DCRT Dutch Center for RNA Therapeutics

2021-2022 REPORT

Pauline de Graaf on behalf of

DEPARTMENT OF HUMAN GENETICS, Leiden University Medical Center, Prof. Annemieke Aartsma-Rus, Prof. Willeke van Roon-Mom

DEPARTMENT OF HUMAN GENETICS, Radboud University Medical Center, Prof. Rob Collin

DEPARTMENT OF CLINICAL GENETICS, Erasmus Medical Center, Prof. Ype Elgersma

https://rnatherapy.nl/

dcrt@lumc.nl

Introduction

On behalf of the consortium we have the pleasure to herewith present the first annual report of the Dutch Center for RNA Therapeutics (DCRT). The DCRT was founded in 2020, just before the COVID pandemic-induced lockdown of the laboratories. As such, our activities in the first year mainly focused on issues such as creating a business and budget plan, a charter and a communication plan, the installation of an advisory board and the design of a logo and website. Furthermore, we had interactions with representatives from regulatory agencies (European Medicines Agency, Medicines Evaluation Board), payers (Zorginstituut Nederland) and the Dutch Ministry of Health, Welfare and Sport. In 2021, with the consortium framework in place and the arrival of a DCRT project manager, postdoc and technician, we were able to start expanding our network and to work on the identification of eligible genes and finding candidate patients. This will hopefully lead to the development and testing of an exon skipping antisense oligonucleotide therapy and the first individualized patient treatment. Our efforts towards that goal are described in the present report.

Background

The Dutch Center for RNA Therapeutics (DCRT) is a non-profit consortium that was officially launched on February 29, 2020 (rare disease day). Seed funding for one year was provided by the Human Genetics department of the LUMC for both operational costs and ASO development. In 2021, all consortium partners committed to financial support for a period of 3 years. The DCRT consortium currently consists of three university medical centers in The Netherlands: LUMC (represented by Annemieke Aartsma-Rus and Willeke van Roon-Mom), Radboudumc (represented by Rob Collin) and ErasmusMC (represented by Ype Elgersma). Each consortium member has its own expertise and area of focus: translational exon skipping therapy development, neurodegenerative diseases, inherited retinal diseases and neurodevelopmental disorders, respectively. However, by sharing results and insights, they work together to reach a common goal: to develop novel therapies for eligible patients with ultra-rare genetic diseases of the brain or the eye. The aim of the DCRT is to develop individualized therapy with locally injected, EMA-FDA approved antisense oligonucleotide (ASO) modalities for eligible patients with a rare genetic disorder harbouring a unique mutation. The eligibility criteria are: brain or eye disease, ultra-rare, hereditary diseases and no alternative treatment available. This ASO treatment should restore or increase production of a missing protein or reduce the expression of toxic proteins resulting in benefit for the patient. The regulatory approved nusinersen application has revealed that local delivery of ASOs is well tolerated and effective and that dosing frequency is low (3-4 times per year). In 2018, at Boston Childrens' Hospital, the identification of a novel mutation in a 6-year old girl with Batten's disease led to the development of an ASO exon skipping drug called 'milasen', named after the patient -Mila- and based on nusinersen (J. Kim et al. 2019). Since then, several other personalized ASO therapies were developed including an RNase H ASO for a specific ALS causing mutation and several exon skipping ASOs for ataxia-telangiectasia mutations.

Inspired by Mila's story and her treatment, Annemieke Aartsma-Rus, Willeke van Roon-Mom and Rob Collin founded the DCRT in 2020 and were joined by Ype Elgersma later that year. DCRT's primary focus is on the development of an exon skipping ASO treatment for eligible patients within

The Netherlands. Therefore, we have first established contact with university medical centers in the Netherlands. To date we have already identified more than 2000 genes and evaluated 750 of which 550 are potentially eligible. They are listed in an overview that can be found on the DCRT website. In order to find eligible patients, the DCRT is creating awareness through collaboration and communication. Our target audience consists of clinical geneticists, pediatricians and patient organizations in The Netherlands and neighbouring countries.

Collaboration

Besides collaboration with neurologists, geneticists, pediatricians and clinicians in our own institutes, the DCRT is involved in the national ZonMW PSIDER consortium on the treatment of neurodevelopmental and neurodegenerative disorders led by DCRT partner ErasmusMC. Furthermore, we have established cooperation with other university medical centers in the Netherlands that are involved in the development of antisense oligonucleotide treatment for ultra-rare diseases. Marlen Lauffer has contacted stakeholders at UMC Utrecht (Peter van Hasselt, Gijs van Haaften), Groningen (Peter van den Akker, Jeroen Bremer, Charlotte Lubout, Dennis Bos, Robert Sietsma, Mario Mauthe, Marina and Tom de Koning), Amsterdam (Clara van Karnebeek, Mieke van Haelst) and Maastricht (Els Vanhoutte).

In Europe, DCRT has connected with research groups and clinicians in Belgium (Ghent and Leuven), Germany (Munich, Marburg, Cologne, Tuebingen) and the UK (London).

Interactions with Tuebingen have resulted in establishing a Europewide network: 1 mutation 1 medicine (1M1M). It focuses on the development of exon skipping ASOs for severely progressive debilitating neurodegenerative disorders. Annemieke Aartsma-Rus and Willeke van Roon-Mom represent the DCRT in 1M1M. Globally N=1 ASO therapy developments are aligned by the N=1 collaborative (N1C), which arose from the N=1 taskforce of the Oligonucleotide Therapeutics Society. Annemieke Aartsma-Rus is representing the DCRT as a member of the board of N1C.

DCRT also regularly touches base with the following non-profit and patient organizations in The Netherlands: VSOP (national patient alliance for rare and genetic diseases), Oogfonds (patient organization for individual with eye disorders), Stichting Usher (through Erwin van Wijk, patient organization for Usher syndrome), Epilepsy NL, Vereniging Angelman Syndroom Nederland (vASN), Stichting Tubereuze Sclerosis Nederland (STSN), Hersenstichting Nederland.

Communication

Through talks and posters at national and international conferences and meetings, DCRT's work is presented to a broad audience of fellow researchers, clinicians and patient organizations with the purpose of creating awareness and forming new collaborations. In 2021, 21 presentations that focused (largely) on DCRT were given and in 2022 more than 38 presentations. A list of these presentations can be found in the section Dissemination.

Upcoming talks at (inter)national events are advertised on the DCRT website (https://rnatherapy.nl/) to enable personal interactions with fellow researchers and other stakeholders. Furthermore, the DCRT website contains information and news items about DCRT-colleagues, a list of genes that we are interested in and an explanation of the process of exon skipping and RNA therapy. News items and events are also posted on Team DCRT's LinkedIn account.

DCRT had planned to organize a yearly symposium for an audience consisting of DCRT colleagues from LUMC, ErasmusMC, Radboudumc, from other University Medical Centers in The Netherlands and further interested parties who are involved in ultra-rare diseases and ASO therapy. Due to the COVID pandemic though, we have unfortunately not been able to organize a DCRT symposium yet. For 2023, the DCRT has teamed up with the organizing committee of the Dutch Antisense Therapeutics Symposium (DATS) to hold a joint event at Radboudumc in Nijmegen. The symposium is scheduled for the 1st and 2nd of June 2023.

On November 3rd, 2022, the DCRT invited LUMC stakeholders from the departments of human genetics, clinical genetics, neurology, clinical pharmacy and the central animal facility for an update on DCRT's activities. Annemieke Aartsma-Rus and Marlen Lauffer presented on external collaborations, progress and research and Tineke Coenen, head of the central animal facility, gave a talk on plans for animal safety studies. In between presentations and after the meeting there was time for discussion and knowledge exchange.

Management

The DCRT executive board is responsible for DCRT governance. The board consists of the following consortium members:

Annemieke Aartsma-Rus, Professor at Leiden University Medical Center (elected chair 2022-2024)

Willeke van Roon-Mom, Professor at Leiden University Medical Center

Rob Collin, Professor at Radboud University Medical Center (elected co-chair 2022-2024, to become chair in 2025)

Ype Elgersma, Professor at Erasmus Medical Center

The executive board meets once monthly online to discuss strategy and progress. Since DCRT's foundation in 2020, a total of 17 meetings took place. The board is supported by project manager Pauline de Graaf, who joined DCRT in May 2022, and DCRT postdoc Marlen Lauffer. Marlen started her work for the DCRT in October 2021 at the same time as technician Anouk Spruit. Together they are responsible for identification of new mutations to pave the way towards clinical testing for future treatments. For retinal diseases, new targets are being discovered and developed in the joint labs of Rob Collin and Alex Garanto at the Radboudumc.

The board has drafted a DCRT consortium agreement this year, which is currently being checked by the technology transfer offices of the partner institutes. As soon as they have approved, we can proceed to collect the necessary signatures.

On April 20, 2022, the first meeting of the DCRT scientific advisory board (SAB) took place. The SAB provides guidance on issues concerning ethics, scientific feasibility, interactions with patients, interaction with the private sector, regulatory aspects and possible reimbursement by health providers. Online meetings with the SAB are held once or twice per year. All SAB members agreed to being contacted individually in case of ad hoc questions in their area of expertise.

The members of the SAB are: Cathalijne van Doorne (European Medicines Agency); Mariette Driessens (VSOP – Patiëntenkoepel voor zeldzame en genetische aandoeningen); Frits Fallaux (Princess Máxima Center for Pediatric Oncology); Richard Geary (Ionis Pharmaceuticals); Carla Hollak (University of Amsterdam); Bart Leroy (Ghent University Hospital & The Children's Hospital of Philadelphia); Anke Pisters-van Roy (medical advisor health insurer CZ); Ghislaine van Thiel (UMC Utrecht); Lonneke Timmers (Zorginstituut Nederland); Tim Yu (Boston Children's Hospital).

DCRT asked for formal advice from the European Medicines Association (EMA). An Innovation Task Force Briefing Meeting (ITF-BM) took place on the 10th of December 2021 about scientific and regulatory topics such as efficacy studies, safety studies, clinical development and patient benefit. See also 'Research progress'. Similar topics were discussed in a more formal setting by 1M1M through the EMA scientific advice process. From these meetings, the DCRT has now formed an updated preclinical and clinical pipeline that incorporates the experts' opinions from EMA and will be used for the first eligible patient identified by the DCRT (see below).

Furthermore, DCRT is actively applying for network funding. Together with the 1M1M consortium we wrote an application for a COST Action and a Marie Skłodowska-Curie Action (Doctoral Networks). Together with UMC Utrecht, DCRT applied successfully for a grant from the already mentioned ZonMW-PSIDER programme.

Towards a development pipeline

On December 10th, 2021, a meeting with the EMA-ITF (European Medicines Agency – Innovation Task Force) was organized to discuss scientific and regulatory topics related to ASO development and safety and efficacy studies (meeting report is available on request).

Participants were:

- from DCRT: *Anouk Spruit, Willeke van Roon-Mom, Annemieke Aartsma-Rus, Marlen Lauffer, Tineke Coenen-de Roo (Leiden University Medical Center); Ype Elgersma (Erasmus Medical Center); Rob Collin (Radboud University Medical Center)*
- from European Medicines Agency: *Falk Ehmann, Wouter Eijkelkamp, Ralf Herold, Panna Vass, Pavel Balabanov, Corina Popescu, Brian Dooley, Laura Fregonese, Alice Cuccagna, Gauri Deoras, Frank Holtkamp, Britt Duijndam, Lutz Wiesner, Mylene Hübecker, Susanne Brendler-Schwaab, Doris Hoeschele, Anna Maria Gerdina Pasmooij, Vedrana Aljinović-Vučić,*

Maria Evandri, Ana Claudia Figueiredo, Rene Thürmer, Panagiota Tsantili, Viktoriia Starokozhko

- from PSIDER consortium: *Ghislaine van Tiel*
- observers from Tuebingen University: *Rebecca Schuele, Holm Graessner, Matthis Synofzik*

From this meeting and interactions from 1M1M with the EMA, we propose the following pathway for N=1 exon skipping ASO development:

First patient eligibility needs to be assessed. This involves four aspects: 1. The mutation must be ultra-rare and eligible for exon skipping, i.e. after skipping a (cryptic) exon a (partially) functional protein needs to be produced. 2. The disease needs to be severely progressive and debilitating disorder (SDLT) where the symptoms most impacting patient quality of life are from the central nervous system or the eye. 3. It must be possible to treat the patient with intrathecal or intravitreal injections. 4. A positive benefit-risk ratio should be expected from ASO treatment and the benefit must be measurable ideally within a time-frame of 48 months.

When a patient meets all the criteria for selection, a treatment board is convened that involves also patient representatives and ethicists. The treatment board makes the final decision whether treatment development should or should not be initiated.

Once a patient is formally selected, preclinical ASO development starts in cultured cells, a lead compound is selected and studied in acute rat/rabbit safety studies and in vitro safety studies, and finally a GMP or GMP-like batch is ordered. In parallel, the "run-in natural history" is obtained with a selected broader pool of outcome measures, which then allows to prioritize the 3-4 individually most promising outcomes at the end of the run for the treatment phase. In addition, start and stop criteria are discussed with the patient and family prior to initiation of treatment. Treatment is initiated starting with a dose known to be tolerable for nusinersen and dosing is escalated based on agreed upon safety and pharmacodynamic parameters. Monitoring of safety and efficacy is performed regularly and the maintenance dosing is based on pharmacodynamic and safety parameters. Notably, patients and families are heavily involved in the decision making, setting start and stop criteria and making decisions based on realistic assessments during the maintenance dosing regimen.

Research progress

LUMC

It takes quite some time and expertise to determine whether rare variants are suitable for RNA therapy. To facilitate this process, Iris Huitink, one of DCRT's students, has developed the ExonCheck tool which works for single variants. It gives an overview of all the information for a single variant to decide yes or no on exon-skipping. The tool is currently being tested and will be expanded to include a web application with the help of Sowmiyaa Kumar, a new student who will continue the work of Iris Huitink. As part of the ongoing collaboration with Tim Yu's group at Boston Children's Hospital

we are also investigating to combine the ExonCheck tool with their system (SpliceCheck). Disease relevant outcome measures are essential to determine restoration of protein function after ASO treatment. We are developing an in vitro autophagy assay using p62 and LCIII immuno stainings in a 96 well format to ensure semi high throughput analysis. For in vivo studies it is important to quantify ASO distribution in brain and peripheral organs. Standard is hybridization ELISA ASO quantification, but we are implementing a PCR based assay published in 2021 (Shin et al, Nucleic Acid Therapeutics, 2021).

Radboudumc

Ongoing optimization of ASOs for two intronic *ABCA4* variants in Stargardt disease. One mutation is carried by a pair of siblings, with relatively mild Stargardt disease (thus leaving a window of opportunity), with the brother based on the clinical phenotype being "most eligible". Each six months, he is visiting the hospital for a clinical check-up to build natural history data. Following donation of a skin biopsy, iPSCs have been generated that, following differentiation to retina-like cells, will be exposed to AONs (demonstrated to be efficacious in midigene assays) to identify the lead compound. Similarly, a Turkish girl (in her early teens) with a unique mutation has visited the clinic, and iPSCs have been generated. Protocols on how to prepare for ethical permission are being discussed with experts in the hospital.

ErasmusMC

Ongoing research into ASO treatment for neurodevelopmental disorders and providing proof of principle in both iPSC and mouse models. Besides identifying variants with splice mutations, the lab has focussed on gain of function mutations and developed cellular assays for rapid primary ASO screening. In addition, the lab is working on optimizing an in-silico pipeline by combining published software packages to calculate ASO toxicity as well as RNA secondary structure.

Dissemination

Talks and poster presentations

1/14/2021	Ype / Edwin	Erasmus MC
1/28/2021	Willeke	LUMC clinical genetics department
2/25/2021	Annemieke	Summit meeting
2/28/2021	Willeke	LUMC website
3/27/2021	Annemieke	CSH: RNA therapeutics
3/8/2021	Annemieke	UMass RNA Institute Seminar
3/15/2021	Annemieke	Inserm seminar
3/23/2021	Annemieke/Willeke/Linda	LUMC internal
4/6/2021	Rob	Radboudumc internal
4/9/2021	Rob	15th ProRetina Research Colloquium, virtual
4/13/2021	Annemieke	Undruggable Leaders Forum Europe, virtual
4/21/2021	Annemieke	7th annual Oxford Symposium on antisense oligonucleotides
4/22/2021	Willeke	Proefdiercentrum LUMC

4/22/2021	Annemieke	Cells, Tissues and Organs, LUMC theme meeting
5/20/2021	Annemieke	Online VIG discussion
5/27/2021	Annemieke	Metabolic diseases webinar: RNA therapies for inherited disorders
6/12-15/2021	Annemieke	European Society of Human Genetics, virtual
9 Nov 2021	Annemieke	Netherlands Society of Gene and Cell Therapy meeting, Lunteren
9/7/2021	Annemieke	World Duchenne awareness day
9/26-29/2021	Many people	OTS 17th annual meeting (talk and posters)
October 2021	Annemieke	Webinar of rare neurological diseases (RND) ERN
26 Jan 2022	Annemieke	British Genomics meeting, online
27 Jan 2022	Annemieke	Opening new institute in India, online
29 Feb 2022	All	2 year celebration DCRT
7 Mar 2022	Ype	Joint Dutch/ UK Genetics meeting, Rotterdam
29 Mar 2022	Ype	Nederlandse Vereniging Kindergeneeskunde, EAA congres, Nijmegen
7 April 2022	Ype	Troina Genetics of NDD meeting, Troina, Sicily
11 April 2022	Ype	SFARI meeting, New York
13 May 2022	Ype	Dutch RNA conference, Leiden
26 May 2022	Rob	Israeli Retinal Disease Consortium meeting, Haifa, Israel
8 June 2022	Ype	European Peadiatric Neurology Society, Webinar
3 June 2022	Many people	1st Dutch Antisense Therapeutics Symposium (DATS), Leiden
June 2022	Annemieke	Muscles2Meet symposium, Zeist
June 2022	Marlen	European Society of Human Genetics (ESHG), Vienna
June 2022	Willeke	Società Italiana di Biofisica e Biologia Molecolare (SIBBM), Rome
June 2022	Rob/Irene/Dyah	Netherlands Society of Gene and Cell Therapy meeting, Lunteren
July 2022	Annemieke	Federation of European Biochemical Societies conference, Lisbon
July 2022	Marlen	Neurodevelopment and neurodegeneration course, Vienna
2 August 2022	Ype	Angelman Syndrome Foundation family conference, Austin, Texas
1 Sep 2022	Rob	European Society of Retina specialists (EURETINA) congress, Hamburg
6 Sep 2022	Annemieke	44th congress Sociedad Espanola de Bioquimica y Biologia Molecular
15 Sep 2022	Ype	Angelman Syndrome Alliance (ASA) meeting, Vienna
17 Sep 2022	Rob	European Society for Cataract and Refractive Surgery (ESCRS), Milan
22 Sep 2022	Annemieke	1st AGORA consortium meeting, London
2-5 Oct 2022	Many people	Oligonucleotide Therapeutics Society (OTS), Phoenix
20 Oct 2022	Willeke	Biotech Thursday: Going for the Cure, Utrecht
2 Nov 2022	Anouk, Bianca, Laurie, Marlen	Translational Neuroscience symposium, LUMC
30 Oct 2022	Annemieke	MGC meeting about DNA for the general public, botanical gardens, Leiden
3 Nov 2022	Annemieke, Marlen	Progress meeting for LUMC-stakeholders, Leiden
3 Nov 2022	Ype	EMBO meeting, Bangalore, India
10 Nov 2022	Annemieke	German society for rare neurological diseases, Warburg, Germany
17 Nov 2022	Willeke	VIG symposium on orphan drug development, The Hague
18 Nov 2022	Ype	vASN meeting Utrecht
20 Nov 2022	Ype	SignaLife meeting, Nice

21 Nov 2022	Willeke	Science for Health conference: Advancing Gene Therapy, Brussels
25 Nov 2022	Ype	Angelman meeting, Antwerp
25 Nov 2022	Willeke	Dutch Association Community Genetics and Public Health Genomics
21 Nov 2022	Rob	Biomedical Vision Seminar, University of Bonn
24-Nov	Rob	Dutch Life Science Conference, Leiden
8 Dec 2022	Willeke	Rare-Med 2022 Symposium, Ghent

Awards and other highlights (also posted on the DCRT website)

- DCRT's 2nd anniversary was celebrated on 29th February 2022
- Willeke van Roon-Mom received the 3rd Riccardo Cortese lecture award for her talk on splice modulating RNA targeting therapies for brain disorders at the SIBBM in Rome
- DATS awards for poster and talk on DCRT research by Dyah Karjosukarso and Irene Vazquez-Dominguez (Radboud)
- Marlen Lauffer dedicated a session to the work of DCRT in a course on neurodevelopment and neurodegeneration that she taught at a summer school in Vienna
- OTS prize for Marlen Lauffer's poster on the ExonCheck tool of DCRT
- Nuria Suarez Herrera (Radboudumc) won Best Poster Award at Retina 2022 meeting in Dublin, November 4th 2022

Publications

- 'N of 1' therapies need a better model - Oligonucleotides offer therapeutic potential for patients with genetic disorders carrying unique mutations but developing individualized therapies is not supported by the current process for drug development. A. Aartsma-Rus, May 2021, Annemieke's publication
- Preparing n-of-1 Antisense Oligonucleotide Treatments for Rare Neurological Diseases in Europe: Genetic, Regulatory, and Ethical Perspectives. M. Synofzik et al., September 2021 (collaboration between LUMC and Tuebingen) Matthis' paper
- Consensus Guidelines for the Design and In Vitro Preclinical Efficacy Testing of N-of-1 Exon Skipping Antisense Oligonucleotides. Annemieke Aartsma-Rus et al., 2022, Nucleic Acid Therapeutics. DOI: 10.1089/nat.2022.0060

Budget

	Jaar 1 (2021-2022)	budget	expenses	balance
Personnel	Project Manager (schaal 10, 0.5 fte;	€ 42.000,00	26318,11	€ 15.681,89
	vanaf 1 mei 2022)			
Material	Dissemination	€ 5.000,00	3186,25	€ 1.813,75
	Annual symposium	€ 5.000,00	0	€ 5.000,00
Other	Travel costs	€ 3.000,00	0	€ 3.000,00
	Unforeseen	€ 5.000,00	0	€ 5.000,00
Total		**€ 60.000,00**	**29504,36**	**€ 30.495,64**

Plans for the next year

- Publication of guidelines for variant selection
- Make web application for the ExonCheck tool
- Secure funding for a postdoc to do computational work
- Safety studies at the central animal facility at LUMC/Radboudumc
- Set up an in vitro toxicity panel
- Marie Curie Action (if awarded) – plan activities
- COST Action (if awarded) – plan activities
- Organize a second meeting with the Scientific Advisory Board
- Apply for funding for a networking event together with Tuebingen

Per institute

EMC will (continue to) design and test ASOs for dominant gain of function mutations (both gapmer and exon skipping approach). We will continue to work on (user-friendly) software that identifies suitable ASOs based on predicted toxicity and off-targets. In addition we will continue optimizing our reporter assays that are used as first-pass screen.

For 2023, LUMC aims to assess in vitro proof of concept of correcting mutations that affect PLP1 splicing and identify additional potentially eligible cases through DNA/RNA sequencing of patients with recessive neurodegenerative diseases, where mutations on one allele are found. LUMC aims to establish an advanced diagnostic pipeline to assess undiagnosed cases with a suspected neurogenetic disorder. Therefore, LUMC will set up protocols for transdifferentiating fibroblasts into different CNS cell types to facilitate mutation identification and also ASO testing and treatment effects. LUMC will further develop the ExonCheck application that will facilitate assessing if an exon is eligible for exon skipping. Furthermore, LUMC will continue to work on the SPLINT(R) approach to measure ASO concentrations in tissues. Finally, LUMC will start setting up a panel for in vitro toxicity studies to evaluate how predictive they are for in vivo toxicity.

For 2023, Radboudumc aims to further develop AON treatment for Stargardt disease with an AON correcting the splicing mutation caused by c.859-506G>C, including efficacy data in patient-derived retina-like cells, and immediate safety studies in rabbits. In parallel, we aim to submit an METC application to obtain approval for treatment of a patient harboring this mutation. In addition, opportunities to commence n=1 treatment using AONs in Turkey will be explored. With respect to the identification of novel retinal disease causing mutations, a pipeline will be established to rapidly select results from our diagnostics department to be further developed for n=1 treatment.