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# The Impact of ZonMw's Translational Gene Therapy Research Programme on the Field of Gene Therapy Research in the Netherlands

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# Uitgebreide Nederlandse samenvatting

Het doel van deze evaluatie is het in kaart brengen van de impact van het programma Translationeel Gentherapeutisch Onderzoek (TGO) op het veld van translationele gentherapie in Nederland. Hiervoor zijn interviews uitgevoerd met alle projectleiders en de belangrijkste stakeholders zoals beleidsmakers, biotech industrie, collega onderzoekers, investeringsbedrijven en patiënten. De impact van het programma is gemeten op drie verschillende relevantie gebieden:

- verworven kennis op het gebied van translationeel gentherapie onderzoek en opgebouwde infrastructuur,
- economische impact en
- impact op patiënten.

In december 2003 is het programmavoorstel voor het programma Translationeel Gentherapeutisch Onderzoek (TGO), met een totaal budget van 15,8M€, door het Ministerie van Volksgezondheid, Welzijn en Sport (VWS) goedgekeurd. Het hoofddoel is het stimuleren van klinisch onderzoek na gedegen preklinisch translationeel onderzoek op het terrein van gentherapie. In totaal zijn 15 projecten gehonoreerd met een looptijd van maximaal zes jaar.

Gebaseerd op de ontwikkelingsfase varieert de duur en financiering per project. Voor alle projecten geldt dat er uiteindelijk een klinische fase I/II uitgevoerd moet worden. Door korte en lange projecten te financieren kon er een bredere range aan projecten worden gehonoreerd, waarbij de financiering zo goed mogelijk afgestemd is op de behoefte van het individuele project.

Twee eerdere evaluaties (2005 en 2011) hebben al geconcludeerd dat voldoende onderzoeksprojecten van hoge kwaliteit zijn ingediend waardoor de meest kansrijke projecten konden worden geselecteerd. En ondanks dat de meeste projecten om verschillende redenen grote vertraging hebben opgelopen, is het hoofddoel 'Stimuleren van klinisch gentherapie onderzoek', ruimschoots behaald. Ten tijde van de tweede evaluatie waren twee projecten zeer succesvol, exon skipping therapie voor Duchenne Muscular Dystrophy en de op AAV-gebaseerde gentherapie voor LPL deficiëntie. Beide projecten hadden hun eerste klinische studies succesvol afgerond en werden verder ontwikkeld binnen de private sector.

*Huidige status projecten*
De 15 gehonoreerde projecten kunnen op basis van aandoening in drie clusters worden ingedeeld: oncologische-, erfelijke-, en infectieuze aandoeningen. Acht projecten richten zich op gentherapie voor kanker, zes projecten op erfelijke aandoeningen en één project richt zich op de behandeling van HIV (infectieuze aandoening).

*Oncologische aandoeningen*
Twee van de acht gehonoreerde projecten oncologie projecten zijn succesvol afgerond, terwijl één project vroegtijdig gestopt is door onoverkomelijke problemen. Drie projecten bevinden zich in de klinische fase, één project is gestart met GMP productie van de vector en één project bevindt zich nog in de preklinische fase. Alle acht projecten hebben een vertraging opgelopen, met een gemiddelde duur van vier jaar. Meer details over het verloop en de huidige status van de projecten staan in de eerste bijlage.

*Erfelijke aandoeningen*
Twee van de zes projecten op het gebied van erfelijke ziektes zijn succesvol afgesloten, terwijl één project voortijdig beëindigt is door onoverkomelijke problemen. Twee projecten bevinden zich in de farmaceutische fase en één project bevindt zich nog in de preklinische fase. Opmerkelijk is dat de twee succesvol afgeronde projecten zonder vertraging zijn verlopen. Terwijl de andere projecten meer dan vier jaar vertraging hebben opgelopen.

*Infectieuze ziekte*
Het enige project op het gebied van infectieuze ziekten heeft een vertraging van meer dan vier jaar opgelopen. Meer preklinisch onderzoek is nodig voordat het project verder kan naar de farmaceutische fase en uiteindelijk de klinische fase. Meer details over het verloop en de huidige status van het project staat in bijlage 1.

Binnen de projecten wordt gebruik gemaakt van verschillende technieken. De meeste projecten (11) maken gebruik van retro-, lenti- of adenovirale (AAV) vectoren voor gen overdracht in doel cellen. Eén project richt zich op het ontwikkelen van een DNA vaccin door gebruik te maken van 'naakt DNA'. Eén project maakt gebruik van Clostridia bacteriën om specifiek hypoxische delen van tumoren aan te vallen. En tenslotte maakt één project gebruik van exon skipping door middel van anti-sense RNA en één van RNA interferentie techniek waarbij HIV proteïne expressie onderdrukt wordt.

*Impact op kennis en infrastructuur*
De primaire output van academisch onderzoek is kennis, deze kan het best gekwantificeerd worden door het aantal en de kwaliteit van publicaties in wetenschappelijke literatuur. De 15 projecten hebben tot nu toe 140 publicaties in 'peer reviewed' wetenschappelijke tijdschriften opgeleverd. De algemene kwaliteit van deze publicaties, op basis van impact factoren van de tijdschriften, is hoog en bevat verschillende publicaties in vermaarde tijdschriften als Nature, Nature Biotechnology en de New England Journal of Medicine. Daarnaast zijn er verschillende bijdragen vanuit de projecten aan nationale en internationale leerboek hoofdstukken, keynote, podium en poster presentaties op wetenschappelijke congressen en symposia, proefschriften en heeft het programma verschillende octrooien opgeleverd.

Het programma heeft op verschillende professionele niveaus gezorgd voor toename van kennis op het gebied van (translationeel) gentherapie. Bij de projecten die een preklinische fase bevatten zijn 16 promovendi betrokken, vijf daarvan moeten hun thesis nog verdedigen. Tien van de 11 gepromoveerden hebben hun carrière voortgezet binnen het gentherapie veld. De ene uitzondering keert na een Post doc in een ander veld binnenkort terug naar het gentherapie veld. Het merendeel van de gepromoveerde onderzoekers zijn in Nederland gebleven, hieruit blijkt dat er voldoende mogelijkheden zijn voor jonge onderzoekers binnen het gentherapieveld.

Buiten de kennis op het gebied van gentherapie is er ook een steile leercurve te zien op het gebied van regelgeving voor GMP en klinische studies onder de projectleiders, ziekenhuisapothekers en onderzoeksstaf. Een zelfde leercurve is zichtbaar bij de toezichthouders. De projectleiders van de eerste klinische studies bemerkten een tekortkoming in de expertise op het gebied van gentherapie bij de toezichthouders en een gebrek aan bereidheid om de studies vooraf te bespreken. Door de komst van het loketgentherapie en de mogelijkheid om de studie vooraf met de toezichthouders te bespreken zorgen ervoor dat de projectleiders meer vertrouwen hebben in het regulatoire proces. Punt van zorg blijft de aanvraag voor de benodigde milieuvergunning bij het ministerie I&M en bureau GGO.

De volledige kosten voor de GMP productie en de klinische studie worden niet volledig gedekt door de toegekende subsidie. Om de klinische translatie te kunnen maken is het vinden van aanvullende financiering een voorwaarde voor alle projecten. Bijna alle projecten zijn hierin succesvol geweest en de overall matching is 1:1. Daarnaast is de steun vanuit patiënten(organisaties) voor veel projecten van cruciaal belang voor het succes van het project.

Samenwerking met farmaceutische industrie is ook een belangrijke bron van financiering, met name universitaire spin-off bedrijven zijn hierbij belangrijk. Hierdoor is het mogelijk om private investeerders aan te trekken. De drie meest succesvolle samenwerkingen op dit gebied zijn: LPL deficiëntie met AMT (nu UniQure), Duchenne Muscular Dystrophy met Prosensa (nu BioMarin Pharmaceuticals) en Metastasized melanoma met T-cel Factory BV (nu Kite Pharma). Deze drie bedrijven werken nog steeds vanuit Nederland en hebben zich gecommitteerd aan nauwe samenwerking met de universiteiten van waaruit ze ooit opgericht

zijn. De huidige waarde van UniQure is meer dan 500M€ (september 2015). Prosensa en T-cell Factory zijn overgenomen voor respectievelijk $680M en 20M€ plus milestone betalingen. Recentelijk heeft Prof. Kuball 7 M€ serie A subsidie ontvangen om de stap naar de kliniek mogelijk te maken.

Het doel van het programma is het ontwikkelen van (betere) behandelingen en het verhogen van kwaliteit van leven van patiënten. Vier projecten hebben succesvol fase I/II studies afgerond en een aantal projecten hebben geleid tot vervolg studies en meer dan 400 patiënten zijn behandeld. Het grootste gedeelte van de projecten moet nog starten met fase I/II studie, de verwachting is dat het merendeel van deze projecten de translatie naar de kliniek gaat maken.

*Discussie*
Het programma Translationeel Gentherapeutisch Onderzoek is opgezet in een moeilijke tijd voor gentherapie onderzoek. Na een beloftevolle start waren er een aantal voorvallen waardoor (geplande) klinische studies (tijdelijk) gestopt werden. Gedurende de loop van het programma is er voor vele nieuwe therapieën de translatie naar de kliniek gemaakt, waar nu de vruchten van geplukt kunnen worden. Met name de toelating van de eerste gentherapie op de Europese markt, een therapie die mede ontwikkeld is binnen TGO, is een mijlpaal in het gentherapie veld. Maar ook andere projecten binnen het programma zijn succesvol geweest, zoals het exon skipping project van het LUMC met een product dat nog steeds zicht heeft om op de markt toegelaten te worden. Echter naast deze successen, hebben veel projecten ook vertraging opgelopen en hebben een aantal projecten de klinische fase nog niet bereikt.

De redenen voor de opgelopen vertraging betreft voor de meeste projecten obstakels in de regelgeving, problemen met GMP productie en nieuwe ontwikkelingen waardoor meer preklinisch onderzoek nodig is. Hoewel obstakels in de regelgeving het vaakst door projectleiders genoemd worden, blijkt de meeste vertraging veroorzaakt te worden door nieuwe ontwikkelingen, waardoor de huidige behandeling niet meer de meest optimale lijkt te zijn, en de stap naar de kliniek uitgesteld wordt.

Vooral voor de projecten die direct aan het begin van het programma de klinische fase starten waren de obstakels voor het verkrijgen van vergunningen het grootst. Eén project is het niet gelukt om toestemming van de regelgevende instanties te verkrijgen, ondanks een tijdsinvestering van zes jaar. In de loop van het programma is het regulatoire proces voor gentherapie studies gestroomlijnd, vooral de komst van het loket gentherapie heeft hier aan bijgedragen. Ondanks de verbeteringen blijft het regulatoire proces tijdrovend en lijkt het voor de onderzoekers een gefragmenteerd proces waar meerdere verschillende partijen voor risico inventarisatie op verschillende niveaus bij betrokken zijn, zoals CCMO, COGEM, ministerie I&M en bureau GGO.

Twee projecten binnen het TGO programma zijn er niet in geslaagd de klinische fase te behalen en zijn voortijdig gestopt door onoverkomelijke problemen. Het project ‘terugkerende bloed- en lymfklierkanker’ heeft grote problemen gehad met het regulatoire proces en kon niet worden voortgezet doordat de externe GMP productie partner stopte met de vector productie. Het tweede project ‘primaire immunodeficiëntie XLA, SCID’ is voortijdig gestopt door problemen met de veiligheid van de vector. Echter na verbeteringen is het project binnen het ZonMw programma Priority Medicines zeldzame ziekten en weesgeneesmiddelen (PM-Rare) voortgezet.

Voor negen projecten is het nog te vroeg om een eindconclusie te kunnen trekken, deze projecten lopen op dit moment nog. De vertraging die deze projecten hebben opgelopen is een punt van zorg. Vertragingen kunnen kostbaar zijn en het gevaar met zich meedragen dat het onderzoek overbodig wordt. Echter, de onderzoeken van de deze TGO projecten zijn nog steeds relevant doordat in de loop van de tijd de ‘unmet medical need’ voor deze ziektes is blijven bestaan. Daarnaast zijn de meeste projecten in staat om het project budget-neutraal te verlengen of financiering van derden binnen te halen.

Het meest in het oog springende resultaat is behaald door de onderzoekers i.s.m. het Amsterdamse Spin off bedrijf AMT (nu UniQure) van het project 'LPL deficiëntie' door het ontwikkelen van de eerste gentherapie die markt autorisatie heeft behaald. Daarnaast is het project van de Leidse onderzoekers en Prosensa (Duchenne Muscular Dystrophy) succesvol voortgezet en is er kans dat de therapie op termijn de markt komt. Terwijl de fase I/II studie van het project 'metastaserende melanoma' nog loopt, is het spin off bedrijf (T cell Factory) van de projectleider overgenomen door Kite Pharma. Bovengenoemde bedrijven werken nog steeds vanuit Nederland en hebben zich gecommitteerd aan nauwe samenwerking met de universiteiten van waaruit ze ooit opgericht zijn.

*Conclusie*
Het programma Translationeel Gentherapeutisch Onderzoek is opvallend succesvol geweest en heeft het Nederlandse gentherapie veld een impuls gegeven. Het programma heeft impact op gehad op kennisontwikkeling bij zowel onderzoekers als beleidsmakers. Daarnaast heeft het programma een substantiële bijdrage geleverd aan de opbouw van de infrastructuur. Mede door deze financiële impuls heeft het gentherapie zich binnen Nederland kunnen ontwikkelen tot leidende positie binnen het Internationale veld. De eerste goedgekeurde gentherapie op de Europese markt, Glybera (UniQure) is binnen het programma succesvol getest in fase I/II studie. Het fundamenteel nieuwe concept (exon skipping) voor Duchenne muscular dystrophy, is binnen het programma ontwikkeld van proof-of-principle naar eerste toepassing in de kliniek. Daarnaast zijn er meerdere spin off bedrijven opgestart, een stimulans voor de Nederlandse kennis economie.

*Toekomstvisie*
Voor veel ziekten waar nu alleen nog suboptimale behandelingen beschikbaar zijn of behandelingen geheel ontbreken, is gentherapie de hoop op genezing. Nieuw ontwikkelde technologieën en grote doorbraken zoals de goedkeuring van de eerste gentherapie door de EMA kunnen zorgen voor een fundamentele verandering in het denken over gentherapie, alhoewel er nog een lange weg te gaan is voor de technologie beschikbaar komt voor verschillende en brede populaties.

De kwaliteit van klinisch en translationeel onderzoek in Nederlandse academische instellingen is van zeer hoge kwaliteit. In de laatste 10 jaar zijn veel nieuwe faciliteiten, interdisciplinaire samenwerking, protocollen en procedures opgezet voor het faciliteren van de ontwikkeling van verschillende Advanced Medicinal Therapy Products (ATMPs), waaronder gentherapie, cellulaire immuuntherapie (kanker) en tissue engineering. Op dit moment zijn er veel nieuwe therapieën in ontwikkeling in de Nederlandse academische instellingen die op dit moment nog niet interessant zijn voor de grotere farmaceutische bedrijven, maar die al wel de belofte inhouden om curatieve behandelingen te leveren voor patiënten met 'unmet medical needs'. Nu is het moment voor een vervolgprogramma zodat de opgebouwde kennis en infrastructuur maximaal wordt gebruikt.

# Summary

In December 2003 the Dutch Ministry of Health, Welfare and Sport (*Dutch:* VWS) approved the Translational Gene Therapy Research (*Dutch:* TGO) programme. The programme was established to provide financial support for the development of novel gene therapies, specifically in order to stimulate the transition from laboratory to first clinical trials. A total of 15 projects have been funded for a maximum of six years with a combined budget of €15.8 million, covering many different technologies for the treatment of oncological, heritable and infectious diseases.

Aim of this study is to assess the impact of the TGO programme on the field of translational gene therapy research in the Netherlands. Three separate pillars are analysed: the knowledge gained and infrastructure built, the economic impact and the impact on patients. To this end, interviews were conducted with the project leaders of the research projects awarded grants, as well as with other major stakeholders including regulators, the biotech industry, fellow researchers, venture capital companies and patients.

Previous evaluations of the programme in 2005 and 2011 concluded that there were sufficient high-quality research proposals to ensure a selection of only outstanding projects for the TGO programme and that the programme is on track to fulfil its main objective of stimulating translational gene therapy research.

There have been two remarkably successful projects in the TGO programme. The project *LPL deficiency* has led to the development of the first EMA-approved gene therapy, Glybera. In the project *Duchenne muscular dystrophy*, a revolutionary concept has been developed from a proof-of-principle to a clinical grade treatment (PRO051). The treatment has now been tested in more than 300 patients and is currently awaiting FDA approval.

Most of the 15 projects in the TGO programme have encountered significant delays and have not yet completed the clinical testing of the treatments under development. Notably, however, most project extensions were budget neutral and so far only two projects have had to be cancelled because of insurmountable problems. However, some projects are at risk of falling behind, with no prospect of achieving their goals anytime soon.

Overall, the TGO programme has had a large impact in terms of all the parameters under review: knowledge-wise, economically and for patients. More than 140 publications in scientific peer-reviewed journals, 14 PhD theses and several patents have been produced. Trained staff, including researchers and newly graduated PhD students, are continuing their careers in the field of translational gene therapy in the Netherlands. The programme has given

a significant boost to academia-industry collaborations and contributed to the creation of several new biotech companies in the Netherlands exceeding a billion euros in company valuations and acquisitions. So far, over 40 patients have been treated directly under the programme and a further 400 or more in subsequent follow-up trials.

The significant amount of delay in most projects is due to only a small number of factors: regulatory hurdles, GMP production issues and catching up with new findings in the respective fields. While the need for additional preclinical research in order to deal with new findings and GMP production problems are hard to foresee and even harder to prevent, there might be room for improvement regarding the regulatory hurdles.

Many steps have been taken in the right direction to enhance regulatory processes since this programme started 11 years ago. They include establishing a gene therapy office (*Dutch:* loket gentherapie) to streamline individual appraisal processes. However, the regulatory process as a whole is still fragmented and researchers and the biopharmaceutical industry would greatly appreciate legislative change to integrate certain competencies in order to reduce unnecessary bureaucracy and accelerate the appraisal processes both nationally and at the EU level.

The field of gene therapy research has finally matured from a purely experimental to a medical discipline. Major breakthroughs have recently been achieved, including EMA approval of Glybera and large-scale trials of PRO051. New gene therapy concepts currently under development hold the promise of a clinical cure for diseases with high unmet medical needs.

An emerging biomedical technology for precise gene editing, CRISPER-Cas9, has recently reached sufficient maturity for translational medical research and is believed by many to hold great promise for the future of gene therapy research. CRISPR-Cas9 builds upon existing gene therapy delivery systems, but instead of inserting a therapeutic gene at a random location into the genome within the patient's target cells, it allows for precise correction of the affected faulty genes.

Given the remarkable successes of the current Translational Gene Therapy Research programme and the overall high research quality in Dutch university medical centres, the Ministry (VWS) and ZonMw are advised to organise and finance a follow-up TGO programme. Gene therapy research as a whole has progressed significantly, both nationally and internationally. Early phase clinical trials have demonstrated efficacy in genetic disease but also in acquired diseases such as cancer and cardiovascular disorders, elevating interest from industry and academia alike. A follow-up funding programme would help to further strengthen the Dutch position in the field, maintain the momentum of the Netherlands' knowledge economy and, most importantly, it could in the long run benefit many patients with high unmet medical needs. The overall structure of the programme should remain largely the same,

but professional drug development experts might be a valuable addition to the programme committee for early feasibility analysis and additional support for the clinical development of new treatments.

# Introduction

In December 2003 the Dutch Ministry of Health, Welfare and Sport (*Dutch: Ministerie van Volksgezondheid, Welzijn en Sport*; VWS) approved the Translational Gene Therapy Research (*Dutch: Translationeel Gentherapeutisch Onderzoek*; TGO) programme. The programme is designed to provide financial support for the preclinical and clinical development of novel gene therapies, specifically in order to stimulate the transition from the laboratory to phase I/II clinical trials. These first clinical trials are crucial to test the safety of the therapies in human volunteers and establish the maximum tolerated doses. Only when the new therapies are safe can their clinical efficacy be explored in subsequent larger-scale phase II and III trials. However, transition from a new medical entity to the clinics traditionally highlights a shortfall in funding, as the further clinical development of therapies may be too expensive for academic institutions. Moreover, these centres often fail to attract additional funding from the private sector, e.g. venture capitalists or pharmaceutical companies, due to uncertainties surrounding the product.

While this financial dilemma holds true for all kinds of clinical applications, gene therapies had a particularly rough time some 15 years ago. Back then, the field had just matured from a purely preclinical discipline to the first clinical trials in humans. However, after some initial successes and growing enthusiasm within the scientific community, things went wrong. In 1999, an 18-year-old boy suffering from ornithine transcarbamylase deficiency, an X-linked genetic disorder, died only four days after an injection of an adenoviral vector due to a severe immunological response. An investigation by the American Food and Drug Administration (FDA) concluded that the trial investigators had violated several rules of conduct and that the patient in question, a substitute for another patient who had dropped out, should never have been included according to the study protocol.[1]

In an earlier trial infants born with X-linked severe combined immunodeficiency (X-SCID) were treated with a new gene therapy. X-SCID is an inherited immunodeficiency which, if not treated with a matched donor, has a very poor prognosis, usually being lethal within the first two years of life. However, even with a partially or non-matched donor there was a high risk of treatment failure. In the gene therapy trial, transplanted stem cells were engineered with a retrovirus that contained a functional gene copy of the disrupted IL2RG gene.[2] While the overall success rate was extremely encouraging, correcting the disease in 18 out of the total 20 patients, three patients eventually developed leukaemia, which eventually resulted in the death of one patient. A later study confirmed the causality between the gene therapy and the development of leukaemia, showing that the viral vector had integrated near the site of LMO2, a known oncogene.[3]

Although the scientific community was quickly able to identify and address the issues that caused the deaths of both young patients in the two different trials, regulators and the public were split about the potential benefits and risks of gene therapies.[45] Despite the promised efficacy, the trials had shown the risks associated with gene therapies. Many of the researchers involved in gene therapy research kept their positive outlook, but at the time there simply were not that many of them.[6] Although the human genome project was completed in 2003, which could have been a major turning point, gene therapy research remained a relatively small niche in the medical sciences.

Due to the early setbacks, only a few countries chose to launch new initiatives to specifically stimulate the clinical development of gene therapies. Besides some smaller and larger initiatives in the US and the UK, the Dutch Ministry of Health, Welfare and Sport (*Dutch:* VWS) authorised and financed ZonMw to launch its *translational gene therapy research programme* at the end of 2003. The ZonMw TGO programme was established to stimulate the translation of new gene therapies to clinical studies by providing funding and building a national network of expertise to assist in overcoming regulatory hurdles.

## Overview of the projects

In total, 15 projects have been awarded financial support of up to €1,400,000 for a maximum of six years. Originally, the anticipated timelines were a maximum of two years' preclinical research followed by a one-year pharmaceutical phase and, finally, a maximum of three years for the clinical phase (usually a phase I/II clinical trial). However, it soon became apparent that one year is not a realistic timeframe for the completion of the pharmaceutical phase. Many projects encountered additional delays, which will be discussed in more detail in this report.

The 15 projects can be classified by disease area into three clusters: oncology, genetic diseases and infectious diseases. A total of eight projects fall into the oncological cluster, six projects target genetic diseases and one aims at the treatment of HIV (infectious diseases). On the technical side, the projects can be characterised by the form of treatment delivery. Most projects (eleven) use either retro-, lenti- or adenoviral vectors for gene transfer to the targeted cells. One project aims at developing a DNA vaccine using 'naked DNA', while in another project Clostridia bacteria are being used to specifically target hypoxic tumour regions. In another project a novel approach called exon-skipping is being developed by the use of anti-sense RNA, and the final project also uses an RNA interference technique to silence HIV protein expression.

Not only is there a large variety of disease areas and gene therapy delivery systems in the TGO programme, the duration and amounts of funding also vary among the projects, based on the

development phase at the outset. All projects were required to complete the clinical phase within two years, but the three-year (maximum) preclinical and one-year pharmaceutical phases were optional. The decision to award funding to both longer and shorter projects allowed for a broader range of project proposals and funding much more tailored to the needs of each individual project.

## Aim of this study and research methods

The primary objective of this study is to evaluate the impact of the TGO programme on the translational gene therapy landscape in the Netherlands. To this end, all projects within the programme have been evaluated in order to determine if ZonMw's pre-defined primary endpoints were achieved. The impact on knowledge, infrastructure, patients and the biotech industry in the Netherlands is also discussed. Finally, a recommendation is given as to how the development of gene therapies in the Netherlands might be further strengthened.

In order to evaluate the impact of the TGO programme, the organisation of the programme itself was reviewed. To this end, the two earlier preliminary evaluations from 2005 and 2011 are reviewed and summarised in this report. The implementation of the suggestions made in those earlier evaluations is assessed in interviews with all project group leaders. Additionally, interviews with the group leaders as well as other major stakeholders were conducted to provide a conclusive picture of the main challenges during the lifetime of the programme and to identify potential room for improvement.

Ultimately, the success of the programme will be determined by the success of the individual projects. The achievements and struggles of the individual projects are therefore assessed at different levels, e.g. the scientific knowledge gained and institutional infrastructure built, follow-up projects or newly formed biotech spin-offs and societal value, such as patients successfully treated and newly created jobs, and also failure to obtain regulatory approval, project stagnation and the actual relevance of the results achieved.

# Results

## Previous evaluations

The TGO programme was evaluated in 2005 to ensure that the selection of the projects was in accordance with the predefined criteria, and later in 2011 to create an inventory of the project results, identify major hurdles and expected breakthroughs, and propose a potential structure

for a new gene therapy funding programme. It was already clear in 2005 that the programme might take slightly longer than the originally anticipated end date of 2013, and in the 2011 evaluation further delays were reported, pushing the expected end date back to 2015. However, these delays did not cause any direct budgetary constraints nor did they endanger the main objectives of the TGO programme. A summary of the main findings of the two evaluations is presented below.

## Evaluation of the selection procedure in 2005

At the time of the evaluation of the selection procedure by the TGO committee, eight projects had been awarded funding in the first two selection rounds in 2004 and 2005. Seven more projects would later be selected in the subsequent rounds in 2006 and 2007. The number and quality of the grant proposals submitted had vastly exceeded the expectations of the committee. This was interpreted as a sign of a large unmet need for funding of translational gene therapy research and as confirmation that there is plenty of high-quality research being conducted in the Netherlands, which allowed only the projects very high or outstanding quality to be selected. Furthermore, the selected projects met the expectations regarding interdisciplinary collaborations between non-clinical and clinical researchers, as well as knowledge transfer with 'user committees' and patient organisations. Most project leaders were able to attract additional funding and new facilities were being built at UMC Utrecht and the Dutch Cancer Institute (*Dutch: Nederlandse Kanker Instituut*; NKI). Overall, the selection procedure was found to be consistent with the predefined criteria and the recommendation was given to proceed with the TGO programme as planned.

## Midterm evaluation in 2011

In 2011, six years after the first projects started, the first midterm review of the TGO programme was conducted by the TGO committee and its chairman Prof. Sixma and published on the ZonMw website. The report provided an overview of all 15 projects and their progress with an emphasis on the two most successful projects to date. Bottlenecks current at that time were also identified and measures were described to better facilitate clinical gene therapy research in the Netherlands.

The report highlighted the diversity of the individual projects, which roughly fell into three categories: oncological, heritable and infectious diseases. The techniques used to tackle the different diseases were equally diverse. Several different cellular immunotherapies, lenti-, retro- and AAV vectors and non-viral therapies were under development in the TGO programme. The two most successful projects at that time were the exon-skipping gene

therapy for Duchenne muscular dystrophy and the AAV-based gene therapy for LPL deficiency. Both of these projects had successfully completed their first clinical trials and were now being developed further in the private sector. Interestingly, the TGO committee noted that the involvement of private parties had had a profound positive effect on the regulatory side of these two projects.

Unlike the two very successful projects, most other projects in the TGO programme were found to be delayed for various reasons. While in some projects the researchers simply needed more time for preclinical testing and vector optimisations, many others struggled with regulatory hurdles. Most notably, the requirements for approval from the Office for Genetically Modified Organisms (GMO office) at the Ministry of Infrastructure and the Environment (*Dutch:* IenM) were often perceived as unclear and the safety requirements as exaggerated, causing additional delays. Furthermore, the Central Committee on Research Involving Human Subjects (*Dutch: Centrale Commissie Mensgebonden Onderzoek*; CCMO), which must approve all clinical trials, was found to provide too little advice for researchers prior to their entering the lengthy review process by officially submitting their dossiers. This left researchers in the dark until the authorities announced their decision. Another delaying factor was the limited translational research experience of many researchers.

Finally, the report concluded that the return of clinical gene therapy research after the deaths of young clinical trial participants in 1999 and 2002 had raised deep concerns about its safety. Alongside the two major breakthroughs in the TGO programme, four promising foreign projects were described involving Leber's congenital amaurosis, Parkinson's disease, adenosine deaminase-deficiency (ADD) and X-linked adrenoleukodystrophy (ALD). There have been funding programmes for translational gene therapy research in other countries similar to the TGO programme in the Netherlands, most notably in the USA, UK, Germany, Switzerland and France. The report finishes with a recommendation to the Dutch Ministry of Health, Welfare and Sports for a follow-up programme to the current TGO programme, providing two alternative funding schemes worth €28 million and €17 million, both with a proposed starting date of 2012.

## Achievements and current status of all 15 projects

As noted in the earlier assessments of the TGO programme, there have some remarkably successful projects, and a few others that face seemingly unresolvable challenges. The majority of the projects are more or less on target to eventually test their technology in phase I/II clinical trials, but with significant delays. A brief overview of the current status of all projects, the main reasons for their delay and a future outlook are provided below. As in earlier reports,

the projects are grouped by disease indication into the following three categories: oncological (eight projects), heritable (six projects) and infectious diseases (one project).

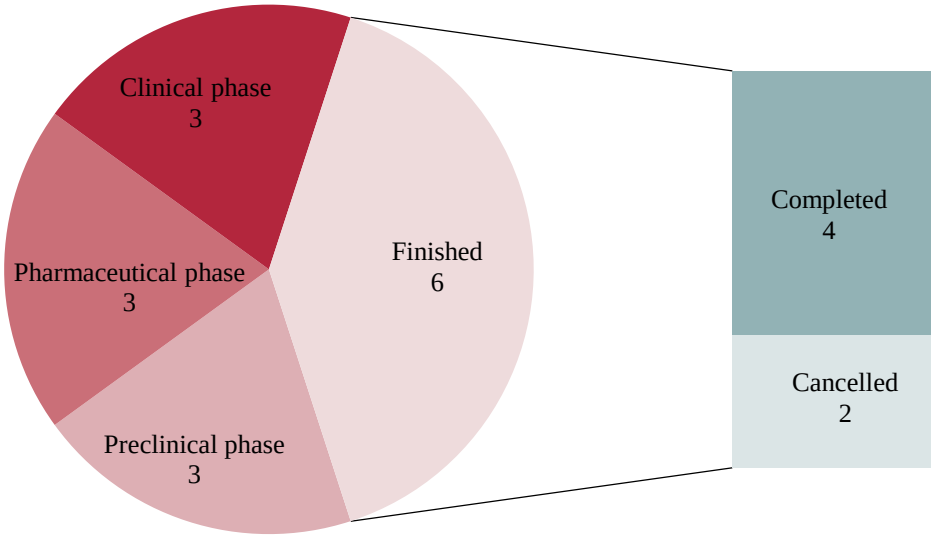


***Figure 1:*** *Current phase of the 15 projects in the TGO programme. Four projects have been completed successfully, while two were cancelled. The remaining nine projects are ongoing and equally distributed among the preclinical, pharmaceutical and clinical phases, with three projects in each of the phases.*

## Oncology projects

Only two of the eight oncology projects have been completed successfully, while one project was cancelled after experiencing insurmountable problems. Another three projects have entered but not yet completed the clinical phase, one has finally been able to start GMP vector production (pharmaceutical phase) and the last project is still in the preclinical phase, as regulatory requirements for proceeding to the clinics remain unclear to the project lead. On average, each oncological project is almost four years behind schedule and no project, either completed or ongoing, has been entirely free of delay. In the following section, all eight oncology projects and their recent progress are summarised and discussed briefly. For a more detailed summary and discussion of each individual project please see Appendix 1.

In the project ***Metastatic melanoma***, a highly melanoma-reactive T-cell receptor (TCR) was identified and a retroviral vector system developed to transfect autologous T-cells *ex vivo*. After initial problems during the toxicity studies in the pharmaceutical phase and an

unexpected immunological response in the first patient treated in the clinical trial, the project is now nearing its end. A total of four patients have been treated and the results of the clinical study will be published soon after its completion. However, this specific TCR vector is unlikely ever to be used in a broader range of patients, let alone achieve market authorisation. A more potent vector is already under development at the NKI spin-off T-Cell Factory BV, which has recently been acquired by the US biotechnology company Kite Pharma.

The project ***Recurrent blood and lymph node cancer*** focused on the development of a retroviral vector for the gene transfer of T-cell receptors recognising minor histocompatibility antigens to virus-specific T-cells as a cellular immunotherapy for patients with haematological malignancies after allogeneic stem cell transplantation. However, the researchers were not able to test the vector in the clinic as regulatory obstacles significantly delayed the approval process for the clinical trial and eventually the external GMP production partner stopped providing new vector batches due to an organisational change. Lacking the required financial resources to seek another GMP production partner, the researchers decided to discontinue the project.

Similar to the project described above, the project ***Haematological malignancies*** has encountered significant problems during GMP vector production. However, the project did eventually proceed to the clinical evaluation of its technology. In this project a different retroviral vector was developed for gene transfer of T-cell receptors (TCR) recognising minor histocompatibility antigens to virus-specific T-cells as cellular immunotherapy for patients with haematological malignancies after allogeneic stem cell transplantation. The researchers successfully constructed and tested the safety and efficacy of the retroviral vector in mice and vector production was initiated at EUFETS GmbH in 2012. However, soon after the virus batch had been delivered and the clinical trial was opened, EUFETS suddenly reported concerns regarding the quality of the batch, leading to substantial delays for further safety evaluations. There was additional delay after the first patient treated in the trial died three months later. The trial is still ongoing, but due to a very complex patient inclusion scheme progress is very slow and no date for trial completion has been set.

In the project ***Redirecting T-cells in leukaemia*** at UMC Utrecht, broadly tumour-reactive second-generation T-cell receptors are being developed to redirect autologous immune cells to target haematological malignancies. The researchers have been able to significantly improve the TCR vector through multiple iterations, which has caused about three years' delay in the preclinical phase. However, during this time new intellectual property has been created and protected. Right now, the project is at the end of the pharmaceutical phase, with the final vector batch currently in production at EUFETS. All patents, clinical protocols and regulatory documents are currently under review by a potential industry partner for the clinical development of the technology. Notably, adoptive cellular therapies (ACT) have become a hot

research topic, attracting increasing industry activity. As a result, the project leader's start-up Gadeta received €7 million series A funding to advance the technology into the clinics.

The project ***Glioblastoma multiforme*** is exploring a completely different approach to targeting cancer cells. Instead of using viral vectors to adapt immune cells with tumour-specific TCRs, adeno-associated viruses are being targeted directly at the brain cancer cells. The virotherapy of glioblastoma multiforme uses infectivity-enhanced selectively replication-competent adenoviruses to infect glioblastoma cells for selective replication. This eventually kills the cancer cells, releasing new virus particles to target more cancer cells. When the project started in 2006, the virus had already been produced in the US and the clinical trial was to be performed in duplicate at VUmc and in Houston, Texas. However, by the time the Dutch trial finally started in 2010, having been delayed by regulatory issues in the Netherlands, its US counterpart had already been completed. The vector technology has received FDA orphan drug designation and is currently in clinical development at the young biotech company DNAtrix in collaboration with Merck (MSD).

A similar gene therapy is being developed in the project ***Prostate cancer*** at Erasmus MC in Rotterdam to address the high unmet medical need of numerous prostate cancer patients. The feasibility of an oncolytic adenovirus therapy as neoadjuvant treatment for localised prostate cancer is currently being explored in an ongoing phase I/II clinical trial. The oncolytic virus is administered locally prior to the surgical removal of the prostate. The researchers aim at reducing the likelihood of residual cancer cells after surgery, thus lowering the risk of tumour recurrence. Like the *glioblastoma multiforme* project, this project also encountered significant delay due to regulatory hurdles in the Netherlands after receiving the GMP vector batch produced in the US. Additionally, the pace of patient inclusion for the ongoing trial lagged behind the researchers' expectations.

In the project ***HPV-positive penile and cervical cancer*** the researchers are using a non-viral technique to treat penile and cervical cancer caused by persistent HPV infections. By micro-injection of nonfunctional HPV gene (a DNA vaccination) into the patients' skin, the researchers hope to induce an HOV-specific T-cell immune response. Shortly after the project started in 2006, it became apparent that the DNA vaccine previously developed was not safe for use in humans and needed to be revised. ZonMw allowed a shift from a pharmaceutical + clinical project to a preclinical + pharmaceutical phase project, if it was able to obtain funding for the clinical trial elsewhere. In the end, the researchers were able to develop a new, safer DNA vaccine, which was produced in-house at the Dutch Cancer Institute, and secured additional funding for the phase I/II clinical trial from the European Union. So far, twelve patients have been treated and the data is currently being analysed. However, this vaccine is unlikely to be developed any further, as there is no IP to allow for commercialisation of the

technology. The researchers have already moved on to working on the next improved version of the DNA vaccine.

The last of the eight oncology projects is ***Anti-tumour therapy using Clostridia***. Researchers at the MAASTRO clinic are exploring the possibility of using non-pathogenic engineered clostridia as a delivery vector for toxic gene products to the tumour. The bacteria will migrate and proliferate at the hypoxic areas on the inside of a solid tumour and locally convert the prodrug to its active form. The idea of administering bacteria into the blood stream as a cancer treatment might seem dangerous and far-fetched, but according to the lead researcher it can be done in a controlled fashion and terminated at any time using common antibiotics. The project started in 2009 and has made great progress in testing multiple prodrug-enzyme combinations and a method of engineering clostridia without the use of antibiotics resistance has been developed . However, the project is still in the preclinical phase, due mainly to two reasons. Firstly, given the uniqueness of this approach, a failure in the clinical trial will most likely signify the end of this technology, as it might be impossible to attract new funding for future improvements. Secondly, the researchers have been unable to agree terms in early discussions with regulators about the proposed clinical trial. The required safety data and protocols are not yet available, as the researchers feel unable to commit to a prodrug-enzyme-clostridia combination to initiate the expensive toxicology studies. However, these are required to obtain clinical trial approval.

## Heritable disease projects

A total of six projects in the ZonMw TGO programme are focusing on heritable (genetic) diseases. Two projects have been completed successfully (*LPL deficiency* and *Duchenne muscular dystrophy*), while one project was cancelled due to major safety concerns (*Primary immunodeficiencies XLA, SCID*). The remaining three projects have yet to enter the clinical phase, with two projects now in the pharmaceutical phase (*Leber's congenital amaurosis* and *Crigler-Najjar syndrome*) and one still in preclinical testing after a change in project leadership (*Pompe disease and Hurler syndrome*). Notably, the two successfully completed projects proceeded without any delay, while the rest are an average of >4 years behind schedule. The following section briefly discusses and summarises all six heritable disease projects and their recent progress. For a more detailed summary and discussion of each individual project please see Appendix 1.

The project ***LPL deficiency*** is arguably the most successful project of the entire TGO programme to date. The adeno-associated virus (AVV) vector for treatment of the ultra-orphan disease lipoprotein lipase (LPL) deficiency has been developed and successfully tested in the clinics at the Academic Medical Center in Amsterdam in collaboration with the biotech spin-off

AMT (now uniQure). Now marketed as Glybera, it has become the first EMA-approved gene therapy. Notably, the project was the first in the TGO programme to move into the clinics and despite many regulatory hurdles (to the frustration of the researchers), the project was completed without any significant delay.

The project ***Duchenne muscular dystrophy*** was also remarkably successful. In this five-year project comprising preclinical, pharmaceutical and clinical phases, researchers at LUMC developed an exon-skipping gene therapy for Duchenne muscular dystrophy (DMD). This exon-skipping therapy is not dependent on a viral delivery system. Instead, a small oligonucleotide is used to alter splicing of the pre-messenger RNA of the mutated dystrophin gene, removing the mutated part of the gene. This produces a truncated but largely functional gene, comparable to the less severe Becker's muscular dystrophy. Interestingly, LUMC has a long tradition of research into DMD: from the identification of the underlying gene mutation in dystrophin in 1983 to the first proof-of-principle of exon-skipping in 1993 to this ZonMw project in 2006. The researchers have developed the technology in a collaboration with the Leiden-based biotech company Prosensa. A subsequent attempt to obtain marketing authorisation with GlaxoSmithKline was unsuccessful and all licensing rights were returned to Prosensa. Recently, Prosensa was acquired by the US pharmaceutical company BioMarin, which has committed to bringing the drug to market. Notably, BioMarin not only acquired the marketing rights, but has set up its European operations in Leiden, maintaining and expanding Prosensa's close collaboration with LUMC.

In the project ***Crigler-Najjar syndrome***, an adeno-associated virus (AAV)-mediated liver directed gene therapy is being developed for the treatment of a specific form of inherited unconjugated hyperbilirubinaemia known as Crigler-Najjar syndrome (CNS). Patients born with CNS are unable to break down bilirubin, a heme metabolite from red blood cells, which leads to severe jaundice and potential brain damage and mental retardation. The researchers have been able to cure the disease in its respective animal model, the Gunn Rat. However, the first version of the vector seemed unsuitable for large-scale production and had to be revised, causing significant delay. Right now, the improved vector is in GMP production at Genethon in France. Additionally, several new collaborations have been established to test the vector in multiple centres across Europe, for which a Horizon2020 grant application has been submitted. Luckily, regardless of whether the grant is awarded, the researchers at AMC will be able to conduct a small-scale phase I/II trial on their own, as the required funding has already been provided by ZonMw and others. The current plan is to obtain regulatory approval by the end of 2016 and start the clinical trial shortly thereafter.

The goal of the project ***Leber's congenital amaurosis*** (LCA) is to develop an AAV-based gene therapy to fight one of the subtypes of LCA inheritable blindness in patients with a

mutation in the CRB1gene (LCA subtype 8). In the preclinical phases there has been some delay due to optimisation of the mouse model required to reliably mimic the human disease phenotype and in order to reduce the vector size to increase its stability. As of today, a proof of principle has been achieved, patented and published. The vector is ready for GMP production and the clinical trial's starting date is planned for 2016. Currently, the project leaders are contemplating whether a commercial framework for the clinical trial (e.g. in a spin-off company) might help to increase the chances of successful clinical development of the treatment. However, this is not an easy issue, since there are many implications to considered.

The project ***Pompe disease and Hurler syndrome*** is developing two new gene therapies for lysosomal storage diseases. One will be tested in the clinics as part of the ZonMw TGO programme. Both diseases lead to the accumulation of metabolic byproducts in the blood, causing musculoskeletal degeneration and neurological problems. Current disease management consists of enzyme replacement therapy, which needs to be continued lifelong, has various limitations and is extremely costly. The proposed gene therapies use a lentiviral delivery system to transfect haematological stem cells with a healthy copy of the respective nonfunctional gene. Proof of principle has been established for both diseases and the vectors are ready for GMP production and subsequent clinical testing. However, after the retirement of the project leader, which led to a two-year pause in the project, the new principle investigator has decided to further improve the vector for Pompe disease before initiating GMP production in order to maximise the chances of a successful clinical trial. Despite the significant delay, ZonMw has granted two more years for preclinical optimisation, but the project will need to secure a new source of funding for the pharmaceutical and clinical phases.

The last project involving a heritable disease is ***Primary immunodeficiencies XLA, SCID***. In this project, self-inactivating lentiviral vectors are to be developed for treatment of two X-linked severe combined immune deficiencies (SCID) and for the X-linked agammaglobulinaemia (XLA). Although the lentiviral vectors used in this project have been significantly improved in many ways compared to the vectors of the infamous gene therapy trials some 15 years ago, where several young patients developed leukaemia, preclinical testing of these new vectors still showed an unacceptably high risk of insertional mutagenesis near oncogenes. Extensive additional preclinical revisions would have been required to address this problem and the project was therefore discontinued. However, a few years later the researchers were able to restart the project under ZonMw's Priority Medicines for Rare Conditions and Orphan Drugs programme.

## Infectious disease projects

The only project in the TGO programme within the infectious disease category is titled ***RNAi gene therapy for HIV/AIDS***. Researchers at the Academic Medical Center in Amsterdam are developing a lentiviral vector to tackle HIV-1 infections by transfecting CD34+ haematopoietic stem cells *ex vivo* with multiple short hairpin RNAs (shRNAs) against a crucial HIV-1 gene. Despite a successful proof of principle in cell culture and the Human Immune System (HIS) mouse model, there is the possibility that the HIV-1 virus may become resistant relatively quickly due to silent mutations within the siRNA-targeted regions. Initiating the pharmaceutical phase to move towards clinical testing of this vector might be premature and would require additional financial resources that have not yet been secured. This therefore poses a dilemma for both ZonMw and the researchers. Allowing use of ZonMw resources reserved for the pharmaceutical and clinical phases to further enhance the vector may in the end help to attract more funding (e.g. additional grants or through licensing agreements with a biotech company) and may eventually lead to the clinical development of a promising new treatment. However, the amount of funding required for the clinical evaluation will be even larger once the reserved ZonMw resources have been used for additional preclinical work. The future of this project therefore remains uncertain at this point.

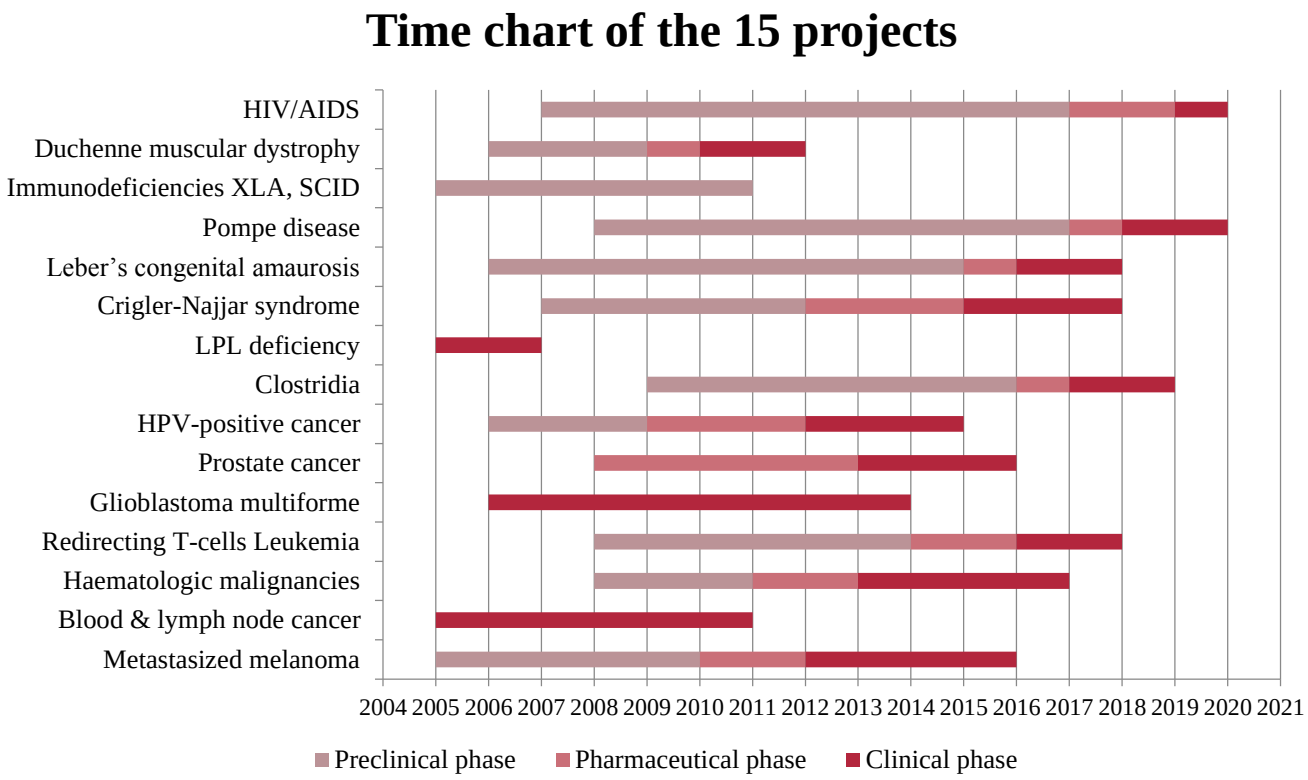


***Figure 2:*** *Time chart showing the actual time spend in each of the three phases of all 15 projects in the TGO programme as of August 2015. Data beyond 2016 based on projections by the respective project leaders.*

# Impact of the Translational Gene Therapy Research programme

As a funding programme for translational research, the main goal of the ZonMw Translational Gene Therapy Research (*Dutch*: TGO) programme has been defined as to stimulate 'the transition from the laboratories to the clinics' or, to use more popular language: '*research from bench to bedside*'. However, analysing the impact of the TGO programme simply based on whether translation to the clinics has been achieved might be too shortsighted. The impact of the TGO programme is therefore discussed on the basis of three separate factors: the impact on knowledge and infrastructure, the economic impact and the impact on patients.

## Impact on knowledge and infrastructure

The performance of academic researchers and the impact of their research are often measured by means of the number and quality of publications. Other indicators of knowledge gained are the staff involved, most notably PhD students who graduate in the projects funded by the TGO

programme, but also personnel who are only partly involved, such as hospital pharmacists and fellow clinicians. In addition, the physical infrastructure plays a crucial role in the quality of research. However, only when the researchers and physical infrastructure involved are connected by well-established protocols, good communication between all parties involved and well-coordinated procedures, can high-quality translational research be guaranteed. Another important factor is industry collaborations, which are discussed in the next section.

The primary output of academic research is knowledge, which is best quantified by the number and quality of publications in the scientific literature. The project leaders of the 15 TGO projects have reported to the ZonMw TGO programme committee a total of 140 publications in international peer-reviewed scientific journals. The overall quality of the published articles as indicated by the journal's impact factor is high and includes multiple publications in renowned journals such as *Nature*, *Nature Biotechnology* and the *New England Journal of Medicine*. Various contributions have also been made to national and international textbooks; there have been keynote, podium and poster presentations at scientific congresses and symposia; and PhD theses and several patents have been published.

Significant knowledge gains can be observed at various professional levels in the Translational Gene Therapy Research programme, including clinicians, primary researchers, hospital pharmacists, post-docs/young investigators and PhD students. The level of involvement of different researchers depends on the phase of the project (preclinical, pharmaceutical and clinical phase). The projects in a preclinical phase have involved a total of 16 students hoping to graduate as highly trained experts in their respective gene therapy field; many of them have already successfully defended their thesis. While five PhD candidates still have to defend their theses, all the others except one are continuing their careers in gene therapy and/ or translational medical research. The one exception took a post-doc in a different field, but is about to return to gene therapy research for his second post-doc. Only three former PhD students chose to continue their research in the biotech industry, but all of them were able to find roles where they can leverage the knowledge they obtained. It is important to note that the majority of the PhDs stayed in the Netherlands, indicating that there is opportunity for young talent in the field of gene therapy. Aside from the knowledge obtained about the therapy itself, which is documented in the numerous publications, there has been a steep learning curve in regulatory matters for GMP production and clinical trials among project leaders, hospital pharmacists and research staff.

Like the learning curve for academics, the regulators themselves seem to have evolved, too. The project leaders of the early trials in the TGO programme complained about a lack of expertise regarding gene therapies and a low degree of willingness to discuss critical issues among regulators. With the Gene Therapy Office now in place and new possibilities to engage

in early discussion with regulators, project leaders are increasingly confident about the regulatory processes. However, a common source of frustration among the project leaders is dealing with the Ministry of Infrastructure and the Environment (*Dutch*: IenM) and the Office for Genetically Modified Organisms (GMO Office) as regards the evaluation of the environmental risks. Both the researchers and the regulators at the CCMO have proposed authorising the CCMO to evaluate the risks to the environment, thereby reducing the total number of parties involved in the regulatory process. However, this is not a simple solution, as it requires legislative changes.

## Economic impact

Most project leaders have described the funding provided by ZonMw's Translational Gene Therapy Research programme as essential to the project. However, the funding provided was not intended to fully cover the costs of GMP production and clinical trials. Additional (matching) funding was therefore a requirement for each project in order to accomplish the clinical translation of the treatment. An earlier report stated that the project leaders had been very successful in securing the additional funding from various sources, generally matching the ZonMw funding 1:1.[7] Besides financial support provided by the research institutes themselves, funding was obtained from the Dutch Cancer Society and various foundations and patient organisations. Support by patients and patient organisations has been a crucial factor in the success of many research projects in and outside the ZonMw programme, providing active engagement and long-term funding. However, some projects are struggling to obtain the funding to move to the next phase of development. Fortunately, no project has had to be cancelled yet simply for a lack of financial resources.

Another important source of funding is industry collaborations. However, large pharmaceutical companies remain very hesitant and rarely provide funding in return for exclusive licensing rights. Furthermore, universities and university medical centres are by their very nature not suited to commercial drug development. An alternative way of facilitating drug development and allowing for private investments is to found a university spin-off. This approach has been taken successfully in two projects in the TGO programme: *LPL deficiency* with Amsterdam Molecular Therapeutics (now uniQure) and *Duchenne muscular dystrophy* with Prosensa (now a division of BioMarin Pharmaceuticals). Similarly, as the project *Metastasized melanoma* was nearing completion the project leader established the spin-off company T-Cell Factory BV to improve the T-cell receptor gene therapy. Shortly thereafter, the US biotech company Kite Pharma acquired the company.

The three companies mentioned above - Prosensa, uniQure and T-Cell Factory - either directly or indirectly resulted from a project funded by the Translational Gene Therapy Research

programme. UniQure alone has a current market capitalisation of more than €500 million (as of September 2015). Prosensa and T-cell Factory were acquired for $680 million and €20 million plus milestone payments respectively. Additionally, the Utrecht-based start-up company Gadeta, co-founded by project leader Prof. Kuball, just received €7 million series A funding to facilitate the step towards clinical translation. Most notably, all four companies still operate from the Netherlands and have committed to continuing their close collaboration with the universities where they were founded.

## Impact on patients

The goal of all research in the medical sciences is ultimately to develop better treatments and to increase patients' well-being. Therefore, the impact of the Translational Gene Therapy Research programme on knowledge, infrastructure and economic parameters can only be seen as transient endpoints towards the development of new treatments. Four projects In the TGO programme have successfully completed their phase I/II clinical trial. Some of these projects have led to follow-up trials and more than 400 patients have been treated. Additionally, three more projects have just started or are about to start treating the first patients. The majority of the projects - nine in total - have yet to enter the clinical phase due to significant delays in the preclinical and pharmaceutical phases. However, some of these projects will presumably also achieve the transition into the clinics, eventually reaching patients.

One treatment developed in the TGO programme is from the project *LPL deficiency*, alipogene tiparvovec (Glybera), which was not only tested successfully in clinical trials, but also received EMA approval in 2102. This is a major breakthrough for the entire field of gene therapy research, and also for patients with heritable diseases in general. All Dutch patients with familial lipoprotein lipase (LPL) deficiency meeting the inclusion criteria for the clinical trials have been treated, plus some eligible patients in Canada. The commercial rollout of Glybera in Europe, originally planned for 2014, as well as FDA approval for the US, are still pending. Nevertheless, the impact of this project and the forerunner role it might have for similar therapies is a remarkable success for patients worldwide.

The exon-skipping treatment drisapersen (*formerly:* PRO051) that was developed in the project *Duchenne muscular dystrophy* at LUMC in Leiden has been tested in multiple clinical trials. So far, more than 300 patients have been treated and the treatment has received fast track and breakthrough designation in the US, allowing for a faster FDA review process and potentially faster marketing authorisation. The project has been very close to market authorisation once before in a collaboration between Prosensa and GlaxoSmithKline, but the primary endpoints were not reached in a large scale phase III clinical trial. While drisapersen is clearly not a panacea for all Duchenne patients, there seems to be a subset of patients who

benefit from the treatment. Prosensa, BioMarin and LUMC are currently working hard on several other oligonucleotides for other Duchenne patient subgroups.

While most projects in the TGO programme have yet to enter the clinical phase, there are two more projects that have successfully completed their first clinical trials: *Glioblastoma multiforme* and *HPV-positive penile and cervical cancer*. In the project *Glioblastoma multiforme*, an oncolytic conditionally replicative adenovirus (CRAd) was injected through a special convection-enhanced system for directed delivery onto the brains of 15 patients. Unfortunately, the trial data (yet to be published) are not likely to be of any relevance to the company developing the product, because of the delay prior to the start of the trial in the Netherlands. In the project *HPV-positive penile and cervical cancer* at the National Cancer Institute (*Dutch:* NKI) in Amsterdam, a total of 12 patients have been treated with the DNA vaccine produced at the NKI's own GMP facilities. The data from the first trial is still being analysed. However, thanks to the in-house GMP production facilities, the researchers at the NKI are able to iterate on their products to produce and test more effective DNA vaccines in the near future.

# Discussion

The Translational Gene Therapy Research programme was set up at a difficult time for gene therapy research when promising new treatments had just caused leukaemia in several adolescent patients, putting many planned or ongoing clinical efforts on hold. During the lifespan of the TGO programme, many promising new therapies have been translated to clinical trials and the field is starting to reap the first benefits. Most notably, the first gene therapy to gain marketing approval in either Europe or the United States has been developed in the TGO programme. Other projects have been very successful, too. One of these projects, to develop an exon-skipping technology for the treatment of Duchenne muscular dystrophy, still has the potential to reach the market, too. However, there are also projects that have not yet proved so successful. Significant delays have occurred in most projects and despite the fact that the TGO programme was originally planned to end in 2014, most projects have yet to complete the clinical phase.

## Reasons for delay: regulation, GMP production and catching up

The reasons for the significant amount of delay in the majority of the projects are very similar: regulatory hurdles, problems during (external) GMP production and new findings that require

additional preclinical research. While regulatory hurdles were mentioned most frequently by the vast majority of the project leaders, they do not actually account for the lion's share of the delay. Similarly, GMP production and toxicology studies in the pharmaceutical phase accounted for only about 20% of total postponements. The largest proportion of the delay came about due to additional preclinical research required whenever new findings indicated that the current treatment might not be the best option for the transition to the clinics. Frankly, the higher level of frustration among the researchers about seemingly unnecessary or exaggerated regulatory requirements and GMP production problems at external partners is understandable. Unlike the additional time spent to optimise the treatments, dealing with regulators or GMP production delays means idle time spent waiting. However, part of the frustration about dealing with regulators also stems from inefficiencies that may or may not be necessary.

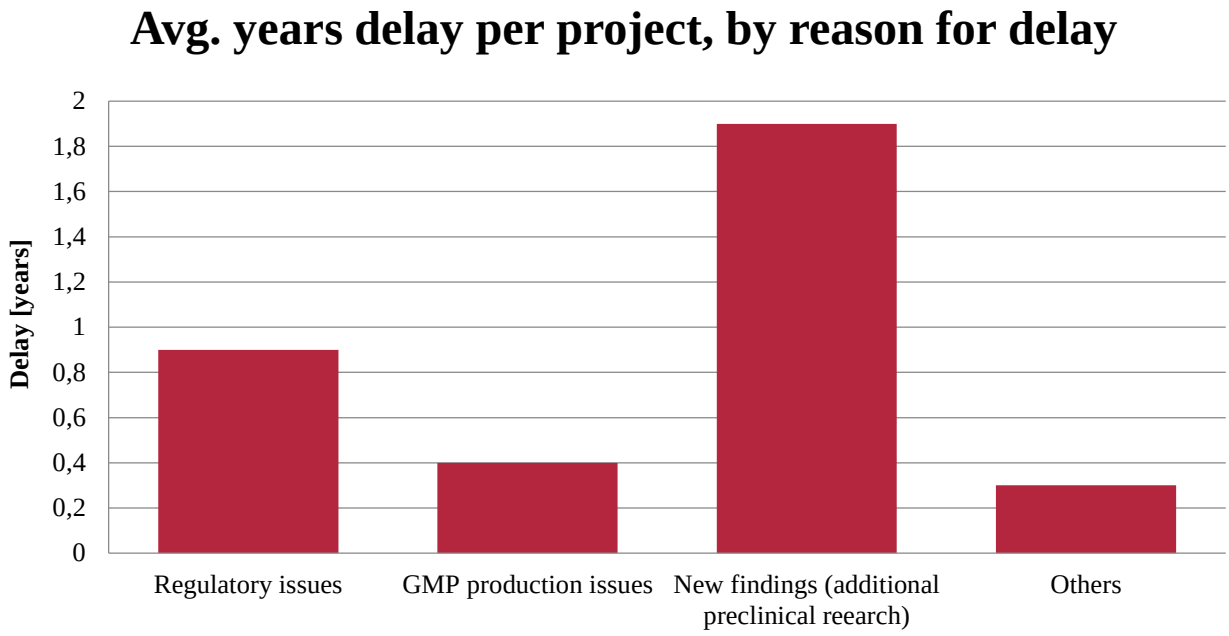


***Figure 3:*** *Reasons for delay, showing the average amount of delay per project in years as indicated by researcher.*

## Regulatory processes have become clearer, but many hurdles persist

Many researchers reported their frustration about regulatory procedures for obtaining approval for clinical trials. This has been a particular issue for the projects that entered the clinical phase relatively early in the lifetime of the TGO programme. While most of these projects were eventually able to obtain regulatory approval, one project (*Recurrent blood and lymph node cancer*) failed to do so, leading to the discontinuation of the project after a six-year struggle. Now, several years after the very first projects entered the clinical phase, many things

have been changed to make regulatory processes easier and most researchers that have yet to submit their requests feel confident about obtaining regulatory approval in a timely manner. However, some hurdles remain, not only causing delays and frustration, but potentially having wider implications for the entire field of gene therapy research in the Netherlands.

When the first projects in the TGO programme started preparing their dossiers for the regulatory approval process in order to conduct the first clinical trials in human subjects in 2005, there was a substantial amount of confusion among researchers about the exact procedures and processes. Similarly, regulators were confronted with the need to update the safety requirements from conventional drugs to fit gene therapies with unique safety implications. Since gene therapies are fundamentally different from small molecule drugs and other biologicals, such as hormones or therapeutic antibodies, realistic requirements to demonstrate the safety of these new treatments had yet to be established. Since then, a lot has changed. Besides the new guidelines from the EMA for the approval process for gene therapies, the Dutch authorities have also started providing much clearer instructions. The Central Committee on Research Involving Human Subjects (*Dutch:* CCMO) has established a dedicated approval process for gene therapy trials with all requirements specified in detail in sample documents. Similarly, the Netherlands Commission on Genetic Modification (*Dutch:* COGEM), the Office for Genetically Modified Organisms (GMO office) and the Ministry of Infrastructure and the Environment (*Dutch:* IenM) have all put together more specific requirements to streamline the approval processes. Very recently, plans have been made to promote the option of early discussion with all major regulators before officially submitting the dossier for a clinical trial approval. Furthermore, the Gene Therapy Office (*Dutch:* Loket Gentherapie) has been established during the course of the TGO programme to provide a single contact point for information about all regulators in order to further streamline the approval process.

Despite many efforts to streamline the regulatory process for gene therapy trials, most notably by the Gene Therapy Office, some issues remain unresolved. The regulatory process is very time-consuming and appears fragmented to many researchers, involving several different parties for risk appraisals, such as CCMO, COGEM, the Ministry and the GMO office.[8]

# Evaluating the success of the TGO programme

The primary objective of the Translational Gene Therapy Research programme was to stimulate translational research in the field of gene therapy research in the Netherlands. This has been defined as either preclinical research to explore the safety and efficacy of new treatments with the intention of conducting a subsequent clinical trial, or a phase I/II clinical trial of a new gene therapy, after the safety and efficacy has been demonstrated in preclinical

studies. The overall success of the TGO programme can be assessed by the combined impact of the individual 15 research projects on the field of gene therapy research in the Netherlands, their combined financial/economic impact and the impact on patients. There have been some remarkable success stories. For the majority of the projects, however, it is still unclear whether additional breakthroughs are to be expected.

Probably the most significant achievement has been accomplished by the researchers in the *LPL deficiency* project, who have developed the world's first gene therapy to obtain marketing authorisation in either Europe or the United States. The researchers at the Academic Medical Center in Amsterdam, working with the biotech spin-off Amsterdam Molecular Therapeutics (AMT; now uniQure), have successfully completed a phase I/II clinical trial to demonstrate the safety and efficiency of the gene therapy alipogene tiparvovec (trade name Glybera) for patients suffering from the ultra-orphan disease lipoprotein lipase deficiency, also known as familial chylomicronaemia syndrome. Moreover, the resulting Dutch biotech company uniQure now has several other gene therapy products in the pipeline, with initial positive results for their haemophilia B product from early clinical trials in Canada, UK and the Netherlands.[9] However, this has not been the only remarkable achievement of the TGO programme. In the project *Duchenne muscular dystrophy*, researchers at LUMC in Leiden, working with the spin-off company Prosensa, have been able to successfully bring their gene therapy treatment drisapersen, also known as PRO051 and GSK2402968, into the clinics. Prosensa has now been acquired by the US biotech company BioMarin for up to $840 million, but it continues to operate its facilities in Leiden with the goal of bringing the FDA fast track and designated breakthrough treatment to the market. While the phase I/II clinical trial of the project *Metastasized melanoma* is still ongoing, the project leader's spin-off company that specialises in the development of adoptive T-cell receptor cancer therapies has been acquired by Kite Pharma to push the technology forward towards the market.

On the flip side, two projects in the TGO programme failed to achieve clinical translation and had to be cancelled after running into insurmountable problems. The project *Recurrent blood and lymph node cancer* had a long struggle to achieve regulatory approval for the proposed clinical trial and was eventually cancelled when the external GMP manufacturing partner ceased vector production. In the project *Primary immunodeficiencies XLA, SCID*, next generation lentiviruses with an enhanced safety profile have been developed for two severe combined immune deficiencies (SCID) and X-linked agammaglobulinaemia (XLA). Despite improvements in the vector, there was a remaining risk that the treatment would induce leukaemia in humans and the project was discontinued. Although this project has fallen short of clinical translation and, therefore, of the primary goal of this programme, the project leaders have secured new funding for a restart and remain positive that the treatment will eventually reach the clinics. The project *Glioblastoma multiforme* also took a rather unsatisfactory

course. While the virus therapy under development was ultimately tested in the clinics, it took the researchers many years to get the trials going, effectively rendering the results useless for the clinical development of the product, as another trial in the US with the same vector was long completed by then and a follow-up trial had already been organised in Spain.

For the nine other projects in the TGO programme it is impossible to draw any final conclusions yet as the research is still ongoing. Nonetheless, the significant delay in these projects is certainly a significant concern. Delays are often costly and risk rendering the potential treatment obsolete as other research groups might progress faster. However, the research in these nine projects remains highly relevant, as the unmet medical needs associated with the diseases in question has persisted over time. Furthermore, most projects have been able to extend their research in a budget-neutral manner, covering incidental extra costs for personnel and material from various different sources. Up to 90% of the entire costs of a programme are accrued in the pharmaceutical and clinical phase. It may therefore be reasonable in many cases to extend the preclinical phase whenever the technology does not yet seem ready for testing in human subjects.

## A new star on the horizon: DNA editing with CRISPR-Cas9

The current TGO programme is characterised by great diversity in terms of therapeutic areas and technologies to restore gene functions or to use genetic material to target cancer cells in the patient. New concepts have been developed and tested in human subjects for the first time, potentially marking a slow but important paradigm shift in medicine. In the meantime other new technologies are emerging, building upon the initial successes with gene therapies. The most striking is the gene editing tool CRISPR-Cas9 that allows for precise genetic modifications in bacterial and eukaryotic cells, including humans. CRISPR-Cas9 could allow genetic defects to be repaired, which would mean fixing the very cause of a genetic disease rather than treating the symptoms. However, this new technology has an enormous range of implications that need to be considered.

In gene therapy for genetic diseases functional genes are introduced into some of the affected somatic cells. In the studies currently in clinical evaluation the therapeutic genes are integrated at random positions into the DNA in the cells, but do not replace the therapeutic genes. This approach carries the risk of unwanted side effects as the therapeutic genes can integrate at undesired positions in the cellular DNA. Gene replacement would be more precise. This technology has already been shown to be feasible. Upon transfer of naked DNA molecules these can replace the host genes by a process called homologous recombination. This is the

technology used to make mouse models of human diseases, for example. The efficiency of the technique is very low and often hundreds of independent clones have to be analysed before a corrected recombinant is identified. It has not therefore been explored much in connection with gene therapy.

The efficiency of the homologous recombination process can be enhanced by creating a double-stranded break in the DNA at the target locus. This requires nucleases with programmable target-site specificity. The first two such nucleases that became widely available were the Zinc-Finger Nucleases (ZFNs) and the TAL-effector nucleases (TALENs). These nucleases could be modified in such a manner that they only made a double-stranded break at the desired locus in the chromosomal DNA. Although these systems enhance the efficiency of gene replacement, one drawback of the ZFNs and TALENs is that the genes encoding these proteins contain a large number of repeats. This leads to genetic instability in these genes in plasmids and viral vectors. As a result, the ZFNs and TALENs have been employed to only a limited extent in gene therapy. One notable exception has been the use of ZFNs to disrupt the CCR5 gene in order to confer HIV resistance in human bone marrow stem cells.[10] Such cells could be used in a potentially life-saving therapeutic strategy for HIV/AIDS patients.

## *DNA editing made easy*

The limitations of ZFNs and TALENS were overcome by using bacterial RNA-guided nucleases. Bacteria harbour a system that provides them with adaptive immunity to bacteriophages. The so-called CRISPR-Cas9 system is based on the bacteria accumulating small DNA sequences of the bacteriophages to synthesise small bacteriophage-specific RNA transcripts to guide nucleases to the genomes of incoming bacteriophages. These nucleases digest the incoming phage DNA while leaving the bacterial DNA unaffected. In 2013 it was shown that the CRISPR-Cas9 system could be modified for use in mammalian cells, allowing the introduction of double-stranded breaks at specific sites in the cells of many species, including mammals.[11]

In the absence of homologous DNA the cells respond to double-stranded breaks in their chromosomal DNA by a repair process called non-homologous end joining. This leads to the loss or insertion of small nucleotide stretches at the site of the break, the so-called ‘indels’. If the break is inside the protein-coding region of a gene the indels frequently result in loss of function in the targeted gene.

## *Gene editing: an evolution in gene therapy*

The CRISPR-Cas9 technology should be seen as an *evolution* in gene therapy. The field is now aiming to repair the molecular defects that cause the disease in the somatic cells, rather than

the gene-addition therapy. The high precision of the modification should reduce adverse events that occur as a result of genetic alterations at loci other than the target genes ('off-target' mutations). Currently a lot of research is being devoted to developing efficient assays for quantifying the magnitude of off-target mutations and estimating the associated risks.

Dutch researchers are actively contributing to these developments. Prime examples include the development of a technique for efficient transfer of native Cas9 proteins into cultured cells at the Hubrecht Institute in Utrecht,[12] and the use of viral vectors as an efficient strategy to prevent off-target integration of the heterologous transgenes at LUMC in Leiden.[13] With this technology it is conceptually straightforward to edit the chromosomal DNA of somatic stem cells in a predictable and precise manner. It therefore seems reasonable to anticipate the initiation of translational research with this technology in the Netherlands within the next two years.

## *Germline editing: a revolution in human genetics?*

So far genetic modification of the human germline has been widely seen as off limits in biomedical research. In practical terms, human germline modification using a gene-addition technology is hampered by the meiotic segregation of chromosomes. This could lead to segregation of the therapeutic gene from the affected gene. The CRISPR-Cas9 technology enables precise editing and thus makes human germline modification more feasible in a practical sense.

The first publication in this area provoked a lot of discussion.[14] Given the ethical and practical considerations, a panel of researchers called for caution.[15] In light of the ethical sensitivity of human germline editing it is vitally important to clearly distinguish the use of the editing for gene-therapy applications in human somatic cells ('gene editing') from the editing the human germline ('germline editing'). Because of the significant ethical implications, thorough discussions between scientists and regulators worldwide will be vital to establish common ground. To this end a summit was held at the US National Academy of Science in Washington DC where leading US researchers met with colleagues from the UK Royal Society and Chinese Academy of Sciences.[16] Such discussions to balance the need to push this technology further towards medical application without rushing into ethically questionable areas are also taking place in the Netherlands.[17] Concerns around gene editing of human embryos, in particular, are being addressed by Dutch scientists and no requests for such studies have been filed yet.[18]

### *Regulation of gene editing*

The gene-therapy applications are seen as a further maturation of the field and as such the practical and ethical aspects of the clinical use of the technology may be based on existing regulations. It should be noted that it is unclear whether somatic cells modified using the CRISPR-Cas9 system should be legally classified as genetically modified organisms. When it comes to human germline editing, it should be noted that the Dutch Embryo Act currently prohibits alterations to genetic material in the nucleus of gametes or embryos.[19]

## The future of gene therapy research in the Netherlands

For many diseases, gene therapies hold the promise of a clinical cure rather than just dealing with symptoms, or no treatment at all. The new technologies currently emerging might signify a major paradigm shift in medicine. The first major breakthroughs have been achieved, such as the EMA approval of Glybera and, very recently, the first positive FDA appraisal of an oncolytic virus marketed as T-VEC in the US.[20] Despite significant progress in the last decade, however, the field as a whole is developing only slowly. Many of the treatments currently under development will probably have to complete multiple development cycles before reaching truly satisfactory levels of efficiency. Furthermore, new treatments and technologies are not as easily transferable to different patient populations or diseases as previously thought. Each new vector, oligonucleotide or cellular product needs to be analysed extensively before it can be tested in the clinics. Consequently, each new treatment has to go through the entire drug development process before market approval.

Back in 2005, the sheer number and quality of project proposals for the Translational Gene Therapy Research programme indicated the bulk of high potential research in the translational gene therapy field in university medical centres across the Netherlands. Similar Dutch Cancer Society and ZonMw translational research programmes focused on advanced therapeutic medicinal products (ATMPs) have seen an equally high demand. While gene therapy research has a very long tradition in the Netherlands, the field has only recently started to mature from a purely experimental to a medical field of research. However, the translation from preclinical proof-of-principles in animal models to the first clinical trials with the GMP-produced therapies is a complex and costly interdisciplinary endeavour. Despite slowly increasing industry activity in the field of gene therapy, only very few emerging treatments and technologies are actually picked up by larger pharmaceutical companies and lack of funding remains a critical issue for many researchers at the Dutch university medical centres.

The Dutch university medical centres in general produce remarkably high-quality clinical and translational research. In the last decade, many new facilities, interdisciplinary collaborations,

protocols and procedures have been established to facilitate the development of various different ATMPs, including gene therapies, cellular cancer immunotherapies and tissue engineering. There are currently plenty of new concepts and technologies under development at Dutch university medical centres that are not yet ready for larger industry investments, but hold the promise of curative treatments for many patients with currently unmet medical needs.

# Conclusion

## Overall, the current TGO programme has been a success

The Translational Gene Therapy Research (*Dutch:* TGO) programme has been a remarkable success. Under this programme, a gene therapy for lipoprotein lipase (LPL) deficiency has been tested successfully in a phase I/II clinical trial, leading to its subsequent clinical development process to become the first EMA-approved gene therapy. A fundamentally new concept in the medical sciences, exon-skipping for Duchenne muscular dystrophy, has been developed from a proof-of-principle to a clinical reality, tested in more than 300 patients and currently awaiting FDA approval. New spin-off companies have been founded, providing a significant boost to the Dutch knowledge economy. Some of these spin-offs have been acquired by larger pharmaceutical companies, but all continue to operate in the Netherlands with close links to the Dutch university medical centres. More than 140 scientific publications document the wealth of knowledge that has been created, alongside several patents, dissertations and presentations at scientific conferences and symposia. Talented new researchers have been trained in the field and the vast majority of PhD graduates from the programme are still engaged in translational and gene therapy research in the Netherlands.

Since the TGO programme started in 2005, the field of gene therapy research in the Netherlands and worldwide has matured to a medical research field that attracts increasing industry interest and funding. Nevertheless, larger pharmaceutical companies hesitate to invest in these new technologies, generally preferring a ‘wait and see’ approach and investing only after clinical trials have demonstrated efficacy. However, these clinical trials and the prior pharmaceutical phases are very costly. Academic researchers wanting to bring their technologies to the clinics therefore continue to rely heavily on larger public grants. Given the impact of the current TGO programme, the amount of high-quality research in the Netherlands and the scarcity of translational research funding, a follow-up programme is the only logical next step. The Dutch Ministry of Health, Welfare and Sport (VWS) and ZonMw are therefore advised to organise and finance a follow-up programme to the current Translational Gene

Therapy Research programme. The exact focus of the programme may be adjusted to specifically target areas with the highest potential for significant breakthroughs.

## Considerations for a follow-up programme

The follow-up funding programme could build on the strengths of the current programme in order to maximise the probability of success. A good potential focus area at the moment might be exploratory gene editing therapies that leverage the novel CRISPR-Cas9 technology. The technology has been successfully tested in various animal models and seems ready for translational medical research. Dutch UMCs and researchers are also in a strong position to build on earlier gene and cell therapy experience and use existing facilities to get a headstart in the global race for medical applications of CRISPR-Cas9. The three-tier flexible programme structure of the current programme with optional preclinical and pharmaceutical phases but a required clinical phase, together with the requirement for obtaining additional matching funding has worked very well. The current programme has shown that the projects with industry partners experienced significantly less delay and were generally more successful. The researchers in these projects accordingly indicated that the commercial parties were much more efficient at dealing with regulators and in planning the drug development process way ahead, foreseeing and preventing potential risks, such as GMP manufacturing issues and regulatory approvals. Drug development experts from industry should therefore be considered a valuable addition to the programme committee for feasibility analysis prior to project selection and as advisors during the development process for the selected projects. While some risk of delay is inevitable when engaging in exploratory research, ZonMw needs to be more exacting to encourage timely project completion.

# Appendices

## Summary and discussion of individual projects

### Metastasized melanoma

| | |
|---|---|
| Full title: | T-cell receptor gene therapy of metastatic melanoma: a phase I clinical trial |
| Project leader: | Prof. A.N.M. (Ton) Schumacher |
| Institute: | Netherlands Cancer Institute |
| Project structure: | preclinical, pharmaceutical and clinical phase |
| Status: | Completed (delay: 4 years) |
| Duration: | 60 months |
| Start: | 2005 |
| ZonMw budget: | €1,137,400 |
| External funding: | €250,000 |
| Reasons for delay: | GMP production issues at EUFETS and serious adverse event in first patient |

**Summary**

The primary goal of this project was to establish the safety and efficacy of T-cell receptor (TCR) gene therapy in mouse models and the feasibility of creating tumour-specific T-cell immunity through TCR gene therapy in patients with metastasized melanoma. As opposed to conventional adoptive T-cell therapy where tumour-specific immune cells are isolated, proliferated and reimplanted, this project aimed to transfer T-cell receptor genes into recipient T-cells. In the first phase of this project a highly melanoma-reactive TCR was identified and characterised. The most suitable vector system for the clinical trial was determined and TCR expression was optimised. In the second phase of the project a GMP retrovirus producer cell line was generated and a high titer retrovirus batch produced. Clinical scale test runs were completed successfully in order to obtain all required permissions for the start of the clinical trial. A total of six patients have been treated since the opening of the trial in 2012.

**Discussion**

After some delay in the pharmaceutical phase due to problems during external GMP virus production and unexpected toxicity of the product in preclinical models, the clinical trial was eventually able to commence. However, the trial was interrupted soon after the first patient developed an acute immune reaction. After rewriting the clinical protocol with a strongly reduced dosage regimen, the trial was resumed and a further five patients have now been treated. Despite these setbacks, the ongoing project can be considered a success. This is not so much because of the product itself (in fact, improved iterations of the vector targeting more

potent tumour antigens are already under development). In fact, the major milestones have been the development of various novel techniques and protocols, the establishment of a new facility to genetically modify T-cells, and the know-how generated, attracting the interest of various parties to this field of research. Evidence that there is indeed a lot of confidence in this approach can be seen in the recent acquisition of co-founder Prof. Schumacher's relatively young biotech spin-off T-Cell Factory BV by 'big pharma' (Kite Pharma, Santa Monica, CA, USA) for approximately € 20 million upfront plus undisclosed milestone payments. Both Kite Pharma and the Netherlands Cancer Institute have committed to expanding their efforts to develop new cellular gene therapies and are aspiring to become industry/academic leaders in this field of research.

# Recurrent blood and lymph node cancer

| | |
|---|---|
| Full title: | Suicide gene therapy of haematological malignancies with herpes simplex virus thymidine kinase gene-transduced allogeneic donor T-lymphocytes in the context of allogeneic stem cell transplantation. A phase I/II clinical feasibility study |
| Project leader: | Prof. Anton Hagenbeek |
| Institute: | UMC Utrecht |
| Project structure: | Clinical phase |
| Status: | Discontinued (2011; delay: 4 years) |
| Duration: | 24 months |
| Start: | 2005 |
| ZonMw budget: | €196,050 (resources used; original budget €550,000) |
| External funding: | €161,000 |
| Reasons for delay: | Numerous regulatory hurdles, GMP virus manufacturer ceased production |

**Summary**

The current standard treatment for most haematological malignancies is transplantation of healthy bone marrow stem cells from a donor. However, in some cases the cancer recurs, giving patients a very poor prognosis. One way to achieve remission once again is to administer lymphocytes from the original donor in order to induce a graft versus leukaemia (GVL) effect. While this immune response can be very effective against the cancer cells, normal cells might also be attacked (graft versus host disease; GVHD). To benefit from the GVL effects and prevent severe GVHD, donor T-cells are transduced *in vitro* with a retroviral vector carrying the herpes simplex virus thymidine kinase (HSV-Tk) suicide gene and the gene encoding a truncated (non-functional) form of the low affinity nerve growth factor receptor (NGF-R). Transduced donor T-cells will be selected *in vitro* on the basis of the expression of NGF-R at their cell surface. A fixed quantity of pure transduced donor T-cells will be infused into patients with a high-risk haematological malignancy (AML, MDS, ALL, Multiple Myeloma, aggressive NHL) who have relapsed after allogeneic SCT. If GVHD emerges, the HSV-Tk gene transduced donor T-cells will be selectively eliminated by treatment with the prodrug ganciclovir. The primary objectives of this clinical phase I/II study are to evaluate the toxicity, survival and alloreactivity of the transduced donor T-cells as well as their *in vivo* response to ganciclovir treatment.

# Haematological malignancies

| | |
|---|---|
| Full title: | Retroviral gene transfer of T-cell receptors recognising minor histocompatibility antigens to virus-specific T-cells as cellular immunotherapy for patients with haematological malignancies after allogeneic stem cell transplantation<br>Project leader: Dr. Mirjam H.M. Heemskerk |
| Institute: | LUM |
| Project structure: | preclinical, pharmaceutical and clinical phase |
| Status: | Ongoing (clinical phase, delay: 2 years) |
| Duration: | 60 months |
| Start: | 2008 |
| ZonMw budget: | €1,129,000 |
| External funding: | €427,500 |
| Reasons for delay: | problems during GMP virus production, complicated patient recruitment scheme |

**Summary**

The primary objective of this project is to investigate the feasibility of minor histocompatibility antigen (MiHA) specific T-cell receptor (TCR) gene transfer to virus-specific T-cells as a cellular anti-tumour immunotherapy with a minimal risk of graft versus host disease. This approach could prove particularly useful for patient with a relapsing haematological malignancy after a human leukocyte antigen (HLA) matched stem cell transplantation (SCT). Ideally, patients receive an HLA-matched SCT from a sibling or suitable donor. In the event of disease recurrence, donor lymphocytes are infused to achieve complete remission. In some cases, however, donor lymphocytes induce severe graft versus host disease (GVHD), even after HLA-matched SCT. Virus-specific T-cells can overcome this problem as they do not recognise foreign cells and therefore do not induce GVHD. Retroviral transfer of the MiHA specific TCR into virus-specific T-cells demonstrated the generation of potent anti-leukaemic T-cells.

During the preclinical phase, several enhancements of the vector were made to improve TCR expression and stability on the cell surface of the modified T-cells. In addition, to prevent dimerization of the introduced TCR chains with other endogenous TCR chains, potentially leading to off-target immune effects (such as GVHD), further modifications were made.

Eventually, the vector went into external GMP production for the clinical trial, which opened by the end of 2012. However, the actual start of the trial had to be postponed after EUFETS GmbH, the external partner for the GMP vector production, reported safety concerns about the vector batch it had produced. After detailed risk analysis, the trial was restarted and the first patient was treated by the end of 2013. The clinical trial is currently ongoing and the fourth patient has just been treated (March 2016).

**Discussion**

As opposed to other projects, the need for additional vector optimisations has led to only minor delays. However, in common with most projects, additional delays occurred during external GMP vector production. The subsequent risk analysis after safety concerns had been reported interrupted the clinical trial for at least another six months. Due to the complicated recruitment scheme in the study protocol the first patient was treated a year after the initial opening of the trial. However, the patient died three months later most likely not as a consequence of the treatment itself, leading to another interruption in the trial. The next two patients were treated without any complications and the project leader remains confident of treating sufficient patients to be able to draw conclusions about the safety and feasibility of the treatment. Notably, the ongoing clinical trial requires significant effort in many quarters to ensure potentially eligible patients and donors are referred and subsequently included in the trial. It therefore remains to be seen if the researchers will be able to complete the clinical trial while it is still relevant.

# Redirecting T-cells in leukaemia

| | |
|---|---|
| Full title: | Redirecting T-cells by broadly tumour-reactive second-generation T-cell receptors |
| Project leader: | Prof. Jürgen Kuball |
| Institute: | UMC Utrecht |
| Project structure: | Preclinical, pharmaceutical and clinical phases |
| Status: | Ongoing (pharmaceutical phase, delay: ~3 years) |
| Duration: | 72 months |
| Start: | 2008 |
| ZonMw budget: | €1,000,000 |
| External funding: | €1,529,308 |
| Reasons for delay: | 'Last-minute' identification of more potent vector, external vector production |

**Summary**

Various adaptive cellular treatments for different malignancies are currently in preclinical and clinical development, but most of them have significant limitations in terms of their potency, efficiency and universality that pose a challenge when it comes to implementation in clinical practice. This project aims to tackle many of these limitations by targeting a more broadly expressed tumour antigen, allowing the therapy to be used against different types of cancer. Additionally, the T-cell receptor used here to redirect the immune cells does not interact with other T-cell receptors, which significantly lowers the risk of severe adverse effects.

**Discussion**

After its launch in 2008, the project experienced multiple delays and continues to face challenges today. However, those delays were mainly due to unexpected beneficial opportunities, and additional funding was secured to keep the project afloat. In 2012, when the project was about to move into the pharmaceutical phase, a more potent vector was discovered. The researchers decided to postpone the clinical-grade GMP production of the old vector in favour of the new one and went back to preclinical safety and efficacy evaluation. Shortly thereafter, GMP production was postponed once more in order to develop a novel transduction and isolation technique. Both delays ultimately led to two new patent applications, offering potential leverage for clinical development in collaboration with a biotech or pharmaceutical company.

In 2014, GMP vector production was initiated at EUFETS, but technical problems have kept vector yields below expectations. To date (August 2015), enough vector particles have been produced for an initial phase I/II clinical trial, but the researchers might opt to hold out for the

final vector batch before moving into the clinics. Furthermore, the project leaders are currently in talks with two potential industry partners for the clinical development of the vector. All patents, protocols and applications to regulators have been reviewed by the interested industry partners to streamline potential further clinical development. In late March 2016 it was announced that the project leader's start-up company Gadeta had obtained €7 million series A funding to help push clinical development. After all, the results of the phase I/II trial – most likely not due before late 2017 – will determine whether the vector proceeds further down the path of clinical development, or the researchers must go back to the drawing board.

Research on adoptive cellular therapies (ACT) using chimeric antigen receptors (CARs) has become 'a race to the finish line', with many competing approaches currently under development in various research groups around the world.[21] Despite the multiple delays, persisted GMP production difficulties and the lack of any clinical data just yet, it must be acknowledged that the knowledge acquired so far has enhanced our understanding of ACTs and that the project remains very promising.

# Glioblastoma multiforme

| | |
|---|---|
| Full title: | Virotherapy of glioblastoma multiforme using an infectivity-enhanced selectively replication-competent adenovirus |
| Project leader: | Dr. Victor W. van Beusechem |
| Institute: | VUmc |
| Project structure: | Clinical phase |
| Status: | Completed (delay: ~6 years) |
| Duration: | 30 months |
| Start: | 2010 (originally 2006) |
| ZonMw budget: | €497,000 |
| External funding: | €630,979 |
| Reasons for delay: | Multiple regulatory hurdles, slow patient inclusion, complications during clinical trial (wound leakage) |

**Summary**

Glioblastoma multiforme (GBM) is a highly invasive brain tumour with a very poor prognosis. It is commonly treated with surgery on the solid tumour parts together with radiotherapy and chemotherapy. Due to its invasiveness and resistance to both radiation and chemotherapy, the tumour quickly recurs after surgery, leading to a median survival of only about 12 months and less than a 10% two-year survival rate. In this project a new generation of infectivity-enhanced oncolytic conditionally replicative adenovirus (CRAd) will be tested in GBM, using a convection-enhanced delivery system. The virus has been tested extensively and will be brought to the clinics at multiple sites in Birmingham and Houston in the US and at VUmc in the Netherlands. While earlier versions of oncolytic viruses have failed to deliver any real therapeutic value, this virus has undergone multiple improvements, including the new delivery system that might help spread the viruses to most parts of the brain.

**Discussion**

The virus has been produced in the US under a grant from the national cancer institute and after significant delays the toxicology reviews were completed in 2007. Two years earlier ZonMw had awarded the grant and the researchers originally aimed to start the clinical trial in 2006, with a planned completion date in 2008. However, it was not until 2010 that the first patient was treated and the second phase of the clinical trial was completed by the end of 2014. The reasons for the significant delay of the clinical trial were manifold: delay in the toxicology studies and new European legislation that required the GMP vector batch produced outside the EU to be reexamined by a qualified person. This trial also marked the first time a replication-competent virus was brought to the clinics in the Netherlands, which raised concerns among regulators, especially at the Ministry of Infrastructure and the Environment, which oversees

the use of genetically modified organisms. After the trial had started, a wound leakage caused an interruption of the trails, which led to another year of delay. When the trial was finally completed in 2014 (about 6 years later than anticipated), the results were no longer relevant for the specific product under evaluation. Nevertheless, it would be short-sighted to conclude that the entire endeavour was a failure. Although DNAtrix, the company which obtain the proprietary rights to the vector, had already moved on based on the results obtained much earlier in Houston, Texas, Dutch regulators and the two participating university hospitals in Amsterdam and Rotterdam went through a steep learning curve, which could be of inestimable value for similar projects in the future. In fact, a recently obtained European grant for the development of a new therapy combining oncolytic viruses and immunotherapy, as well as a planned trial by Prof. Van Beusechem's start-up company ORCA Therapeutics, might prove to be indirect beneficiaries.

# Prostate cancer

| | |
|---|---|
| Full title: | Oncolytic adenovirus therapy as a neoadjuvant treatment for localised prostate cancer |
| Project leader: | Prof. Chris H. Bangma |
| Institute: | Erasmus MC |
| Project structure: | Pharmaceutical and clinical phases |
| Status: | Ongoing (clinical phase, delay: ~5 year) |
| Duration: | 36 months |
| Start: | 2008 |
| ZonMw budget: | €500,000 |
| External funding: | €386,500 |
| Reasons for delay: | Virus production and toxicology report from US had to be validated according to new European laws |

**Summary**

Prostate cancer is the most frequent malignancy in males in the Netherlands. In most cases, the tumour is diagnosed at a localised stage and can be treated by surgical removal, offering a good prognosis. However, in some cases the tumour recurs, eventually spreading to different organs and resulting in a very poor prognosis and ultimately the patient's death. There is therefore a pressing medical need to increase overall treatment efficiency by lowering the risk of residual tumour cells after surgery. This may be achieved by an adjunctive oncolytic adenovirus administered prior to surgical removal of the prostate, which might enhance chances of complete remission, avoiding tumour relapse and improving overall survival.

**Discussion**

The conditionally replicative oncolytic adenovirus, was developed under the European gene therapy programme GIANT and finally produced in the US. However, changes in European legislation mean all GMP products for clinical testing from outside the EU have to undergo extensive toxicological testing in the EU. Given the high costs of such testing, a compromise had to worked out involving a review of the US toxicology reports by qualified personnel in order to obtain permission from the regulator for the product to be used in a clinical trial. Although this compromise was achieved in the end, it had delayed the project by almost five years. Additional delay occurred due to slower than expected patient inclusion. Given the fact that there are about 150-200 radical prostatectomies (surgical removal of the entire prostate gland and some surrounding tissue) per year at Erasmus MC alone, it was assumed that finding 12-18 volunteers would be much easier. Inclusion of the first three patients took over nine months, however, though the next three only took about three weeks, indicating the unpredictability of the inclusion rate. With the active involvement of patients' organisations

now secured, inclusion might become more efficient in the near future. The eighth patient has just been treated, the first one to receive the highest dose in this phase I safety and dose-finding part of the phase I/II clinical trial. So far, the clinical development of the investigational virus has depended entirely on public funding. There are currently no industry partners or spin-offs/start-ups involved, which may in part be due to the absence of patents protecting the intellectual property of the vector. However, the study investigators are currently preparing for the next phase (phase II) of the clinical trial, which is scheduled to start in mid-2016 and will collect crucial data on the immunological response of the virus treatment. If the results meet (or exceed) the expectation of the project leaders, it might be possible to establish industry collaborations in order to push the vector further through clinical development. At this point, however, the exploration of this technology remains a purely academic endeavour and more public founding will be required for its development if the immunological response turns out to be lower than expected. The anticipated clinical trial in Sweden was postponed indefinitely due lack of funding, further highlighting the crucial role that the ZonMw TGO programme has played in this project at Erasmus MC.

# HPV-positive penile and cervical cancer

Full title: Treatment of HPV-positive penile and cervical cancer by induction of HPV E7-specific T-cell immunity through DNA vaccination
Project leader: Prof. John B.A.G. Haanen
Institute: Netherlands Cancer Institute
Project structure: Preclinical and pharmaceutical phases (originally: pharmaceutical and clinical phases)
Status: Completed (delay: 3 years)
Duration: 36 months
Start: 2006
ZonMw budget: €665,000
External funding: €225,000
Reasons for delay: Original DNA vaccine not safe for use in humans, project went back to preclinical testing, little experience with new GMP production process of DNA vaccine

**Summary**
Persistent human papilloma virus (HPV) serotype 16 and 18 infections are strongly associated with squamous cell cancer of the penis and cervix. The carcinogenic transformation of HPV-infected keratinocytes (epidermal cells) requires a persistent expression of the viral proteins E6 and E7. In this project, the goal was to produce and clinically test an E7-based non-oncogenic DNA vaccine in humans. By using a novel micro-injection technique called DNA tattooing, non-functional E7 gene copies are injected into the patient's skin in order to induce an immunological response. If the vaccine does indeed trigger the immune system, not only may already infected cells be cleared out, but the patient might also be protected from future HPV infection.

**Discussion**
Shortly after the project started in 2006, it became clear that the DNA vaccine the researchers intended to use in the clinical trial did not have a favourable safety profile and would need to be adjusted before initiating GMP production. While there are many different ways to render a potentially hazardous gene harmless (point mutations, deletions, etc.), the project leaders chose to use a more rigorous approach called DNA shuffling to try to find an optimum safety/efficacy ratio. It took about three years to try many different approaches and to optimise and test the vaccines to finally identify a safe and effective candidate for testing in human subjects. This eventually altered the project from a pharmaceutical + clinical phase project to a project with preclinical + pharmaceutical phases. Funding for the subsequent clinical trial will therefore no longer be provided by ZonMw, but will need to be obtained elsewhere. The project experienced additional delay during GMP production of the vaccine. A new production process

in the NKI's own GMP facilities was used with the intention – somewhat ironically – of "speeding up" GMP production of small-scale DNA vaccines. Nevertheless, the project achieved its adjusted goal in 2012 when production of the vaccine was completed. As of now (June 2015), the developed DNA vaccine has been tested in twelve patients with vulvar intra-epithelial neoplasia grade III (VIN III), an HPV 16-induced premalignant lesion in a phase I/II clinical trial funded by the European Union. Notably, all regulatory hurdles were taken fairly smoothly (although the CCMO did not allow the NKI to study the vaccine in cancer patients, only in patients with premalignant disease), causing no more significant delays. Currently, the data from the trial are being analysed and it is not yet known if the vaccine was indeed able to induce the desired immune response. However, the study investigators already have an improved version of the DNA vaccine in preclinical development, indicating that additional improvements might be required before this technology can be deployed more broadly. Another limiting factor for rapid clinical development with a commercial partner is the lack of protected intellectual property. The DNA vaccine is therefore likely to stay exclusively in academic development for the near future. In retrospect, the early setbacks in this project as well as the limited potential for a successful commercialisation of the technology might have been foreseeable. Nevertheless, the achievements of this project must be acknowledged. In the end, the vaccine was optimised and tested successfully without relying on an external GMP production company and additional funding was secured to test the vaccine in a clinical setting. Being able to develop, produce and clinically test oncological DNA vaccines puts the NKI in a very favourable position for further DNA vaccine research.

# Anti-tumour therapy using Clostridia

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| Full title: | Use of non-pathogenic engineered clostridia as delivery vector for toxic gene products to the tumour |
| Project leader: | Dr. Jan Theys |
| Institute: | MAASTRO Clinic |
| Project structure: | Preclinical, pharmaceutical and clinical phases |
| Status: | Ongoing (preclinical phase, delay: 3 years) |
| Duration: | 72 months |
| Start: | 2009 |
| ZonMw budget: | €800,000 |
| External funding: | €1,400,000 |
| Reasons for delay: | Optimisation of enzyme/prodrug combination, regulatory hurdles |

**Summary**

There are many different ways to effectively deliver gene therapy to cancer cells. However, this project marks an entirely different approach compared to all other oncological projects in the ZonMw TGO programme. In this project, non-pathogenic engineered clostridia bacteria are being used as a delivery vector in order to specifically target tumours in the body. Clostridium bacteria are strictly anaerobic, meaning they only proliferate in hypoxic/necrotic areas as found within most solid tumours, but not in healthy tissues in the rest of the body. After the bacteria have migrated to those necrotic areas of the tumour, they will start to proliferate locally. Then, a prodrug will be administered systemically that is activated by the clostridia bacteria through an enzymatic reaction of its engineered nitroreductase. This allows for optimal targeting of the tumour, potentially sparing systemic side effects usually seen in most chemotherapies. The clostridia prodrug combination specially targets the hypoxic areas of the tumour (those with a very poor blood supply), which are the most resistant to most common therapies. According to the project leaders, the safety of a treatment involving systemic administration of bacterial spores can be guaranteed by using antibiotics to immediately kill all bacteria and preclude any unexpected side-effects.

**Discussion**

The project, consisting of a preclinical, pharmaceutical and clinical phase, started in 2009 with the aim of identifying and testing suitable enzyme/prodrug combinations and guaranteeing the highest possible degree of safety. Given the lack of clinical experience with this approach, it was expected early on that regulators would be very cautious. In a larger collaboration with a group from Nottingham, UK and a group from New Zealand, the researchers have made a lot of progress in improving and testing the three main components of the treatment: (1) the clostridium strain can be engineered efficiently within its genome without inducing antibiotics

resistance, (2) a second generation of the vector has been developed for improved safety and efficiency and (3) a potential setup for the molecular imaging of the treatment response. However, these improvements have already taken twice as much time as originally anticipated. Right now, many different combinations of these three components have been evaluated, but a decision has yet to be made. The main reasons for the hesitancy are twofold. Firstly, unlike more mainstream approaches that are developed in parallel at several research institutes, the project leaders have little hope of attracting additional funding for reiterating this promising technology if the chosen combination fails to achieve the expected results during the phase I/II clinical trial. Secondly, the lack of clinical experience with this technology causes a lot of uncertainty concerning regulatory hurdles. Earlier, to the frustration of the researchers, the supposedly safe clostridium strain was classified by regulators as “risk class II”, placing many restrictions and safety requirements on the preclinical testing (e.g. additional safety procedures for animal testing). The project leaders are also convinced that the choice of the best NTR/prodrug combination (including potential for proprietary rights development) is of vital importance to attract interest from industry to invest in this technology at such an early stage. Every effort is therefore being made to take this decision before proceeding to the pharmaceutical phase and preparing the submission to the authorities because unless this choice is reasonable and well documented the risk associated with that investment will be considered unreasonably high. However, in order to fulfil the requirements of the ZonMw *translational* gene therapy research programme, a decision needs to be made, and will be made eventually.

# LPL deficiency

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| Full title: | Gene therapy for LPL deficiency |
| Project leader: | Prof. John J.P. Kastelein |
| Institute: | Academic Medical Center (AMC) Amsterdam |
| Project structure: | Pharmaceutical and clinical phases |
| Status: | Completed (no delay) |
| Duration: | 12 months |
| Start: | 2005 |
| ZonMw budget: | €687,000 |
| External funding: | €1,745,000 |
| Reasons for delay: | None |

**Summary**

Lipoprotein lipase (LPL) deficiency (or 'familial chylomicronaemia syndrome') is an ultra-orphan metabolic disease affecting the breakdown of fatty acids. Patients with severe LPL deficiency lack functional lipoprotein lipase enzymes, which leads to accumulation of triglycerides (fatty acids) in the blood, causing abdominal pain and often fatal acute pancreatitis. Prior to this project, the only available treatment for LPL deficiency was a strict dietary restriction of fat intake, which has proven unattainable for most patients in the long run. In this project, the researchers worked on an adeno-associated virus (AAV) gene therapy that restores functional lipoprotein lipase production in LPL-deficient patients, potentially relieving the disease symptoms over a whole lifetime with a single treatment. Due to the extensive preclinical work that had been done at the Academic Medical Center and the gene therapy biotech spin-off Amsterdam Molecular Therapeutics (AMT; now uniQure), among other places, the project was ready for GMP vector production (pharmaceutical phase) and subsequent clinical testing when it commenced in 2005.

**Discussion**

In the first midterm evaluation this project was described as one of the two most promising projects in the TGO programme and its success story has continued over the last four years, albeit not without plenty of challenges. During the period of financial support from ZonMw (2005-2007), the AAV vector was successfully tested at different doses in eight patients at the Academic Medical Center in Amsterdam. The treatment was well tolerated and the primary efficacy endpoint of a 40% reduction in triglycerides was achieved in three patients. Notably, the study showed a sustained effect 26-32 weeks after treatment, justifying additional phase II trials.

Now, eight years later, the AAV gene therapy alipogene tiparvovec described here has received marketing authorisation from the EMA, and is being marketed under the trade name Glybera. UniQure is a mature Dutch/international biotech company headquartered in Amsterdam. Although the financial success of Glybera might still be uncertain, uniQure has secured a broad pipeline of innovative new gene therapies based on the same delivery system of Glybera and has entered the race to develop a treatment for a much larger patient population, with haemophilia B. It is impossible to say whether these developments would have been possible without financial help from ZonMw, but according to the project leaders the grant was crucial as there were no other funding programmes at that time in this therapeutic area.

# Crigler-Najjar syndrome

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| Full title: | AAV-mediated liver-directed gene therapy for Crigler-Najjar syndrome, inherited unconjugated hyperbilirubinaemia |
| Project leader: | Dr. Piter J. Bosma |
| Institute: | Academic Medical Center (AMC) Amsterdam |
| Project structure: | Preclinical, pharmaceutical and clinical phases |
| Status: | Ongoing (pharmaceutical phase, delay: 4+ years) |
| Duration: | 72 months |
| Start: | 2007 |
| ZonMw budget: | €1,350,000 |
| External funding: | €1,800,598 |
| Reasons for delay: | Vector design and GMP vector production |

**Summary**

Crigler-Najjar (CN) syndrome is a rare inherited metabolic liver disorder caused by a missing or non-functional liver enzyme, called UGT1A1, which is responsible for the breakdown of yellow-coloured bilirubin. Patients with CN suffer from severe jaundice and have to undergo up to 14 hours of phototherapy every day to decrease bilirubin blood levels in order to prevent irreversible brain damage. Loss of phototherapy efficacy over time means a liver transplant is an inevitability for most patients. The transplant does cure the disease, but given the scarcity of organ donors, the risks associated with a liver transplant and subsequent lifelong immunosuppression, Crigler-Najjar patients have major unmet medical needs. The goal of this project is to develop an adeno-associated virus (AAV)-mediated gene transfer of a very potent mutant UGT1A1 enzyme into liver cells, thereby restoring liver enzyme production in some cells of the patient's own liver and decreasing blood bilirubin levels, thus relieving the symptoms or even reversing the disease altogether.

**Discussion**

The project leaders were able to develop a vector that is able to cure the disease in the respective animal models and were ready to scale up vector production for the clinical phase in 2012. However, other clinical trials in the meantime indicated a high tolerability of large injected doses, which could potentially lead to greater clinical efficacy. The production scale-up needed to produce larger quantities of vector to allow administration of this higher dose appeared to be much more difficult than expected. Eventually, the researchers decided to switch to a slightly different vector that would be easier to produce in the required larger quantities. Today, the project appears to be back on track, with the new vector now in production at Genethon in France and the clinical trials in preparation. Several new collaborations have also been established, including with French and Italian groups and

patients' organisations. This significantly enhances the prospect of increasing the number of participants in the clinical trials. So far 45 patients, eight of them from the Netherlands, have been screened for eligibility for the clinical trial, approximately half of whom will have to be excluded because of liver damage or pre-existing antibodies against the viral vector. However, orchestrating a multi-centred multinational trial instead of a small single-centred study involves many different parties and requires much more careful planning. Financial support for the Dutch part of the clinical trial is guaranteed thanks to the ZonMw grant, but France and Italy depend partly on a current Horizon2020 grant application. Additional delays are therefore to be expected once the vector batch has been produced and analysed early next year. Nevertheless, the results of the clinical trial will deliver valuable insights and hopefully lead to the clinical development of the desperately-awaited new treatment for CN patients. However, those results might not be available before 2018.

# Leber congenital amaurosis

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| Full title: | Gene therapy to fight Leber congenital amaurosis due to loss of CRB1 function |
| Project leader: | Dr. Jan Wijnholds |
| Institute: | Netherlands Institute for Neurosciences (now at LUMC) |
| Project structure: | Preclinical, pharmaceutical phases and clinical phases |
| Status: | Ongoing (pharmaceutical phase, delay: 6 years) |
| Duration: | 72 months |
| Start: | 2006 |
| ZonMw budget: | €1,400,000 |
| External funding: | €700,000 |
| Reasons for delay: | New mouse model had to be established, AAV vector not suitable for large insertions, new functional CRB1 gene homologue had to be generated |

**Summary**

Leber congenital amaurosis (LCA) is an inherited form of blindness at birth (or shortly thereafter) caused by a mutation in one of the 21 currently known genes associated with the disease. Similarly, retinitis pigmentosa (RP) is an inherited form of blindness caused by a mutation in one of more than 60 currently known genes. Mutations in the CRB1 gene cause early-onset RP, with patients becoming blind before the age of 20 years. In this project, a new treatment will be developed for LCA and RP patients with disease causing mutations in the CRB1 gene. Notably, even when the exact genotype is known at birth, it is impossible to predict which disease phenotype will be developed. However, the progression of both diseases could potentially be halted by the gene therapy under development in this project. In these LCA and RP patients with a mutated CRB1 gene the photoreceptor cells of the retina appear to be unable to make a functional connection to Müller glial cells, which is required for the structural stability of the retina to allow proper neuronal signalling of visual information from the retina to the brain. The researchers developed retina mouse models to test the safety and efficacy of gene transfer of a functional CRB1 gene homologue to the photoreceptor and glial cells. Several different adeno-associated virus (AAV) vector systems were explored and the most promising vector will be selected for GMP virus production and ultimately a phase I clinical trial.

**Discussion**

At the beginning of the project it became apparent that the mouse model did not mimic the disease phenotype seen in humans closely enough to make conclusive predictions about the mechanism of action in humans. Eventually, it was possible to optimise the model by adding another mutation in CRB2, but at the expense of two extra years of preclinical research. Another reason for the significant delay was one that many researchers struggled with at the

time: despite some published evidence suggesting otherwise, it seemed impossible to produce stable AAV vectors larger than around 4.7kB. The vector therefore needed to be reduced in size by several optimisations. A third reason for the delay was that CRB1 had to be replaced by a CRB1 gene homologue. As of today, the proof-of-principle has been achieved, published and patented. The vector is ready for GMP production. The additional funding required for the GMP vector batch and toxicology studies will most likely be provided by an American blindness foundation. The project leaders' current plan is to start GMP vector production and subsequent toxicology studies this year and to open the first phase I/II clinical trial in 2017. If all these steps are successful, the researchers plan to continue clinical development towards marketing authorisation by licensing the technology to industry or through further development by a spin-off company of LUMC, where the group just recently moved. However, one should bear in mind that there are quite a few hurdles to be overcome before phase II/III trials or even commercialisation can be considered.

In the meantime, another Leber congenital amaurosis subtype (LCA2) that is caused by a mutation in the RPE65 gene was treated with positive results in one patient in 2008 and several others in 2009. Using adeno-associated virus serotype 2 (AAV2) RPE65 gene therapy vectors, the groups demonstrated the safety and feasibility of treating LCA patients harbouring RPE65 mutations with gene therapy. In October 2015, the results of a phase III clinical trial at the Children's Hospital Philadelphia were announced. If the primary endpoints are achieved, the treatment will be a significant step closer to FDA approval (marketing authorisation). This development arguably justifies further research into other gene therapies to treat inheritable blindness.

# Pompe disease and Hurler syndrome

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| Full title: | Gene Therapy of Lysosomal Storage Diseases |
| Project leader: | Prof. Gerard Wagemaker & Prof. Ans T. van der Ploeg |
| Institute: | Erasmus MC Rotterdam |
| Project structure: | Preclinical, pharmaceutical phases and clinical phases |
| Status: | Ongoing (preclinical phase, delay: >4 years) |
| Duration: | 72 months |
| Start: | 2008 |
| ZonMw budget: | €1,000,000 |
| External funding: | €2,900,000 |
| Reasons for delay: | Vector selection for GMP production |

**Summary**

Lysosomal storage disorders (LSDs) are progressive inheritable disorders causing major health problems in children and adults. They comprise a group of about 50 different disorders. For most there is no treatment. Disease-specific enzyme replacement therapies have considerably changed the prospects of patients with some LSDs. However, further development of innovative next-generation curative therapies remains necessary. ERT has to be administered weekly or biweekly throughout the patient's life, not all patients respond equally well, ERT cannot cross the blood-brain barrier, and treatment costs are high (up to 400,000 euros per patient per year).

In this project the feasibility, safety and efficacy of self-inactivating lentiviral gene therapy using hematopoietic stem cells is being explored for two representative lysosomal storage disorders, Pompe disease and Hurler disease. Pompe disease (caused by mutations in the GAA gene) presents as a progressive muscular disorder and may also affect the brain. Hurler disease (caused by mutations in IDUA gene) leads to bone deformities and mental retardation. The aim of the project is to develop and select a vector for one disease, followed ultimately by GMP production and clinical testing.

**Discussion**

The results obtained on Pompe disease were most promising and gave reason to focus on this disease for the development of stem cell mediated lentiviral gene therapy. Proof-of-principle of correction of the Pompe disease phenotype was obtained in a knock-out mouse model, with significant reduction of pathology in the main target tissues (skeletal muscle, heart and brain). A robust immune tolerance was observed for the expressed GAA which is considered an additional advantage when it comes to clinical application.

The initial lentiviral vectors generated needed further development to be curative for Pompe disease and have been subject to various modifications aimed at reducing the viral copy number per stem cell and optimisation of the GAA sequence in order to improve the effect. The current research is focused on comparative analysis of different GAA sequences and the search for a weaker and potentially safer promoter. This has recently resulted in the identification of a GAA sequence/promoter combination that is acceptable for clinical application and has proven to be very efficacious at low copy numbers.

The long-term effects are currently being explored in the mouse model for Pompe disease. These final tests will be instrumental for final vector selection for GMP production and clinical implementation.

The subsequent pharmaceutical and clinical phases will require new sponsors. The feasibility of the approach is supported by the promising results of an ongoing clinical trial in another lysosomal storage disorder (metachromatic leukodystrophy, MLD) using a similar lentiviral vector.

The project is benefiting from extensive national and international collaboration and from the pioneering work on enzyme replacement therapy for Pompe disease previously performed by the Center for Lysosomal and Metabolic Disorders at Erasmus MC.

# Primary immunodeficiencies XLA, SCID

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| Full title: | Gene Therapy of Primary Immunodeficiencies |
| Project leader: | Prof. Gerald Wagemaker & Prof. Frank Staal |
| Institute: | Erasmus MC Rotterdam |
| Project structure: | Preclinical, pharmaceutical and clinical phases |
| Status: | Discontinued (preclinical phase, delay: 3 years) |
| Duration: | 72 months |
| Start: | 2005 |
| ZonMw budget: | €1,400,000 |
| External funding: | - € |
| Reasons for delay: | Project cancelled in 2011 due to occurrence of leukaemia in mouse model |

**Summary**

Primary immunodeficiencies are inherited (genetic) disorders that prevent or reduce the formation of a functional immune system. Infants suffering from severe combined immune deficiency (SCID) lack both cellular and antibody immunity and often die of a severe infection within their first year of life . The primary immunodeficiency X-linked a-gamma globulinaemia (XLA) is similar to SCID, although often less severe. Patients with XLA have no functional B-cells and therefore a complete lack of antibodies in their bloodstream, making them prone to severe infections. Current XLA disease management consists of frequent intravenous injections of immunoglobulin (IVIg) extracted and pooled from blood donations. However, IVIg needs to be administered for an entire lifespan, can trigger severe adverse reactions in some patients and often requires additional antibiotics to deal with acute infections. In addition, it is costly but not curative, and patients die in the third to fifth decade of life.

In this project self-inactivating lentiviral vectors (known as third-generation vectors) are to be developed for the treatment of SCID subtypes, RAG1 and RAG2 deficiency, and for the treatment of XLA (X-linked a-gamma globulinaemia), which is caused by a mutation in the Bruton's tyrosine kinase (Btk) gene. After proof-of-principle and subsequent safety profiling, the plan is to clinically test at least one of the vectors for transgene expression in the patient's haematopoietic stem cells.

**Discussion**

Earlier trials in X-linked SCID and adenosine deaminase (ADA) genes initiated around 2000-2002 were very efficient, normalising the immune system in 94% of the patients, but five of the 34 patients treated in the X-linked SCID gene therapy trials developed leukaemia as a direct consequence of the integrated vector. This demonstrated both the promise gene therapy holds

for primary immunodeficiencies, and the associated risks. Like the early gene therapy SCID trials, the vectors developed here showed promising efficacy in their proof-of-principle animal models, but without a similar high risk of insertional mutagenesis. The alternative treatment option, a non-matched bone marrow transplantation, has even poorer outcomes, with a much higher mortality rate. In the meantime, several improvements have been made to the RAG1 and RAG2 deficiency vectors and a European platform for collaborative multicentre development has been established. However, the structure of the ZonMw TGO programme did not allow for several more years of extensive preclinical testing of the new vectors and it was decided to put the project on hold and revoke the funding reserved for the pharmaceutical and clinical phases.

This setback was not the end of the project, however. The European collaboration continued and only a few years later in 2013, project leader Frank Staal was awarded another ZonMw grant worth three million euros under the Priority Medicines for Rare Conditions and Orphan Drugs programme. With this substantial funding, the project is back on track to bring the therapy into the clinics after all. An international multicentre Phase I/II trial is expected to open in 2017. GMP grade RAG1 vector is currently being produced by a pharmaceutical company and regulatory approval has been requested. Importantly, EMA/CAT has granted RAG1 SCID orphan drug designation. Proof-of-principle had been obtained for RAG2 by several groups, but not with clinically suitable vectors. New RAG2 vectors will be produced in the context of a H2020 project, SCID-Net. In addition, gene editing approaches will be attempted for RAG deficiencies during this programme. Gene editing is also the way to go for XLA, as the animal model studies have shown that constitutive expression of BTK carries the risk of oligoclonal  myeloproliferative disease, even with cellular promoters.

In conclusion, while the initial goals were unattainable in the short timespan available, novel ATMPs based on gene-corrected stem cells are likely to reach the clinic in the next couple of years, thanks in large part to the seminal TGO grant.

# Duchenne muscular dystrophy

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| Full title: | Development of exon-skipping gene therapy for Duchenne muscular dystrophy |
| Project leader: | Prof. Gert-Jan B. van Ommen |
| Institute: | LUMC |
| Project structure: | Preclinical, pharmaceutical phases and clinical phases |
| Status: | Completed (no delay) |
| Duration: | 72 months |
| Start: | 2006 |
| ZonMw budget: | €1,350,000 |
| External funding: | €4,300,000 |
| Reasons for delay: | None |

**Summary**

Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disease caused by a mutation in the dystrophin gene. Boys suffering from DMD progressively lose motor functions due to muscle deterioration and are typically wheelchair-dependent before the age of 12 and need assisted ventilation around the age of 20. Muscles continue to deteriorate until total paralysis eventually causes death at around the age of 25-30. There is currently no cure for DMD and treatment focuses on managing symptoms to increase quality of life. This project aimed to develop a potential treatment for DMD by applying a technique called exon-skipping. Exon-skipping is a molecular biology technique that uses antisense oligonucleotides to hide an exon from the splicing machinery, causing it to be spliced out. This will restore the reading frame to allow the production of an internally truncated but largely functional protein. Since most DMD patients have deletions that cluster, multiple patients can be treated with the same antisense oligonucleotide (e.g. the one targeting exon 51).

When the project officially started in 2006, proof-of-principle had already been achieved in animal and *in vitro* studies, demonstrating the feasibility of using exon-skipping to induce dystrophin expression. In the first two years of the project, the safety and efficacy of the antisense oligonucleotide targeting exon 51, now called drisapersen (formerly known as PRO51 and GSK2402968), was confirmed in four patients after local injection into the shin muscle (tibialis anterior). In a subsequent phase I/II clinical trial, a total of 12 patients were treated at different doses. No severe adverse events were reported and all patients eventually received the highest weekly dose in an ongoing extension study. After 6 months of treatment the ten patients who were still ambulant could walk further in six minutes than at the start of the extension study.

**Discussion**

Besides the *Gene therapy for LPL deficiency* project, which eventually led to the development of the first EMA-approved gene therapy alipogene tiparvovec (trade name Glybera), this project is the only project not to have experienced any significant delay. According to the project leader the reasons are relatively simple. Since the beginning of the project there was close collaboration not only between primary researchers and clinicians, but also between academics and the biotech/pharmaceutical industry in the form of the Leiden-based company Prosensa. This collaboration led to a much more streamlined clinical development. Perhaps even more importantly, the industry partner went to great lengths to overcome many regulatory obstacles. Exon-skipping is a fairly unique approach lacking any real reference clinical experience, which significantly complicated many regulatory approval processes, most notably the green light from the CCMO for the clinical trials. While the advantage of having a strong industry partner for an academic research project is often associated primarily with the apparently endless financial resources, which help in the planning, preparation and execution of expensive clinical trials, this was not the case in the first two clinical studies of drisapersen. Only for the later, much larger phase II/III clinical trial did financial responsibility shift entirely to Prosensa. The collaboration with the patient organization (Duchenne Parent Project) also warrants a mention. They were involved in resolving the regulatory issues and co-funded the clinical trials as well as the proof-of-concept and preclinical work ongoing in academia.

After a failed attempt to bring the exon-skipping technology for Duchenne muscular dystrophy (DMD) to the market in a collaboration with big pharma's GlaxoSmithKline, all licensing rights reverted to Prosensa. Despite the initial defeat due to the failure to reach the primary endpoints in the clinical trial, Prosensa continued to push forward for market authorisation. After reevaluating the trial's data and demonstrating efficiency in a subset of patients, the company was incorporated by a takeover from USA-based BioMarin Pharmaceuticals (California, USA). BioMarin has committed to bringing drisapersen and similar drugs for different DMD patient subgroups to the market. The drug is currently under evaluation for marketing authorisation with the European Medicines Agency; the FDA has stated that drisapersen is not ready for approval yet.

While the Prosensa takeover by BioMarin has led to several changes, BioMarin Netherlands continues to operate from the Leiden BioScience Park next to LUMC. The close collaboration between the university hospital in Leiden and BioMarin Pharmaceuticals will continue and is likely to be extended even further in the near future, according to the project leaders at LUMC.

# RNAi gene therapy for HIV/AIDS

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| Full title: | RNAi gene therapy for HIV/AIDS |
| Project leader: | Prof. Ben Berkhout |
| Institute: | AMC Amsterdam |
| Project structure: | Preclinical, pharmaceutical phases and clinical phases |
| Status: | Ongoing (preclinical phase, delay: >5 years) |
| Duration: | 72 months |
| Start: | 2007 |
| ZonMw budget: | €1,350,000 |
| External funding: | €500,000 |
| Reasons for delay: | Lack of efficiency/resistance in *in vivo* studies, discontinued patent and lack of (matching) funding |

**Summary**

More than 35 million people are currently living with HIV/AIDS, most of them in sub-Saharan Africa. The disease can be managed in most cases with antiretroviral treatment, but this needs to be continued lifelong, is very costly and is not without side effects. The unmet medical needs of HIV/AIDS patients therefore remain substantial. In this project, a lentiviral vector is being developed to infect CD34+ haematological stem cells *ex vivo* with a combination of four short hairpin RNAs (shRNA) that prevent the expression of HIV-1 genes, creating HIV-1 resistant immune cells that are reinfused into the patient's blood stream. Multiple shRNAs have been designed and tested in cell culture and in the Human Immune System (HIS) mouse model. Several potent hairpins were identified in previous testing, but the use of a single hairpin was not able to prevent viral escape (production and release of new HIV-1 particles from infected cells). However, in combination viral escape was prevented, thus constituting a durable therapy. The proposed lentiviral gene therapy might therefore offer an alternative single gene therapy option for HIV/AIDS patients who do not respond or who suffer from side-effects with the current standard antiretroviral medication.

**Discussion**

When the project commenced as part of the ZonMw translational gene therapy research programme in 2007, the four stages towards clinical translation were clearly defined in a time-dependent manner: 1) development of a gene construct encoding for multiple shRNAs, 2) development of a safe and effective lentiviral vector which could be produced with high titer yields, 3) preclinical testing of the therapy in cell culture and the safety in a new humanized immune system (HIS) mouse model and finally, 4) the phase I/II clinical trial in six patients who no longer responded to heavy antiretroviral medication. The anticipated timelines, as for all projects in the ZonMw TGO programme, were three years for preclinical research (stage 1-

2), a one-year pharmaceutical phase (stage 3) and two more years for the clinical evaluation (stage 4). However, not unlike many other projects, the preclinical phase took longer than expected and the pharmaceutical phase has yet to commence. This has to do with two connected problems. First, despite strong *in vitro* proof-of-principle that the proposed mechanism could work to efficiently reduce virus loads to a degree that antiretroviral medication could be reduced or even discontinued, the initial *in vivo* results were disappointing. Although full safety was demonstrated, very recent results indicate that HIV-1 suppression is sub-optimal, as if not all shRNAs of the combinatorial therapy were being properly expressed. Second, initiating the pharmaceutical phase to move forward towards the clinical evaluation of the vector requires additional financial resources that have not been secured yet. This poses a dilemma for both ZonMw and the researchers. Allowing the use of ZonMw resources reserved for the pharmaceutical and clinical phases to further enhance the antiviral vector may in the end help to attract more funding (e.g. other grants, or licensing to a biotech company) and lead to the clinical development of a potential new breakthrough treatment. However, the required amount of additional funding for the clinical evaluation has increased as the - once reserved - ZonMw resources have been used for additional preclinical work, including the *in vivo* efficacy tests. The future of this project therefore remains uncertain.

Prof. Joep Lange, who was leading the project to translate this gene therapy to the clinic, tragically died in 2014 in the MH17 air disaster over Ukraine.

(Unfortunately, no interview was held with Prof. Ben Berkhout or another representative of the research team in order to discuss the most recent progress with the project. The information is derived from annual reports and other resources, including publications and publicly available AMC documents.)

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