NVGCT Nederlandse Vereniging voor Gen- & Celtherapie

ANNUAL SYMPOSIUM
June 12th and 13th 2023

Congrescentrum De Werelt

Westhofflaan 2, 6741 KH Lunteren

MONDAY JUNE 12th 2023

9.00 **Registration and Coffee**

9.30 **Welcome and Opening Announcements**

Victor van Beusechem, President of the NVGCT

9.45 **KEYNOTE LECTURE I**

MARIA THEMELI, Amsterdam university medical centers, Amsterdam
Next generation of CAR T cell therapy: improved efficacy and availability

10.30 - 10.45 **EXHIBITOR PITCHES**

Twist Biosciences, Thermo Fisher Scientific, Eurofins, BioTechne, BioLamina, ChemoMetec, Necstgen, Miltenyi Biotec BV

Chair: Sabine van der Sanden

10.45 - 12.00 **SESSION I** **Gene Therapy**

Chair: Ingrid de Visser

10.45 **Bas Blits, Netherlands Institute for Neuroscience, Amsterdam**
Capsid modification for optimization of AAV-mediated gene therapy for rare bleeding disorders

11.15 **Coffee break**

11.30 **Peiyu Li, Amsterdam University Medical Centers, Amsterdam**
Human non-small cell lung cancer tumor models on fertilized chicken eggs for preclinical evaluation of cancer gene therapies

11.45 **Niek van Til, Amsterdam University Medical Centers, Amsterdam**
Gene therapy for vanishing white matter

12.00 – 13.00 **NVGCT & ZonMW YOUNG INVESTIGATOR SPEED DATE CHALLENGE**

Chair: Jan Theys

12.00 Introduction / explanation Speed Date Challenge

12.10 **Jella van de Laak (Maastricht University), Selas Bots (Leiden University Medical Center) & Leon Potgeter (University of Lausanne)**

Combining “inside out” C. sporogenes with “outside in” oncolytic virotherapy to improve anti-tumour efficacy in solid tumours

12.30 Speed Dating for Young Investigators (senior researchers can network)

13.00 - 14.00 **LUNCH**

14.00 - 15.30 **SESSION II** **Cell Therapy**

Chair: Toos Daemen

14.00 **Pim Pijnappel, Erasmus Medical Center, Rotterdam**
Cell- and genetic therapies for lysosomal disorders

14.30 **Renee Raaijmakers, Radboud University Medical Center, Nijmegen**
Healthy primary pericytes mitigates disease characteristics in a 2D myogenic cell model for myotonic dystrophy

14.45 **Monica Raimo, Glycostem Therapeutics, Oss**
Stem cell-derived natural killer cells – The new hope for cancer immunotherapy

15.15 **Ellen Stelloo, Cergentis B.V., Utrecht**
Robust transgene integration site analysis and comprehensive genetic QC of genetically modified T-lymphocyte cells

15.30 **Coffee break**

16.00 - 16.50 **COMPANY PRESENTATIONS**

Chair: Hilde van Tongeren

16.00 **Melissa van Pel, Nectsgen, Leiden**
Process development and testing strategy for cell and gene therapies

16.25 **Amiet Chhatta, Thermo Fisher Scientific, Bleiswijk**
Cell & Gene Therapy Solutions by Thermo Fisher Scientific; closed cell therapy manufacturing & viral vector solutions

17.00 - 17.30 **GENERAL ASSEMBLY NVGCT**

17.30 - 19.00 **LEISURE TIME**

19.00 **DINNER**

20.30 **FAMOUS NVGCT PUBQUIZ**

Quizmaster: **Jan Theys**

TUESDAY JUNE 13th 2023

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

9.05 KEYNOTE LECTURE II

ALBERTO AURICCHIO, Telethon Institute for Genetics & Medicine, Naples
ExpEditing AAV gene therapy

Chair: Rob Collin

9.50 - 10.50 SESSION III Cardiac Diseases

Chair: Max Medina Ramírez

9.50 **Geert Boink, Amsterdam University Medical Centers, Amsterdam**
Gene and cell therapies for cardiac arrhythmias

10.20 **Jianan Wang, Amsterdam University Medical Centers, Amsterdam**
SCN10A-short gene therapy for the treatment of cardiac conduction disorders

10.35 **Timo Jonker, Amsterdam University Medical Centers, Amsterdam**
Transcriptional optimization of cardiac gene therapy

10.50 Coffee break

11.00 - 11.45 SESSION IV DNA & RNA editing

Chair: Enrico Mastrobattista

11.00 **Frauke Coppieters, Ghent University, Ghent**
The quest for novel non-coding therapeutic targets for inherited blindness

11.30 **Elena Herrera Carrillo, Amsterdam University Medical Centers, Amsterdam**
CRISPR-Cas therapy towards a cure for HIV/AIDS

11.45 - 12.15 Greiner Award for Best Thesis 2022

Chair: Rob Collin

12.15 - 12.45 NVGCT & ZonMW YOUNG INVESTIGATOR SPEED DATE CHALLENGE

Chair: Jan Theys

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

12.45- 13.30 Lunch

13.30 - 15.15 **SESSION V** **Cancer**

Chair: Vera Kemp

13.30 **Rob Coppes, University Medical Center Groningen, Groningen**
Transplantation of autologous submandibular gland organoids to ameliorate xerostomia after radiotherapy - The RESTART study

14.00 **Darshak Bhatt, University Medical Center Groningen, Groningen**
Modelling the spatial dynamics of oncolytic virotherapy

14.15 **Tereza Brachlova, Amsterdam University Medical Centers, Amsterdam**
Introducing ORCA-020: construction and characterization of a conditionally replicating oncolytic adenovirus encoding constitutively active GSK3β

14.30 **Merve Yildiz, Maastricht University, Maastricht**
Living anticancer *Clostridium sporogenes* colonizes *in vitro* 3D necrotic spheroids

14.45 **Marco Huberts, Erasmus Medical Center, Rotterdam**
Near-patient models for pancreatic cancer for evaluation of oncolytic efficacy of adenovirus, reovirus, Newcastle Disease virus and measles virus

15.00 **Nicole Dam, Leiden University Medical Center, Leiden**
Zeb1 downregulation sensitizes pancreatic cancer-associated fibroblasts to killing by oncolytic reovirus through upregulation of the reovirus receptor Junction Adhesion Molecule A

15.15 **Coffee break**

15.30 **NVGCT OUTSTANDING ACHIEVEMENT AWARD LECTURE**

Chair: Victor van Beusechem

THIERRY VANDENDRIESSCHE, Free Brussels University, Brussels
Towards a cure for hemophilia: 30 years in the making

16.15 - 16.30 **CLOSING CEREMONY**

16.15 *Announcement winner Thermo Fisher Scientific Best Abstract Award*
Announcement winners ZonMW Speed Date

16.30 Meeting Closure
Victor van Beusechem, President of the NVGCT

WE ARE GRATEFUL TO OUR 2023 CONFERENCE PARTNERS

NVGCT Outstanding Achievement Award Winner

THIERRY VANDENDRIESSCHE

TOWARDS A CURE FOR HEMOPHILIA: 30 YEARS IN THE MAKING

Department of Gene Therapy & Regenerative Medicine, Free University of Brussels (VUB), Brussels, Belgium.

The momentum in the field of gene therapy increasing with several gene therapy products approved for the EU and US markets. Hemophilia has been considered as a trailblazer for gene therapy since the 1990s since it is well characterized monogenic disease caused by a defect in the coagulation factor VIII (FVIII) or factor IX (FIX) genes. The delivery of the clotting factor genes into the desired cells requires sophisticated gene delivery vectors. In 1999 we demonstrated that hemophilia A could be cured in FVIII-deficient mice by gene therapy using viral vectors. Currently, the most promising vectors for gene therapy of hemophilia are adeno-associated viral vectors (AAV) and lentiviral vectors yielding sustained therapeutic levels of FVIII or FIX in preclinical models. The performance of these vectors could be improved by altering their capsid or envelopes to maximize cellular entry, modification of the promoters that boost expression levels and/or altering the FIX or FVIII gene itself to increase production and/or activity, such as the FIX Padua. It is particularly encouraging that relatively sustained therapeutic FVIII and FIX expression levels after gene therapy in patients with severe hemophilia A or B reduced bleeding frequency, obviating the need for repeated clotting factor infusions. Recently, these clinical advances resulted in the first approved gene therapy products for hemophilia A and B. Next-generation gene therapies for hemophilia are now emerging based on targeted genome modification using gene editing based on CRISPR/Cas or designer nucleases. Though these encouraging results highlight the potential of gene therapy as a game changer for treating patients afflicted with this type of bleeding disorders, challenges remain with respect to the immune response directed against the vector, the transduced cells or the transgene product.

Grant funding: EU Horizon 2020 UPGRADE - Unlocking Precision Gene Therapy N°825825, FWO, VUB GOA, VUB IOF

KEYNOTE SPEAKERS

MARIA THEMELI

Next generation CAR T cells: rationale combinations and logic gating

Amsterdam university medical centers, Amsterdam, The Netherlands.

Adoptive immunotherapy with chimeric antigen receptor engineered T cells (CAR T) has emerged as a promising therapeutic tool against cancer. Clinical studies of CAR T cell therapy have established that an important mechanism of treatment failure includes the down-regulation or loss of target antigen expression. Multi-specific targeting using a pool of CARs, bi-cistronic CARs or tandem CARs has been proposed as strategies to overcome tumor heterogeneity and antigen expression loss. These strategies focus on targeting a second tumor-antigen with a second CAR or a second CAR-binding moiety and thus, antigen selection is restricted to antigens with limited off-tumor expression. Furthermore, T cell exhaustion and reduced persistence is another major factor limiting the efficacy of CAR T cells. The addition of costimulatory intracellular components has provided second generation CAR T cells with enhanced activation, expansion and *in vivo* persistence. Multiplex targeting and co-stimulation through co-expression of a CAR and CCR can enhance CAR T cell therapy by concomitantly augmenting cytotoxic efficacy, that includes tumor cells with low antigen expression, as well as persistence. On another note, the widespread application of CAR T cell therapy, especially in solid tumors, is limited by the lack of truly tumor-restricted targets leading to unwanted on-target/off-tumor toxicities on normal tissues. The paucity of tumor-restricted antigens has incited the development of engineering concepts to spatially control CAR T cell function. Designing chimeric receptor systems using rationale combinations of targets, co-stimulatory signals and logic-gating expression circuits can lead to the next generation of CAR T cells with improved efficacy and safety profiles.

ALBERTO AURICCHIO

ExpEditing AAV gene therapy

Telethon Institute for Genetics and Medicine (TIGEM), Naples, Italy.

In vivo gene therapy with adeno-associated viral (AAV) vectors its holding its promise for treatment of genetic diseases, yet some challenges remain that prevent to expand this approach to a larger patients population. These include: AAV cargo capacity limited to about 5 kb which prevents their application to conditions due to mutations in genes with a larger coding sequence; the episomal status of AAV genomes which results in short-term expression in proliferating tissues; efficient approaches to target toxic gain-of-function mutations that do not benefit from traditional gene addition. To overcome these limitations, we have developed platforms based on the co-delivery of 2 AAV vectors that either EXPand AAV transfer capacity or EDIT genomic loci by stably integrating therapeutic donor DNAs. We provide proof-of-concept of the efficacy and safety of these approaches in animal models of human inherited diseases.

INVITED SPEAKERS

BAS BLITS

Capsid modification for optimization of AAV-mediated gene therapy for rare bleeding disorders

B Blits[1], A D'Amico[2], J van den Herik[3], F de Winter[3], P Kaijen[2], B. Hobo[3], J Verhaagen[3], J Voorberg[2] and D Mosmuller[1]

[1]SanaGen B.V. Amsterdam, The Netherlands; [2]Dept. of Molecular Hematology, Sanquin Research and Landsteiner Laboratory of Amsterdam UMC, Amsterdam, The Netherlands; [3]Dept. of Neuroregeneration, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands.

FVII deficiency is a rare autosomal recessive bleeding disorder resulting from variants in the gene encoding FVII with a prevalence in western countries of approximately 1:300.000-500.000 individuals. At the moment, the only available treatment is a recurrent and lifelong factor concentrate infusion, which is both a burden for the patients and is linked to high costs for the healthcare system. A gene therapeutic approach could possibly serve as a lifelong cure for patients suffering from FVII deficiency.

Our proof of concept studies in mice show that it is possible to obtain physiological levels of FVII at relatively high vector dose. To further limit vector dose we set out to modify our gene therapy vector using the following approach. First, we designed a novel expression cassette driving high levels of FVII expression. Secondly, we employed capsid-engineering by inserting ligands known to bind liver cells into the AAV capsid.

Our results suggest that capsid engineering of surface-exposed loops successfully generated several correctly packaged AAV candidates that efficiently targeted a human hepatocyte cell line in vitro. Furthermore, in vitro selection of novel rationally designed liver-specific promoters also showed multiple candidates with high liver specificity. A modified FVII cDNA which lacked a portion of the FVII pro-peptide revealed an increased expression in vitro. Proof-of-concept in vivo studies employing the "short" FVII version showed that AAV-directed gene therapy can successfully express and release biologically active hFVII protein into the circulation. hFVII antigen levels were such that they could potentially rescue the bleeding phenotype.

Our results support further (pre)clinical development of the AAV-directed gene therapy approach for FVII deficiency using AAV8 derived vectors encoding for the short version of the FVII.

NIEK VAN TIL

Gene therapy development for vanishing white matter

Department of Child Neurology, Amsterdam Leukodystrophy Center, Emma Children's Hospital, Amsterdam University Medical Centers, VU University, and Amsterdam Neuroscience, Cellular & Molecular Mechanisms, Amsterdam, The Netherlands.

Vanishing white matter (VWM) is a rare inherited disorder that affects the white matter of the brain. VWM typically begins in early childhood, although it can manifest at any age. It is caused by mutations in one of five genes (EIF2B1-5) encoding subunits of a complex called eukaryotic initiation factor 2B (eIF2B), which plays a crucial role in protein synthesis and the normal functioning of cells. It is characterized by central nervous system (CNS) white matter degeneration, causing slowly progressive ataxia, spasticity and cognitive decline with stress-provoked episodes of rapid and major deterioration. VWM leads to progressive handicap and early death. There is no curative therapy available. We aim to develop gene therapy applications to provide long-lasting therapeutic correction in a well-characterized VWM murine model (Eif2b5R191H/R191H). Hence, we tested state-of-the-art base-editing technology to directly correct the mutation in the CNS to prevent disease symptoms. Adeno-associated virus (AAV) vectors with neurotropic capsid expressing adenine base editors were administered via intracerebroventricular (ICV) injection in neonatal VWM mice. This resulted in partial molecular and phenotype correction. In addition, we also observed off-target base editing and exacerbation of motor function in both VWM mice and healthy controls. In another study, we administered AAV vectors for EIF2B5 gene augmentation, which resulted in amelioration of motor function. This latter approach appears to be a viable option for further efficacy and safety assessment as a potential treatment with curative intent.

PIM PIJNAPPEL

IDS-tagging reveals efficient and cell engraftment-independent correction of brain pathology by lentiviral gene therapy for Mucopolysaccharidosis type II

F. Catalano[1,2,3], E. C. Vlaar[1,2,3], Drosos Katsavelis[1,2,3], Z. Dammou[1,2,3], T. F. Huizer[1,2,3], J. van den Bosh[1,3], M. Hoogeveen-Westerveld[1,3], H. van den Hout[2,3], E. Oussoren[2,3], G. Ruijter[1,3], G. Schaaf [1,2,3], K. Pike-Overzet[4], F. J.T. Staal[4,5], A. T. van der Ploeg[2,3], W.W.M. Pijnappel[1,2,3]

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Mucopolysaccharidosis type II (OMIM 309900) is lysosomal storage disorder (LSD) caused by Iduronate 2-sulfatase (IDS) deficiency and accumulation of glycosaminoglycans, leading to severe progressive neurodegeneration in pediatric patients. As intravenously infused enzyme replacement therapy cannot cross the blood-brain barrier (BBB), it fails to treat the brain, highlighting the unmet medical need to develop alternative therapies. We have tested modified versions of Hematopoietic Stem and Progenitor Cell (HSPC)-mediated Lentiviral Gene Therapy (LVGT) using IDS-tagging in combination with the strong ubiquitous MND promoter to optimize efficacy in brain and to investigate its mechanism of action. We found that tagging of IDS to improve brain-targeting or lysosomal delivery strongly improved correction of brain heparan sulfate and neuroinflammation at clinically-relevant vector copy numbers. HSPC-derived cells engrafted in brain areas with efficiencies that were highest in perivascular areas, lower in choroid plexus and meninges, and lowest in parenchyma. The efficacy of correction of brain pathology was independent of the number of brain-engrafted HSPC-derived cells, but correlated with plasma levels of tagged IDS protein. These results indicate that tagged versions of IDS can outperform untagged IDS in HSPC-LVGT for the correction of brain pathology in MPS II, and they imply that the mechanisms involved include both cell-mediated and protein-mediated passage across the BBB.

MONICA RAIMO

Stem cell derived Natural Killer Cells - The New Hope for Cancer Immunotherapy

Glycostem Therapeutics, Oss, The Netherlands.

Natural Killer (NK) cells are innate immune effectors with the unique property of being able to recognize and kill tumor or infected cells by an array of germline-encoded surface receptors, without prior sensitization. Thanks to their highly cytotoxic, non-MHC-restricted function, NK cells have strong capability to become successful immunotherapies. Adoptive transfer of allogeneic, “off-the-shelf” NK cells has so far been remarkably safe and has shown promising efficacy in hundreds of patients in Phase I-II clinical trials.

Glycostem® Therapeutics is a clinical-stage company focused on the development of NK cell therapies for the treatment of solid and hematological malignancies. Glycostem developed a closed, automated and feeder-free system (called uniK™) for *ex vivo* expansion and differentiation of umbilical cord blood-derived $CD34^+$ stem cells into highly functional, cryopreserved, off-the-shelf NK cells. The first product, non-engineered NK cells (GTA002/inaleucel/oNKord®) is currently being evaluated in a Phase I/II clinical trial in acute myeloid leukemia (AML) patients (WiNK study, NCT04632316). From the dose escalation cohorts, NK cell infusion showed outstanding safety, chimerism in peripheral blood, and Minimal Residual Disease (MRD) conversion.

Glycostem is also very active in developing novel therapeutic products, especially for the treatment of solid tumors, which remain a challenge for cell therapies. Difficulties in homing to the tumor site, to resist the immune suppressive tumor microenvironment and to efficiently kill cancer cells can be overcome using NK cells in combination therapies and/or by genetic engineering. Combination with antibodies can unleash the potential of NK cells to mediate Antibody-Dependent Cellular Cytotoxicity (ADCC) through surface CD16; similarly, NK Cell Engagers (NKCEs) induce the co-engagement of NK cell receptors with tumor cell ligands, facilitating interaction and killing of targets. Preclinical *in vitro* and *in vivo* data with non-engineered NK cells show great potential of developing combination therapies against glioblastoma, ovarian and breast cancer. In parallel, genetic engineering of NK cells (viveNK™) with Chimeric Antigen Receptor molecules (CAR-NK) is also a favourable strategy; from *in vitro* data, NK-resistant B cell lymphoma cell lines can be efficiently killed by CAR-mediated CD19 antigen targeting on the tumor cell surface.

In summary, Glycostem’s mission is to improve cancer patients’ lives by the use of NK cell therapies, unleashing their unique potential for a next-generation of cancer immunotherapies.

GEERT BOINK

AAV-mediated biological pacemaker activity in a porcine model of complete atrioventricular block

Departments of Cardiology and Medical Biology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers, Amsterdam, The Netherlands.

Background: In an effort to develop hardware-free pacemakers, we have engaged into the development of gene therapy-based biological pacemakers by means of ion channel overexpression. To this end, we report our progress with regard to Hcn2/SkM1 gene delivery and related strategies, mediated by adeno associated virus (AAV) based delivery.

Objective: To assess the long-term efficacy of biological pacing based on AAV-mediated gene transfer of Hcn2/SkM1.

Methods: Functional biological pacemaker studies were conducted in a porcine model of radiofrequency ablation-induced complete atrioventricular block. Four weeks after the ablation, animals were distributed in four different groups: non-injected, saline, and AAV6-Hcn2/SkM1 with or without immunosuppression. All animals were followed for another four to six weeks to evaluate in vivo biological pacemaker performance and then hearts were harvested to assess transduction efficiency.

Results: One week after gene transfer, Hcn2/SkM1-transduced animals displayed a notable increase in maximal beating rates above 100 beats/min, mean heart rates only modestly trended up, yet a remarkable reduction of electronically paced beats was evident with a backup pacing of less than 60%. Quantification of gene transfer efficiency in the injection sites showed considerable expression levels of both transgenes (Hcn2 and SkM1) in transduced animals at four and six weeks post-injection.

Conclusion: AAV-mediated Hcn2/SkM1 gene transfer in CAVB pigs resulted in a biphasic pacing response with an acute and a chronic component. We hypothesize that the acute component largely stems from ion channel overexpression in combination with a local tissue inflammatory response. The latter was likely also an important contributor to the phenotype observed in preceding studies with adenoviral vector-mediated gene transfer.

FRAUKE COPPIETERS

The quest for novel non-coding therapeutic targets for inherited blindness

M. Bouckaert[1], A. Dueñas Rey[1], Marta del Pozo Valero[1,2], F. Van den Broeck[3,4], M. Daich Varela[5,6], Genomics England Research Consortium, G. Menschaert[7], C. Rivolta[8,9], C. Ayuso[2,10], J. De Zaeytijd[3,4], B.P. Leroy[1,3,4], K. Remaut[11], E. De Baere[1], F. Coppieters[1,11]

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Antisense oligonucleotides (ASOs) are an emerging therapeutic strategy due to their easy synthesis, high specificity, and relatively fast clinical implementation. Most clinically approved ASOs modulate gene expression through either mRNA degradation or splice modulation, and many ASOs are in pre-clinical development. A selection of ASOs is directed against single mutations or exons, which limits wide applicability for inherited diseases displaying high allelic heterogeneity.

Here, we aimed to identify novel, non-coding therapeutic targets for ASOs, specifically located in the 5' untranslated region (5'UTR). The 5'UTR is a major determinant of post-transcriptional control and translation efficiency. 5'UTRs harbor numerous *cis*-regulatory elements which can recruit ribosomes and initiate translation, as well secondary structures which influence mRNA translation. Using inherited retinal disease (IRD) as a proof of concept, we identified and validated translation-regulatory elements in IRD disease genes in the human retina and retinal pigment epithelium. Furthermore, we investigated 5'UTR variation in over 4,000 IRD patients, resulting in the identification and functional characterization of potential pathogenic variants affecting translation efficiency. Overall, these translation-regulating elements represent novel ASO targets to modulate protein translation.

ROB COPPES

Transplantation of Autologous Submandibular Gland Organoids to Ameliorate Xerostomia After Radiotherapy – The RESTART study

Departments of Radiation Oncology and Biomedical Sciences of Cell & Systems, Section Molecular Cell Biology; University Medical Centrum Groningen, University of Groningen, Groningen, The Netherlands.

Severe hyposalivation and consequential xerostomia (dry mouth syndrome) are common, often irreversible side effects of radiotherapy treatment for head-and-neck cancer. Symptoms include alterations in speech and taste, difficulties with mastication and deglutition, and an increased risk of developing oral infections and dental caries. Xerostomia severely hampers the quality of affected patients' lives. Currently, no successful treatment exists - radiation-induced hyposalivation results from an inability of the tissue's stem cells to produce enough mature functional cells. The aim was to develop a stem cell therapy to treat radiation-induced hyposalivation.

We developed a GMP-compliant protocol for isolating and expanding human-derived salivary gland organoids derived from patient submandibular gland biopsies taken before radiotherapy treatment with an efficiency comparable to current non-GMP research-based protocols. The functionality of salivary gland-derived cells is maintained after cryopreservation allowing the protocol to be adapted to the radiotherapy treatment schedule of the patients. This presentation will show the developmental path taken to come to the first in human application of autologous organoid derived cell transplantation in head and neck cancer patients,

Funding: Supported by the Dutch Cancer Society (KWF, grant No. 10650 and 12092) and The Netherlands Organization for Health Research and Development (ZonMw, Grant nrs. 11.600.1023 and 40-43600-98-14003).

SELECTED ABSTRACTS

Human Non-Small Cell Lung Cancer Tumor Models on Fertilized Chicken Eggs For Preclinical Evaluation of Cancer Gene Therapies

Jing Li[1], Peiyu Li[1], Ida van der Meulen Muileman[1], Tereza Brachtlova[1,2], Puck Veen[1], Stijn Kleerebezem[1], Idris Bahce[3], Victor W. van Beusechem[1,2]

Amsterdam UMC location Vrije Universiteit Amsterdam, [1]Medical Oncology, [3]Pulmonary Diseases, Cancer Center Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands; [2]ORCA Therapeutics B.V., Onderwijsboulevard 225, 5223 DE 's-Hertogenbosch, The Netherlands.

Background: Non-small-cell lung cancer (NSCLC) has a high disease burden and is a major cause of cancer death worldwide. With current treatments falling short, there is a high need to develop more effective therapies. Relevant preclinical models are necessary to test new treatment options, such as experimental gene therapies. To this end, we developed 5 different human NSCLC xenograft tumor models on fertilized chicken eggs.
Methods: A549, H292, H460, H1299 and SW1573 NSCLC cell suspensions in Matrigel were inoculated on the chorioallantoic membrane (CAM) of fertilized chicken eggs at embryo development day (EDD)6. Tumor engraftment was assessed on EDD9. Tumor growth, histology and embryo survival were analyzed until EDD18. Cisplatin/pemetrexed chemotherapy (often used to treat patients with advanced NSCLC) was given systemically and tumor growth inhibition was measured. Gene delivery methods (intratumoral injection and systemic delivery via the CAM) were tested using lentiviral vectors and adenoviral vectors expressing Firefly luciferase (LV-Luc and AdV-Luc). Luminescence was measured by Ivis imaging. Viral vector biodistribution was measured using qPCR.
Results: Tumor take rates were above 80% for all 5 NSCLC cell lines and >90% embryos remained vital until the end of experiments at EDD18. The NSCLC-CAM tumors displayed different growth rates, with A549 and H460 showing the slowest and fastest growth, respectively. Chemotherapy significantly inhibited A549 and H460 tumor growth, validating the NSCLC-CAM tumor models for preclinical therapy evaluation. Firefly luciferase gene delivery to A549 and H460 tumors was successful via both intratumoral injection and administration onto the CAM using either gene transfer vector. However, gene delivery efficiency was at least 100-fold higher using AdV-Luc than could be reached with LV-Luc. When AdV-Luc was used, also luminescence in CAM tissue was observed reproducibly and in embryo liver and kidney occasionally. AdV-Luc biodistribution analysis revealed that the vector was also delivered to embryo lung, heart, liver, kidney and spleen tissues, albeit 100-1000-fold less efficiently than to NSCLC tumor and CAM.
Conclusion: Human NSCLC tumors can be established on the CAM with high take rate, providing convenient *in vivo* models for preclinical testing of experimental anti-tumor therapies. Gene delivery into these tumors using adenovirus vectors is very efficient. Currently, we are using primary lung cancer cells from patient tissue biopsies to develop patient-derived NSCLC-CAM tumor models. This should maximize the relevance of preclinical research findings, guiding clinical development of new treatment for patients afflicted by NSCLC.

Healthy primary pericytes mitigates disease characteristics in a 2D myogenic cell model for myotonic dystrophy

Renée H. L. Raaijmakers[1], C. Rosanne. M. Ausems[1], Marieke Willemse[2], Walther J. A. A. van den Broek[2], Baziel G. M. van Engelen[3], Hans van Bokhoven[1], Derick G. Wansink[2]

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Background: Myotonic dystrophy type 1 (DM1) is the most common form of adult muscular dystrophy caused by an expanded CTG repeat in *DMPK*. This toxic expanded repeat leads to the generation of repeat RNA-containing nuclear foci, which in turn sequester MBNL1 protein, leading to a cascade of mis-spliced proteins. We aim to set up a personalized cell therapy to alleviate the muscular phenotype by addition of pericytes, a cell type that can be isolated from post-natal tissue with the unique capacity to travel through the circulation to fuse with damaged muscle, giving rise to the possibility of an intra-arterial cell therapy approach. Previously, we isolated pericytes, a distinct class of muscle progenitor cells, from quadriceps muscle of six DM1 patients and two healthy individuals[1]. Here, we report on a detailed analysis of their myogenic behavior and effects on DM1 hallmarks in differentiating 2D co-cultures with myoblasts.
Methods: We differentiated pericytes to myotubes and determined *DMPK* and *MHC* expression by RT-qPCR. Furthermore, we looked at RNA foci with RNA FISH and MBNL1-antibody staining. Co-cultures of primary pericytes with DM1 myoblasts were performed to determine myogenic fusion capacity. RNA foci and nuclear MBNL1 protein behavior was examined by CSLM in composite myotubes.
Results: During differentiation of pericytes the increase in myogenic differentiation marker *MHC* coincided with higher expression of *DMPK*. Quantification of RNA foci in nuclei of MHC-negative cells and in MHC-positive myotubes showed a significant increase in RNA- and MBNL1-positive foci for patient pericyte-derived myotubes, but not for control pericyte-derived myotubes. We also found a significant difference between the fusion capacity of healthy and disease pericyte populations. Furthermore, we found a decrease in the amount of RNA foci and a gain in their size in DM1 nuclei with an increasing percentage of healthy pericytes in the myotubes. When looking at free nuclear MBNL1 as a proxy for disease severity, we found that mixed myotubes made up of >11% healthy pericyte nuclei displayed a significantly higher free MBNL1 concentration in DM11 nuclei after 5 days in culture (C1: $p = 0.0018$; C2: $p = 0.0003$).
Conclusion: We show that differentiated DM1 pericyte cultures exhibit disease hallmarks such as RNA- and MBNL1 foci. Healthy primary pericytes are better able to fuse with DM1 myotubes, providing evidence that healthy pericytes have a higher intrinsic myogenic capacity than those from DM1 patients. This underscores the importance of genetic correction of patient cells in a cell therapy. Furthermore, the decreased amount of RNA foci and increased amount of free MBNL1 in DM1 nuclei in mixed co-cultures with healthy pericytes build the foundation for our cell therapy approach to alleviate the muscle phenotype in DM1 patients.

Robust transgene integration site analysis and comprehensive genetic QC of genetically modified T-lymphocyte cells

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Background: Retroviral and lentiviral vectors are commonly used to genetically engineer T cells due to their high rates of transduction and long-lasting stable transgene expression. Since these vectors permanently integrate into the host cell's genomic DNA, thorough genetic characterization and QC of integration sites is essential to ensure the safety, efficacy, and stability of T-cell therapy products. Here, we genetically characterized transgenic cell lines by Targeted Locus Amplification (TLA).
Methods: The NIST genome editing consortium provided 5 reference T-cell lines with zero to four lentiviral vector integrations per cell (samples VCN0-5)[1]. Through serial dilutions with non-transgenic cells, samples with three different proportions of the transgenic cells (0.1%, 1%, 100%) were produced. TLA, a NGS technology based on proximity ligation, was performed on these samples with primer sets specific to the vector sequence to generate broad sequencing coverage into the host genome for hypothesis-free integration site detection. A peak detection tool for automatic and unbiased detection of these coverage peaks across the whole genome was used to identify integration sites. Small or structural variants in the vector sequence or in the host genome around integration sites were also assessed in the NGS data set. Alongside, droplet digital PCR (ddPCR) was performed to accurately determine the vector copy number (VCN) per cell.
Results: Based on the broad coverage surrounding the integration sites, the peak detection tool identified all ten integration sites down to 1% and some even down to 0.1%. The previously reported integration on chromosome 4[1], placed in a region with non-unique sequences could not be confirmed. Instead, supported by broad coverage across the integration locus, TLA could unambiguously place this integration site on chromosome 16. The coverage also indicated a 648kb genomic deletion near the integration site on chromosome 14, whereas no genomic structural variants were observed near or at the other integration sites. The integration site detection on nucleotide level allowed to assess potential insertional oncogenesis. Seven of the ten integration sites occurred intragenic, however none of the affected genes were listed as cancer genes in the *NCG7.0 network of cancer genes*. Vector integrity analysis revealed a small sequence variant in the integrated vector sequence of three samples. Based on the variant frequency, these variants are only present in one of the integrated vector copies. Finally, ddPCR confirmed all integration sites and showed 1-4 vector copies per cell in the VCN samples.
Conclusion: Altogether, the data obtained for the NIST reference samples indicates that TLA is a powerful technology that can address several key genetic characteristics simultaneously in clonal and heterogeneous T-cell populations, with high accuracy and specificity. Such genetic QC efforts are relevant for genetically engineered cell products, which usually have limited quantities, while extensive characterization is required.

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[1] Paugh, B.S., Baranyi, L., Roy, A. et al. Reference standards for accurate validation and optimization of assays that determine integrated lentiviral vector copy number in transduced cells. Sci Rep 11, 389 (2021).

SCN10A-short Gene Therapy for the Treatment of Cardiac Conduction Disorders

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The cardiac voltage-gated sodium channel $Na_V1.5$ is an important therapeutic target to treat cardiac conduction disorders, such as Brugada syndrome and isolated cardiac conduction defect. However, the large size of *SCN5A* encoding $Na_V1.5$ imposes a substantial challenge to gene therapeutic interventions. Alternatively, small proteins that modulating sodium current can be good gene therapeutic targets. We previously reported that a naturally occurring short transcript encoding a C-terminal portion of neuronal *SCN10A (SCN10A-short)* increases sodium current in an *SCN5A*-dependent manner. In the present study we developed an AAV-based *SCN10A-short* gene therapy for the treatment of cardiac conduction disorders.

First, we studied the effect of *SCN10A-short* overexpression in wild-type mouse cardiomyocytes using single cell patch-clamp. *SCN10A-short* overexpression resulted in a significantly larger sodium current and faster action potential upstroke. We next studied whether *SCN10A-short* gene therapy could improve cardiac conduction in $Na_V1.5$ haploinsufficient mice. Heterozygous *Scn5a* mutant mice were treated with either moderate dose or high dose *SCN10A-short* gene therapy and mice that received treatment with moderate dose GFP gene therapy were used as control. Two weeks post injection, hearts were isolated and perfused on a Langendorff setup, where optical mapping was performed to measure epicardial conduction. Compared with controls, mice receiving moderate dose *SCN10A-short* gene therapy showed significantly increased conduction velocity in the left ventricle. Strikingly, the high dose *SCN10A-short* gene therapy led to faster conduction velocities in both ventricles, which was comparable to wild-type hearts. Electrocardiogram (ECG) analyses revealed significantly shorter QRS duration which is similar to wild-type, in high dose *SCN10A-short* treated animals as compared with controls, reflecting the improved cardiac conduction.

To validate our gene therapy in human cells, we also tested the effect of *SCN10A-short* overexpression in cardiomyocytes obtained from human induced pluripotent stem cells. Single cell patch-clamp experiments showed a significantly higher action potential upstroke velocity compared with GFP-treated control cells, demonstrating a substantial increase in *SCN10A-short* induced sodium current in a human background. Finally, we tested the *in silico* effects of *SCN10A-short* on human ventricular conduction velocity and excitability in case these were affected by a severe loss-of-function mutation in *SCN5A*. In a linear strand of left ventricular cells, the conduction slowing and excitability loss caused by the mutation were largely restored with *SCN10A-short.* In a linear strand model with multiple branches, the mutation-induced partial conduction block in the branched part of the strand was fully restored by the application of *SCN10A-short.* In summary, our results highlight the potential of *SCN10A-short* gene therapy as an effective therapeutic intervention to normalize cardiac conduction in inherited and acquired conduction disorders.

Transcriptional optimization of cardiac gene therapy

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Background: In the past decade, gene therapy has taken a leap from the lab to the clinic with several therapies being approved for clinical applications. The field of cardiac gene therapy has also seen several clinical trials, among others aimed at heart failure. However, these trials have not had satisfactory outcomes, despite encouraging pre-clinical outcomes in the treatment of heart failure. The challenges in cardiac gene therapy development have been attributed to several factors, from transgene ineffectiveness to immune responses and lower-than-expected expression. These hurdles call for optimization of all aspects of gene therapy interventions. In this project, our aim is to optimize the promoter of cardiac gene therapy vectors beyond the current state of art.
Methods: We have selected promoters from a set of highly expressed cardiac genes, and have tested these in vitro in a cardiomyocyte cell line using luciferase assays. A further selected set was produced as AAV9 reporter vectors in mouse. 5*10^11 vg/animal was systemically injected and luciferase assays on tissue homogenates were performed after 4 weeks to measure expression.
Results: In vitro screenings revealed 8 novel promoter candidates with significantly higher expression in a cardiomyocyte cell line compared to the benchmark TNNT2 promoter, while all were significantly weaker compared to the non-specific CMV promoter. Subsequent in vivo injection of a subset of 6 novel promoter and luciferase assays on tissue homogenates revealed none of them was stronger, nor more specific than the benchmark TNNT2 promoter. An additional finding was vector build-up in the liver compared to other organs, while other work has also shown leaky expression of the TNNT2 promoter in this organ. These off-target effects may necessitate further optimization of the capsid and transcriptional properties of cardiac-targeted gene therapy vectors.
Conclusion: Out of tested promoters, the CMV candidate yields strongest, but non-specific, expression in the heart after systemic injection. The TNNT2 promoter yielded 2.5x lower expression, however the expression was more specific, with over 10x higher heart/liver ratio. No novel tested candidates outperformed the CMV or TNNT2 promoter.

CRISPR-Cas therapy towards a cure for HIV/AIDS

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Background: Many potent antiviral drugs are currently available to treat HIV infection and combination therapy has saved many lives. However, antiviral therapy has to be continued life-long because HIV rebounds from an established reservoir when therapy is interrupted. HIV DNA in the reservoir can be targeted by the CRISPR-Cas genome-editing tool. This system consists of the Cas nuclease that cuts double-stranded DNA and a guide RNA (gRNA) that directs Cas to a complementary sequence in the DNA. Our laboratory demonstrated potent and durable HIV inhibition in a combinatorial attack with two gRNAs. Although remarkable, the large size of CRISPR-spCas9 transgene cassettes impedes their implementation in gene therapy applications with vectors that have a limited packaging capacity, including lentiviral vectors. There is a serious need for more simple/smaller CRISPR-Cas vector designs.
Methods: We propose to minimize the size of the lentiviral vector by: a) adopting the small H1 Pol-III promoter that we recently found to exhibit both Pol-III and Pol-II promoter activity for the production of both the gRNA and Cas9-encoding mRNA and b) incorporating a smaller saCas9 or cjCas9 nuclease. These measures will reduce the vector size and likely increase the vector titer.
Results: We have compared the different CRISPR-Cas systems for their efficiency in terms of antiviral activity and viral titer. Virus inhibition was tested in HIV replication studies which allow us to test for viral escape (in long-term cultures) and a potential CURE of the infected cells. The viral gRNA-targets were sequenced to elucidate the mechanism of viral escape or the means of provirus inactivation in case of a CURE. Superior antiviral activity is reported for saCas9 compared to cjCas9, which can achieve full HIV inactivation in cell culture with only a single gRNA. We also disclose that DNA cleavage by the saCas9 and cjCas9 endonucleases and subsequent DNA repair cause mutations with a sequence profile similar to spCas9.
Conclusions: We demonstrated that reduction of the vector size (smaller Cas9 nuclease and dual-polymerase active H1 promoter) increases the vector titer. This greatly facilitates the use of viral vectors with a limited packaging capacity. These results are important in the path towards formulation of a highly effective cure strategy.

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Modelling the spatial dynamics of oncolytic virotherapy

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Background: Oncolytic virotherapy is a promising form of cancer treatment that uses native or genetically engineered viruses to target, infect and kill cancer cells. Unfortunately, this form of therapy is not effective in a substantial proportion of cancer patients, partly due to the occurrence of infection-resistant tumour cells.
Methods: To shed new light on the mechanisms underlying therapeutic failure and to discover strategies that improve therapeutic efficacy we designed a cell-based model of viral infection. The model allows us to investigate the dynamics of infection-sensitive and infection-resistant cells in tumour tissue in the presence of oncolytic viruses. To reflect the importance of the spatial configuration of the tumour on the efficacy of virotherapy, we compare three variants of the model: two 2D models of a monolayer of tumour cells and a 3D model. By analyzing the model systematically, we demonstrate the importance of spatial interactions, the effects of viral properties (such as replication rate and range of infection), the properties of virus-resistant cancer cells, and the sensitivity of healthy cells towards viral infection.
Results: The models predict various therapeutic outcomes when resistant cancer cells arise at low frequency in the tumour. These outcomes depend in an intricate but predictable way on the death rate of infected cells, where faster death leads to rapid virus clearance and cancer persistence. Our simulations reveal three different causes of therapy failure: rapid clearance of the virus, rapid selection of resistant cancer cells, and a low rate of viral spread due to the presence of infection-resistant healthy cells. Our models suggest that improved therapeutic efficacy can be achieved by sensitizing healthy stromal cells to infection, although this remedy has to be weighed against the toxicity induced in the healthy tissue.
Conclusion: Our cell-based model provides new insights into the mechanisms underlying therapeutic failure in oncolytic virotherapy, and suggests potential strategies for improving therapeutic efficacy.

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Introducing ORCA-020: construction and characterization of a conditionally replicating oncolytic adenovirus encoding constitutively active GSK3β

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Background: Conditionally replicating oncolytic adenoviruses (CRAds) not only have the capacity to lyse tumor cells, they can also help combat immune suppression in the tumor microenvironment (TME) by stimulating the host's immune response. This dual activity can lead to effective in-vivo vaccination, eliciting an antitumor T-cell response. As such, CRAds may potentiate the efficacy of immune checkpoint blockade (ICB), which in advanced melanoma has so far shown only limited success with durable responses in a minority of patients. Arming CRAds with different transgenes has previously proven successful and could improve their oncolytic and immune activating potency. Here, we present a novel oncolytic adenovirus ORCA-020, which contains an expression cassette for constitutively active Glycogen synthase kinase-3β (CA.GSK3β) in the ORCA-010 backbone. We previously reported on the generation and oncolytic efficacy of ORCA-010, carrying the safety-enhancing E1AΔ24 deletion, the potency-enhancing T1 mutation, and the infectivity-enhancing fiber RGD modification. Active GSK3β has a critical involvement in oncogenesis inhibition by promoting the degradation of β-catenin. Interestingly, β-catenin was shown to be responsible for decreased secretion of CXCL9 and CXCL10 by tumor-infiltrating dendritic cells (DCs), resulting in lack of T-cell infiltration into the melanoma TME and hampered ICB. By local expression of the CA.GSK3b transgene, we aim to overcome melanoma-induced suppression of myeloid cells and provide a multifactorial immune activating approach.
Methods: An SCP1 promoter-driven CA.GSK3β transgene was introduced in the ORCA-010 backbone by homologous recombination, followed by virus production in A549 cells and characterization of purified virus by sequencing, restriction analysis, PCR and Western Blot. Oncolytic potency of ORCA-020 was assessed on different melanoma cell lines and compared to that of a panel of other CRAds. The immune modulatory effect of ORCA-020 on melanoma-conditioned monocyte-to-DC differentiation was tested in co-cultures of monocytes and a panel of established as well as patient-derived melanoma cell lines.
Results: The successful construction and integrity of ORCA-020 was confirmed by molecular methods and the expression of the transgene was demonstrated in multiple melanoma cell lines infected with the new virus. ORCA-020 showed equal or enhanced oncolytic potency when compared with ORCA-010 and AdΔ24RGD. Moreover, the immune stimulatory capacity of ORCA-020 on DC development and activation was demonstrated by observing a reduced melanoma-induced M2-like macrophage differentiation and increased expression of co-stimulatory markers like CD83 and CD86 in monocyte and melanoma co-cultures. Additionally, these results coincided with a reduction of VEGF secreted levels by melanoma cells infected with ORCA-020.
Conclusion: The novel ORCA-020 CRAd encoding CA.GSK3β appears to be an effective tool to overcome melanoma-induced suppression of myeloid cells in the TME and together with its oncolytic properties may work in tandem with ICB for future enhanced oncolytic immunotherapy.

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Living anticancer *Clostridium sporogenes* colonizes *in vitro* 3D necrotic spheroids

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Background: Most solid cancers contain areas of necrosis resulting from chronic hypoxic injury. These high-risk tumour characteristics are associated with poor prognosis due to increased risk of metastasis and resistance to chemotherapy and radiotherapy. Strikingly, this necrotic environment also provides an opportunity for targeted therapy, such as our alternative anti-cancer approach that uses the harmless anaerobic bacteria *Clostridium sporogenes (C. sporogenes)*. Upon injection as spores, this species selectively penetrates necrotic areas and germinates into metabolically active vegetative bacteria that can produce anticancer effects inside the tumour. We have developed innovative genetic tools to further enhance these effects by "arming" *C. sporogenes* with therapeutic genes such as immune checkpoint inhibitors and cytokines. Novel therapeutic strains can be generated effectively in a short period of time. Therefore, it would be essential to evaluate and validate them in robust systems prior to *in vivo* studies because considering the Three Rs principle of animal research, the use of animals should be reduced or replaced if possible. It would thereby be beneficial to develop an *in vitro* validation method that can mimic tumour necrosis as an intermediate step.
Methods: The Lewis Lung Carcinoma (LLC) cell line was used to generate 3D spheroids. Spheroids were first investigated on the development of necrotic fractions. Next, 5,000 colony-forming unit per millilitre (CFU/mL) of spores were added on different days to determine and optimize the experimental time frame of our spheroid model. The CFU/mL in spheroids was determined for each time point. Microscopic images were taken every day to follow-up spheroid growth. Furthermore, a *C. sporogenes* strain expressing the fluorescent reporter gene unaG was investigated as a live imaging tool in the spheroids.
Results: LLC spheroids developed a necrotic core on day 5 after seeding. When spores were added on this day, *C. sporogenes* colonized the spheroids for 24, 48, and 72 hours, with 48 and 72 hours showing the highest number of bacteria inside the spheroids. Colonized spheroids started to disintegrate around 96 hours due to the antitumour effects of *C. sporogenes*.
The fluorescent unaG strain was live imaged in the spheroid model. Fluorescent intensity positively correlated to CFU/mL and fluorescent signal was diminished after spheroid disintegration.
Conclusion: These promising results show the high potential of 3D *in vitro* models that can potentially serve as important intermediate validation step before embarking *in vivo* studies. These models will be applied for testing therapeutic efficacy and antibiotic sensitivity of our *C. sporogenes* strains.

Near-Patient Models for Pancreatic Cancer for Evaluation of Oncolytic Efficacy of Adenovirus, Reovirus, Newcastle Disease Virus and Measles Virus

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Background: Pancreatic ductal adenocarcinoma (PDAC) is the fourth most deadly cancer in the world and current treatment options are limited. One of PDAC-related challenges include intra-patient heterogeneity that often results in treatments to be effective in a small portion of the patient population. Therefore, novel treatment options are needed and require a personalized medicine approach. A new domain of treatment options for PDAC is viro-immunotherapy. Viro-immunotherapy consists mainly of oncolytic viruses (OVs) that selectively replicate in tumor cells leading to oncolysis and the induction of an antitumor immune response. However, the translational efficacy of viro-immunotherapies is low and assessment of sensitivity of tumors to viro-immunotherapy requires a near-patient model with high predictive value, such a PDAC patient-derived organoids (PDOs). Here, we aimed to determine the sensitivity of PDAC organoids to a panel of OVs, including oncolytic Newcastle Disease Virus (NDV), Adenovirus (AdV), Reovirus (ReoV), and Measles Virus (MeV). Subsequently, we correlated the observed sensitivity to the transcriptome of the PDAC organoids to find genetic markers representing sensitivity to OV treatment resulting in a personalized medicine approach.
Methods: Ten PDAC PDOs were established and each PDO was inoculated with a range of multiplicities of infection (MOIs) between 0.1 and 1000 for each OV. The panel of OV strains included NDV LaSota, Human AdV Type B isolate GoraVir, ReoV Type 3 Dearing Jin-3 and MeV–Edmonston (Edm). Five days after inoculation, cell viability was measured and the concentration at which 50% of the cells were killed, also known as the EC_{50} value, was determined. In parallel, transcriptomes of each PDAC PDO was generated using RNA sequencing and a set of genetic markers representing PDAC PDO sensitivity to OVs were found *in silico*.
Results: Based on the sensitivity of PDAC PDOs to OVs, the PDAC PDOs were ranked as high, medium or low sensitivity. Overall, the ten PDAC PDOs were more sensitive to NDV than to ReoV Jin-3, Goravir and MeV-Edm. *In silico* analysis to identify genetic markers representing sensitivity to OVs is ongoing.
Conclusion: This study shows the variability in responses of PDAC PDOs to a panel of OVs, fortifying the need for personalized medicine approaches to combat solid tumors like PDAC.

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Zeb1 downregulation sensitizes pancreatic cancer-associated fibroblasts to killing by oncolytic reovirus through upregulation of the reovirus receptor Junction Adhesion Molecule A

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Background: Pancreatic tumors display an abundance of cancer-associated fibroblasts (CAFs), which negatively affect prognosis and therapy response. Oncolytic virotherapy exploits viruses that preferentially lyse epithelial cancer cells as opposed to normal cells. Interestingly, we have observed that oncolytic reoviruses are able to infect and lyse CAFs, in addition to epithelial cancer cells. Targeting CAFs, in addition to cancer cells, could be advantageous to increase therapy effectiveness. It could serve as a conduit for viral spread and simultaneously disrupt the desmoplastic barrier around tumors, thereby also accelerating the influx of other therapeutics and immune cells. We previously found that the proneness of CAFs to lysis by oncolytic reovirus correlates with the cell surface expression levels of the reovirus entry receptor junction adhesion molecule A (JAM-A). However, most pancreatic CAFs do not express JAM-A. Therefore, we aimed to identify and subsequently modify regulators of JAM-A expression, to sensitize CAFs to reovirus.
Methods: A genome-wide CRISPR/Cas9 screen was employed to identify the genes regulating JAM-A expression on fibroblasts. Pancreatic stellate cells with a moderate JAM-A expression level were transduced with a gRNA library making a knockout of one gene per cell. The highest and lowest JAM-A expressing cells were sorted and sequenced to identify the gRNAs that regulate JAM-A expression. Clonal CRISPR/Cas9-generated knockouts of a top negative regulator were generated and infected with reovirus, followed by cell viability assays to quantify their susceptibilities to reovirus-induced cell death.
Results: F11R, the gene encoding JAM-A, was identified as the top positive regulator of JAM-A expression in the CRISPR/Cas9 screen, verifying the validity of the screen. The top negative regulators identified were Fibroblast Growth Factor Receptor 1 (FGFR1) and Zinc finger E-box Binding homeobox 1 (Zeb1), thereby serving as potential therapeutic targets to sensitize CAFs to reovirus treatment. Using clonal Zeb1 knock-outs, Zeb1 was confirmed as a strong regulator of JAM-A expression. Zeb1 knockout in JAM-A negative pancreatic fibroblasts caused a robust upregulation of JAM-A and sensitized these inherently resistant fibroblasts to reovirus-directed cytolysis. Additionally, the clinically approved drug Mocetinostat, previously described to inhibit Zeb1, upregulated JAM-A expression on CAFs and increased cell lysis by reovirus.
Conclusion: Altogether, our data show that Zeb1 is a strong negative regulator of JAM-A expression on fibroblasts and that Zeb1 inhibition can sensitize CAFs to reovirus-induced cell death. This research provides a rationale for combining Zeb1 inhibitory drugs with oncolytic reovirus treatment to improve killing of CAFs, which in turn could boost overall tumor eradication.

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