses (OAds) emerged as promising treatment option for cancer. Studies have demonstrated that virus-induced anti-tumor immune responses play a pivotal role in achieving OAd treatment efficacy. Here, we present findings from a Phase I/IIa trial with ORCA-010, a potent OAd, in early-stage treatment-naïve prostate cancer patients (NCT04097002).
In the Phase I dose-escalation study, newly diagnosed patients with localized prostate cancer received a single intra-prostatic administration of ORCA-010 (at 1E^11, 5E^11, or 1.5E^12vp), followed by a one-year follow-up. The Phase IIa part was divided into two parts: 3 patients who underwent two administrations of highest dose (1.5E^12vp) ORCA-010 with a two-week interval and were followed for one year; and 9 high-risk patients receiving the same treatment before radical prostatectomy eight weeks later. The primary objectives included assessment of ORCA-010's safety and its capacity to activate anti-tumor immunity.
Preliminary results confirm the safety of ORCA-010, with no dose-limiting toxicities observed in any of the patients. Biopsies taken one year post-treatment revealed a significant increase in immune-cell infiltration, with high increases in CD8+ and CD8+PD-1+ T-cell. In prostatectomy samples eight weeks after treatment, increases similar to the Phase I patients were seen, in particular adjacent to tumor positive areas. Analysis of immune response in blood samples shows significant increase in activated and Ki67+ proliferating CD8+ T-cells post-treatment compared to before treatment. Furthermore, analyses demonstrated that T-cell repertoire of patients post-treatment presented with significantly enhanced T-cells responsive to prostate cancer-associated antigens.
Our findings show that ORCA-010 treatment exhibits an excellent safety profile, moreover it transforms the immunologically "cold" tumor microenvironment (TME) into immunologically "hot" TME's in prostate cancer. Our results further demonstrate that ORCA-010 induces an increase specific anti-tumor immune response in circulating immune cells. Together, these data support the development of ORCA-010 in prostate cancer.

CISSE VERMEER

ASO-mediated Exon skipping in intact human skin pointing towards a treatment for RDEB

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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe genetic skin condition stemming from a null variant in COL7A1. No curative treatments are currently available. Antisense oligonucleotides (ASOs) have emerged as a potential solution, demonstrating the ability to induce exon skipping of COL7A1 mRNA in human skin models. In order to bring this therapy to the patient, factors such as the safety and distribution of these ASOs need to be assessed. Our research aims to investigate the distribution of ASOs tailored for inducing exon 105 skipping and subsequent exon-skipped mRNA in a 3D intact human skin model. Through Basescope/miRNA-scope in-situ hybridization, we examined ASO distribution and exon skipping. Exon skipping efficiency of the ASO treated human skin model was assessed using TaqMan qPCR assays. Exon skipping was observed in human skin donors treated with ASOs using Basescope. Following intradermal injection, ASOs exhibited migration into the epidermis with nuclear localization in basal keratinocytes, as evidenced by miRNA-scope. Furthermore, ASO induced exon skipping was confirmed in cDNA from ASO-treated healthy skin using qPCR. In summary, our findings support exon skipping therapy as a viable strategy for treating pathogenic null variants of COL7A1. The observed induction of exon skipping in intact human skin suggests its potential as a systemic therapy for RDEB. Future steps will involve replicating similar experiments and quantifying exon skipped mRNA on human skin xenografts derived from patient keratinocytes and fibroblasts containing a COL7A1 null variant, alongside evaluating type VII collagen restoration.

P1 YUNIATHI DINH-SUTARDO

qPCR-based genome copy content assay qualification for AAV5-based gene therapy product

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Quantitative PCR (qPCR) – based genome copy (gc) content assay is historically the most used method within uniQure to determine the number of genome-containing particles of an Adenovirus-Associated Virus (AAV) from in-process to Drug Substance (DS) and Drug Product (DP). Because DP is ultimately used for dosing patients, titer quantification of gene therapy is among the analytical methods mandatory for product characterization and quality control, thus requires qualification and eventually validation. The poster will evaluate a case study of a qPCR-based genome copy content assay qualification at uniQure's Analytical Development (AD) department showing the approach and results obtained for specificity, linearity, precision, range, accuracy, matrix verification and robustness of the method.
The main steps in the genome copy content assay involve a DNAse treatment followed by capsid lysis and qPCR using primers-probe amplifying a specific target region. In an example of an AAV5-based product, assay specificity is confirmed by the lack of amplification in potential impurities (e.g. SF+ genomic DNA and baculovirus DNA residuals from production). Assay precision is demonstrated by the outcome of repeatability (%RSD ≤ 12.4%), intermediate precision (%RSD ≤ 14.0%) and reproducibility (% difference ≤ 13.1%) assessment. Assay linearity is evaluated and the result dictates the range of the assay, i.e. 2.4E+07 – 2.4E+13 gc/mL. Assay accuracy is calculated once precision, linearity and specificity have been established and shown to be between 95 – 106%. Spike recovery is performed to check matrix interference and shown to be between 77 – 115%. Finally, the assay is shown to remain unaffected by variations in primer annealing temperature, reagent lots, equipment and storage of isolated DNA. Altogether, the results demonstrate that the method is specific, linear, precise, accurate and robust for genome copy quantification of the AAV5-based product.

P2 DAAN PERIC-HUPKES

SuRE™-based identification of a strong human promoter induced upon T-cell activation

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The efficacy and safety of gene and cell therapy strategies are in part determined by the expression profile of the therapeutic transgene. Optimal expression profiles are defined by several factors, including tissue specificity, response to stimuli, and resistance to silencing. Many current therapies rely on generic, often constitutive and viral promoters, which often score poorly on several of these factors. The human genome contains hundreds of thousands of Gene Regulatory Elements (GREs) that regulate gene expression throughout the human body and these GREs can be used to achieve correct expression profiles in gene and cell therapy as well. Here we show how we combined a genome-wide SuRE™ screen for GREs that were induced upon T-cell activation, followed by a SuRE™ screen to identify optimal pairwise combinations between these GREs. These experiments yielded a conditional promoter for T-cell activation that outperforms the benchmark NFAT promoter with regards to inducibility and expression level after induction. This work illustrates the strength of our screening approach to identify conditionally active promoters. Moreover, we believe the identified promoter will be a valuable asset for armored CAR-T cell therapies.

P3 CHRISTINE van HATTEM

Patient access to cell and gene therapies in Europe: a scoping review of impeding factors

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Cell and gene therapies have the potential to treat diseases with high unmet medical needs. So far, however, only few have successfully navigated the medicine lifecycle, suggesting that there are factors that impede the pathway from development to patient access. Here, we aimed to provide an overview of factors that impede patient access to cell and gene therapies in Europe. To that aim, we performed a scoping review of scientific and grey literature (2008-2023). Documents were systematically collected and selected through databases and expert input. From this selection, information was extracted on factors reported to impede patient access in Europe, and by and about which stakeholders those were reported. Data were thematically analysed, deductively and inductively, according to commonly described stages of the medicine lifecycle. For all lifecycle stages, the specific impeding factors that were reported concern the following themes: for i) preclinical development: concerning translation to clinical development; ii) clinical development: concerning appropriate clinical trial design and conduct; iii) marketing authorisation and iv) reimbursement: concerning adequacy of evidence, clarity of procedures and predictability of decision-making; and v) use in clinical practice: concerning placement in treatment pathway and safe use in clinical practice, and accredited centres of expertise. Factors reported for multiple stages concern harmonisation, production and logistics, the hospital exemption pathway, expertise and capacity amongst stakeholders, and public opinion. In conclusion, we identified factors specifically impeding patient access to cell and gene therapies in Europe, as well as factors relevant for medicines in general. These factors should be addressed to improve patient access to these promising therapies. To complement our findings, we aim to elicit impeding factors from the symposium attendants and learn if they (still) recognise the reported factors.

P4 NIEK van der ZEE

Development of a clinical CAR-CCR T cell therapy against multiple myeloma

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Multiple myeloma (MM) is an untreatable hematological tumor of the plasma cells. Chimeric antigen receptor (CAR) T cell therapy, an autologous cell therapy aimed at restoring cancer patient T cell function and targeting tumor cells, has shown great clinical success in MM and might actually be curative. Current MM CAR-T cell therapies are only commercially available, and their high costs have prevented reimbursement in the Netherlands, meaning they will not be available to most Dutch patients. In addition, these CAR-T therapies have long production times and are sensitive to tumor antigen escape and CAR-T cell exhaustion. The purpose of the LSMB is to develop a more potent, affordable MM CAR-T cell therapy for use in clinical trials in the Netherlands that addresses these drawbacks. Tumor antigen escape and CAR-T cell exhaustion are ameliorated by the implementation of a dual CAR-CCR (chimeric costimulatory receptor) molecule. We have developed a closed-system, GMP-compatible production system using CD3/C28 Dynabeads (Thermo Fisher Scientific) and the Cocoon bioreactor (Lonza). We established a 10-day manufacturing procedure that yields multiple patient doses of the final CAR-CCR T cell product. This project contributes to current treatment options for MM patients by establishing a more affordable and effective CAR-T cell therapy with reduced manufacturing complexity.

P5 IDA van der MEULEN

Adenovirus infection-neutralizing and infection-promoting activities in humans treated with RGD-modified oncolytic adenovirus

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Oncolytic adenoviruses used for cancer immunotherapy are commonly derived from Ad5. Neutralizing antibodies (NAbs) might attenuate the efficacy of oncolytic adenovirus treatment. Several oncolytic adenoviruses that are currently in clinical development have modified capsids to increase their infectivity. This includes Ad5-based viruses with a cyclic RGD peptide insert in the fiber protein. The effect of this modification on neutralizing activities in humans is largely unknown. Here, we analyzed NAb titers in the serum of 19 patients with glioblastoma multiforme who were treated with oncolytic adenovirus Ad5-D24.RGD by delivery to the tumor and surrounding brain. Pre-existing and elicited neutralization was determined using an Ad5 vector and an RGD-modified Ad5 vector, to distinguish between neutralization of the two alternative cell entry pathways used by Ad5-D24.RGD. The majority of patients had detectable pre-existing neutralizing activity in their blood against both viruses (generally higher against Ad5 than against Ad5RGD). Upon infusion of Ad5-D24.RGD, Ad5 NAb titers but not Ad5RGD NAb titers rose significantly. In cerebrospinal fluid, neutralizing activity against in particular RGD-mediated infection remained very low. Hence, the RGD-mediated component of virus uptake into cells was less affected by neutralizing immune responses than was natural Ad5 infection. This suggests that repeat administration of RGD-modified oncolytic adenovirus could be efficacious. Interestingly, in the course of our experiments we discovered that the majority of humans have an unidentified factor in the blood that promotes RGD-mediated uptake of recombinant adenovirus in human cells. Partial characterization of this factor suggests that it is distinct from serum proteins known to promote Ad5 infection. It was present in serum and plasma; and heat-inactivation augmented the infection-promoting effect of serum. The putative infection-promoting factor could be separated from neutralizing activity via size exclusion. Full characterization of this blood component might aid in predicting success of oncolytic virotherapy with RGD-modified viruses.