Gene therapy in The Netherlands: highlights from the Low Countries

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Abstract
Gene therapy is an active research area in The Netherlands and Dutch scientists involved in fundamental and clinical gene therapy research significantly contribute to the progresses made in this field. This ranges from the establishment of the 293, 911 and PER.C6 cell lines, which are used worldwide for the production of replication-defective adenoviral vectors, to the development of targeted viral vectors and T lymphocytes as well as of non-viral vectors. Several milestones have been achieved in Dutch clinical gene therapy trials, including the first treatment worldwide of patients with adenosine deaminase deficiency with genetically corrected hematopoietic stem cells in collaboration with French and British scientists. Until now, about 230 patients with various diseases have been treated with viral and non-viral gene therapy in this country. Ongoing and upcoming Dutch clinical trials focus on the translation of new developments in gene therapy research, including the restoration of genetic defects other than SCID, and the use of oncolytic adenoviruses and targeted T cells for the treatment of cancer. The growing commercial interest in Dutch clinical gene therapy is reflected by the involvement of two Dutch companies in ongoing trials as well as the participation of Dutch clinical centres in large phase III international multicenter immuno-gene therapy trials on prostate cancer sponsored by an American company. Translational gene therapy research in The Netherlands is boosted at a governmental level by the Dutch Ministry of Health via a dedicated funding programme. This paper presents an overview on milestones in Dutch basic gene therapy research as well as on past, present and future clinical gene therapy trials in The Netherlands. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords gene therapy; The Netherlands; fundamental research; clinical trial; overview

Introduction
The bulb fields are important eye catchers in The Netherlands. Besides their visible attractiveness they have been of large economical importance. For this reason agricultural sciences traditionally flourished in The Netherlands. This included microbiology (or better bacteriology) to study and prevent contagious infections in plants. It was this setting that led Martinus Willem Beijerinck in 1898 to discover that a prevalent mosaic disease of tobacco, which was at that time an important crop, was caused by a filterable agent, that he called ‘contagium vivum fluidum’. He coined the term ‘virus’ for this agent, that now is known as tobacco mosaic virus [1]. This was the start of the virology research tradition in The Netherlands. Up to today, virology is still a main research area in the biological sciences. The fundamental branch of Dutch gene therapy research was born out of this virology field. Progressions
-made by gene therapy scientists in The Netherlands have had a significant impact on the translation of gene therapy into the clinic both at a national and international level. The expertise of Dutch gene therapy scientists is also recognized at an international level, as demonstrated by the fact that various Dutch research groups participate in several of the pre-clinical and clinical-oriented European gene therapy projects supported by the Framework Programmes of the European Union (Table 1). This paper presents a brief overview on some milestones in Dutch basic gene therapy research as well as on past, present and future clinical gene therapy trials in The Netherlands.

### Fundamental gene therapy research in The Netherlands

#### Adenoviral vectors

Basic gene therapy research was initiated in the lab of Alex van der Eb, who chose adenoviruses as a research model in the mid-1960s. Adenoviruses were first identified by Rowe and colleagues in 1953 when they tried to establish cell lines from adenoidal tissue and tonsils [2]. A transmissible agent was found responsible for degeneration of the cultures. More than 50 serotypes of human adenoviruses (HAdV) have been described to date, which have been classified in six ‘species’, A through F (formerly called ‘subgroups’). The evolution and classification of adenoviruses has recently been reviewed [3].

Adenoviruses attracted much attention with the discovery by Trentin et al. [4] and Huebner et al. [5] that certain serotypes (i.e. serotypes 12 and 18 of species A) can induce tumors in newborn hamsters, whereas others (i.e. serotypes 2 and 5 of species C) are not or only very weakly oncogenic. These observations triggered Van der Eb in Leiden to use adenoviruses as a model for animal studies on cellular transformation in vitro and tumor induction in vivo. This led to the observation that the difference in oncogenicity correlates with the capacity of serotype-12, but not serotype-5, to down-regulate antigen presentation by MHC class I [6,7]. This illustrated how important the immune system is for preventing tumor growth.

As such, adenoviruses have played an important role in fundamental biomedical research. They provided the model systems for studying the organization of eukaryotic genes and the regulation of their expression, as well as for studying the mechanisms of DNA replication in mammalian cells [8]. The latter studies were performed predominantly in the lab of John Sussenbach and Peter Van der Vliet in Utrecht. Such studies not only led to detailed insight in many cellular and viral processes, but also yielded various broadly applicable tools and techniques.

The ‘Graham & van der Eb’ paper describing the calcium technique for efficient transfer of naked adenovirus DNA into cultured cells [9] has been cited more than 8500 times and this cheap and efficient technique for DNA transfer is routine in virtually all cell biology laboratories. This technique was used by Frank Graham in Alex van der Eb’s lab to show that human cells, too, can be transformed and immortalized with naked adenovirus DNA. This is exemplified by the ‘293’ cell line obtained after transformation of embryonal kidney cells with sheared adenovirus serotype-5 DNA [10]. Later, the Leiden group generated the ‘911’ cell line, by transfer of plasmids carrying defined adenovirus type 5 sequences into human embryonal retinoblasts [11]. The observation that such adenovirus-transformed cells can be used for propagating viruses with defects in their Early Region 1 (E1) gene [12] facilitated the development and use of replication-defective adenovirus vectors. The relatively easy generation of E1-deleted replication-defective vectors made these vectors a prime choice for many gene therapists.

Although very useful, both the 293 and the 911 cell lines have the same intrinsic problem. During propagation of replication-defective E1-deleted adenovirus vectors replication-competent adenoviruses (RCA) are being generated with high frequency. RCA are reverted vectors that re-acquire the E1 region while losing the transgene cassette. This is the result of recombination between homologous sequences integrated in the helper cells and in the vector backbone [13]. In an attempt to

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**Table 1. Dutch Participation in the European Sixth Framework Programme on gene therapy**

| Project              | Project description                                      | Project type | Participating Dutch institute(s) | Website                        |
|----------------------|----------------------------------------------------------|--------------|---------------------------------|--------------------------------|
| ATTACK               | Develops specific T cells for tumor cell killing         | Pre-clinical | Erasmus MC (Rotterdam)          | www.attack-cancer.org           |
| CONSORT              | Improves safety and efficacy of gene therapy for hereditary diseases | Pre-clinical and clinical | Erasmus MC (Rotterdam)          | www.gene-therapy.eu             |
| EPI-vector           | Develops episomal vectors for clinical gene therapy      | Pre-clinical | University of Amsterdam         | www.ls.manchester.ac.uk/genevector |
| GIANT                | Improves safety and efficacy of gene therapy for cancer  | Pre-clinical and clinical | Erasmus MC (Rotterdam) Leiden University Medical Center | www.giant.eu.com                |
| IMPROVED             | Develops gene therapy for cystic fibrosis                | Pre-clinical | Erasmus MC (Rotterdam)          | www.improved-precision.com      |
| Tumor-Host Genomics  | Develops gene therapy focused on the interaction of the tumor cell and its environment | Pre-clinical | Leiden University Medical Center | research.med.helsinki.fi/tumorhostgenomics |

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**ATTACK** Develops specific T cells for tumor cell killing Pre-clinical Erasmus MC (Rotterdam) www.attack-cancer.org

**CONSORT** Improves safety and efficacy of gene therapy for hereditary diseases Pre-clinical and clinical Erasmus MC (Rotterdam) www.gene-therapy.eu

**EPI-vector** Develops episomal vectors for clinical gene therapy Pre-clinical University of Amsterdam www.ls.manchester.ac.uk/genevector

**GIANT** Improves safety and efficacy of gene therapy for cancer Pre-clinical and clinical Erasmus MC (Rotterdam) Leiden University Medical Center www.giant.eu.com

**IMPROVED** Develops gene therapy for cystic fibrosis Pre-clinical Erasmus MC (Rotterdam) www.improved-precision.com

**Tumor-Host Genomics** Develops gene therapy focused on the interaction of the tumor cell and its environment Pre-clinical Leiden University Medical Center research.med.helsinki.fi/tumorhostgenomics

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solve the problem the Leiden group, in collaboration with scientists at Introgene (now Crucell), generated the PER.C6 cell line [14]. This line was established after transfer of a minimal portion of the adenovirus serotype 5 genome (bp 457–3510) into human embryonal retinoblasts, again using the calcium technique. In the process GLP conditions were used and the cell line, and the vectors produced on it, were extensively characterized [14,15]. This cell system has been used for the production of clinical-grade adenovirus-vector batches [16,17]. The use of this cell line in combination with matched E1-deleted vectors that do not have sequence homology largely solved the issue of RCA generation by homologous recombination.

**Retroviral and AAV vectors**

Also scientists from other fields embraced the gene therapy approach. While working as a PhD student in Alex van der Eb’s group in Leiden, Dinko Valerio in 1983 was the first to report cloning of the human adenosine deaminase (ADA) cDNA and the corresponding gene [18]. He embraced the retroviral vector technology and eventually used these vectors to transfer the ADA cDNA into hematopoietic stem cells of three ADA patients in a study performed with French and British scientists [19]. This represented the first study in which hematopoietic stem cells of ADA patients were modified. In addition, Valerio founded the company IntroGene, now called Crucell. Crucell has been one of the key players in the adenoviral-vector field and pioneered the use of fiber-modified and adenovirus serotype 35 derived vectors to overcome the paucity of the adenovirus receptor CAR and circumvent pre-existing immunity, respectively [20].

The relation between retroviral vectors and cancer was studied in several other labs. Anton Berns in Amsterdam examined how murine leukemia viruses (MLV) induced tumors upon infection in newborn mice. He recognized that the retroviruses found in tumors often had integrated into common sites. In these sites the proviruses disturbed the expression of nearby genes [21,22]. The commonality of the integration sites pointed to the association of the nearby genes with the induction of the leukemic state. Berns’ analyses of common integration sites led to the identification of several genes that can be involved in leukaemia. The use of MLV infection in transgenic animals further delineated the pathways in virus-induced leukaemia [23,24]. The relevance of these observations to gene therapy became all too apparent with the development of leukaemia in four infants born with X-linked severe combined immunodeficiency (SCID) gene who were successfully treated with an infusion of autologous hematopoietic stem cells transduced with a defective retroviral vector [25]. This has been attributed to deregulation of expression of the LMO-2 gene by the integrated vector provirus.

In Amsterdam the use of adeno-associated vectors (AAV) has been thoroughly exploited. The company Amsterdam Molecular Therapeutics (AMT) was founded to generate and produce state-of-the-art AAV for clinical studies. This greatly facilitated scientists to move from the lab to the clinic and the operations of AMT boosted translational gene therapy research in the Netherlands.

**Retargeting of viral vectors**

To date, research on viral vectors has significantly expanded in The Netherlands. Various labs in Rotterdam, Amsterdam, Groningen, and Leiden are exploring diverse targeting strategies to increase the selectivity of adenoviruses as oncolytic agents. Transductional targeting is studied by Van Beusechem and collaborators, who replaced the adenovirus fiber with sequences of the spike protein of a reovirus [26], and by Hoeben and collaborators who developed the adenovirus minor capsid protein IX as an anchor for targeting ligands [27]. Transcriptional targeting of the vectors to glioblastoma cells is employed by Sillevis Smitt and collaborators [28], to cholangiocarcinoma cells in Bosma’s group [29], and to prostate cancer cells by Kraaij and collaborators [30]. Optimized delivery methods and enhanced-efficiency vectors are being evaluated in cell culture, spheroid cell aggregates [31], tumor explants [32], tissue slices [33], and in small-animal models [34]. These approaches are not limited to adenovirus vectors. Also RNA viruses such as reoviruses, alphaviruses and coronaviruses are being evaluated for use as in anticancer strategies. Kranenburg and collaborators linked the capacity of reovirus to induce tumor-cell-specific cell death to the propensity of reovirus to sensitize ras-transformed cells to apoptosis [35]. Wilschut pioneered the use of alphaviruses and used these for eradication of pre-established tumors in an immunization protocol [36]. In a collaborative project Rottier’s and Gerritsen’s groups redirected the mouse hepatitis coronavirus to human tumor cells using a monoclonal-antibody dependent targeting strategy. This led to efficient syncytium formation in the tumor cells, which may eventually enhance the antitumor efficiency of this approach [37].

In addition, retroviral vectors are used in studies aiming at targeting T lymphocytes to tumors. In these studies adoptive transfer of autologous T lymphocytes that have been genetically modified to express antigen-specific receptors may provide tumor-specific immunity to cancer patients [38–40]. This approach is being clinically evaluated in Rotterdam, and other studies will be initiated soon in Amsterdam and Leiden. In Utrecht a strategy to manage Graft-versus-Host disease after donor lymphocyte infusions has been developed. Several of these approaches have been brought to clinical evaluation (see below). In Nijmegen, van den Berg and collaborators have developed vectors modified to express genes specifically in response to inflammatory signals for fine-tuned gene therapy in rheumatoid arthritis [41].
Non-viral vectors

Because synthetic and non-viral vectors may be preferable over viral vectors in particular gene therapy applications, new vector systems are being developed. In Groningen, the use of so-called virosomes is being evaluated by Wilschut and collaborators. Virosomes are virus-like particles, consisting of reconstituted virus envelopes, lacking any genetic material of the native virus. These studies provide insight into how macromolecules can be transferred across the cytoplasmic membrane and how the macromolecules can be released from the endosomes to the cytoplasm [36]. In Utrecht, Hennink and collaborators are exploring the use of cationic polymer based gene delivery systems. The approach here is two-sided: on the one hand new polymers are generated and characterized, and on the other hand cell biology is used to pinpoint which intracellular processes

Table 2. Completed clinical gene therapy trials in The Netherlands

| Clinical centre | Medical condition | Trial concept | Number of patients |
|-----------------|-------------------|--------------|--------------------|
| **1. Correction of a genetic defect** | | | |
| LUMC (IM 91-008/93-008) | SCID | Correction of ADA deficiency by transplantation of autologous bone marrow cells transduced with retroviral vector encoding ADA gene [19] | 3 |
| EMC (IM 98-007) | Malignant glioma | Killing tumor cells by intratumoral administration of adenoviral vector encoding herpes simplex thymidine kinase gene followed by ganciclovir treatment [50] | 14 |
| **2. Suicide gene therapy for cancer** | | | |
| EMC (IM 99-015) | Prostate cancer | Killing tumor cells prior to surgery by intratumoral administration of adenoviral vector encoding herpes simplex thymidine kinase gene followed by GCV treatment [46] | 12 |
| UMCG & LUMC (IM 95-004/95-015) | Glioblastoma multiforme | Killing tumor cells by intratumoral administration of cells producing retroviral vector encoding herpes simplex thymidine kinase gene followed by GCV treatment [48,51] | 48 + 248 |
| VUmc (IM 01-001) | Liver cancer | Killing tumor cells by intratumoral administration of adenoviral vector encoding nitroreductase gene (CTL102) followed by treatment with CB1954 [52] | 3 (out of 18 in a multicentre trial) |
| **3. Immuno-gene therapy for cancer** | | | |
| LUMC (IM 01-009) | Metastasized melanoma | Induction anti-tumor immune response by subcutaneous vaccination with allogeneic melanoma cell line transduced with plasmid encoding interleukin-2 gene [45] | 33 |
| LUMC (not available) | Metastasized colorectal cancer | Induction anti-tumor immune response by intravenous administration of canarypox viral vector encoding p53 gene [53] | 16 |
| LUMC (IM 99-018/01-005) | Melanoma | Induction anti-tumor immune response by intradermal and intracutaneous vaccination with canarypox viral vector encoding minIAGE-1/3 [54] | 1 (out of 40 in a multicentre trial) |
| NKI (not available) | Metastasized melanoma | Induction anti-tumor immune response by intra- and subcutaneous vaccination with autologous tumor cells transduced with retroviral vector encoding GM-CSF gene [55] | 28 |
| UMCG (IM 95-010) | Superficial solid tumors | Induction anti-tumor immune response by intratumoral administration of canarypox viral vector encoding IL-2 gene [56] | 3 (out of 15 in a multicentre trial) |
| UMCG (IM 96-007) | Superficial solid tumors | Killing tumor cells by intratumoral administration of adenoviral vector encoding p53 gene [57] | 2 (out of 6 in a multicentre trial) |
| **4. Other gene therapy applications** | | | |
| AMC (not available) | Crohn’s disease | Modulation inflammation by oral administration of transgenic Lactococcus lactis bacteria expressing IL-10 gene [58] | 10 |
| UMCG (IM 99-010***) | End-stage coronary artery disease | Enhancement vascularization by intramyocardial administration of naked DNA encoding VEGF2 gene [59] | 10 |
| UMCG & LUMC (IM 99-012***) | Critical limb ischaemia in diabetes mellitus | Enhancement vascularization by intramuscular administration of naked DNA encoding VEGF2 gene [49] | 54 |

This table is based on Table 3 from the article [Gene therapy in The Netherlands: past, presence and future] by E.A.M. Schenk-Braat et al. accepted for publication in the Nederlands Tijdschrift voor Geneeskunde with permission from that journal. The database from the Ministry of Housing, Spatial Planning and the Environment (VROM) containing approvals for biotechnology applications after environmental risk assessment (http://www.vrom.nl/ggo-vergunningverlening) is currently the only public source of information on Dutch clinical gene therapy trials. This database has therefore been used for the overviews presented in Tables 2–4. The protocol ID refers to the dossier number assigned by the Ministry of VROM. Information on completed gene therapy trials has also been retrieved from Entrez PubMed. The listed trials are phase I or phase II trials, unless indicated otherwise. *This protocol concerns a multicentre phase I trial and a multicentre phase III trial in which two Dutch clinical centres have participated. The respective total number of included patients is indicated. **This protocol concerns a randomized trial in which gene therapy was compared to standard medical treatment. ***This protocol concerns a small phase III double-blind, placebo-controlled randomized trial.

Abbreviations: ADA: adenosine deaminase, AMC: Academic Medical Center Amsterdam; CB1954: 5-(aziridine-1-yl)-2,4-dinitrobenzamide, EMC: Erasmus Medical Center Rotterdam, GCV: ganciclovir, GM-CSF: granulocyte macrophage colony stimulating factor, IL-2: interleukin-2, IL-10: interleukin-10, LUMC: Leiden University Medical Center, NKI: Netherlands Cancer Institute (Amsterdam), SCID: severe combined immuno-deficiency, UMCG: University Medical Center Groningen, VEGF2: vascular endothelial growth factor 2, VUmc: VU University Medical Center Amsterdam.
Table 3. Ongoing clinical gene therapy trials in The Netherlands

| Clinical centre (protocol ID) | Medical condition | Trial concept | Number of patients |
|-------------------------------|-------------------|--------------|-------------------|
| AMC (IM 05-001)*# | Genetic lipoprotein lipase deficiency | Correction LPL deficiency by intramuscular administration of AMT-010, an AAV vector encoding lipoprotein lipase variant S447X (LPL S447X) | Inclusion of 8 out of 50 patients completed |
| LUMC (not available**#) | Duchenne muscular dystrophy | Restoration dystrophin production by intramuscular administration of antisense oligoribonucleotide (exon skipping) | Inclusion of 4 patients completed |
| LUMC (IM 03-001) | Loosened hip protheses | Killing interface tissue around implant by intrarticular administration of adenoviral vector encoding nitroreductase gene followed by treatment with CB1954 | Inclusion of 12 patients completed |
| UMCU (IM 97-020)* | Hematological malignancies | Modulation GvHD and GvL disease after allogeneic stem cell transplantation by transplantation of allogeneic donor T lymphocytes transduced with retroviral vector encoding herpes simplex thymidine kinase gene followed by timed GCV treatment | No patients included yet (10 patients projected) |
| EMC (IM 97-014) | Metastasized renal cell carcinoma | Killing tumor cells by transplantation of autologous T lymphocytes retargeted against carbonic anhydrase IX using a retroviral vector | Inclusion of 5 out of 30 patients completed**# |
| VUmc (IM 03–008) | Metastasized prostate cancer | Induction anti-tumor immune response by vaccination with two prostate cancer cell lines transduced with AAV vector encoding GM-CSF gene (CG1940 and CG8711) in combination with MDX-010 immunotherapy | Inclusion of 12 out of 41 patients completed |

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*This trial is conducted in collaboration with the Dutch company Amsterdam Molecular Therapeutics (Amsterdam).

**This protocol is conducted in collaboration with the Dutch company Prosensa B.V. (Leiden). The study does not involve a genetically modified organism and therefore does not require approval from the Ministry of VROM. #These projects are funded by the ZonMW Programme Translational Gene Therapy Research.

##The first three patients were treated according to the original protocol. Due to liver toxicity, the protocol has been revised [47] and until now two patients have been included according to this revised protocol.

Abbreviations: AMC: Academic Medical Center Amsterdam, CB1954: 5-(aziridine-1-yl)-2,4-dinitrobenzamide, CG1940: PC-3 derived prostate cancer cell line expressing human GM-CSF, CG8711: LNCaP derived prostate cancer cell line expressing human GM-CSF, EMC: Erasmus Medical Center Rotterdam, GCV: ganciclovir, GM-CSF: granulocyte macrophage colony stimulating factor, GvHD: graft-versus-host disease, GvL: graft-versus-leukemia disease, LUMC: Leiden University Medical Center, MDX-010: monoclonal antibody against CTLA-4 on surface activated T cells that enhances T cell responses UMCU: University Medical Center Utrecht, VUmc: VU University Medical Center Amsterdam.

Clinical gene therapy in The Netherlands

An overview of completed, ongoing and future clinical gene therapy trials for the treatment of various diseases in The Netherlands is presented in Tables 2–4. In total, 14 trials have been completed (Table 2), 6 trials are ongoing (Table 3), and 13 protocols will be started in the near future (Table 4). Dutch scientists have been in the front line in the field of clinical gene therapy as well. As mentioned earlier, the first clinical trial worldwide on the treatment of patients with ADA deficiency with autologous stem cells genetically modified with a retroviral vector was conducted by collaborating Dutch, French and British scientists [19]. Susanne Osanto and collaborators in Leiden were amongst the first in Europe to treat patients with cancer with IL-2 gene-modified allogeneic melanoma cells that express high levels of HLA-A1 and -A2 [45]. Furthermore, Chris Bangma in Rotterdam performed a clinical study on adenovirus-mediated suicide gene therapy for prostate cancer on an outpatient basis [46]. Various gene therapy concepts developed by Dutch research groups are translated into the clinic. An example is the Rotterdam trial (Table 3, protocol IM 97-014) studying T lymphocytes retargeted to carbonic anhydrase IX as a treatment for metastasized renal cell carcinoma. This protein is overexpressed on these tumor cells. In addition, in Amsterdam AAV-mediated gene therapy for patients with lipoprotein lipase deficiency is being tested (Table 3, protocol IM 05-001) and exon skipping by an antisense oligoribonucleotide to restore dystrophin production in Duchenne muscular dystrophy patients is being clinically evaluated in Leiden (Table 3, no protocol ID).

Most of the Dutch clinical gene therapy trials are phase I studies aimed at the assessment of safety.
Table 4. Future clinical gene therapy trials in The Netherlands

| Clinical centre (protocol ID) | Medical condition | Trial concept |
|------------------------------|-------------------|--------------|
| **1. Correction of a genetic defect** | | |
| AMC (not available yet) | Crigler-Najjar syndrome | Correction of deficiency of the hepatic enzyme UGT1A1 using an AAV vector |
| EMC (not available yet) | XLA and SCID | Correction of genetic defect in autologous stem cells using a retroviral vector |
| IOI (not available yet) | Leber congenital amaurosis | Correction of CRB1 gene expression in the retina using an AAV vector |
| **2. Suicide gene therapy for cancer** | | |
| AZM (IM 01–010) | Solid tumors | Killing of tumor cells using *Salmonella typhimurium* bacteria expressing cytosine deaminase gene followed by 5-fluorouracil treatment |
| **3. Immuno-gene therapy for cancer** | | |
| EMC (not available yet) | Melanoma | Killing tumor cells by transplantation of autologous T lymphocytes retargeted against major histocompatibility class I and II-restricted MAGE epitopes using a retroviral vector |
| LUMC (not available yet) | Relapsed hematological malignancies after allogeneic stem cell transplantation | Killing tumor cells by transfection with virus-specific T cells reprogrammed into leukemia-specific T cells via retargeting to minor histocompatibility antigens using a retroviral vector |
| NKI (not available yet) | Metastasized melanoma | Killing tumor cells by transplantation of autologous T lymphocytes retargeted against melanoma antigens using a retroviral vector |
| NKI (not available yet) | HPV-positive penile or cervical cancer | Induction of anti-tumor immune response by vaccination with DNA plasmid encoding the HPV E7 gene |
| UMCG & AZM & UMCR (IM 06-001/06-003/06-010**) | Metastasized prostate cancer | Induction of anti-tumor immune response by vaccination with two prostate cancer cell lines transduced with AAV encoding the GM-CSF gene (CG1940 and CG8711) compared to treatment with docetaxel and prednisone |
| **4. Oncolytic adenovirus therapy for cancer** | | |
| EMC (not available yet) | Localized prostate cancer | Killing tumor cells prior to surgery by a targeted replicating adenovirus |
| VUMc (not available yet) | Glioblastoma multiforme | Killing tumor cells by a targeted replicating adenovirus |
| **5. Other gene therapy applications** | | |
| AMC (not available yet) | HIV infection and AIDS | Inhibition of HIV replication by HIV-1-specific short hairpin RNAs delivered via a lentiviral vector |

This table is based on Table 4 from the article [Gene therapy in The Netherlands: past, presence and future] by E.A.M. Schenk-Braat et al. accepted for publication in the Nederlands Tijdschrift voor Geneeskunde with permission from that journal. The listed trials are phase I trials, except for protocols IM 06-001, IM 06-003, IM 06–010, and IM 06–011 (see **). The protocol ID refers to the dossier number assigned by the Ministry of VROM (see legends of Table 2).

*This trial will be conducted in collaboration with the Dutch company Amsterdam Molecular Therapeutics (Amsterdam).

**These protocols are related to multicentre Phase III randomized, open-label studies sponsored by Cell Genesys, Inc., and involve four Dutch clinical centres.

# These projects are funded by the ZonMw Translational Gene Therapy Research Programme.

This project is part of the European Commission Sixth Framework Programme project GIANT (Gene therapy: an Integrated Approach for Neoplastic Treatment).

Abbreviations: AMC: Academic Medical Center Amsterdam, AZM: Academic Hospital Maastricht, CB1954: 5-(aziridine-1-yl)-2,4-dinitrobenzamide, CG1940: PC-3 derived prostate cancer cell line expressing human GM-CSF, CG8711: LNCaP derived prostate cancer cell line expressing human GM-CSF, CRB1: Crumbs Homologue 1, EMC: Erasmus Medical Center Rotterdam, GCV: ganciclovir, GM-CSF: granulocyte macrophage colony stimulating factor, GvHD: graft-versus-host disease, GvL: graft-versus-leukemia disease, HIV: human immunodeficiency virus, HPV: Human Papilloma Virus, IOI: The Netherlands Ophthalmic Research Institute (Amsterdam), LUMC: Leiden University Medical Center, MDX-010: monoclonal antibody against CTLA-4 on surface activated T cells that enhances T cell responses, NKI: Netherlands Cancer Institute (Amsterdam), UGT1A1: bilirubin UDP glucuronosyltransferase, UMCG: University Medical Center Groningen, UMC: University Medical Center St. Radboud (Nijmegen), UMC: University Medical Center Amsterdam, XLA: X-linked agammaglobulinemia.

The results from these trials confirm the general observations that gene therapy is well tolerated without major complications. In the Rotterdam trial on T lymphocytes retargeted to renal cell carcinoma described above (Table 3, protocol IM 97-014), liver toxicity was encountered in the first three patients. The trial was allowed to continue according to an amended protocol [47] and two patients have now been included without side effects. In accordance to trials conducted in other countries, clear clinical efficacy of gene therapy has not been demonstrated yet in Dutch trials. In the two randomized and placebo-controlled phase III trials on gene therapy for brain tumors (Table 2, protocols IM 95-004/95-015) and for critical limb ischemia in diabetes mellitus, efficacy could not be established [48] or the clinical effect was only minor [49]. The first results reported for the Leiden trial studying suicide gene therapy in patients with a loosened hip prosthesis (Table 3, protocol IM 03-001) are promising. In this trial, interface tissue around the implant is destroyed by suicide gene therapy prior to fixation of the prosthesis with cement. Ongoing and upcoming clinical trials now
focus on the translation of new developments in gene therapy research aimed at improvement of efficacy and safety of this treatment modality. The first Dutch trials on oncolytic adenovirus therapy for cancer will be carried out in Amsterdam and Rotterdam (Table 4). Furthermore, the restoration of genetic defects other than SCID is addressed, including genetic lipoprotein lipase deficiency, Duchenne muscular dystrophy, Leber congenital amaurosis causing blindness and Crigler Najjar syndrome causing irreversible brain damage due to hyperbilirubinemia (Tables 3 and 4).

The Dutch Ministry of Health has committed to gene therapy trials by approving a 15.6 M€ funding the ‘Translational Gene Therapy Research Programme’ that has been developed and is executed by the funding agency ZonMw. The programme aims to improve the translation of pre-clinical gene therapy research to the clinic (www.zonmw.nl/genetherapy). So far, 11 phase I/II trials described in Tables 3 and 4 have been supported by this programme. ZonMw stimulates the cooperation of researchers and commercial enterprises. Besides investigator-initiated clinical studies, commercial interest in clinical gene therapy in The Netherlands is increasing. The phase I/II clinical study in Leiden involving the suicide gene therapy approach for prosthesis refixation has been sponsored by Innovata Ltd. (Ruddington, UK). Currently, Amsterdam Molecular Therapeutics is participating in the Amsterdam trial on lipoprotein lipase deficiency (Table 3, protocol IM 05-001). Another Dutch company, Prosensa B.V. based in Leiden, is involved in the Leiden trial on Duchenne muscular dystrophy (Table 3, no protocol ID). The Dutch drug development company ORCA Therapeutics B.V. in Amsterdam is currently preparing its lead product, an oncolytic adenovirus-expressing tumor suppressor p53, for clinical evaluation in cancer patients. Another company, Arthrogen B.V. (Amsterdam), is developing AAV-based gene therapy for rheumatoid arthritis for future clinical application. Furthermore, at least four Dutch clinical centres will participate in large phase III international multicenter immuno-gene therapy trials on prostate cancer sponsored by Cell Genesys, Inc. (San Francisco, USA) (Table 4, protocols IM 06-001/06-003/06-010/06-011).

Conclusions

Dutch scientists involved in fundamental and clinical gene therapy research are significantly contributing to the progress made in this research area, including the translation of innovative developments into the clinic. The latter process has been greatly stimulated by the Translational Gene Therapy Research Programme supported by ZonMw. Many of the Dutch academic research centers have now made long-term commitment to clinical research involving gene therapy, which has resulted in the establishment of various gene transfer facilities. The national regulatory conundrum has precipitated into the establishment of the Gene Therapy Office, which coordinates the assessment of the clinical protocols by the various regulatory bodies. This should aid the applicants and should warrant efficient communication between and amongst the pertinent regulatory bodies and the applicants (see paper by Bleijs et al., this issue). The atmosphere surrounding Dutch gene therapy may also be evident from the fact that several active Dutch scientists have accepted invitations to serve on review panels and advisory boards that are involved in the regulation of clinical gene therapy. Also the Dutch Society of Gene Therapy has a formal role in the evaluation of the Gene Therapy Office. This tight network of interrelations ensures efficient communication between the Dutch gene therapy community and bodies that should facilitate safe and efficient progress in this powerful field.

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