Gene therapy and cement injection for the treatment of hip prosthesis loosening in elderly patients

Jolanda de Poorter

Gene therapy and cement injection for the treatment of hip prosthesis loosening in elderly patients

Jolanda Johanna de Poorter

PhD Thesis, Leiden University Medical Center, Leiden, The Netherlands

ISBN/EAN: 978-90-9024975-9

Lay-out:	Grafisch bureau Christine van der Ven, Voorschoten
Cover photographs:	Gerrit Kracht
Printed by:	Buijten en Schipperheijn, Amsterdam

Publication of this thesis was financially supported by de Nederlandse Orthopaedische Vereniging, Stichting Anna Fonds, Link Nederland, Implantcast Benelux en Bauerfeind benelux BV.

Gene therapy and cement injection for the treatment of hip prosthesis loosening in elderly patients

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 28 januari 2010
klokke 16.15 uur

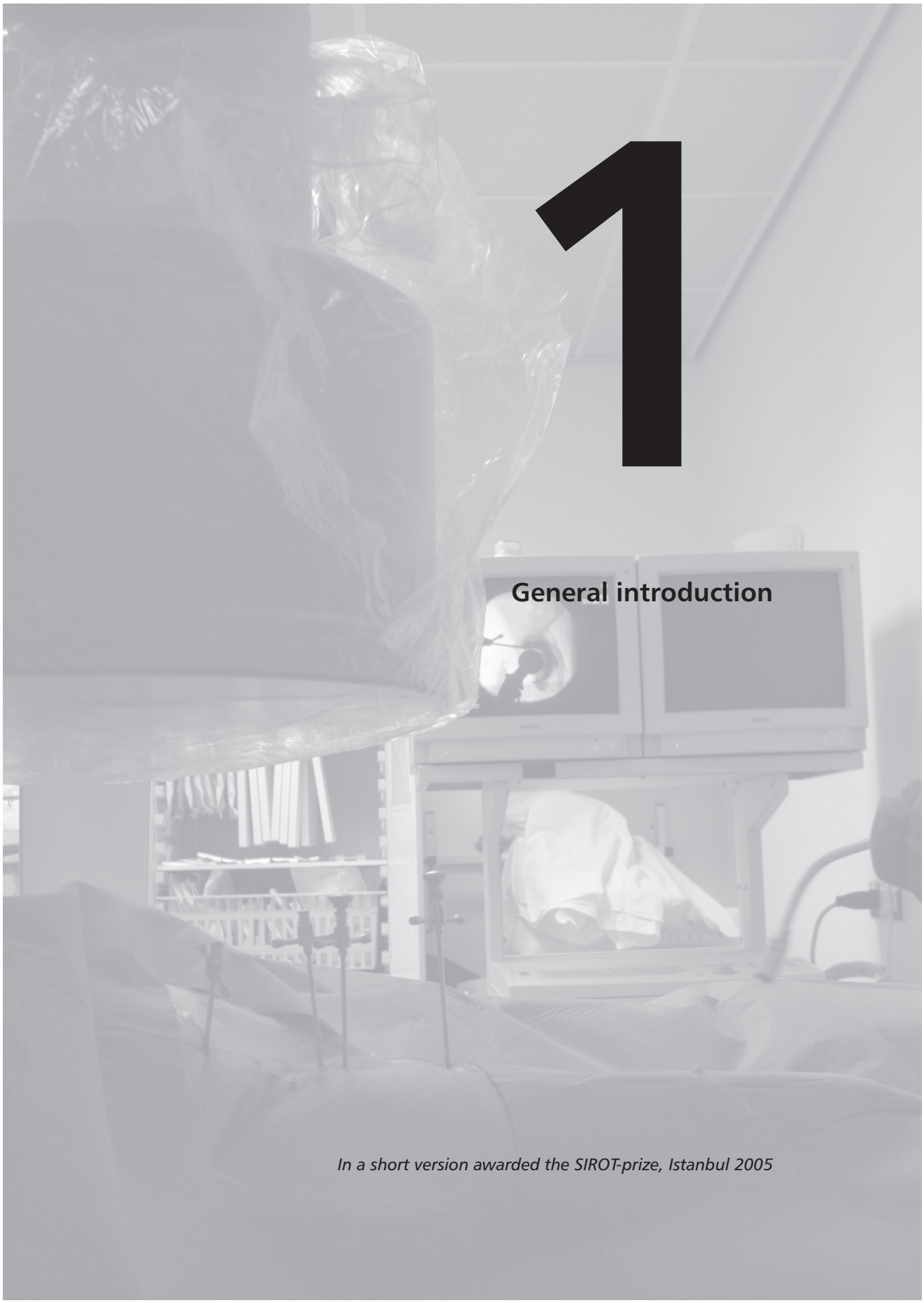
door
Jolanda de Poorter
geboren te Eindhoven in 1976

PROMOTIECOMMISSIE

Promotores: Prof. dr. R.G.H.H. Nelissen
Prof. dr. T.W.J. Huizinga

Overige leden: Prof. dr. S.K. Bulstra (Universitair Medisch Centrum Groningen)
Prof. dr. R.C. Hoeben
Prof. dr. L.W. van Rhijn (Maastricht Universitair Medisch Centrum)
Dr. R.E.M. Toes
Prof. dr. D. Valerio
Dr. ir. E.R. Valstar

Contents



1

General introduction

In a short version awarded the SIROT-prize, Istanbul 2005

Hip prosthesis loosening and the problems in elderly patients

Approximately one million total hip replacement operations are performed worldwide annually, and this number is likely to increase considerably in the next decades. A major complication in total hip arthroplasties is loosening of the prosthesis leading to pain and walking difficulties and a higher risk for dislocations and pathological fractures.[54] Within ten years of primary hip replacement 7-13 percent of patients need revision surgery due to loosening of the implant.[78] Revision surgery has a high morbidity and mortality rate, especially in elderly patients with comorbidity. In the United States Medicare Population 5.3% of 3,165 patients undergoing revision surgery at age 80 and older died within 90 days of surgery. This was 1.9 times higher than a comparable Medicare cohort that had not undergone revision total hip replacement.[77] Strehle *et al*.[114] registered complications and social outcome in a cohort of 53 patients undergoing revision total hip arthroplasty older than age 80 years. They reported a total mean blood loss of 4,730mL and a mean duration of the procedure of 200 minutes. Eleven patients (21%) were admitted postoperatively to the intensive care unit, and mean hospital stay was 30 days. Complication rate was higher in patients with comorbidity. Of the 53 patients followed, three patients died during hospital stay, ten patients formerly living alone in a house or an apartment went to nursing care institutions and five patients became dependent on outside help from family members, neighbours or health care institutions. These figures indicate that revision surgery can be a heavy burden for elderly patients and the indication needs to be reconsidered thoroughly before these patients can be operated. Consequently, there remains a group of elderly patients with comorbidity who are not eligible for surgery and experience incapacitating pain and dependency in activities of daily living. Currently there are no alternative treatments for revision surgery.

Aseptic loosening

Aseptic loosening by particulate-induced osteolysis is the most common cause of implant failure. Wear particles, such as particles of polyethylene and metal, are phagocytosed by macrophages, leading to secretion of inflammatory cytokines.[43] The resulting chronic inflammation eventually produces a pseudomembrane of synovium-like interface tissue with activated macrophages, fibroblasts, giant cells and osteoclasts.

At present, experimental approaches to the aseptic loosening problem are preventative rather than therapeutic. Preclinical studies have shown that bisphosphonates

might be useful to prevent aseptic loosening,[108] but up till now clinical evidence is missing that bisphosphonates will prevent aseptic loosening at longer term. An alternative preventative approach for aseptic loosening involves gene therapy, e.g. using an osteoclast inhibitory protein, osteoprotegerin, delivered by a vector that delivers the gene inside the cell, such as an adeno-associated vector.[120] Osteoprotegerin serves as a competitive inhibitor for the differentiation of osteoclasts, thereby preventing osteoclast activation. The vector to express the active ingredient was delivered by intramuscular injection into the quadriceps muscles of mice. The effect on osteoclasts was therefore systemic and this appeared to be successful in inhibiting the osteolysis that was seen in untreated controls.[120] Although the results of these experimental animal data are interesting, it is unclear what the long-term systemic effects of prolonged elevations in serum osteoprotegerin might be. Before clinical application a deleterious effect on normal osteoclast function needs to be excluded.

In summary, experimental studies on alternatives for revision surgery are primary preventative and not yet in clinical trials.

Removal of interface tissue

This thesis describes an approach to stabilise loosened hip prostheses as an alternative to regular revision surgery. The technique involves, among other things, injection of bone cement around the loosened prosthesis percutaneously, while the prosthesis remains in place. Before bone cement can be injected to stabilise the prosthesis, interface tissue preferably needs to be removed to leave space for the cement. As the periprosthetic space is a more or less closed compartment local application of a toxic component could be a good option. Non-surgical removal of interface tissue has not been described in the literature. However, from the early 1950s several chemical agents have been used for intra-articular chemical synovectomy in patients with rheumatoid arthritis. Synovial tissue has the histological and histochemical characteristics of interface tissue,[43] and results of studies with synovial tissue could therefore be an indicator for outcome of studies with interface tissue. Chemical agents as osmic acid, nitrogen mustard, and thiopeta have been shown successful in non-controlled studies, but not in controlled studies and are currently not in use because of potential hazards.[25] Cytostatics that are used in cancer therapy act by inhibition of cell division. As the cells in interface tissue are only slowly dividing cells, the use of cytostatics to remove interface tissue is not a good option. Another approach to killing pathological cells is to introduce a gene into the target cells that encodes an enzyme capable of converting a prodrug of relatively low toxicity into a potent cytotoxic drug. As the prodrug is converted

to the toxic derivative locally, occurrence of systemic adverse effects will be low. This approach is known as gene-directed enzyme prodrug therapy (GDEPT).[23]

Gene therapy

The definition of gene therapy (according to a medical dictionary) is the insertion of normal DNA directly into cells to correct a genetic defect. It involves the treatment of disease by replacing, altering, or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for a disease.

The first attempt for human gene therapy was reported in 1975 when Rogers and Terheggen combined their knowledge on the Shope rabbit papilloma virus that induces arginase,[104] and on the disease argininemia, a genetic disease involving a low arginase-level, causing spastic diplegia, epileptic seizures, and mental retardation.[119] They inoculated fibroblasts from humans with arginase deficiency with the Shope virus, resulting in an induction in arginase activity.[105] However, in a clinical trial in three patients, intravenous injection of the Slope virus was unsuccessful.[118]

In recent years the potential role of gene therapy has been expanded to gene therapy as a tool for delivering individual proteins to specific tissues and cells. This also encompasses delivering proteins to kill cells (e.g. in cancer) and introducing therapeutic proteins locally for a longer period of time (e.g. in rheumatoid arthritis). The most common method to introduce the gene into the cell is by using a virus as a vector, as viruses are known to deliver their DNA into the host cell for replication. The adenoviral (Ad) vectors are popular for gene therapy since they are very efficient at infecting dividing as well as non-dividing cells, are easy to produce in large titers,[38] and provide ample space for transgenes. Adenoviral vectors deliver their gene outside the host cell genome, thereby minimising the risk for disturbing normal cellular gene expression. The expression of their inserted gene is transient due to cellular and humoral immune responses[130] which could actually be an advantage when only transient expression is required for adequate therapy. The presence of anti-Ad neutralising antibodies tends to be ubiquitous in human adults and greatly reduces virus dissemination while peak transgen expression in the targeted tissue is only minimally reduced.[15] Moreover, the virus has a high particle size which, when introduced in a more or less closed compartment, prevents most of it from diffusing into other tissues. Thus, an adenoviral vector can ideally be used to deliver a gene to the interface tissue in the periprosthetic space. When using adenovirus 5 as a vector to express the gene Escherichia coli nitroreductase (Ntr), infected cells become extremely sensitive to the prodrug CB1954. This prodrug causes death of the infected cells.[34] In a study by Goossens *et al.*,[46] it was demon-

strated that genes can be transferred to synovial tissue *in vivo* in rhesus monkeys by direct injection into the joint, and that the synoviocytes can be killed with injection of a specific prodrug. In our laboratory, previous experiments have shown the efficacy of the infection and destroying of synoviocytes and fibroblasts from interface tissue by HAdV-5-Ntr and CB1954.

Cement injection

One of the major difficulties in revision surgery is the removal of cement from the femoral shaft without fracturing the femur which may be eggshell thin. Therefore debate exists whether this cement should be removed completely before a new stem can be cemented. Chapchal *et al*.[20] advocated in 1973 to remove all old bone cement despite it being time-consuming and hazardous. Later, this advise was more differentiated by Charnley *et al*.[22] who stated that the difficulties of complete removal, the risk of fracture, of interrupting blood supply and of reduction in the amount of cancellous bone may together represent a greater risk than that of bond failure at an old-new cement interface. They recommended, in the replacement of a non-infected femoral prosthesis, to ream out sufficient cement to permit a loose fit of the new prosthesis. Biomechanical studies showed that recementing over old cement is a practical alternative when all the blood is removed from the old cement, the old cement is rasped and the newly inserted cement is as fresh as possible to assure the presence of non-activated monomer that can be activated by the benzoyl peroxide activator still present in the old cement giving a greater interface strength.[49] Lieberman *et al*.[73] showed this in clinical practice in 19 patients where a new prosthesis was cemented in an old cement mantle. The technique involved rasping and drying of the surface before applying fresh cement, to increase interface strength between the old and the new cement.

Since the mid 1980s percutaneous cement injection is used for vertebroplasty in patients with painful vertebral lesions to relieve pain and provide strength.[60] For this purpose low viscosity PMMA-cement with additional radio-opacity has been developed together with cement-guns and needles for injecting the cement. As aseptic loosening results in a radiolucent line around the prosthesis and the pain from loosening is caused by movement of the prosthesis within the bone, it would be worthwhile trying to inject cement in the periprosthetic zone percutaneously. Ideally three or more injection sites should be used to allow stabilisation in a 3D-space. In this way the prosthesis could again be stabilised in the bone, leading to decrease in pain and improvement in walking. Furthermore, the mechanical stress on the bone by the loose prosthesis is reduced, thus allowing for reconstituting of the resorbed bone[14]. Percutaneous cement

injection in the periprosthetic space has not been described in the literature. To our knowledge this procedure has not been studied as an alternative to revision surgery. To assess if the periprosthetic space is accessible to bone cement an arthrography of the hip can be useful. With arthrography, the periprosthetic space can be visualised when the contrast medium is easily distributed around the prosthesis. However, arthrography of the hip in a loosened prosthesis often shows just a small line (i.e. <1mm) of contrast medium between bone and cement. It should be questioned if this area is large enough to inject a sufficient amount of cement to stabilise the prosthesis, particularly since the cement has a higher viscosity than the contrast medium. Therefore, before cement can be injected, the interface tissue preferably has to be removed.

Aims and outline of this Thesis

The aim of this thesis was to evaluate risks and benefits of revision hip arthroplasty in a retrospective cohort of patients 80 years and older and to develop and assess an alternative treatment for revision hip arthroplasty for elderly patients with a high risk for complications due to serious comorbidity or a low bone stock (i.e. high likelihood of femoral fracture) . In **Chapter 2** we analysed the risks of revision hip arthroplasty in elderly patients. We studied the burden of hospital stay and occurrence of complications and the benefits of improvement in social outcome. We assessed social outcome of 145 patients 80 years and older undergoing 183 hospital admittances for revision surgery of their hip prostheses. Primary objective was to investigate whether hip revision surgery in elderly patients could improve social outcome (housing situation and independency in ADL (activities in daily living)). Secondary objectives were occurrence of complications during hospital stay, patient survival, and use of walking aids before and after revision surgery.

In **Chapter 3** we studied whether cells from the interface tissue between prosthesis and bone could be killed by Gene-directed Enzyme Prodrug Therapy (GDEPT). We investigated whether these cells could be transduced by a human adenoviral-5 vector carrying the *E.coli*-derived nitroreductase gene (CTL102) and sensitised to the prodrug CB1954. First, we exposed the cells to various concentrations of Ad.CMV.LacZ to determine the infectivity of interface cells. In the next experiment, interface cells were exposed to various concentrations of CTL102 and subsequently to various concentrations of CB1954 to study cell-killing potential of the Ntr/CB1954 GDEPT. In this chapter we also discuss the influence of iodide-containing contrast medium on adenovirus-mediated gene transfer.

Chapter 4 describes two alternative methods to optimise short-term transgene

expression. In clinical studies it is essential to have a predictable and adequate expression. When the gene expression can be made more efficient and predictable, the vector dose can be decreased. This has several advantages, including less evocation of an immune response, a smaller demand for the production of clinical grade adenovirus, and less adverse events. A Ubiquitous Chromatin Opening Element (UCOE) was inserted in an Ad.CMV.Luc vector, and sodium butyrate (NaB) was added to the culture medium of interface cells in various concentrations to study the effect on transgene expression. Both these methods have a theoretical potential to enhance expression without increasing the amount of viral particles. The two methods were tested individually and in combination to evaluate their effect on transgene expression.

For the clinical use of intra-articular treatments a good exposure of the target tissue is essential and the injected active ingredient must remain in the joint for a sufficient amount of time to ensure adequate therapy. Beside size of the therapeutic particle, the integrity of the surrounding joint tissue (containment) is important in retaining active particles within the joint space. Efficacy of intra-articular therapy is also dependent on the joint volume, because this determines the concentration of the therapeutic ingredient. **Chapter 5** shows a retrospective analysis of 221 hip arthrograms performed for diagnosis of prosthesis loosening. All arthrograms were studied for leakage of contrast medium and injected volume. This analysis was performed to determine the percentage of hip prostheses that would be eligible for therapy using intra-articular delivery of genes and proteins.

After these pre-clinical studies, a phase-1 clinical gene therapy approach was designed to destroy the periprosthetic loosening membrane, and enable refixing of the hip prosthesis with percutaneous bone cement injections under radiological guidance. In this phase-1/2 dose escalating gene therapy trial twelve patients were treated. **Chapter 6** shows the protocol of the phase-1 clinical study on gene therapy in aseptic prosthetic replacement loosening, carried out in the Leiden University Medical Center between June 2004 and February 2006.

The results of the clinical study are described in two chapters. As the study is a phase-1 clinical study, safety is the primary objective. **Chapter 7** describes all adverse events of the clinical study and their possible relations to gene therapy, cement injection or other features of the study. **Chapter 8** describes the secondary objectives of the clinical study. Virus shedding was quantitatively measured by analysis of urine, stool, blood, and nose and throat swabs. Biopsies from periprosthetic interface tissue, taken during the cementing procedure, were investigated for apoptotic and necrotic tissue. X-rays of the hip before and after the cementing procedure were analysed for increase in cement thickness. Finally, for clinical evaluation, Harris Hip Score and Visual Analogue Scales for pain, walking distance, and independency for activities in daily liv-

ing, were done pretreatment, and three and six weeks, and three and six months after therapy.

In **Chapter 9** a small case series is described in which percutaneous peri-prothetic cement injection is performed in elderly patients with hip prosthesis loosening, without previous gene therapy to remove the interface tissue. This series was performed to study whether cement injection is feasible without previous interface tissue removal.

Finally, in **Chapter 10**, a general discussion is given on percutaneous peri-prosthetic cement injection with or without gene therapy as an alternative to revision surgery, based on the work presented in this thesis.

2

Revision Hip Arthroplasty in patients over 80 years of age. Implications on social life and activities in daily living

Jolanda J. de Poorter[1]
Evert J. van Langelaan[2]
Mo E. van Ark[1]
Tom W.J. Huizinga[3]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Orthopaedics, Rijnland Ziekenhuis, Leiderdorp, The Netherlands
[3]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

Abstract

We retrospectively reviewed social outcome of all octogenarians undergoing revision total hip arthroplasty in two hospitals in the Netherlands. A total of 183 hospital admittances in 145 patients were identified. Overall in 58% of hospital admittances the patient returned to the previous social situation, 35% had a worsening in social situation, and 8% had an improvement. 59% of patients living in a house or apartment went to a nursing institution for rehabilitation after discharge. Presence of a spouse was the only predictor for returning home immediately after discharge. There was a mean rate of 1.3 medical complications per patient with statistical differences between ASA-categories. 29% of patients needed less walking aids after revision surgery.

Revision surgery gives a high rate of complications in octogenarians, with patients with a higher ASA-category having more complications. However, this does not affect returning to previous housing situations after discharge.

Introduction

The number of revision surgeries in elderly patients is likely to increase considerably in the next decades, due to the tendency to insert orthopaedic implants at younger ages and the longer life expectancy of patients. Revision surgery has a high complication rate in elderly patients[6,92,99,114] and is associated with less improvement in social outcome, compared to primary hip arthroplasty in patients of all ages.[103] The patients themselves often don't realise the technical limitations of revision surgery and expect the same result as in primary total hip arthroplasty.[51] Consequently, overall satisfaction in patients after revision total hip arthroplasty is 3.7 times lower than after primary hip arthroplasty.[36] To stress the burden of revision hip arthroplasty for elderly patients we studied the social outcome after surgery in all patients 80 years and older who had revision THP in two hospitals in the Netherlands between 1994 and 2007. Primary objective was to investigate whether hip revision surgery in elderly patients could improve social outcome (housing situation and independency in ADL (activities in daily living)). Secondary objectives were occurrence of complications during hospital stay, patient survival, and use of walking aids before and after revision surgery.

Patients and methods

To evaluate outcome of revision hip arthroplasty in octogenarians, all patients who were 80 years and older when undergoing removal and/ or insertion of an acetabular and/ or femoral component of a hip prosthesis in two hospitals in the Netherlands between 1994 and 2007 were included.

Preoperative data, surgery data and data regarding the hospital admission were collected from the patients' hospital chart. The patients' general practitioners were asked if and when the patient had died. The physical status of the patient was classified by the anaesthesiologists according to ASA-classification (American Society of Anesthesiologists)[121] (Table 1).

Information regarding complications that occurred was gathered from the hospital charts as recorded by the doctors as well as by the nurses. Prophylactic antibiotics (cephalosporin) were given routinely 30 minutes before the procedure and this was repeated when the surgery time was more than 3 h, and when blood loss was more than two litres. Post-operative antibiotics were not given routinely. Thrombosis prophylaxis was given until six weeks post-operatively, first as low-molecular-weight heparin, in some cases followed by coumarins. To study the impact of different kinds of revi-

Table 1. American Society of Anesthesiologists' Physical Status Classification

ASA-1	A normal healthy patient
ASA-2	A patient with mild systemic disease (mild diabetes mellitus, controlled hypertension, anemia, chronic bronchitis, morbid obesity)
ASA-3	A patient with severe systemic disease that limits activity (angina pectoris, obstructive pulmonary disease, prior myocardial infarction)
ASA-4	A patient with an incapacitating disease that is a constant threat to life (congestive heart failure, renal failure)
ASA-5	A moribund patient not expected to survive >24 h (ruptured aortic aneurysm, head trauma with increased intracranial pressure)

sion operations, the procedures were divided in 7 groups (liner revision, cup revision, stem revision, total hip revision, hemi-arthroplasty revised to total hip prosthesis, only components removed for infection, and other). For these groups, operative time, blood loss, number of complications, duration of hospital stay, and return to home were analysed. Also, a distinction was made in removal of cemented and uncemented stems for the same parameters as noted above.

To get an impression on pre-operative dependency the Katz-ADL-index was measured.[63] This index is a tool for assessing a patient's ability to perform activities of daily living in the areas of bathing, dressing, toileting, transferring, continence, and feeding. In each category, a score of one indicates complete independence in performing the activity and zero indicates that assistance is required, so that the total score ranges from zero to six.

Primary objective

Social situation of the patients was pre-operatively recorded by the nurses. They recorded housing situation (house/apartment, home for the elderly or nursing home), the presence or absence of a spouse or child living with the patient, and the kind of help the patient received in activities of daily living (no help, informal care from children living nearby, house keeper, or home care). The postoperative situation was defined as the most favourable situation the patient achieved in social situation after the revision surgery. These data were gathered from post-operative correspondence between general practitioner and hospital and from the nurses' charts on consecutive hospital admittances. For patients who went to a nursing home for rehabilitation the nursing homes were called to ask for how long the patient had stayed there and whether they could return to their own homes after rehabilitation.

Secondary objectives

Survival after revision surgery was measured for each patient. For patients who had multiple revisions only the latest revision was taken into account for the survival analysis. Kaplan Meier-analysis was performed to compare survival in different ASA-groups (American Society of Anesthesiologists) (Table 1)[121], with date of death as end point, and date of end of follow-up as sensored data.

The hospital charts were studied for occurrence of complications. The charts made by the doctors as well as the charts made by the nurses were studied. A distinction was made between orthopaedic complications and medical complications. The list of medical complications was subdivided in cardiovascular, renal, gastro-intestinal, pulmonary, neurological, and other complications. Some complications were defined as major: death, pulmonary embolism, myocardial infarction, pneumonia, stroke, deep infection, shock, peri-prosthetic fractures, and renal failure. The patient group was divided by ASA-category to determine the differences in occurrence of complications in the comorbidity-groups. Pressure sores were registered as such when there was at least disruption of the skin (grade II pressure ulcer, according to the National Pressure Ulcer Advisory Panel).[1] Delirium was defined as severe confusion (with interference of normal function) during at least one day, that could not be corrected, and with inaccessibility to normal contact. Anorexia, and nausea and vomiting were only registered when parenteral feeding was necessary.

The use of walking-aids by the patients was registered by the nurse at hospital admittance. For the post-operative use of walking aids the most favourable situation was recorded. These data were gathered from the outpatients' clinic's chart, regarding post-operative controls and follow-ups. The categories for walking aids were: no aids, cane, one crutch, two crutches, walker, wheelchair, or bedridden.

Statistical Methods

Kaplan Meier analysis was performed for analysis of patient survival after revision surgery, with date of death as the end point and end of follow-up as sensored data. Log-rank test was used to compare between ASA-categories. A oneway ANOVA with Bonferroni test was used to compare between different revision operations. A Student T-test was used to compare parameters between cemented and uncemented stems. Logistic regression was used to find predictive factors for patients living in a house or apartment to return to their own homes.

A statistical p-value of <0.05 was chosen as the level of significance. SPSS version 16.0 was used for statistical analyses.

Results

Patients and hospital admittances

183 hospital admittances for revision hip arthroplasties were reported between 1994 and 2007 in the two Dutch hospitals. A total number of 145 patients had 183 hospital admittances (1.26 admittances per patient).Table 2 shows demographic characteristics for hospital admittances where at least one component of the hip prosthesis was removed or placed. In 41 of 183 admittances the patient went to the ICU (Intensive Care Unit) postoperatively, mean length of stay was 2.3 days. Figure 1 shows means (and standard deviations) for operative time, blood loss, number of complications and duration of hospital stay per type of revision. Operative time was longer in patients where a cemented stem had to be removed (mean 190 min, sd 75 min), compared to an uncemented stem (mean 149 min, sd 86 min) ($p = 0.01$). No differences were found in blood loss, number of complications, and length of hospital stay, between removal of cemented and uncemented stems.

Social outcome

Figure 2 shows social housing situation before and after hospital admittance for hip revision surgery. For the post-operative situation the most favourable situation was taken. For example, when patients went post-operatively to a nursing home for rehabilitation and returned to home after several months, the latter situation was recorded. For all patients information on pre-operative situation was available. In 143 of 183 hospital admittances patients lived pre-operatively in a house or apartment (78%), in 21 the patient lived in a home for the elderly (10%), and in 19 the patient lived in a nursing home (11%). Four patients died during hospital stay. In the remaining of hospital admittances 138 times (75%) the patient could return to their previous housing situation, in 22 cases (12%) there was a worsening in the housing situation, and in 7 cases (4%) there was an improvement.

In the cases where the patient was living alone in a house or apartment, 39% returned to the same social situation regarding outside help, 58% had a worsening in the situation and 3% had an improvement. In the cases where the patient lived with a spouse in a house or apartment, 70% returned to the same social situation regarding outside help, in 24% there was a worsening in the situation, and in 5% there was an improvement.

Overall (housing situations and help in activities of daily living) 58% remained in the same social situation, 35% had a worsening in social situation, and 8% had an improvement.

Table 2. Demographic Characteristics of the Patients and Outcomes

Parameter	Total 183 Hips
Age (years)*	83.9 (Range 80.1 – 97.9; sd 2.9)
Men / women	24 / 159 (13.1% / 86.9%)
ASA score	
ASA – 1	4 (2.2%)
ASA – 2	105 (57.4%)
ASA – 3	67 (36.1%)
ASA – 4	7 (3.8%)
Indication for revision	
Aseptic loosening	121 (66.1%)
Periprosthetic infection	29 (15.8%)
(Periprosthetic) fracture	12 (6.6%)
(Recurrent) dislocations	18 (9.8%)
Fausse route	1 (0.5%)
Persisting pain	1 (0.5%)
Titanium debris	1 (0.5%)
Component removed	
None	15 (8.2%)
Stem	45 (24.6%)
Cup	46 (25.1%)
Both components	77 (42.1%)
Component inserted	
None	35 (19.1%)
Stem	19 (10.4%)
Cup	47 (25.7%)
Both components	82 (44.8%)
Operative time (hours)*	2.7 (Range 0.6 – 8.0; sd 1.3)
Blood loss (Liter)*	1.6 (Range 0.15 – 6.5; sd 1.2)
Length of hospital stay (days)*	34 (Range 2 – 197; sd 28)

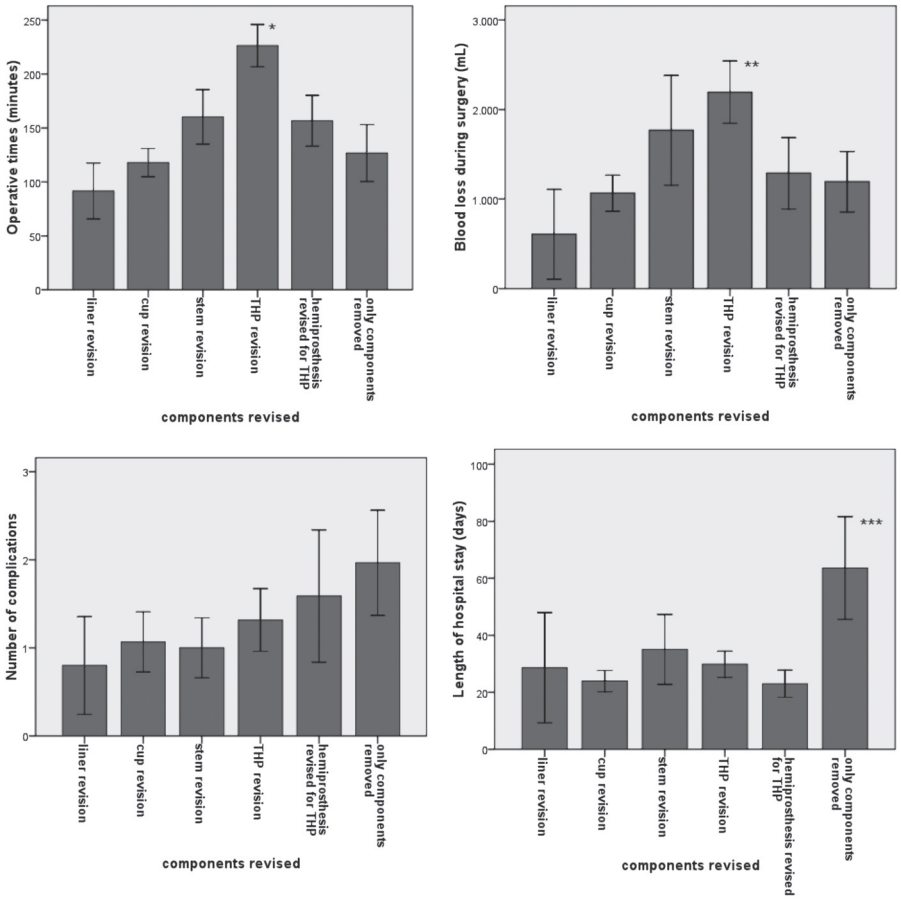
* Data are given as mean with range and standard deviations in brackets

Of the 143 hospital admittances where a patient lived in a house or apartment pre-operatively, in 84 (59%) cases the patient went post-operatively to some sort of rehabilitation facility. Of these cases 64 (76%) went to a nursing home (mean period of stay was 114 days), 19 (23%) went to a home for the elderly (mean period of stay was 44 days), and 1 patient went temporarily to her daughter's house. 11 Patients never returned to their homes.

Predictors for returning to home in patients living in a house or apartment

To predict which patients could return to their home and which patients had to go to

Figure 1. Bar chart of means and standard deviation for operative time, blood loss during surgery, number of complications, and length of hospital stay per component revised.



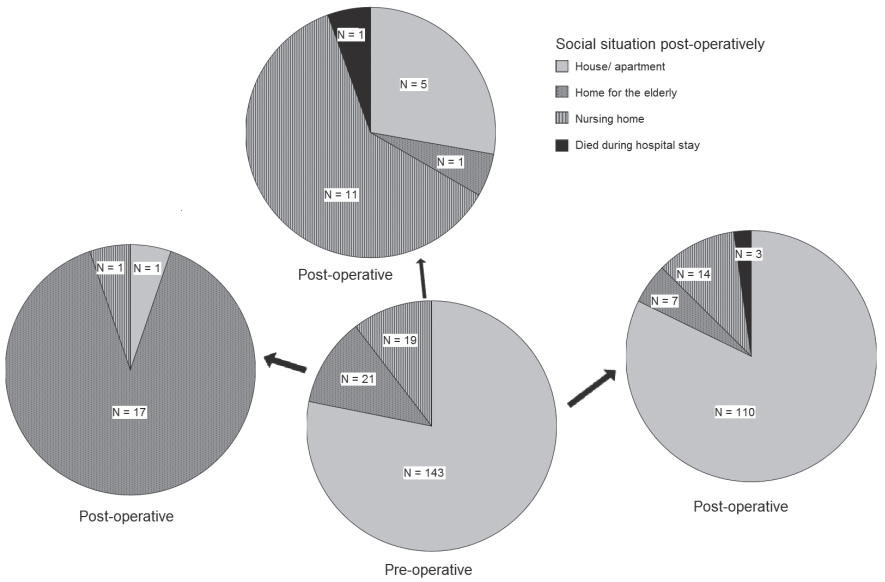
* Operative time for a total hip revision was significantly longer than for the other revisions ($p < 0.01$).

** Blood loss was significantly higher in the total hip revision group compared to all other groups ($p < 0.03$) except for stem revision only ($p > 0.95$). There were no differences in number of complications occurring between the revision groups.

*** Duration of hospital stay was significantly longer in the patients who only underwent removal of components compared to the other groups.

a nursing home or a home for the elderly for rehabilitation or permanent stay after discharge, a logistic regression analysis was performed. First, the factors we expected to be a potential predictor of returning home were tested individually with univariate logistic regression. The factors tested were: sex (male/female Odds-ratio for returning home was 6.0, p <0.01); ASA-category (ASA1-2 / ASA 3-4 OR = 0.74, $p = 0.40$);

Figure 2. Social housing situation before and after hospital admittance for revision hip arthroplasty.



The central pie shows the pre-operative situation. The pies on the side show the distribution of post-operative situations per pre-operative situation. For example the pie on the right shows the post-operative situation of the patients who pre-operatively lived in a house or apartment.

Katz-ADL-index (OR = 1.1; p = 0.47); indication for surgery (p = 0.84); surgery time (OR = 1.0; p = 0.87); blood loss during surgery (OR = 1.0; p = 0.94); components revised (p = 0.63); removal of cemented or uncemented stem (p = 0.59), presence of a spouse (with spouse/ alone OR = 24; $p < 0.01$); outside help (p = 0.047, with significant difference between no help/housekeeper OR = 0.34, p = 0.014; no difference between no help/home care OR = 0.65, p = 0.33; and no difference between housekeeper/ home care OR = 1.9, p = 0.13). The parameters entered in the logistic regression model were: sex, presence of a spouse, and outside help. With these three parameters in the model, only the presence of a spouse was a predictive value for return to home (OR = 36, p <0.01). As we expected sex to be a confounder of the presence of a spouse we verified this by doing a crosstabs in which we found that 87% of male patients had a spouse at home, while only 17% of female patients had a spouse. We also noticed that the coefficient for sex in the equation changed remarkably when correcting for spouse, proving that sex is a confounder of spouse. To determine whether the presence of outside help in patients without a spouse would predict returning to home

we excluded the patients with a spouse and did a logistic regression analysis in patients living alone in a house or apartment. In these patients the presence of outside help was not a predictor for returning home ($p = 0.84$). In conclusion, the only predictor for returning to their home for patients living in a house or apartment was the presence of a spouse, with sex being a strong confounder, as the majority of male patients lived with a spouse while the majority of female patients lived without a spouse.

Patient survival

54 Patients died during follow-up. Mean survival after revision surgery in these patients was 44 months (range 0 – 135, sd 42). 8 Patients (5.5%) died within 90 days of surgery. Figure 3 shows the survival curve of all patients after their latest revision surgery per ASA-category. Differences in patient survival between the ASA-categories were significant for all groups (Log-rank test: $p < 0.01$).

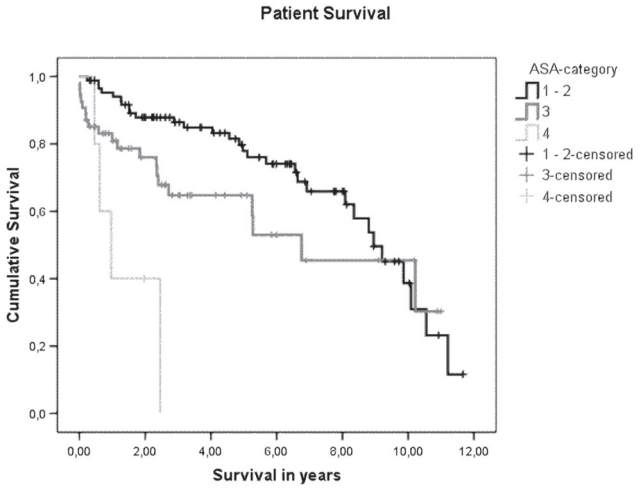
Complications

Table 3 shows the incidence of medical complications during hospital stay per ASA-group. A total number of 239 complications occurred during 183 hospital admittances (mean: 1.3 complications per admittance). Patients in ASA 1 and 2 had 1.0 complication per admittance, patients in ASA 3 had 1.7, and patients in ASA 4 had 2.6 complications per hospital admittance. This difference in number of complications between ASA-groups was statistically significant ($p < 0.01$). In 22% of hospital admittances for revision hips arthroplasty no complications occurred. Table 4 shows the incidence of orthopaedic complications in the first six months after surgery. Orthopaedic complications occurred in 25% of hospital admittances with no statistical difference in total of complications between the ASA-groups ($p = 0.61$). The differences in kind of orthopaedic complications was not statistically significant (Chi-square) between the ASA-groups (dislocations $p = 0.07$; fractures $p = 0.10$)

Walking aids

Table 5 shows pre-operative and post-operative use of walking aids by the patients. For post-operative use of walking aids the most favourable situation was registered. For example, when the patient used a walker after discharge from the hospital and used a cane six months after surgery until the end of follow-up, 'cane' was chosen as the post-operative use of walking aids. The table shows changes in the use of walking aids after revision surgery. In 1 patient the use of walking aids before revision surgery was

Figure 3. Kaplan Meier analysis of patient survival per ASA-category. ASA-1 and ASA-2 are taken together as 1 category. Differences in survival between ASA-categories was statistically significant ($p < 0.01$).



unknown and in 6 patients the post-operative data were missing. In 86 of 176 cases (49%) the use of walking aids remained the same before and after revision surgery, in 39 cases (22%) the situation in use of walking aids had worsened, and in 51 cases (29%) the situation improved.

Discussion

This study describes the social outcome of patients 80 years and older after hospital admittance for revision hip arthroplasty. In total there were 183 hospital admittances in 145 patients. Table 2 shows peri-operative data of the current study. Mean operative time was 2.7 h, mean blood loss was 1.6 litres, and mean length of hospital stay was 34 days. These data resemble the data in previous studies[6,99,114], except for the study by Parvizi which showed a much shorter hospital stay.[92] Patients who had revision of both components had a longer operative time and more blood loss, than patients who had revision of 1 component or only removal of components. Patients who only had removal of components, due to infection of the prosthesis, had a longer hospital stay than patients who had a revision. This can be explained by the fact that in these

Table 3. Incidence of medical complications during hospital stays, per ASA-category and in total. Complications are divided per organ system. The table shows the incidence of the complication, with the percentage in the patient group in brackets.

Medical complications during hospital stay	ASA 1-2 (109 hips)	ASA 3 (67 hips)	ASA 4 (7 hips)	Total (183 hips)
No complications (medical or orthopaedic)	29 (27%)	12 (18%)	0	41 (22%)
Cardiovascular	13 (12%)	20 (30%)	1 (14%)	34 (19%)
Myocardial infarction	3 (2.8%)	2 (3.0%)	0	5 (2.7%)
Congestive cardiac failure	3 (2.8%)	8 (12%)	0	11 (6.0%)
Shock	2 (1.8%)	6 (9.0%)	0	8 (4.4%)
Hypovolemic	1 (0.9%)	4 (6.0%)	0	5 (2.7%)
Septic	1 (0.9%)	2 (3.0%)	0	3 (1.6%)
Hypotension	1 (0.9%)	2 (3.0%)	0	3 (1.6%)
Arrhythmia	2 (1.8%)	1 (1.5%)	1 (14%)	4 (2.2%)
Arterial occlusion in both legs	0	1 (1.5%)	0	1 (0.5%)
Collaps	1 (0.9%)	0	0	1 (0.5%)
Pulmonary embolism	1 (0.9%)	0	0	1 (0.5%)
Renal	8 (7.3%)	14 (21%)	2 (29%)	24 (13%)
Urinary retention	4 (3.7%)	5 (7.5%)	2 (29%)	11 (6.0%)
Urinary tract infection	4 (3.7%)	6 (9.0%)	0	10 (5.5%)
Kidney failure	0	2 (3.0%)	0	2 (1.1%)
Decrease in renal function	0	1 (1.5%)	0	1 (0.5%)
Gastrointestinal	3 (2.8%)	8 (12%)	3 (43%)	14 (7.7%)
Bleeding	1 (0.9%)	2 (3.0%)	0	3 (1.6%)
Severe nausea and vomiting	2 (1.8%)	0	1 (14%)	3 (1.6%)
Anorexia	0	2 (3.0%)	0	2 (1.1%)
Pancreatitis	0	2 (3.0%)	0	2 (1.1%)
Diarrhea	0	1 (1.5%)	2 (29%)	3 (1.6%)
Ileus	0	1 (1.5%)	0	1 (0.5%)
Pulmonary	2 (1.8%)	2 (3.0%)	0	4 (2.2%)
Pneumonia	1 (0.9%)	2 (3.0%)	0	3 (1.6%)
Exacerbation of bronchitis	1 (0.9%)	0	0	1 (0.5%)
Neurological	30 (28%)	23 (34%)	5 (71%)	58 (32%)
Delirium	25 (23%)	19 (28%)	4 (57%)	48 (26%)
Foot drop	3 (2.8%)	1 (1.5%)	0	4 (2.2%)
Femoral neuropathy	1 (0.9%)	1 (1.5%)	0	2 (1.1%)
Transient ischemic attack (TIA)	0	1 (1.5%)	1 (14%)	2 (1.1%)
Cerebrovascular accident (CVA)	0	1 (1.5%)	0	1 (0.5%)
Carpal tunnel syndrome (CTS)	1 (0.9%)	0	0	1 (0.5%)

Table 3. *Continued*

Medical complications during hospital stay	ASA 1-2 (109 hips)	ASA 3 (67 hips)	ASA 4 (7 hips)	Total (183 hips)
Other	53 (49%)	45 (67%)	7 (100%)	105 (57%)
Pressure sore	50 (46%)	35 (52%)	5 (71%)	90 (50%)
Death	0	4 (6.0%)	0	4 (2.2%)
Hyponatremia	1 (0.9%)	2 (3.0%)	1 (14%)	4 (2.2%)
Vaginal infection	0	1 (1.5%)	1 (14%)	2 (1.1%)
Hypokalemia	0	1 (1.5%)	0	1 (0.5%)
Osteomyelitis of MT5	1 (0.9%)	0	0	1 (0.5%)
Polyarthritis	1 (0.9%)	0	0	1 (0.5%)
Allergic reaction to antibiotics	0	1 (1.5%)	0	1 (0.5%)
Tung necrosis	0	1 (1.5%)	0	1 (0.5%)
Total	**109 (100%)**	**112 (167%)**	**18 (257%)**	**239 (131%)**

Table 4. Incidence of orthopedic complications within the first six months after surgery, per ASA-category and in total. The table shows the incidence of the complication, with the percentage in the patient group in brackets.

Orthopaedic complications within six months	ASA 1-2 (109 hips)	ASA 3 (67 hips)	ASA 4 (7 hips)	Total (183 hips)
No complications (orthopaedic)	82 (75%)	51 (75%)	4 (57%)	137 (75%)
Peri-prosthetic fracture	5 (4.6%)	9 (13%)	1 (14%)	15 (8.2%)
Wrong head-size	0	1 (1.5%)	0	1 (0.5%)
Dislocation	17 (16%)	4 (6.0%)	2 (29%)	23 (13%)
Infection	3 (2.8%)	3 (4.5%)	0	6 (3.3%)
Superficial	2 (1.8%)	0	0	2 (1.1%)
Deep	1 (0.9%)	3 (4.5%)	0	4 (2.2%)
Arterial bleeding (embolisation needed)	1 (0.9%)	0	0	1 (0.5%)
Total	**27 (25%)**	**17 (25%)**	**3 (43%)**	**47 (26%)**

cases repeated surgery for debridement of the infected area was performed, weight bearing was usually not allowed and even impossible due to leg length discrepancy. Furthermore, most of these patients had bed-rest with their leg in traction to prevent shortening of the leg. In our study neither blood loss, nor operation time correlated with social outcome.

In the current study four patients died during hospital stay. In the remaining of hospital admittances 138 times (75%) the patient could return to their previous housing

Table 5. Pre- and postoperative use of walking aids by the patients. The numbers in the light gray cells represent no change in use of walking aids. The numbers in darker gray cells represent worsening in the use of walking aids and the numbers in white cells represent improvement in use of walking aids.

	Support post-operatively									
Support preoperatively	None	Cane	crutch	2 crutches	Walker	Wheel-chair	bedridden	Died during hospital stay	Unknown	Total
None	2	2	0	1	1	1	0	0	0	7
Cane	2	14	3	3	9	1	0	1	1	34
Crutch	2	2	1	1	1	0	0	0	0	7
2 Crutches	1	10	5	6	6	1	0	1	1	31
Walker	0	5	2	5	48	6	0	1	4	71
Wheelchair	1	2	1	0	10	15	0	0	0	29
Bedridden	0	1	1	0	0	1	0	0	0	3
Unknown	0	0	0	0	0	1	0	0	0	1
Total	8	36	13	16	75	26	0	3	6	183

situation, in 22 cases (12%) there was a worsening in the housing situation, and in 7 cases (4%) there was an improvement. In 12 cases the post-operative situation was unknown. Strehle *et al*. [114] also compared pre-operative and ultimate post-operative situations. They reported that 80% of patients could return to their original environment eventually and 20% (all patients pre-operatively living in a house or apartment) had to move to a home for the elderly or nursing home. Ballard *et al*. [6] reported that of 27 octogenarians 11 went to a nursing care institution after discharge for revision hip arthroplasty, none of them could return home after rehabilitation.

The only predictor for returning home in patients living in a house or apartment in the current study was the presence of a spouse, with gender being a strong confounder as most of the male patients lived with a spouse and only a minority of female patients did. Strehle *et al*. [114] also compared social situation in patients living with and without a spouse. They found that 95% of patients living with a spouse could return to their homes compared to 70% of patients living without a spouse. No statistical testing was reported.

Several studies reported where patients went after discharge from the hospital, which was not always the ultimate situation. In the current study 59% of patients living in a house or apartment pre-operatively went to a nursing home or home for the elderly after discharge from the hospital for rehabilitation. The majority of these patients could return to their own homes after a mean rehabilitation period of 106 days.

In the current study, 8 patients (5.5%) died within 90 days of surgery. This corresponds with the findings by Mahomed *et al*. [77] who studied survival of octogenarians after revision hip arthroplasty and found that 168 of 3165 patients (5.3%) died within 90 days of surgery and with the findings of Parvizi *et al*., [92] who had 7 deaths in 159 patients (4.4%) within 90 days.

Patient survival differed significantly between ASA-groups, with all the patients in ASA-4 having died within two and a half years after surgery and the biggest differences in survival between patients in ASA-2 and ASA-3 occurring in the first six months post-operatively. Prause *et al*. [98] showed in a large study of over 16,000 patients that ASA-category is a good predictor for peri-operative mortality.

In our study there were 239 medical complications in 183 hospital admittances (1.3 complications per case), with 1.0 complication per hospital admittance in patients in ASA-1 and ASA-2, 1.7 in ASA-3 and 2.6 in ASA-4. There was no difference in the number of complications observed between various kinds (i.e. total hip versus partial components) of revision surgery. Delirium (26% of cases) and pressure sore grade 2 or more (50%) were the most common complications, followed by urinary retention, urinary tract infection and congestive cardiac failure (all in 6% of cases). In a study

on primary THP and TKP in patients 89 years and older all patients had a delirium post-operatively.[10] Ballard *et al*. [6] found 1.5 medical complications per patient (41 in 27 patients), with urinary retention as the most common (56% of patients). Raut *et al*.[99] found 42 complications in 56 patients (0.8 complications per patient), with major complications only occurring in patients with ASA-3. They observed a correlation between seriousness of complications and ASA-category. Brander *et al*. [16] studied primary hip and knee arthroplasty in octogenarians compared to patients aged 65-80 years. They concluded that the number of comorbidities did not correlate with the occurrence of complications. However, patients 80 years and older who had no comorbidities had a lower chance to have complications. Parvizi *et al*. [92] studied 170 hips in 159 patients aged 80 or older undergoing revision hip arthroplasty and compared them to a gender-matched control group of patients aged less than 70 years. They found a mean complication rate of 0.3 complications per patient, with arrhythmia as the most common (3.5%). Leung *et al*. [71] found ASA-classification to be a predictor for complications in a multivariate analysis (besides emergency surgery and pre-operative tachycardia. Compared to previous studies our study shows a high percentage of delirium and pressure sores. Probably, these complications can be prevented. Pressure sores can sometimes be prevented by using a special mattress. In the two hospitals where the study was done these special mattresses were only used when pressure sores were already present (as a hospital policy), but because of the high occurrence of pressure sores in these octogenarians with a relatively long period of bed rest it would be worthwhile to use an anti-pressure sore mattress as a preventive measure instead of a treatment. Delirium can sometimes be prevented by keeping the patient in an as best as possible condition. In a randomised controlled trial by Marcantonio *et al.*, [80] the occurrence of delirium in patients after orthopaedic surgery could be reduced significantly by proactive geriatrics consultation. A geriatrician made daily visits and made targeted recommendations based on a structured protocol. Introducing these measures will probably reduce the occurrence and seriousness of delirium.

In the current study orthopaedic complications occurred in 25% of cases. 13% of hips had one or more dislocations post-operatively within 6 months, 8.2% had peri-prosthetic fractures (mostly per-operatively), and 2.2% had a deep infection. A large study of complications after revision hip arthroplasty within 6 months in almost 13,000 patients of all ages showed 1.1% infections and 14% dislocations.[94] In Ballard's study 4 of 27 octogenarians (15%) had dislocations post-operatively and there were no infections.[6] Raut *et al*.[99] found dislocations in 4 (7%) patients and infections in 2 (4%). There were no periprosthetic fractures. In the study by Parvizi *et al*. [92] 1.8% periprosthetic infections occurred in the octogenarians group, which was the same amount for the con-

trol group of patients aged 70 years and younger. Fractures occurred in 7.6% of elderly patients compared to 1.8% in the younger group ($p = 0.006$), and this difference was attributed to a lower bone stock in elderly patients, predisposing to fractures. On the other hand dislocations occurred more in the younger patient group (9.4%, compared to 2.4% in the elderly patients) ($p = 0.01$), and this was explained by the fact that for the elderly patients more constrained liners were used. In our study there seemed to be a difference in occurrence of the kind of complications between the ASA-groups, but this was not statistically significant. There were 16 hips (16%) with dislocations in the ASA-2 group and 4 (6%) in the ASA-3 group ($p = 0.07$). In contradistinction to Parvizi's study the same liners were used in all ASA-categories. We explain the higher percentage of dislocations in the ASA-2 group by the supposition that these people are usually more active in physical activities. In our study there were 5 hips (4.6%) with periprosthetic fractures in the ASA-2 group and 9 (13%) in the ASA-3 group ($p = 0.10$). We agree with Parvizi that peri-prosthetic fractures are more likely to occur in patients with a poor bone-quality and these patients are probably more represented in the higher ASA-groups. Probably, a larger group of patients would have given significant differences in the kind of complications in our study.

In the current study the use of walking aids was reported pre- and postoperatively. In 49% of cases the use of walking aids remained the same before and after revision surgery, in 22% the situation in use of walking aids had worsened, and in 29% the situation improved. This implicates that there may be a slight improvement in use of walking aids after revision surgery. No other study reported on difference in use of walking aids before and after revision hip arthroplasty in octogenarians.

Conclusion

Revision hip arthroplasty can be a heavy burden in octogenarians. This study reports the social outcome of 183 hospital admittances for revision hip arthroplasty in 145 patients over 80 years. Some of the very common complications occurring in this study (pressure sores and delirium) can probably be minimised by preventive measures such as special mattresses and early consultation of a geriatrician.

Patients with higher ASA-categories had a higher number of complications. However, this had no influence on whether the patient could be discharged to his or her own home or that the patient had to be discharged to a home for the elderly or nursing home for rehabilitation or definitive stay. The only predictor for returning home was the presence of a spouse at home with gender being a strong confounder.

3

Towards gene therapy in prosthesis loosening: Efficient killing of interface cells by Gene-Directed Enzyme Prodrug Therapy with nitroreductase and the prodrug CB1954

Jolanda J. de Poorter[1]
Tanja C.A. Tolboom[2]
Martijn J.W.E. Rabelink[3]
Elsbet Pieterman[2]
Rob C. Hoeben[3]
Rob G.H.H. Nelissen[1]
Tom W.J. Huizinga[2]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

Journal of Gene Medicine, 2005; 7: 1421-8

Abstract

Background

Loosening is a major complication in prosthesis surgery. To stabilise loosened orthopaedic implants, the interface tissue surrounding the implant must be removed. As an alternative to manual removal, we explored the possibility of removing the tissue by gene-directed enzyme prodrug therapy. In the current study we investigated whether interface cells can be transduced by an HAdV-5 vector carrying the *E.coli*-derived nitroreductase gene and sensitised to the prodrug CB1954.

Methods

The gene transfer efficiency into cultures of diploid human interface cells was tested by exposing these cells to various concentrations of Ad.CMV.LacZ. Subsequently, we studied the susceptibility of cells to the Ntr/CB1954 combination.

Results

X-gal staining of the Ad.CMV.LacZ-transduced cell cultures revealed that at 200 plaque forming units (pfu)/cell, 74% of the cells expressed the LacZ gene. Infection with an Ntr construct in interface cell lines resulted in a 60-fold sensitisation to the prodrug CB1954. In addition we observed that iotrolan (Isovist) contrast medium had no effect on viability of the cells. However, the presence of the contrast medium completely inhibited adenovirus-mediated gene transfer.

Conclusions

From these data we conclude that HAdV-5-based vectors carrying nitroreductase can be used to sensitise interface tissue. Instead of contrast medium the clinical protocol will use an alternative visualisation procedure.

Introduction

Approximately 1 million total hip replacement operations are carried out worldwide annually for degenerative joint disease, mostly osteoarthritis and rheumatoid arthritis. Of these prostheses 7-13% will have loosening within 10 years, causing pain and difficulty in walking.[54,78,112] The current treatment for prosthesis loosening is revision surgery, which has a high mortality and morbidity rate, especially in elderly patients with comorbidity.[114] For these patients revision surgery is not an option and there remains a need for effective treatment of implant loosening in this patient population.

Loosening of orthopaedic implants is for the greater part caused by an inflammatory reaction to wear particles (mostly polyethylene).[5] The inflammatory reaction causes a periprosthetic tissue to be formed, consisting of fibroblasts and macrophages, which is called interface tissue.[61,62] Before a loosened prosthesis can be refixed, the interface tissue and the prosthesis need to be removed; thereafter a new prosthesis is implanted. This revision surgery can often be extensive (3-8 h surgery), due to the necessity of removing all interface tissue and the prosthesis, leading to high morbidity rates. Alternatively, the interface tissue could be removed by introduction of a toxic component.

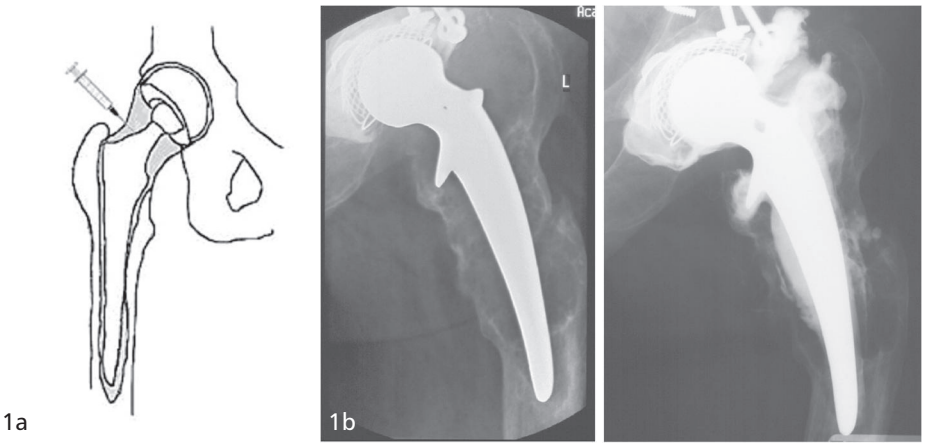
In the current study we tested whether interface cells can be sensitised by nitroreductase (Ntr) to the prodrug CB1954. The prodrug CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide) is a weak monofunctional alkylating agent, which is converted by the *Escherichia coli* enzyme Ntr to a cytotoxic derivative.[68] Cells containing Ntr convert CB1954 into a bifunctional alkylating agent which is capable of forming DNA interstrand cross links, resulting in apoptosis or cell death.[18,34]

Since there is no human homologue to Ntr[23], only cells expressing the nitroreductase gene are killed when exposed to CB1954. In the present study the therapeutic window for interface cells is determined to find out which CB1954 concentrations are safe to use in interface tissue, preventing killing of non-transduced cells.

To refix a loosened joint prosthesis, the interface cells in the periprosthetic space need to be eradicated. The interface cells are located in the joint space, which is a closed compartment. This has the advantage that with a high local concentration of vector the systemic exposure may be minimal (Figure 1a).

Contrast medium may be used to visualise the cavity by radiological images. To assure that the vector is administered in the joint space, the position of the needle in the joint space can be monitored by injecting a small amount of contrast medium into the cavity, while making fluoroscopic images (Figure 1b). Before the contrast medium can be used in a clinical study together with CTL102 and CB1954, its effect on the efficiency of transduction and killing should be tested.

Figure 1. Schematic representation of a hip joint with a loosened prosthesis (a). Arthrogram of a hip joint with a loosened prosthesis (b).



(a) The gray area indicates the joint space, which is continuous with the periprosthetic space. When injecting a fluid into the joint space, this will spread through the area that is marked gray in the image.
(b) The contrast medium is injected into the joint space under fluoroscopic guidance. The picture shows that a part of the area around the prosthesis (periprosthetic space) is filled with contrast medium. This proves that the prosthesis is loose in that area.

The effect of contrast medium on fibroblastic cells has been studied previously.[69,131] In various studies it was shown that exposure of cells to low-osmolarity contrast media has no significant influence on cell proliferation and apoptosis. These studies show that exposure of the interface cells to contrast media with low osmolarity will probably have no effect on the cells themselves. However, the influence of contrast medium on viral transduction of cells by a HAdV-5-vector has not been reported. In this study the influence of low-osmolarity contrast medium on adenoviral transduction is tested.

Materials and Methods

Adenoviral vector

The adenoviral vector CTL102 was constructed by homologous recombination in PER.C6 helper cells,[38,114] as described in previous studies.[34] CTL102 carries the *E. coli* Ntr gene under control of the cytomegalovirus (CMV) promoter/enhancer. The complete Ntr expression cassette is cloned into an E1-deleted HAdV-5 adenovirus transfer vector. The Ad.CMV.LacZ[122] vector is identical to CTL102, but the *E.coli* lacZ gene replaces the

Ntr gene. In the rAd5F35.CMV.LacZ vector the shaft and knob of the HAdV-5 fiber were replaced by the homologous regions of the HAdV-35 fiber.[100]

Interface tissue samples

For all experiments described, interface cells were used. Interface tissue was removed from the periprosthetic space during revision surgery by an orthopaedic surgeon and collected in sterile phosphate-buffered saline (PBS). Connective tissue and fat were removed thoroughly and the interface tissue was digested for at least 2 h at 37°C using collagenase 1A (1 mg/ml; Sigma, St Louis, MO, USA). Cells were then harvested by filtering the tissue/collagenase substance through a 200 μm filter (NPBI, Emmer-Compascuum, The Netherlands). The cells were cultured in 75 cm^2 flasks (Cellstar, Greiner, Alphen aan de Rijn, The Netherlands) with Iscove's modified Dulbecco's medium (IMDM; Biowitthaker, Verviers, Belgium), supplemented with glutamax (GibcoBRL, Paisley, UK), penicillin and streptomycin (Boehringer Mannheim, Germany), and 10% fetal calf serum (FCS; GibcoBRL) at 37°C and 5% CO_2.

Before each experiment interface cells were detached from the flasks using 0.25% trypsin (GibcoBRL). The cells were counted in a Bürker counter and death cells were excluded by trypan blue. Cells were seeded in a 96-well plate (flat bottomed) at a density of 5000 cells/ well. Cells were incubated overnight to allow attachment to the bottom. Before each experiment the wells were washed twice with IMDM. For the experiments passage 2 to 4 interface cells were used. Light microscopy indicated that more than 95% of the cells were interface cells.

The human hepatoma cell line HepG2[67] was used as a control for experiments with contrast medium and sodium iodide (NaI).

Potential of cell-killing by Ntr/CB1954 GDEPT (Gene-Directed Enzyme Prodrug Therapy)

To investigate cell-killing potential, interface cells were infected by CTL102 (Cobra Biomanufacturing plc, Keele, UK) in different concentrations (0, 25, 100, and 200 plaque forming units (pfu)/cell) in IMDM/10% FCS for 24 h, washed twice with IMDM, and then incubated with the prodrug CB1954 (Oxford Asymmetry Ltd, Abingdon, UK) in different concentrations (0, 25, 50, and 100 μM) in IMDM/10% FCS for 24 h. The cells were washed again and then cultured in IMDM/10% FCS for 1 day, after which the cell viability was measured using cell proliferation reagent WST-1 (Roche, Mannheim, Germany) according to manufacturer's instructions with a 2 h incubation period .

To study the infectivity of interface cells by HAdV-5, interface cells were infected with Ad.CMV.LacZ vector (in concentrations of 0, 25, 50, 100, 200, 400 pfu/cell). Twen-

ty-four hours post-infection the cells were washed twice with IMDM, and cultured for 2 days. Medium was refreshed each day. On day 3, the monolayer cultures were washed twice with PBS and fixed with 0.2% glutaraldehyde and 2% formaldehyde in PBS for 10 minutes at 4°C. Subsequently, cells were washed once with PBS and stained for β-galactosidase activity in 50 μl of reaction mix (1 mg/ml X-gal (Eurogentec, Seraing, Belgium), 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$ in PBS) for 2 h at 37 °C. The percentage of transduced cells was assessed by counting at least 100 interface cells, using light microscopy. All conditions were tested in duplicate.

CB1954 toxicity

To assess CB1954 toxicity in interface cells that do not express nitroreductase, interface cells were exposed to CB1954 in various concentrations (0-4000 μM) in IMDM/10% FCS for 24 h, in duplicate. Cells were then washed twice with 100 μl IMDM, and cultured in IMDM/10% FCS for a further 2 days. On the third day, cell viability was measured using cell proliferation reagent WST-1 (Roche) according to manufacturer's instructions with a 2 h incubation period.

Effect of contrast medium on interface cells

Interface cells and HepG2 cells were seeded in 96-well plates. Into each well 50 μl of IMDM/ 20% FCS and 50 μl of a solution containing contrast medium and 0.9% NaCl in various concentrations (0, 12.5, 25, and 50% contrast medium) were added. The contrast media used were the low-osmolarity, non-ionic dimer iotrolan (Isovist; Schering, Berlin, Germany) with an iodide concentration of 300 mg/mL and the low-osmolarity, ionic dimer ioxaglate meglumine – ioxaglate sodium (Hexabrix, Guerbet, Aulnay-sous-Bois, France) with an iodide concentration of 320 mg/mL. As a control, sodium-iodide (NaI) was used in concentrations of 0 to 150 mM. After 4 h exposure to the contrast medium, the cells were washed twice and incubated in IMDM/10% FCS. The cells were cultured for a further 2 days, changing the culture medium every day. On day 4, cell viability was determined with the WST-1 cell viability assay kit (Roche) according to the manufacturer's protocol.

Effect of contrast medium on HAdV-5 transduction of interface cells

Interface cells and HepG2 cells were seeded in 96-well plates. After overnight incubation cells were infected with Ad.CMV.LacZ (concentrations of 0, 25, 100, and 200 pfu/

cell) in IMDM/ 20% FCS, 25 μl per well. Then 25 μl of iotrolan (Isovist) or ioxaglate meglumine – ioxaglate sodium (Hexabrix) in 0.9% NaCl were added in concentrations of 0, 25, 50, and 100%. (When diluted in the culture medium these concentrations decreased to 0, 12.5, 25, and 50%.). As a control for iodide activity, NaI was added in concentrations of 0 to 150 mM (0, 1, 2.5, 5, 10, 25, 50, 100 and 150 mM). Four hours after infection, the cells were washed twice with IMDM and incubated for the rest of the day in IMDM/10% FCS at 37°C and 5% CO_2. The Ad.CMV.LacZ transduced cells were cultured for 2 days after removal of the vector and contrast medium. Subsequently, the cells were fixed and stained for β-gal activity. The transduction rate was assessed as described above.

To investigate the transient effect of contrast medium on transduction of interface cells, cells were first exposed to iotrolan for different periods of time. Subsequently, the contrast medium was removed, the cells were washed twice, and the vector CTL102 was added. The interval between removal of the contrast medium and addition of the vector was varied. Twenty-four hours post-infection cells were washed twice and CB1954 was added. The medium with CB1954 was removed after 24 h, and replaced with fresh medium without CB1954. Cell viability was determined after addition of the fresh medium.

Statistical analysis

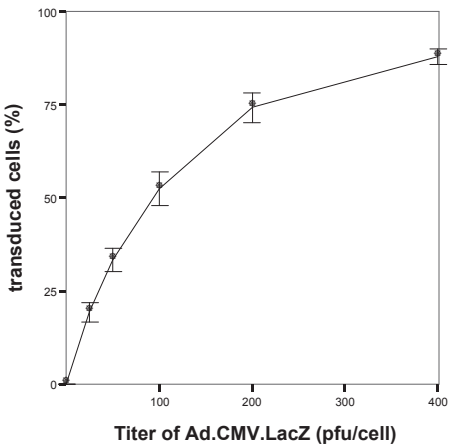
A univariate analysis of variance and Spearman's correlation was used to study the interaction between vector and prodrug and between vector and contrast medium and to study the effect of CB1954 on viability of the cells. A Mann-Whitney test for independent groups was performed to determine the difference in cell killing between the cells that were exposed to contrast medium and the non-exposed cells. In the experiment to study the effect of transient exposure to contrast medium on transduction of HAdV-5-vector Spearman's correlation between contact time and viability and between delay time and viability was tested. For all statistical analyses $p < 0.05$ was the level of statistical significance.

Results

Transduction and killing of interface cells by HAdV-5 vectors

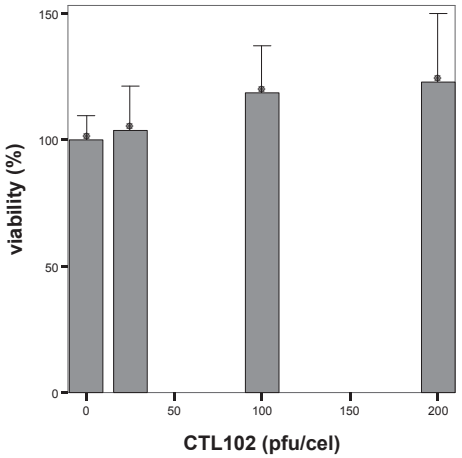
To test the susceptibility of interface cells to HAdV-5 vectors, primary cultures of interface cells were exposed to the HAdV-5 vector Ad.CMV.LacZ. Twenty-four hours post-infection the cells were stained with X-gal solution for β-galactosidase reporter

Figure 2. Transduction of interface cells by Ad.CMV.LacZ in six concentrations (0, 25, 50, 100, 200 and 400 pfu/ cell).



After 3 days, cells were fixed and stained with X-gal reaction mix. The percentage of transduced (blue) cells was counted. The figure shows the means and standard errors of 12 independent experiments

Figure 3. Toxicity of adenoviral vector CTL102 in interface cells.



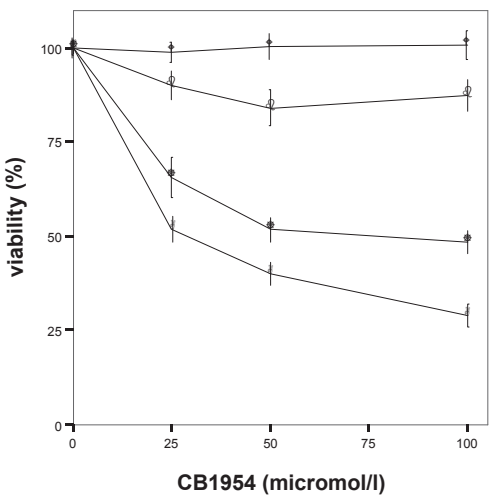
Cells were infected with CTL102 in concentrations of 0, 50, 100, and 200 pfu/ cell, without subsequent addition of prodrug. After 3 days cell viability was measured. The figure shows the means and standard deviations of 12 independent experiments.

gene expression. The transduction efficiency increased with increasing vector concentration. At 400 pfu/cell the percentage of cells expressing the reporter gene was 88% (standard deviation (sd) 4.0) (Figure 2). Thus HAdV-5 vectors can transduce interface cells.

To test whether the cells can be sensitised to the prodrug CB1954, interface cells were exposed to increasing concentrations of the HAdV-5 vector CTL102, which carries the *E.coli*-derived Ntr gene. After transduction, the cells were exposed to CB1954 and cell viability was assessed with the WST-1 viability assay kit. Cells that were transduced with the vector CTL102 without addition of prodrug showed a significant enhancement of cell viability with increasing vector concentration ($p < 0.001$; Figure 3).

In the calculations for the effect of CB1954 we corrected for this effect of CTL102 on viability. The level of sensitisation to CB1954 increased with increasing vector titer. At 200 pfu/cell the mean IC_{50} value was 25 μM (Figure 4). There is a significant correlation (Spearman) between the viability of the cells and the concentration of CTL102 (corr. –0.444, $p < 0.001$) and CB1954 (corr. –0.445, $p < 0.001$; Figure 4). CTL102 and CB1954 showed interaction in their effect on viability ($p < 0.001$). These experiments show that interface cells can be killed using the Ntr/CB1954 approach.

Figure 4. Viability of interface cells after GDEPT using CTL102 and CB1954.



Cells were transduced with CTL102 and, after 24 h, CB1954 was added. Viability of interface cells was determined using WST-1 reagent. Cells were transduced with four concentrations of CTL102: (●) 0 pfu/ cell; (×) 25 pfu/ cell; (■) 100 pfu/ cell; (○) 200 pfu/ cell. (means and standard error of 12 independent experiments)

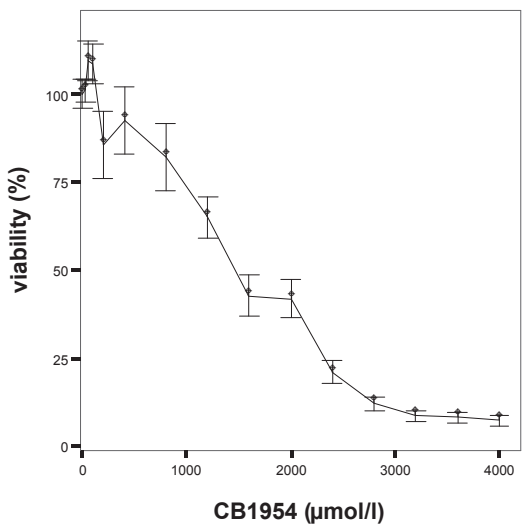
CB1954 toxicity

The toxicity of the prodrug for untransduced cells was tested by a dose ranging study. Interface cells were exposed to various concentrations of CB1954 and their viability was evaluated by WST-1 cell viability assay. In this assay, the absorbance was converted into viability by setting the absorbance of the wells containing interface cells without CB1954 as 100%, and the negative controls, in which no cells were present, as 0% viability. In these assays, the absorbance first increased at low concentrations of CB1954, reaching a maximum at 50-100 μM CB1954 (Figure 5). At higher concentrations, absorbance gradually decreased to a minimum of 0.211 (sd 0.011), corresponding to a cell viability of around 8.2% (sd 5.4). At a CB1954 concentration of 1500 μM 50% of the non-transduced cells were killed. The effect of CB1954 on cell viability was statistically significant ($p < 0.001$). In CTL102-transduced cells a CB1954 concentration of 25 μM revealed killing of 50% of the cells. Transduction of interface cells results in a 60-fold sensitisation to the prodrug CB1954.

Effect of contrast medium on interface cells

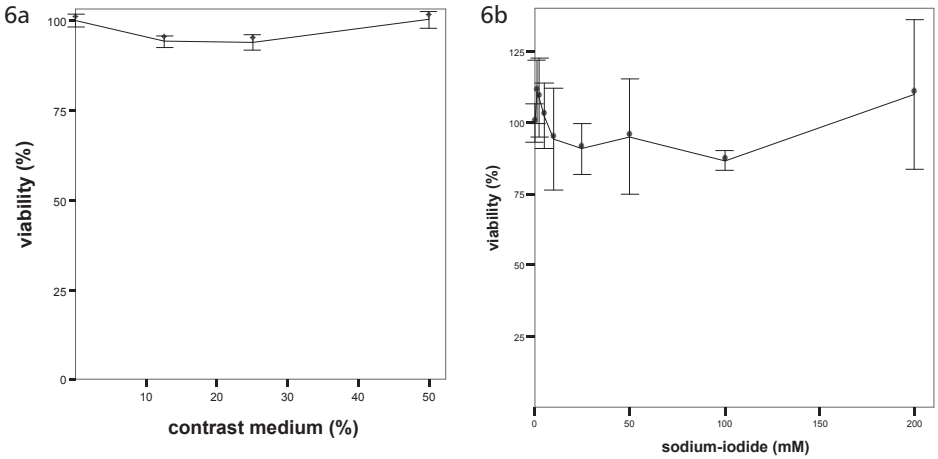
The toxicity of contrast medium (iotrolan) on interface cells was evaluated (Figure 6a). Iotrolan does not affect the viability of the cells at any concentration ($p = 0.563$). Ad-

Figure 5. Toxicity of CB1954 on interface cells.



Cells were exposed to a range of CB1954 concentrations for 24 h. Cell viability was measured using WST-1 reagent. (n=14)

Figure 6. Toxicity of (a) iotrolan (Isovist) contrast medium and (b) sodium iodide on interface cells.



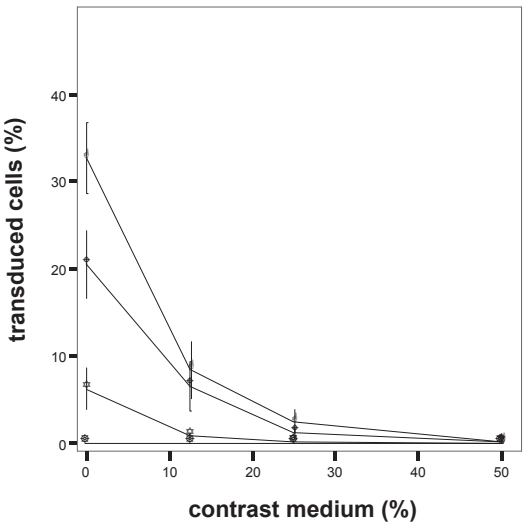
(a) Interface cells were exposed to contrast medium (iotrolan) for 4 h. After 3 days of cell culturing viability of the cells was measured. (n=12)
(b) Toxicity of sodium iodide (NaI) on interface cells and HepG2 cells. Cells were exposed to NaI for 4 h. Viability was tested after 3 days. (n=6)

dition of contrast medium to the interface cells for 4 h did not lead to killing of the cells. As a control for toxicity of possible iodide release the previous experiment was repeated with NaI instead of iotrolan (Figure 6b). There was no effect of NaI on viability of the cells ($p = 0.903$).

Effect of contrast medium on HAdV-5 transduction of interface cells

The effect of contrast medium (iotrolan) on HAdV-5 transduction of interface cells was investigated with Ad.CMV.LacZ. Infectivity of the cells increases with the concentration of HAdV-5 vector. However, the contrast medium has a restraining influence on the transduction efficiency. With higher concentrations of iotrolan, the HAdV-5 vector concentration has less effect on gene transfer efficiency. At a contrast medium concentration of 50% none of the cells were transduced (Figure 7). Between patients (n=6), interindividual differences were observed. The effect of iotrolan on the transduction is statistically significant ($p < 0.001$). Hexabrix was tested simultaneously with Isovist in two interface cell lines, and in HepG2 cells for both an Ad.CMV.LacZ and an rAd5F35.CMV.LacZ vector. Although the rAd5F35.CMV.LacZ vector was much more efficient, the results for Hexabrix and Isovist were similar for both viruses.

Figure 7. Effect of iotrolan on HAdV-5 transduction of interface cells.

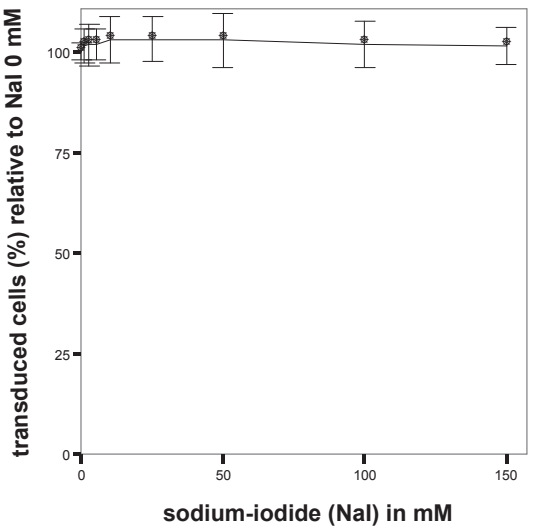


Cells were exposed to a combination of different concentrations of Ad.CMV.LacZ and contrast medium for 4 h and then washed and cultured in IMDM/10% FCS. After 3 days the cells were fixed and stained with X-gal. Percentage of transduced cells was determined by counting blue cells. Ad.CMV.LacZ used: (■) 0 pfu/cell; (▼) 25 pfu/cell; (●) 100 pfu/cell; (□) 200 pfu/cell. (n=12)

To investigate whether the effect of contrast medium on cell transduction by HAdV-5 vectors was caused by free iodide, NaI was used as a control. Sodium iodide had no effect on transduction by HAdV-5 vectors (Figure 8).

In summary, the results from these experiments suggest that iodide-containing contrast medium cannot be used simultaneously with the currently used HAdV-5 vectors. Therefore, the influence of transient exposure to contrast medium on the transduction of interface cells was investigated. Interface cells were exposed to contrast medium for 0 to 120 minutes and the period between washing away of the contrast medium and performing the Ntr/CB1954 cell killing approach was varied. Cell killing was not correlated with contact time (corr –0.033, $p = 0.691$) or length of the period between washing away of the contrast medium and addition of the vector (corr –0.004, $p = 0.962$). After removal of the contrast medium no influence on cell killing by Ntr/CB1954 was observed.

Figure 8. Effect of sodium-iodide (NaI) on transduction of two interface cell lines and HepG2 cells.



Cells were exposed to adenoviral vector and various concentrations of NaI in NaCl for 4 h. Cells were cultured for 3 days and fixed and stained with X-gal. Transduction rates are relative to percentage of transduced cells with NaI concentration of 0 mM. Ad.CMV.LacZ as well as rAd5F35.CMV.LacZ were used to infect the cells

Discussion

This study demonstrates that interface cells can be transduced by an HAdV-5 vector and killed by the Ntr/CB1954 approach.

Human adenovirus 5 is capable of infecting a broad range of dividing and non-dividing human cells including fibroblasts and macrophages.[34] To our knowledge peri-prosthetic interface cells have not been used previously as target cells for gene therapy. As interface tissue has the histological and histochemical characteristics of synovial tissue,[43] results of studies with synovial tissue could be an indicator of outcome in similar studies with interface tissue. The results of the current study are consistent with previous studies that have shown that synovial cells can be successfully transduced by HAdV-5 vectors.[45,87]

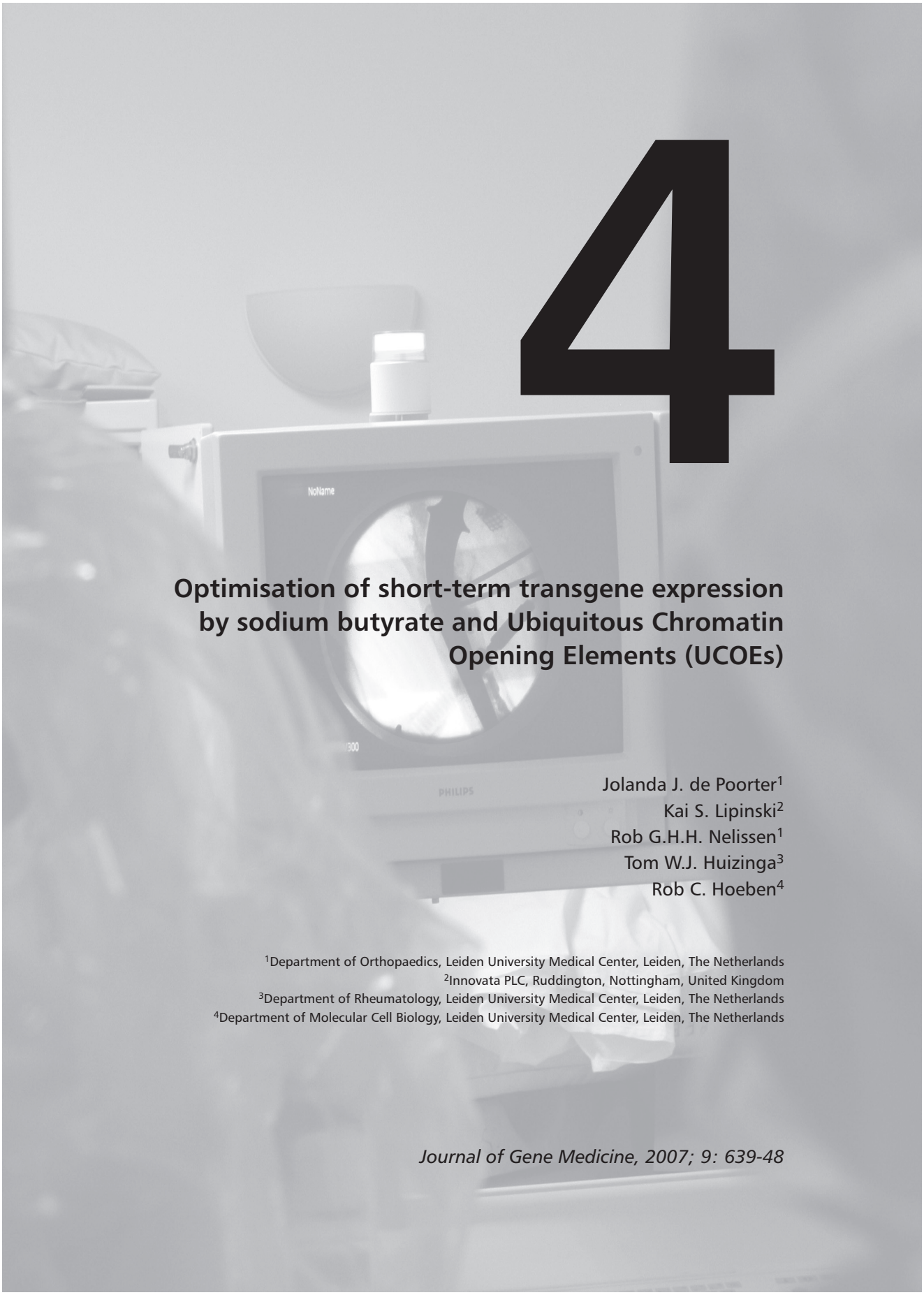
Infection of interface cells with the vector CTL102 without subsequent addition of prodrug leads to an increase in cell viability. This anti-apoptotic effect has been described previously in lung epithelial cells.[39] As the cell viability increases with vector concentrations we conclude that the concentrations used in this experiment are not toxic for the interface cells.

Killing of cells by Gene-directed Enzyme Prodrug Therapy (GDEPT) has been studied before in various cell lines, using various approaches. The Ntr/CB1954 approach is attractive for clinical evaluation for several reasons: (1) it generates a toxic agent that can kill both dividing and non-dividing cells; (2) induction of cell death occurs by a p53-independent mechanism; and (3) CB1954 is well tolerated in humans.[34] Cell killing by the Ntr/CB1954 approach has been proved effective in a variety of human cancer cells,[13,23,48,83,109,125,127] but has not been studied in synovial or interface cells. The current study shows that interface cells can be effectively killed by the Ntr/CB1954 approach. The doses used are assumed to represent concentrations that can be achieved in a clinical study.

For the current study passage 2 to 4 interface cells were used. These passages were used to maximally reduce culture artifacts. On the one hand, in very low passages (0 and 1) there is a risk of presence of contaminating cells (especially macrophages), which decreases with higher passages. On the other hand, at higher passages, the risk of substantial *in vitro* alteration/ growth selection exists (especially at passages higher than 4).[132] In the current study, cultured interface cells of different patients were used. For the interpretation of the results the data of all patients were pooled. However, it must be noted that interindividual differences in infectivity were observed.

In this study the influence of contrast medium on transduction by an adenoviral vector was investigated in view of future clinical studies. Results show that the contrast medium does not seem to have any influence on viability of the interface cells. However, transduction of the cells by an adenoviral vector, in the presence of contrast medium, is almost negligible. The vector is assumed to be inactivated by the contrast medium. This effect was seen in an ionic (Hexabrix) and a non-ionic (Isovist) low-osmolarity contrast medium . The mechanism of transduction inactivation by iotrolan (Isovist) and ioxaglate meglumine – ioxaglate sodium (Hexabrix) remains unclear. The effect is not caused by a change of the cells themselves as transient exposure to contrast medium does not lead to inactivation of Ad.CMV.LacZ. The effect is independent of receptor since rAd5F35, an adenovirus that binds CD46 as receptor, is inhibited by Isovist and Hexabrix as well. The inactivation is not caused by iodide itself as exposure of the cells and vector to NaI did not lead to decrease in transduction efficacy. In a putative clinical study the viral vector will be injected in the joint space. Normally, contrast medium is used to verify the position of the needle in the joint. The results of this study however show that the use of contrast medium in combination with a viral vector is dissuaded. Thus, for a clinical study, we propose that alternative methods for the visualisation of the needle should be employed such as injection of air to create an "air-arthrogram".

In conclusion, the results of this study show that interface cells can be killed by the Ntr/CB1954 enzyme prodrug approach. These data are essential as preclinical work for the starting of a clinical study to kill interface tissue *in vivo*. In such a study, the currently employed contrast media cannot be used in one solution with an HAdV-5 vector given the effect on the transduction of cells.



4

Optimisation of short-term transgene expression by sodium butyrate and Ubiquitous Chromatin Opening Elements (UCOEs)

Jolanda J. de Poorter[1]
Kai S. Lipinski[2]
Rob G.H.H. Nelissen[1]
Tom W.J. Huizinga[3]
Rob C. Hoeben[4]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Innovata PLC, Ruddington, Nottingham, United Kingdom
[3]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[4]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

Journal of Gene Medicine, 2007; 9: 639-48

Abstract

Background

Predictable and adequate transgene expression is essential for clinical gene therapy. Several studies have focused on optimisation of transgene expression. In this study the effect of sodium butyrate (NaB) and a ubiquitous chromatin opening element (UCOE) on short-term gene expression after adenovirus-mediated gene transfer in fibroblastic interface cells from periprosthetic tissue in loosened orthopaedic implants is investigated.

Methods

Cultures of diploid human interface cells from four patients were infected with an adenovirus type-5 vector that carries the luciferase gene driven by the cytomegalovirus (CMV) promoter as a reporter. In addition, viruses with a UCOE were evaluated. Twenty-four hours after infection NaB was added in concentrations of 0 to 9 mM. Luciferase activity was tested after a further 24 h.

Results

NaB in a concentration of 6 mM caused a 7- to 16-fold increase in reporter gene expression compared to control condition. There was no difference in reporter gene expression when cells were infected with Ad.1.5UCOE-CMV.Luc compared to Ad.CMV.Luc. A combination of NaB and a UCOE had no advantage over NaB alone.

Conclusions

Addition of NaB results in a marked increase in transgene expression in cultured cells. This would allow the enhancement of the expression of the transgene, without requiring a higher vector dose. Butyrate administration could not be substituted by inclusion of UCOEs in the vector. It remains to be established whether the effective concentrations of butyrate can be obtained *in vivo*.

Introduction

Adenoviral vectors have been used in clinical gene therapy trials for various applications, including disorders of the lungs, cardiovascular diseases, and cancer, and have been proven well tolerated in local doses of 2.5 x 10^{13} particles.[55,101] The transgene is not inserted into the host chromosome. Therefore, the expression of the transgene is usually transient. This makes adenoviral vectors especially suitable for those applications where short-term expression of the transgene is desirable, such as cancer gene therapy. We are currently investigating the possibilities of killing fibroblastic cells in the periprosthetic tissue of loosened hip prostheses by a gene-directed enzyme-prodrug therapy, using a human adenovirus type-5 (HAdV-5) vector, as an alternative for revision surgery in patients with a high mortality risk.

A common problem in clinical studies is that patients respond differently to treatments. These inter-individual differences can lead to situations in which some patients show low and others high response to the same treatment. Consequently, the occurrence of adverse events can be difficult to predict. Furthermore, several studies have shown that some cell types, including bladder cancer cells, vascular cells, macrophages and fibroblasts are more difficult to transduce with adenoviral vectors, than other cell types.[44,66,89,93] Fibroblastic cells from interface tissue from loosened orthopaedic implants can be transduced *in vitro*, but relatively high HAdV-5 titers are needed.[30] When the gene expression can be made more efficient and predictable, the vector dose can be decreased. This has several advantages, including less evocation of a host immune response and a smaller demand for the production of clinical grade adenovirus.[66]

In the last decades it has become clear that chromatin structure plays an important role in modulation of gene expression.[41] The basic subunit of chromatin, the nucleosome, is composed of about 147 base pairs of DNA wrapped around a complex of eight histone proteins.[41] The organisation of chromatin prevents the transcription machinery from interacting with promoter DNA sequences. This can be overcome by chromatin-remodeling enzymes that alter the folding, fluidity, and basic structure of chromatin.[41] Acetylation of histones is one of the mechanisms that alters chromatin structure and increases gene expression. The equilibrium of histone acetylation is determined by the net activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Sodium butyrate (NaB) inhibits the activity of HDACs, while the function of HATs is continued, thereby leading to hyperacetylation of the histones and an increase in activation of gene expression.[27,116,117] The exact mode of action of NaB on gene expression is still unknown. Alternatively, gene expression has been augmented by *cis*-acting DNA sequences inserted in the vector DNA. UCOEs (ubiquitous

chromatin opening elements) are methylation-free CpG islands commonly associated with dual divergently transcribing promoters, which possess a dominant chromatin-opening function preventing heterochromatin formation. It has been suggested that these elements should be able to provide stable long-term, high-level gene expression in a gene therapy context.[4,126] The advantage of a UCOE would be that the effect is generated locally, thereby minimising systemical adverse events. Furthermore, only the promoter of the transgene is stimulated, while the other elements in the host cell remain unaffected.

The goal of this study is to determine the influence of NaB on short-term gene expression in fibroblastic interface cells from the periprosthetic tissue in loosened orthopaedic implants. Furthermore, the study tries to discover whether the insertion of a 1.5 kb UCOE fragment in an HAdV-5 vector gives a more stable expression in interface cells compared to the vector without the UCOE fragment. Finally, the influence of NaB in a vector with a UCOE fragment is investigated.

Materials and Methods

Cells and cell culture

Cultures of diploid human interface cells were used for all experiments. Interface tissue was removed from the periprosthetic space during revision surgery by an orthopaedic surgeon and collected in sterile phosphate-buffered saline (PBS). Connective tissue and fat were removed thoroughly and the interface tissue was digested for at least 2 h at 37°C using collagenase 1A (1 mg/ml; Sigma, St Louis, MO, USA). Cells were then harvested by filtering the tissue/collagenase substance through a 200 μm filter (NPBI, Emmer-Compascuum, The Netherlands). The cells were cultured in 75 cm^2 flasks (Cellstar, Greiner, Alphen aan de Rijn, The Netherlands) with Iscove's modified Dulbecco's medium (IMDM; Biowitthaker, Verviers, Belgium), supplemented with glutamax (GibcoBRL, Paisley, UK), penicillin and streptomycin (Boehringer Mannheim, Germany), and 10% fetal calf serum (FCS; GibcoBRL, Paisley, UK) at 37°C and 5% CO_2.

Before each experiment interface cells were detached from the flasks using 0.25% trypsin (GibcoBRL). The cells were counted in a Bürker counter and dead cells were excluded by trypan blue. Cells were seeded in a 96-well plate (flat bottomed) at a density of 5000 cells per well. Cells were incubated overnight to allow attachment to the bottom. Before each experiment the wells were washed twice with IMDM. Light microscopy indicated that more than 95% of the cells were fibroblasts. Cell viability was measured using cell proliferation reagent WST-1 (Roche, Mannheim, Germany) according to the manufacturer's instructions with a 2 h incubation period.

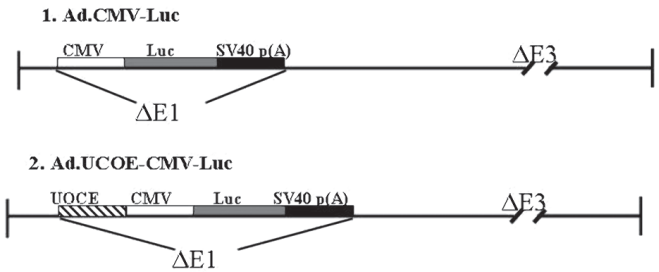
Viral vector construction

PGL3basic was obtained from Promega (Madison, WI, USA) and contains the Luciferase-SV40p(A) cassette downstream from the multiple cloning site. The human cytomegalovirus (CMV) immediate early enhancer/promoter (0.9 kb) was cloned into SmaI digested pGL3basic to generate pGL3/CMV-Luc-SV40p(A). To generate pGL3/1.5kbUCOE-CMV-SV40p(A), the 1.5 kb UCOE Esp3I fragment was blunted with T4 DNA polymerase (NEB, Beverly, MA, USA) and then ligated into NheI digested and T4 blunted pGL3basic/CMV-Luc-SV40p(A). The UCOE comprises a CpG-rich island containing dual bi-directional promoters and is derived from the human HNRPA2B1-CBX3 gene locus spanning a 1.5kb Esp3I fragment.[126]

To construct pPS1128/CMV-Luc-SV40p(A) and pPS1128/1.5kbUCOE-CMV-Luc-SV40p(A), the expression cassette was cut out from pGL3/CMV-Luc-SV40p(A) by PvuI/NheI/BamHI digest and from pGL3/1.5kbUCOE-CMV-Luc-SV40p(A) by KpnI/BamHI digest; both cassettes were then blunted and cloned into SpeI digested and blunted pPS1128, which contains the E1-deleted right part of the HAdV-5 genome. The integrity and identity of the plasmids was verified by restriction enzyme analyses.

The viruses Ad.CMV-Luc-SV40p(A) and Ad.UCOE-CMV-Luc-SV40p(A) were constructed by homologous recombination in PER.C6[38] using pPS1128/CMV-Luc-SV40p(A) and pPS1128/1.5kbUCOE-CMV-Luc-SV40p(A), respectively, and the overlapping adenoviral backbone vector pPS1160.[33] The recombinant adenoviruses were amplified, isolated, CsCl-purified, and titered as described elsewhere[75] (Figure 1).

Figure 1. Schematic representation of the structures of the adenoviral constructs Ad.CMV.Luc-SV40p(A) and Ad.1.5kbUCOE-CMV-Luc-SV40p(A).



Both vectors are derived from adenovirus serotype 5. The luciferase expression cassettes were inserted into the E1 region in a left to right orientation. The E1 and E3 regions are deleted from the viruses. CMV (cytomegalovirus) is the human CMV immediate early enhancer/promoter. SV40p(A) is the SV40 virus late polyadenylation signal from pGL3basic (Promega).

Transduction efficacy with and without NaB

Interface cells from four patients were infected with Ad.CMV.Luc in different concentrations (0, 8, 20 and 80 plaque forming units (pfu)/cell) in IMDM/10%FCS, 40 μl per well. After 2 h 60 μl IMDM/10% FCS was added to the wells. After Ad.CMV.Luc-infection, cells were washed once with IMDM. An interval of 24 h was chosen as this time delay was shown to be effective in a previous study.[32] The cells were then incubated with sodium butyrate (NaB) (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) in concentrations of 0, 3, 6, and 9 mM in IMDM/10% FCS for 24 h. Then luciferase activity was measured. All conditions were tested in triplicate.

Transduction efficacy with and without UCOE

Interface cells from four patients were infected with Ad.CMV.Luc or with Ad.1.5UCOE-CMV.Luc in concentrations of 0, 8, 20, and 80 pfu/cell in IMDM/10% FCS, 40 μl/well. After 2 h 60 μl IMDM/10%FCS was added to the wells. Medium was refreshed after 24 h. Luciferase activity was measured 48 h after infection. All conditions were tested in triplicate.

Combined effect of sodium butyrate and UCOE

To investigate the effect of NaB in the presence of a UCOE, the experiment for transduction efficacy with and without NaB was repeated with Ad.1.5UCOE-CMV.Luc.

Luciferase assay

Cells were washed twice in PBS, and 100 μl cell culture lysis reagent (Promega) was added to the wells. This was left for 10-30 min, and then transferred into another well. The luciferase activity present in 10 μl lysate was measured by the addition of 100 μl of Luciferase assay reagent (Promega). After 10 s of preincubation the produced light was measured for 10 s in a luminometer (Lumat LB 9507, Berthold Technologies, Bad Wildbad, Germany).

Statistical analyses

A univariate analysis of variance (ANOVA) was used to determine the effect of NaB and UCOE on transduction of interface cells. Also interaction of NaB and UCOE was tested with univariate ANOVA. For all statistical analyses a value of $p < 0.05$ was the level of statistical significance.

Results

Aseptic loosening by particulate-induced osteolysis is the most common cause of orthopaedic implant failure. Wear particles, such as particles of polyethylene and metal, are phagocytosed by macrophages, leading to secretion of inflammatory cytokines.[43] The resulting chronic inflammation eventually produces a pseudomembrane of synovium-like interface tissue with activated macrophages, fibroblasts, giant cells and osteoclasts. The interface cells (Figures 2a and 2b) are located surrounding the prosthesis and in the joint space, which is a closed compartment. As an alternative to manual removal, we explored the possibility of removing the tissue by gene-directed enzyme-prodrug therapy. Here we study how we can increase transgene expression without increasing the virus dose.

Effects of NaB on reporter gene expression

Interface cells from four patients were infected with Ad.CMV.Luc at multiplicities of infections (MOIs) of 0, 8, 20 and 80 and, 24 h later, NaB was added to the tissue culture medium in concentrations of 0, 3, 6, and 9 mM. Figure 3a shows the luciferase activity 48 h post-infection. Luciferase activity increases with rising concentrations of NaB. In patients 2 and 3 maximal effect of NaB is reached at a concentration of 6 mM. The stimulating effect of NaB on expression is seen at all MOIs tested. Figures 3b and 3c show the single effect of either the vector Ad.CMV.Luc at an MOI of 80 or NaB treatment with a concentration of 6 mM. These figures show that infection with the adenovirus vector or butyrate alone does not cause any detectable effect on cell viability.

Figure 3d shows the induction of reporter-gene expression by NaB per dose group per patient. This ratio is obtained by dividing the luciferase activity in the NaB-stimulated cells by the activity in the absence of NaB. The mean and standard deviation are then calculated for each NaB concentration. Consequently, the induction factor for NaB 0 mM is always 1. The differences between the ratios are statistically significant ($p < 0.05$) in all patients except for the differences between the ratios of NaB 6mM and NaB 9 mM in patients 2 and 3 (respectively $p = 0.717$ and $p = 0.732$). The influence of the amount of Ad.CMV.Luc on the ratios did not reach statistical significance ($p = 0.086$). Fluorescence-activated cell sorting (FACS) analyses demonstrated that the amounts of coxsackie-adenovirus receptor (CAR) on these cells were very low and not affected by butyrate treatment (data not shown). This is consistent with the relatively inefficient transduction of these cells by HAdV-5 vectors. The luciferase activity achieved is approximately 2 orders of magnitude lower than is achieved in reference tumour cell lines HeLa, HepG2, and Hep2, and equals the activity achieved in diploid skin fibroblast cultures (Figure 3e).

Figure 2. Photomicrographs of interface cells.

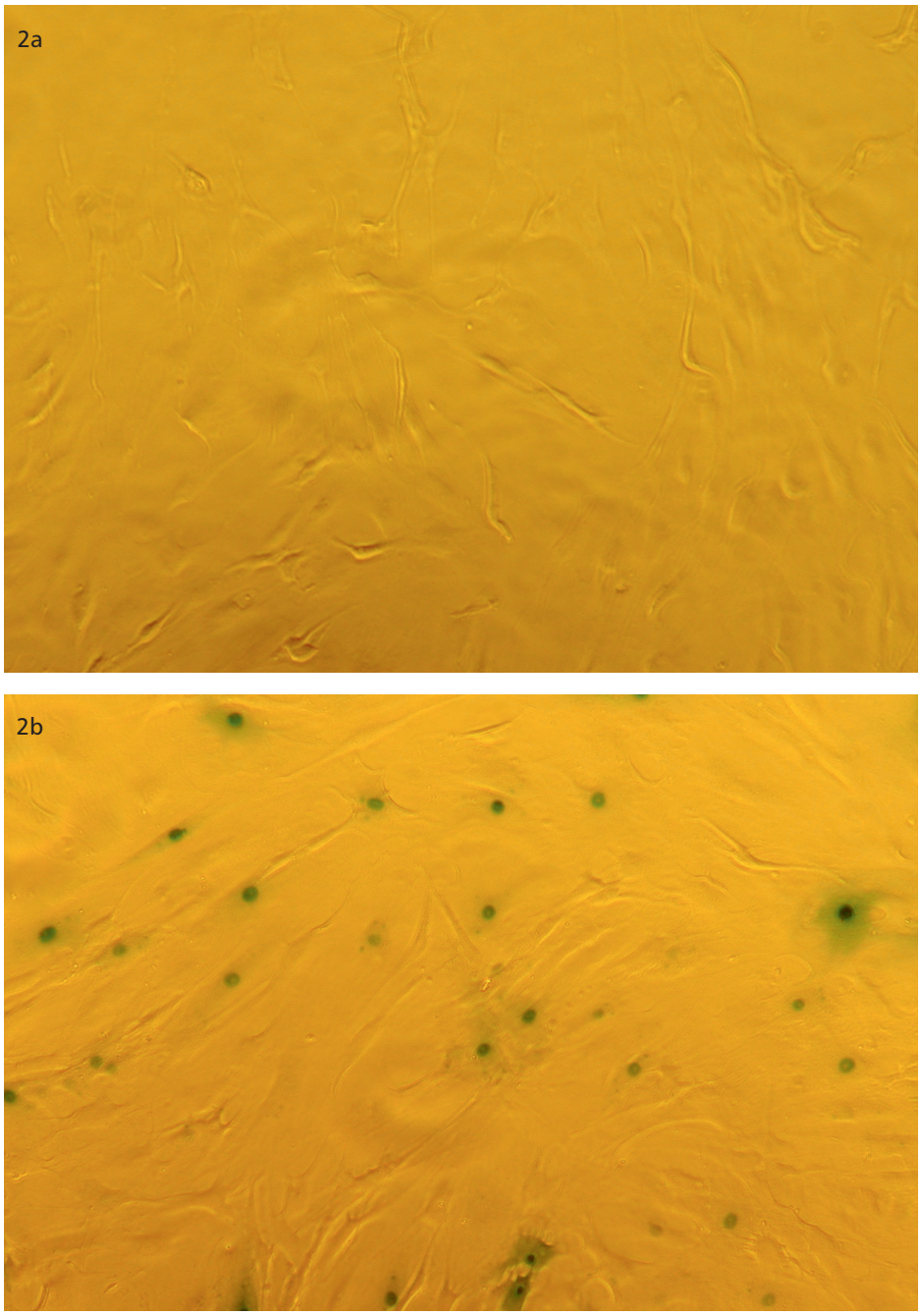
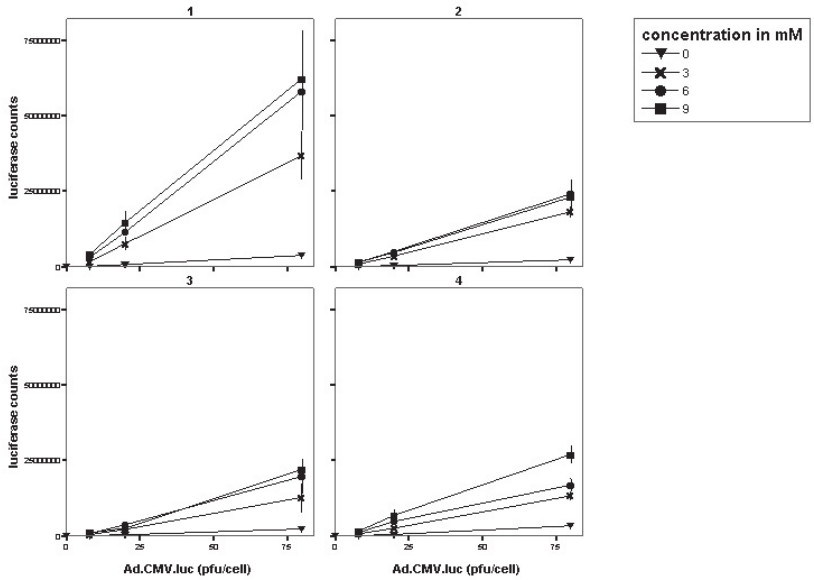


Figure 2a shows non-transduced cells, figure 2b shows interface cells 48 h after transduction with Ad5.LacZ at MOI of 25.

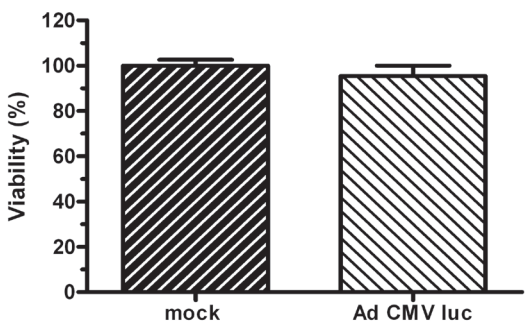
Figure 3. Effect of sodium butyrate (NaB)on transduction of interface cells by Ad.CMV.luc (a). Effect of the vector Ad5.CMV.Luc on cell viability (b). Effect of the NaB on cell viability (c). Influence of different concentrations of NaB on induction of reporter-gene expression in interface cells transduced by Ad.CMV.luc relative to a control condition without NaB (d).Comparison of luciferase activity after infection of three types of cells with Ad.CMV.luc (e).

3a



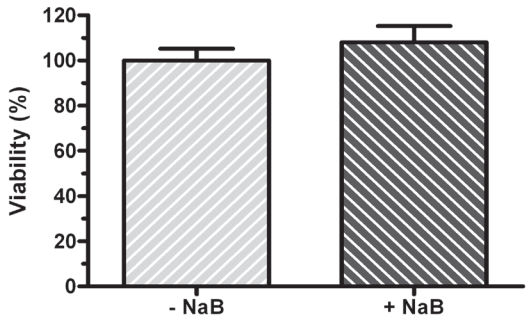
Interface cells of four patients were infected with Ad.CMV.Luc in four concentrations (0, 8, 20, and 80 pfu/ cell). Twenty-four hours after infection four concentrations of NaB (0, 3, 6, and 9mM) were added. All conditions were tested in triplicate. The figure shows luciferase activity (mean and standard deviation) for different concentrations of vector and NaB. Each graph represents the results from one patient.

3b



Ad5.CMV.Luc was added to the cells at an MOI of 80 and, 24 h post-infection, cell viability was determined with the WST cell-viability assay.

3c



NaB was added to cultures of near-confluent cells at a concentration of 6 mM. Twenty-four hours post-infection cell viability was assayed with the WST cell-viability assay.

3d

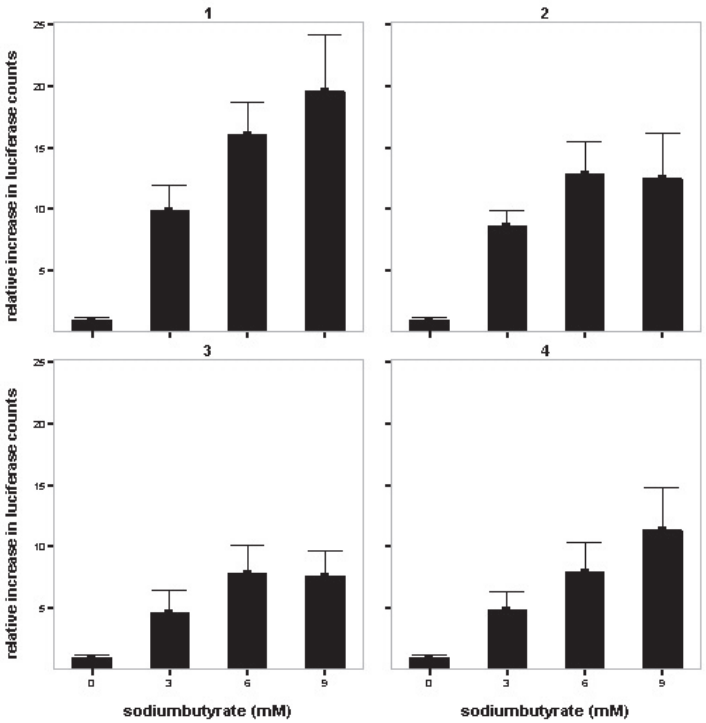
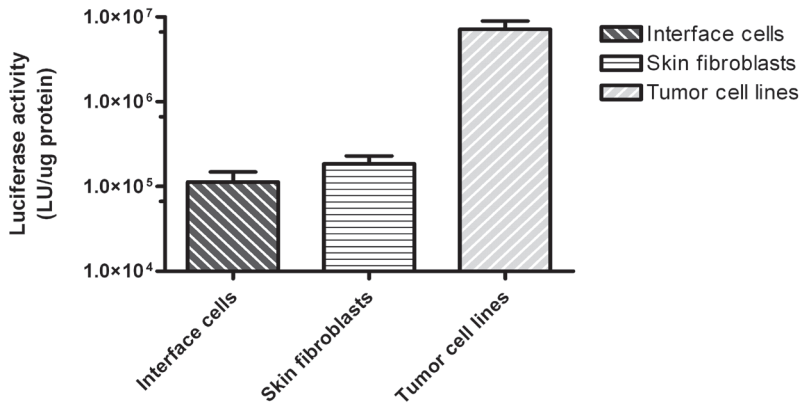


Figure 3d: The figure shows the induction ratio (mean and standard deviation) of luciferase activity between different concentrations of NaB (0, 3, 6, and 9 mM) compared to a control of NaB 0 mM. Cells were infected with Ad.CMV.Luc in concentrations of 8, 20, and 80 pfu/ cell. Each graph represents the results of one patient.

3e



Interface cells derived from three patients, human skin fibroblasts, and three tumour cell lines (HeLa, HepG2, and Hep2 cells) were infected with Ad.CMV.Luc at an MOI of 20. The figure shows luciferase activity in these cells 36 h post-infection.

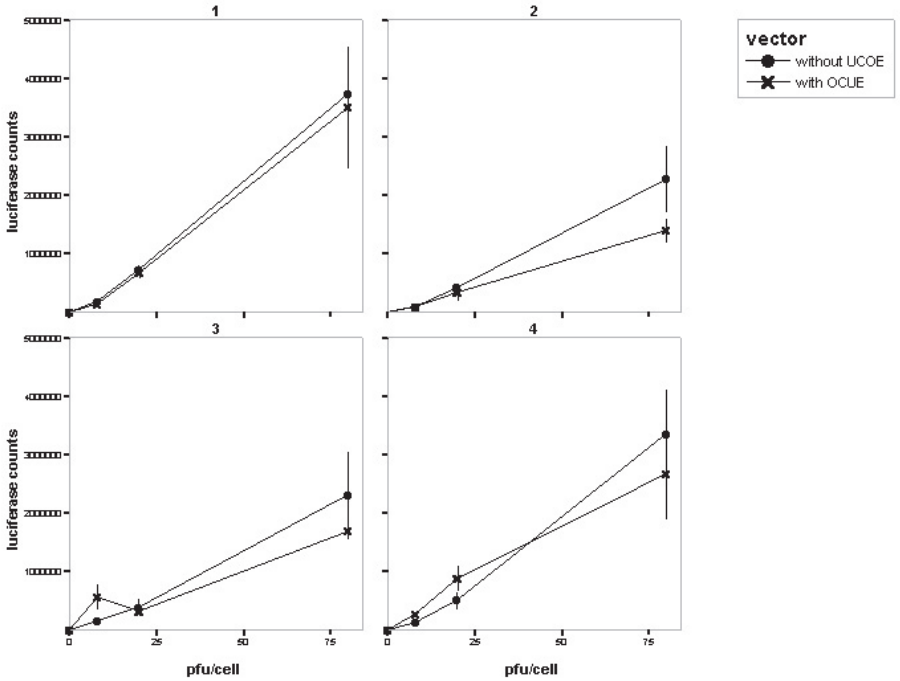
Influence of UCOE on reporter gene expression

Enhancing reporter gene expression by NaB may be a method to increase the efficacy of gene therapy without increasing the vector dose. However, it may not be trivial to achieve locally sufficient concentrations of butyrate. Therefore, we analysed whether UCOEs could be used as *cis*-acting enhancers of gene expression. To study their effect interface cells from four patients were infected at MOI 0, 8, 20, and 80 with either Ad.CMV.Luc or Ad.1.5UCOE-CMV.Luc and grown in IMDM/10%FCS for 48 h. Figure 4 shows the luciferase activity for both vectors per patient. There is no effect of UCOE on reporter gene expression ($p = 0.251$). When the influence of vector type on reporter gene expression is considered per patient Ad.CMV.Luc gives a significantly higher expression of luciferase than Ad.1.5UCOE-CMV.Luc in patient 2 ($p = 0.049$). In the other patients there were no significant differences between the two vectors (patient 1: $p = 0.594$; patient 3: $p = 0.597$; patient 4: $p = 0.798$).

Combined effect of UCOEs and NaB

Butyrate acts indirectly on transgene expression by inhibiting the histone deacetylases. To study whether NaB can also activate the expression of promoters that are linked to UCOEs the effects of butyrate in the presence and absence of the UCOEs were compared. In Figure 5a luciferase activity is shown for Ad.CMV.Luc and Ad.1.5UCOE-CMV.Luc at MOI of 80 with different concentrations of NaB. In the case of a combined effect between the two possible gene expression enhancers the regression coefficients would be different leading to non-parallel regression-lines. Figure 5b shows that the regres-

Figure 4. Transduction of interface cells by vectors Ad.CMV.Luc and Ad.UCOE-CMV.Luc.



The figure shows luciferase activity (mean and standard deviation) in interface cells from four patients infected with four concentrations of vector (0, 8, 20, and 80 pfu/ cell). Two vectors were used, the UCOE-containing Ad.1.5UCOE-CMV.Luc and, as a control, Ad. CMV.Luc. Each graph represents the results from one patient.

sion lines for the two vectors at MOI of 80 are more or less parallel. From these data we conclude that there is no difference in effect of NaB in the presence and absence of UCOEs ($p = 0.424$ at all MOIs).

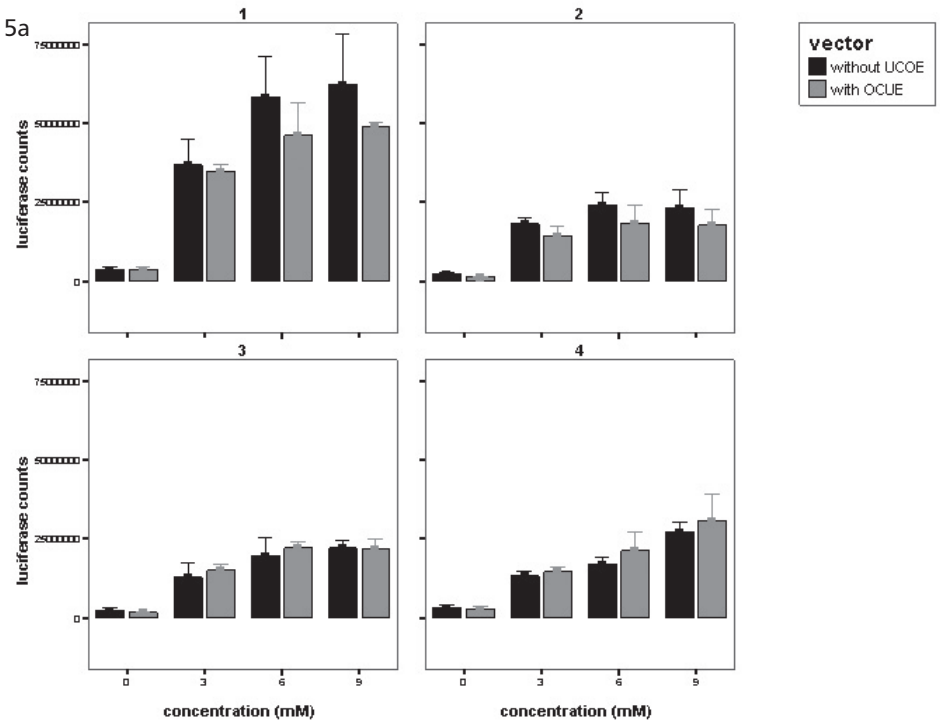
Discussion

Results of this study show that sodium butyrate (NaB) at a concentration of 6 mM increases reporter gene expression by Ad.CMV.Luc with a factor 7 to 16 compared to a control condition without NaB. The insertion of a UCOE in the vector did not increase reporter gene expression. Also, there was no additive effect when NaB was used in combination with a UCOE.

Dion *et al*.[32] studied the effect of NaB on transgene expression. They suggested that butyrate amplification is both time- and concentration-dependent and found that

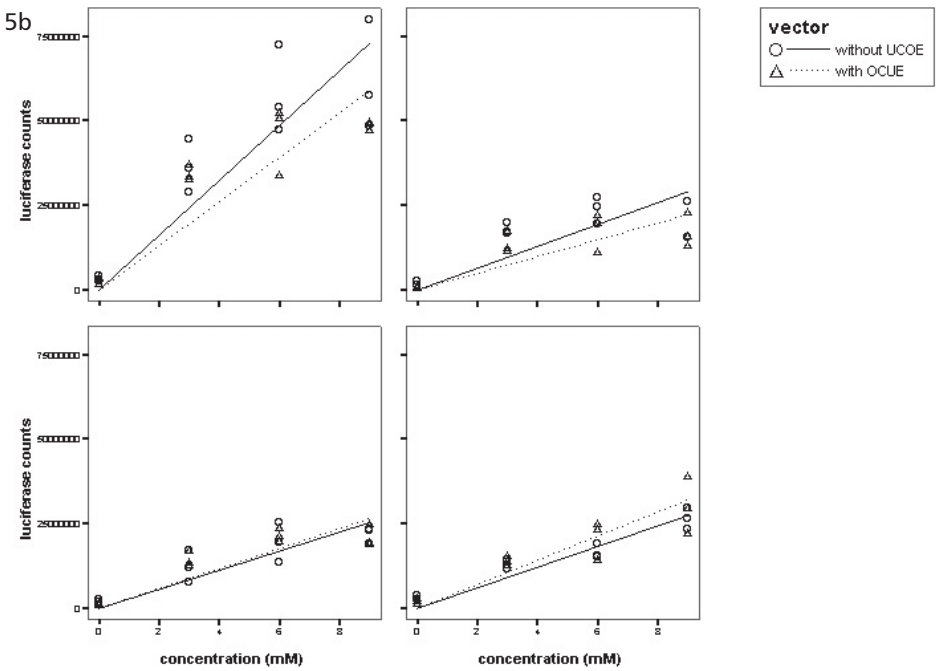
Figure 5. Influence of different concentrations of sodium butyrate (NaB) on transduction of interface cells by Ad.CMV.Luc and Ad.1.5UCOE-CMV.Luc (a). Combined effect of NaB and the different vectors (with and without UCOE). In these scatter plots the luciferase activity for different concentrations of NaB are shown for Ad.CMV.Luc and for Ad.1.5UCOE-CMV.Luc at MOI 80 and their regression lines (b).

5a



The figure shows the luciferase activity at different concentrations (mean and standard deviation) of NaB (0, 3, 6, and 9 mM) for both vectors at an MOI of 80. For reasons of clarity only MOI 80 is shown in this graph. In the statistical analyses all MOIs were taken into account. Each graph represents the results from one patient.

optimal NaB concentration was 0.5 to 5 mM and the exposure time to butyrate should preferably be 24 h or longer. In our study we used an exposure time of 24 h and found 6 mM to be the optimal concentration. Sodium butyrate has been used in similar studies to increase and prolong Ad-LacZ activity in rat fibroblasts.[117] In that study by Taura *et al*.[117] a strong correlation was found between the amount of hyperacetylation and the β-galactosidase activity used as reporter, suggesting that hyperacetylation of histones was responsible for the increase of transcription of the reporter gene LacZ. In another study by Lee *et al*.[70] the effect of NaB on transduction of bladder tumour cells



The figure shows that the lines for the two vectors are more or less parallel suggesting no combined effect of NaB and UCOE. For reasons of clarity only MOI 80 is shown in this graph. In the statistical analyses all MOIs were taken into account. Each graph represents the results from one patient.

was attributed to a higher CAR expression, but this could not be reproduced by Taura *et al*.[117] Since in our study the infection precedes the NaB treatment by 24 h, and since there is no induction of CAR expression, enhanced transduction is unlikely to play a significant role in the enhancement of luciferase expression. However, formal demonstration of a direct effect on transcription in the interface cells is thwarted by the inability to transfer DNA into the cultured interface cells by any of the commercially available transfection reagents. Furthermore, a mechanism in which NaB operates by enhancing transcription via inhibition of histone deacetylase is fully consistent with the data of Dion *et al*.[32]

Sodium butyrate is a short-chain fatty acid and was found to be rapidly metabolised *in vivo*, making it difficult to increase or maintain at an effective therapeutic level. In a study by Miller *et al*.[84] NaB was administered intravenously at a dosage of 500 mg/kg/day as continuous infusion over 10 days. No toxicity was encountered. Due to a half-life of 6.1 min only plasma concentrations of 39-59 μM could be reached. Therefore, esterified butyrate derivatives with longer half-lives have been developed that might be more effective *in vivo* as agents for amplification of adenoviral transgenes.[88,96,97] As

an enema, NaB has been shown to be effective and safe in a double-blind clinical study in a combinational therapy with 5-aminosalicylates (5-ASA) in patients with refractory distal ulcerative colitis.[124] Although effective *in vitro*, the use of NaB for optimisation of transgene expression *in vivo* remains a challenge. However, when a high concentration of NaB can be achieved locally (e.g. in intra-articular injection), NaB can probably be advantageous to increase transgene expression *in vivo*. With injection in an artificial joint in patients with a prosthetic implant, the cells that will most likely be affected will be synovium-like interface cells and osseous cells. Schroeder *et al*.[106] studied the effect of histone deacetylase inhibitors (HDIs) on osseous cells. They showed that HDIs, like TSA and NaB, promote osteoblast maturation and mineralisation *in vitro* and *in vivo* in calvarial tissues. However, they added that additional studies are needed to examine the long-term effects of HDIs on bone formation.

In an attempt to increase local effect on transgene expression while keeping systemical effects at a minimum, a vector was developed with a ubiquitous chromatin opening element (UCOE) inserted in the vector DNA. The mode of action of the UCOE is that the promoter (CMV) is packed in a DNA sequence containing methylation-free CpG islands, making the promoter resistant to heterochromatin-mediated silencing of these genes. This will cause substantial improvements in the level of expression and proportion of transduced cells that express at detectable levels.[74,126] Abdullah *et al.*[2] tested the influence of a UCOE in a plasmid vector on transgene expression in mouse airways. Their results showed that in cells with UCOEs the reporter gene expression in the first 2 days did not differ from the control group. After 28 days there was a four times higher expression in the cells with UCOEs. The effectiveness of this UCOE in enhancing expression of CMV-driven cDNA cassette has been demonstrated by Benton and collaborators in CHO cell lines after stable transfection, although it should be noted that in this study a larger UCOE fragment was used.[9] In the current study we also found no difference in expression between the vector with and without UCOE. The non-integrated CMV promoter is probably not silenced in our target cells within the first days after infection, rendering no effect of an anti-silencer insert. Possibly, the UCOE could have increased reporter-gene expression in the current study in the longer term, but this would be irrelevant in this study, as expression of the transgene is only needed for 2 to 3 days. As NaB does activate the reporter gene expression in contrast to the UCOE, this suggests that NaB increases reporter gene expression not only by activating the activity of the CMV promoter in the transgene, but that other mechanisms are also involved. The upregulation of transcription factors by NaB has been discussed by other authors.[11,17,27,42] This upregulation could be an explanation for the increase in reporter gene expression by NaB.

An alternative method to increase expression is to facilitate binding and infection of

the cell by making changes in the adenoviral vector. This method has previously been described by our research group.[44] Fibroblastic cells have low levels of CAR expression and are therefore hard to infect with a HAdV-5 vector. To increase gene expression the adenoviral vector was changed by replacing the fibers by those of other serotypes. Especially the fibers of the subgroup B (i.e. serotypes 11, 16 and 35) increased the reporter gene expression significantly.[44]

This study shows that the insertion of a UCOE does not increase short-term reporter gene expression in fibroblastic interface cells. Sodium butyrate increases expression by a factor of 7-16, but the feasibility for use *in vivo* remains to be established.

5

Arthrography in loosened hip prostheses. Assessment of possibilities for intra-articular therapy

Jolanda J. de Poorter[1]
Willem R. Obermann[2]
Tom W.J. Huizinga[3]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Radiology, University Medical Center, Leiden, The Netherlands
[3]Department of Rheumatology, University Medical Center, Leiden, The Netherlands

Joint Bone Spine, 2006; 73: 684-90

Abstract

Background

Loosening is a major complication in prosthesis surgery. Less invasive alternatives to revision surgery are required to prevent and treat prosthesis loosening. Some experimental therapies investigating alternative treatments exploit the intra-articular space as a route of administration. For efficient, local delivery of therapeutic agents a contained joint space is required. Furthermore, the volume of the joint space determines the concentration of the therapeutic ingredient in the joint.

Methods

A retrospective analysis of 221 hip arthrograms performed between 1994 and 2004 for diagnosis of prosthesis loosening was performed. All arthrograms were studied for leakage of contrast medium and the volume of injected contrast medium.

Results

There was a contained joint in 164 arthrograms (74%). The volume in these hips was 31 ± 12.7 mL. Male patients had a larger joint volume than female patients ($p = 0.019$). There was no difference in containment and joint volume between hips with a primary and with a revised prosthesis.

Conclusions

For successful intra-articular therapy it is necessary that the injected agent remains in the joint space. As leakage of contrast medium was shown in about a quarter of hips, this study shows that an arthrogram may be useful in the inclusion procedure for intra-articular studies to determine containment of the joint and also the volume that can be injected.

Introduction

For patients undergoing replacement of joints with orthopaedic implants, subsequent loosening of the prosthesis is common at long-term follow-up. In addition, joint prostheses are increasingly scheduled at a younger age and with increases in the average age of developed populations replacement joints experience increased activity over longer life spans. Overall loosening of hip prostheses occurs in 7-13% of cases within 10 years,[54,78,112] this rate is higher in male patients, patients younger than 55 years of age and in cemented prostheses (when adjusted for age).[78] Revision surgery has a 5.7% post-operative mortality (during hospital stay) in elderly patients, and in patients with cardiac insufficiency (ASA3 - American Society of Anesthesiologists) there is an increased risk of major complications such as myocardial failure or coronary artery disease. These individuals are therefore ineligible for revision surgery[114] and currently have no other treatment options. For these patients there is a clear need for alternative therapies that are less invasive.

Studies of alternative treatments for prosthesis loosening are currently ongoing, in particular intra-articular delivery of therapeutic proteins or genes.[37,128] These protocols involve the inhibition of inflammatory processes within the joint to prevent or retard periprosthetic osteolysis[19,115,129] or, killing and removal of interface tissue with gene-directed enzyme prodrug therapy (GDEPT), with subsequent injection of bone cement to refix loosened prostheses.

In both such studies good exposure of the target tissue is essential and the injected active ingredient must remain in the joint for a sufficiently long period. Beside size of the therapeutic particle,[46,90] the integrity of the surrounding joint tissue (containment) is important in retaining active particles within the joint space. Leakage of injected fluid can be visualised by arthrography with contrast medium. The presence of bursae corresponding with the joint space is a common feature, especially in patients with hip prostheses.[12,81] Leakage can also occur in the absence of bursae e.g. along the psoas muscle. In hips with leakage from the joint, exposure of the target tissue to the therapeutic agent will be sub-optimal and there will be an increased risk of exposure to surrounding tissues. Thus, before a patient can undergo an experimental intra-articular treatment it is important to confirm containment of fluid within the joint and demonstrate that the tissue surrounding the joint forms a continuous, closed compartment .

Efficacy of intra-articular therapy is also dependent on the joint volume. In large joint spaces the active ingredient will be more diluted than in smaller joint spaces or a higher dose will be required with the potential for increased toxicity. Joint spaces can also be too small for intra-articular therapy where delivery of the required amount of

active ingredient is limited or the local concentrations too high increasing the potential for normal tissue toxicity.

Therefore, for the clinical use of intra-articular treatments it is relevant to demonstrate containment of injected solutions within the joint space and determine the volume of that space to optimise the delivery of therapeutic agents to joint tissue. The aim of this study was therefore to determine the number of hip prostheses with clinical loosening that would be eligible for therapy using intra-articular delivery of proteins or genes.

Methods

Using the hospital database all hip arthrographies performed between January 31st 1994 and September 30th 2004 were identified. The radiological reports were studied for the indication for which the arthrography was made. All arthrographies made in hips with a prosthesis in situ were included. Exclusion criteria were a suspected infection of the prosthesis, dislocation of the prosthesis, procedures in which no contrast medium was injected, and arthrographies that could not be found in the archives of the radiological department. All arthrograms were performed or supervised by a radiologist with arthrography experience. The patient was positioned supine on the examination table. The hip region was covered with sterile drapes and the skin was infiltrated with a local anaesthetic. Under fluoroscopic guidance a needle directed at the femoral neck was inserted into the joint and an attempt was made to aspirate some of the synovial fluid for culturing of microorganisms. Ioxaglate sodium meglumine (Hexabrix® 320; Guerbet, Aulnay-sous-Bois, France) contrast medium was injected through the needle under fluoroscopic guidance. Injection of contrast medium was stopped when one of the following events occurred: Rapid increase of pressure while injecting the contrast medium; the patient complained of pain in the hip; or presence of contrast medium in the lymphatic system on the fluoroscopic images.

For retrospective analysis of indication for arthrography and the results of the examination, the radiological reports and the patient's medical records were used. From these records the following data were gathered: age, sex, injected volume during arthrography, time span between the total hip arthroplasty and the arthrography, whether the prosthesis was a primary hip prosthesis or a revision procedure, and, when applicable, findings during subsequent surgery. The results of the arthrography were qualified as loose or well-fixed for both the femoral and the acetabular components, respectively. The criterion for loosening of the acetabular component was leakage of contrast medium in all acetabular zones or in zone I and II or II and III. The criterion for loosening

of the femoral part was extension of contrast medium past the intertrochanteric line of the standard stem and halfway down the stem of the long-stemmed component.[3]

Primary surgery and revisions

Some patients had multiple arthrograms. To compare containment of contrast medium and volume of the hip joint after primary and (multiple) revision surgery two subgroups of arthrographies were made. In the primary hip group the latest arthrogram before surgery was considered. In the revision surgery group the latest arthrogram after the most recent revision procedure was considered. In patients with multiple arthrograms each hip (right and left) could only be in each group once.

Containment of contrast Medium

All arthrographies made for diagnosis of aseptic loosening of the hip prosthesis were reviewed to qualify containment of the joint. Containment of the joint was defined as contained or non-contained, depending on leakage of contrast medium from the joint into the surrounding tissues. Presence and localisation of a bursa were noted. To investigate containment of the joint space, the spreading of the contrast medium was evaluated on the arthrogram prints. When the contrast medium was located in the joint space in all prints the joint was considered contained. When the prints showed leakage of contrast medium to surrounding tissues the joint was non-contained. There was never a contained joint in the presence of a bursa. In some cases a small amount of contrast medium leaked out of the joint when the needle was removed, which did not count as non-contained.

Sensitivity of arthrogram for loosening

Patients who underwent revision surgery within 2 years after arthrography were included in a sensitivity analysis. To study the occurrence of false-negative results loosening diagnosed with arthrography was compared to findings of loosening during surgery. The prosthesis was considered loose during surgery when there was macroscopic movement of the prosthetic component evaluated by means of traction and rotation.

Volume

The volume of the joint was assessed from the radiological report. In hips with a non-contained joint, injection of the contrast medium was terminated as contrast medium

was leaking out of the joint space. The amount of contrast medium injected in these cases is probably not the maximal volume. The volumes in non-contained hips were therefore not taken into account for determination of mean volumes in patients with a clinically loosened prosthesis.

Statistical Methods

Distribution of contained and non-contained joints was calculated for all arthrograms together and for different subgroups. Chi-Square tests and Student's *t*-tests were used to study the differences in containment.

For all arthrograms together and for subgroups, mean volume of the joint capacity and standard deviations were calculated. Univariate analysis of variance was used to investigate the differences in volume. For all statistical analyses $p < 0.05$ was the level of statistical significance.

Results

Arthrographies

Between January 31st 1994 and September 30th 2004 484 hip arthrograms were performed at the radiology department of one hospital. In 320 arthrographies a hip prosthesis was in situ when the arthrogram was made. 62 arthrographies were excluded from the study. In 36 of these an infection was suspected. In 11 patients fluoroscopic images were made without injection of contrast medium to analyse mechanical problems associated with the hip prosthesis (e.g. dislocation tendency). Dislocation of the prosthesis was found in two patients. In one patient only a low volume of contrast medium was injected to demonstrate polyethylene wear. Intra-articular injection was unsuccessful in two patients. Ten arthrograms could not be found in the hospital archives. Thus 258 arthrograms were included in the study.

125 Patients had an arthrogram made after primary hip replacement in one of their hips. The hospital archives from 1994 showed arthrogram of one hip after revision surgery in 53 patients. In these patients there were no data on arthrograms before the revision surgery. Ten patients had an arthrogram both before and after revision surgery. In six patients there was an arthrogram after primary hip prosthesis in the right and the left hip and four had an arthrogram in both hips after revision surgery. One patient had an arthrogram of both hips after primary hip replacement and of one of the hips after revision surgery. 26 Patients had more than one arthrogram performed without intervening surgery. In these cases the most recent

Table 1. Comparison of various parameters between hips with primary and revised prostheses.

	Primary prostheses (N=149)	**Revised prostheses** (N=72)	***p*-value**
Sex male (%)	35/149 (24%)	24/72 (33%)	0.121
Age in years (mean ± SD)	68 ± 13	68 ± 11	0.670
Side left (%)	71/149 (48%)	34/72 (47%)	0.952
Loose stem (%)	36/149 (24%)	22/72 (31%)	0.311
Loose cup (%)	64/137 (47%)	38/72 (53%)	0.405
Mean survival time stem (± SD)	103 ± 78 months	105 ± 72 months	0.853
Mean survival time cup (± SD)	108 ± 78 months	87 ± 67 months	0.047

Mean (and standard deviation) of age and survival of prosthesis components are reported for both groups and *p*-values for the differences between the groups are calculated. Distribution of sex and percentage of loose components in both groups and their *p*-values are showed.

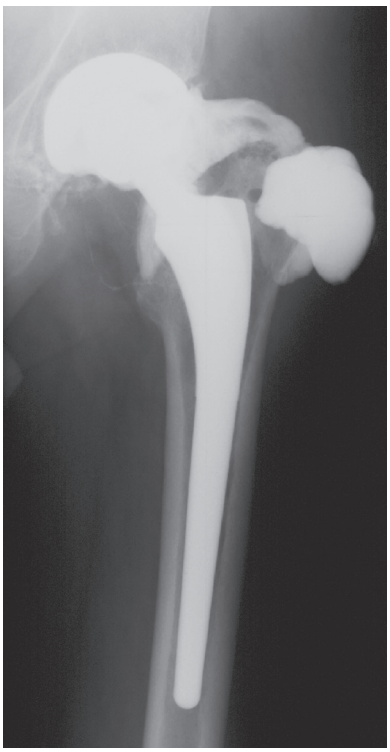
arthrogram was taken into account and the other images were marked as duplicates. There were 31 duplicate arthrograms, which were excluded. In six cases it was not possible to determine if the arthrogram was of a prosthesis following primary or revision surgery. In total 149 arthrograms were included for analysis of primary prostheses and 72 for analysis of revision prostheses. Data regarding sex and age of the patient, and side, loosening and survival time of the components are displayed in table 1.

Containment of contrast medium

Of the 149 arthrograms in primary hip arthroplasties 113 (76%) joints were contained. 36 (24%) arthrograms showed a joint that was not contained, 20 (56%) of which had a bursa (Figure 1, 2A). In the other arthrograms with a non-contained joint the leakage was mainly into the muscles (Figure 3). In the revision hip arthroplasties there was a contained joint in 51 of 72 hips (71%). Twenty-one hips were not contained and 16 (76%) of them had a bursa.

A number of parameters for differences between the containment groups were analysed for primary and revised prostheses. These data are shown in table 2a and 2b. There was no significant difference in sex distribution and in age between the groups. The percentage of hips with a loosened component was higher in the contained group (Table 2).

Figure 1. Fluoroscopic image of an arthrography of a hip with clinical loosening of the prosthesis.



Example of a hip with a subtrochanteric bursa. Left hip of a 58-year old male patient with an uncemented Mallory head prosthesis implanted five months before the arthrography was done. 70 mL contrast medium could be injected, no loosening could be shown.

Table 2a. Comparison of various parameters between contained and non-contained hips for primary hip prostheses.

	Containment good (N=113)	Containment poor (N=36)	p-value
Sex male (%)	29/113 (26%)	6/36 (17%)	0.267
Age in years (mean ± SD)	68 ± 13	66 ± 14	0.387
Loosening of at least one of the components	65/113 (58%)	14/36 (39%)	0.051
Loose stem (%)	30/113 (27%)	6/36 (17%)	0.228
Loose cup (%)	53/103 (52%)	11/34 (32%)	0.053
Mean survival time stem (± SD)	106 ± 77	92 ± 81	0.356
Mean survival time cup (± SD)	114 ± 76	89 ± 82	0.129

Mean (and standard deviation) of age and survival of prosthesis components are reported for both groups and *p*-values for the differences between the groups are calculated. Distribution of sex and percentage of loose components in both groups and their *p*-values are shown.

Table 2b. Comparison of various parameters between contained and non-contained hips for revised prostheses.

	Containment good (N=51)	Containment poor (N=21)	p-value
Sex male (%)	14/51 (28%)	10/21 (48%)	0.099
Age in years (mean ± SD)	67 ± 11	72 ±9	0.068
Loosening of at least one of the components	35/51 (69%)	13/21 (62%)	0.582
Loose stem (%)	20/51 (39%)	2/21 (10%)	0.013*
Loose cup (%)	25/51 (49%)	13/21 (62%)	0.320
Mean survival time stem (± SD)	110 ± 70	93 ± 79	0.388
Mean survival time cup (± SD)	91 ± 62	77 ± 77	0.470

Mean (and standard deviation) of age and survival of prosthesis components are reported for both groups and *p*-values for the differences between the groups are calculated. Distribution of sex and percentage of loose components in both groups and their *p*-values are shown.

Figure 2. Schematic representation of procedure to find percentage of patients with contained and non-contained joints (2A). Schematic representation of procedure to give an idea of the number of patients that can be included in a clinical trial based on arthrography data (2B).

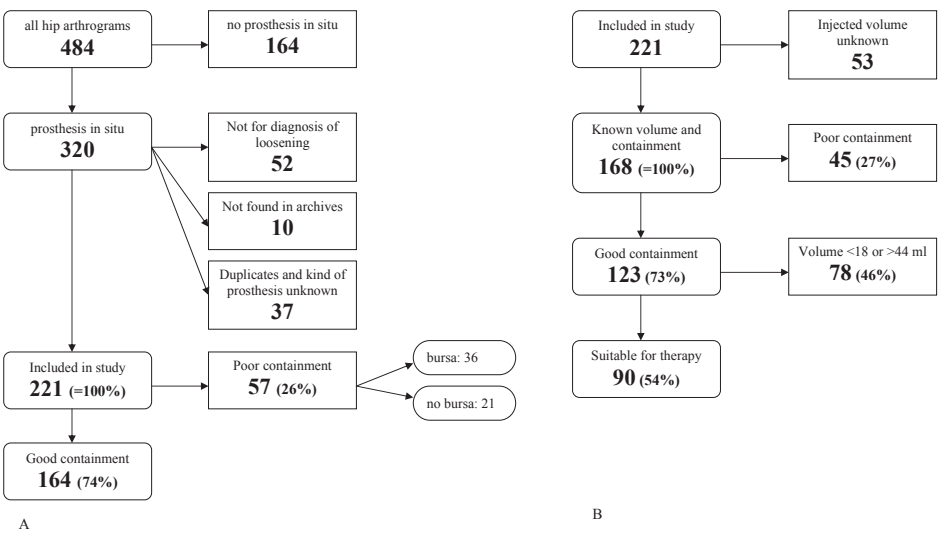
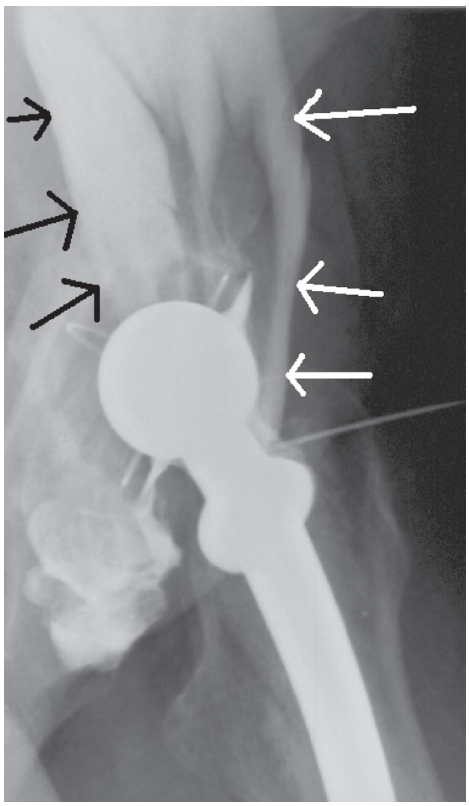


Figure 3. Fluoroscopic image during arthrography in a hip with clinically loose prosthesis.

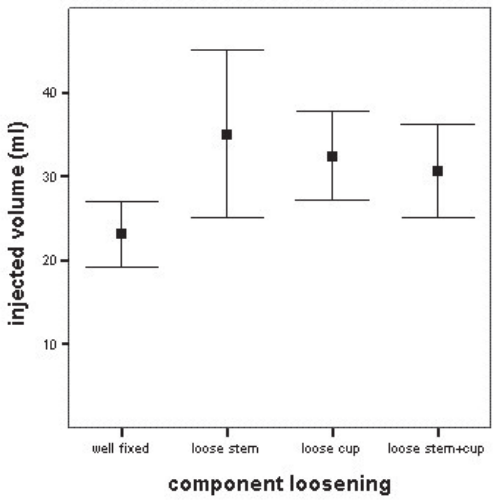


Example of a hip with a poor containment and leakage of contrast medium along the psoas muscle. Left hip of a 50-year-old female patient with a cemented Stanmore prosthesis implanted six months before the arthrography was done. 40 mL of contrast medium was injected. Loosening could not be shown on the arthrogram. Arrows show leakage of contrast medium along the psoas muscle.

Sensitivity of arthrogram for loosening

To investigate whether arthrograms with poor containment exhibited a higher percentage of false-negative results, sensitivity of the arthrogram for loosening was calculated. One hundred and two patients underwent surgery of the hip within 2 years after arthrography. The mean delay between arthrography and surgery was 6.8 ± 5.4 months. Of these 102 patients 21 (21%) were not contained and 81 (79%) were contained. In the non-contained group 11 hips had loosening of the stem as observed during surgery, six of which showed loosening on the arthrogram (sensitivity 55%). In the contained group 41 hips had loosening of the stem as observed during surgery, 28 of which had loosening on the arthrogram (sensitivity 68%). For the cups sensitiv-

Figure 4. Graphic representation of joint capacity in different grades of prosthesis loosening.



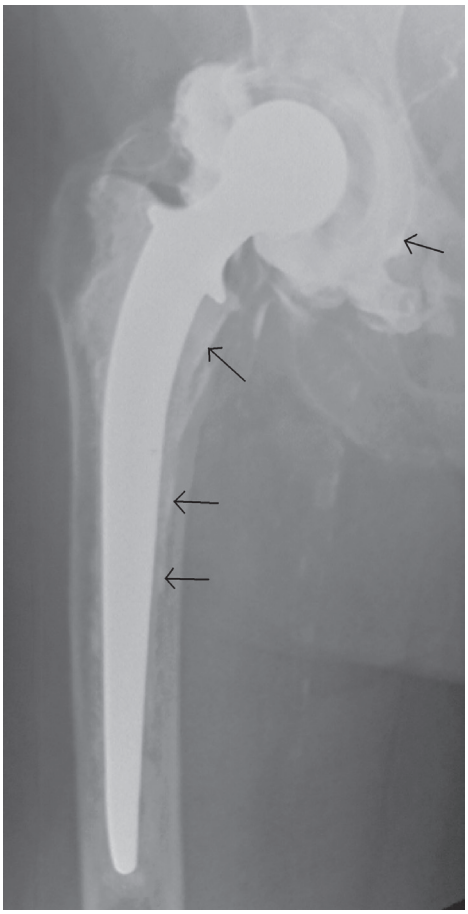
Error bars represent means and their 95% Confidence intervals for four categories of loosening. The mean volume of hips with well-fixed prostheses is significantly lower than the volumes in all other groups ($p = 0.003$). There is no difference in volumes between the three groups that show loosening ($p = 0.171$).

ity was 77% in the group with non-contained joints and 81% in the contained hips. Overall sensitivity and specificity for loosening of the stems was 65% and 96%, respectively. For the cups overall sensitivity was 80% and specificity was 93%. There were no differences in sensitivity and specificity between primary and revised hips.

Volume

Of the primary hip prostheses 113 had a contained joint, in 88 of which a joint volume was noted in the radiological reports. Of the revised hip prostheses 51 had a contained joint of which 35 had a known volume. In total 123 arthrograms were contained and had a known joint volume. The mean injected volume in the contained hips was 29 ± 12.4 mL (range, 2-60 mL) for the primary prostheses and 34 ± 13.2 mL (range 13-60 mL) for the revised prostheses ($p = 0.085$). Male patients had a larger volume than females when corrected for primary or revision surgery (34 ± 13.5 mL and 29 ± 12.3 mL respectively) ($p = 0.019$). There was no association between age and the injected volume ($p = 0.192$). Mean volumes and the 95% confidence intervals for hips with loosening of one or two components are shown in Figure 4.

Figure 5. Fluoroscopic image during arthrography in a hip with clinically loose prosthesis.



Example of a contained hip with average volume. Right hip of a 76-year-old female patient with a cemented Stanmore prosthesis implanted twelve years before the arthrography was done. 20 mL of contrast medium was injected. There is loosening of both cup and stem. Arrows show leakage of contrast medium in the periprosthetic space.

In hips with at least one loose component (diagnosed by arthrography and surgery) the injected volume was larger than in hips with no loosened components (33 ± 12.0 mL; and 26 ± 12.6 mL respectively) ($p = 0.003$) (Figure 5). There were no significant differences between hips with only loosening of the stem or cup, or loosening of both components with respect to volume of the arthrogram ($p = 0.171$). These results were similar for loosening diagnosed by arthrography alone.

Discussion

In this study joint space containment and volume of hips with a clinically loose prosthesis were evaluated. Of 221 hips, 164 (74%) had a contained joint and 123 of these had a known volume. In these hip joints the mean volume was 31 mL. Containment of hip joints in patients with a clinically loosened prosthesis has been studied before. Barentsz *et al*.[7] studied arthrographies of 24 patients with clinically loosened prostheses. Four of 24 patients showed leakage of the contrast medium in the surrounding tissues, one of them had a bursa. In another study of 178 arthrographies a bursa was found in 35% of cases and a non-bursal cavity in seven percent of hips. In total 43% of these hip joints had leakage of the contrast medium outside the joint.[12,81]

The volume of the joint cavity is dependent on the method of measurement used. In the current study volume was defined as the amount of contrast medium that could be injected into the joint until rapid increase of pressure, pain, or lymphatic filling. Hendrix *et al.*[58] used the same criteria and found volumes of 7-70 mL in 31 patients. In the same way Barentsz *et al*.[7] measured a mean volume of 12 mL (6-30 mL). Seelen *et al*.[107] performed an arthrography in 30 patients with cementless hip prostheses with clinical suspicion for loosening. The mean volume was 18 mL (10-30 mL). Maus *et al*.[81] found a small pseudocapsule (0-10 mL) in 32% of arthrographies; a medium size (11-25 mL) in 47% and a large pseudocapsule (>25 mL) in 21% of 178 arthrograms. Volumes found in the current study cannot be compared with studies in which volume of the joint is measured by a different method.

The results of our study show that there is a higher percentage of loosening in hips with a contained joint than in non-contained hips. The association between non-contained joints (bursae and non-bursal communicating cavities) and loosening has not been consistent in the literature. Some authors find a higher amount of loosening in hips with a bursa.[95] Others conclude that the presence of bursal opacification does not have predictive value in identifying loosening or infection.[81] However, it must be noted that in the case of a bursa or leakage in the soft tissues, the pressure required for leakage of contrast medium from the periprosthetic space, cannot be built up. This can lead to false-negative results.[7] In this study the intra-articular pressure required to demonstrate loosening could not be achieved in joints with poor containment. Findings of loosening during revision surgery revealed that in non-contained joints sensitivity of the arthrography for loosening of the stem and cup was lower than in the contained group.

A drawback of the current study is that the injected volume was not always mentioned in the report of the arthrography by the radiologist.

Arthrography can be used to calculate the concentration and dose of the active

ingredient in patients that are considered for intra-articular therapy for hip prosthesis loosening and to exclude patients with leakage from the joint. The volumes that would be suitable for intra-articular injections in clinical studies will differ from study to study. The range of volumes to be included in a clinical study depend on several parameters, including toxicity and the predicted therapeutic window of the injected agent.

The data in this study can be used to predict how many patients will be excluded from intra-articular therapy protocols based on arthrography results. When hips from this study with a known volume are considered 27% (45 of 168) of cases will be excluded due to leakage of contrast medium. If there is also a limitation to the volume of active ingredient to be injected, the number of excluded patients will be larger. When a volume of mean ± 1 S.D. is considered to provide a drug concentration within the therapeutic range, without a risk for toxic side effects, the volumes to be included in the patients in this study would be 18-44 mL, corresponding to a percentage of included patients of 54% (Figure 2B).

Given the above-mentioned limitation, this study shows that 27% of hips with a clinically loosened prosthesis have a non-contained joint.

Conclusion

In this study containment of injected solutions within the joint space and the volume of that space is evaluated to optimise the delivery of therapeutic agents to joint tissue. The results of the study show that about one quarter of the hips with a clinically loosened prosthesis has a non-contained joint. When a clinical study with an intra-articular therapy is considered, an arthrogram is useful in the inclusion procedure to determine containment of the joint and the volume that can be injected.

6

Clinical protocol
Gene Therapy in aseptic prosthetic replacement loosening. A phase 1 study

Jolanda J. de Poorter[1]
Tom W.J. Huizinga[2]
Rob C. Hoeben[3]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

Scheme

Entry criteria

Elderly patients with debilitating pain from aseptic loosening of the hip prosthesis, as proven with arthrography, who are ineligible for surgery due to significant comorbidity

Registration

History, physical examination, radiological studies, laboratory data.

Treatment plan

Hospital admittance for 11 days. Intra-articular HAdV-5 vector injection on day 1, followed by intra-articular prodrug injection on day 3. Periprosthetic bone cement injection 4-7 days after prodrug injection under local anaesthesia (spinal). Mobilisation and discharge one day after cement injection.

Dose escalation

The first three patients will be injected with 3×10^9 HAdV-5 vector particles. Dose escalation will proceed to 1×10^{10}, 3×10^{10}, and 1×10^{11} HAdV-5 vector particles for three patients per dose group or until dose limiting toxicity occurs.

Evaluation

Laboratory toxicity evaluation during first six weeks follow up. Clinical evaluation (Harris Hip Score and VAS) at 3 and 6 weeks follow up, 3 and 6 months and every year. X-rays one day after cement injection, at 6 weeks and 6 months follow up and every year. Collection of excreta for analysis of shedding from 24 h after vector-injection until negative results.

Duration

One period of hospital admittance of 11 days with one single injection of vector and prodrug. Follow-up period of 5 years.

Introduction

In the Netherlands about 20,000 total hip prostheses operations are performed annually. In the minority of total hip replacements, loosening of the femoral stem and acetabulum occurs within 10 years due to aseptic loosening.[24,56] Wear particles, which are released from the polyethylene acetabular component, travel to all sites accessible for joint fluid (including periprosthetic spaces) and are phagocytosed by macrophages. This causes an aseptic inflammatory reaction with stimulation of osteoclast activity. Activation of osteoclasts in turn causes periprosthetic osteolysis and loosening of the implant. Apart from this direct effect mediators released by macrophages stimulate fibroblast-like cells to invade and destroy the bone covering the joint prosthesis. The inflammatory process takes place in interface tissue, which is located periprosthetically. Interface tissue from patients with loosened prostheses exhibits similar behavior as synovial tissue from rheumatoid arthritic joints. When prosthesis loosening occurs, patients will experience more pain and walking difficulty and have a higher risk for dislocations and pathological fractures.[54]

After 10 years of follow-up 7-13% of patients need revision of their prosthesis due to loosening of the implant.[79,112] Revision surgery has a high morbidity rate especially in elderly patients with co-morbidity. In a study by Strehle *et al.*[114] postoperative mortality (during hospital stay) in elderly patients was 5.7%. Especially patients in ASA 3 (grading of mortality risk according to American Society of Anesthesiologists)[121] with cardiac insufficiency had major complications such as myocardial failure or coronary artery disease. In some patients revision-surgery is not an option at all, because benefits don't balance the tremendously high mortality risk.

In order to minimise morbidity due to revision surgery we searched for alternative treatments. In knees (and in a few hips) radiotherapy has been investigated as a means to kill synovial tissue in rheumatoid arthritis. In these studies yttrium 90 is injected into the joint to destroy the synovial tissue. Yttrium 90 was chosen as the best agent for this therapy, because it is a β-emitting radionucleotide (good tissue penetration, but not too extensive) with a relatively large particle size (prevents leaking out of the joint) and a short half-life (reduces radiation exposure to non-target tissues).[25] The results of the studies with yttrium, and other radionucleotides, are diverse. However, in a meta-analysis of randomised controlled trials, no clear evidence of the efficacy of yttrium synovectomy is shown.[59]

Gene therapy is a tool for delivering individual proteins to specific tissues and cells. With gene therapy, target cells can be identified, and then a gene can be delivered to these target cells to produce a corrective or killing protein.[52] Human Adenovirus 5 is a virus that infects cells from interface tissue with a high efficiency.

Moreover, the virus has a high particle size which, in combination with the relatively closed joint space, prevents most of it from diffusing into other tissues. When using human adenovirus 5 as a vector to express the gene Escherichia coli *nitroreductase* (Ntr), infected cells become extremely sensitive to the prodrug CB1954. The activated prodrug causes death of the infected cells.[34] In a study by Goossens *et al.*[46] it was demonstrated that genes can be transferred to synovial tissue *in vivo* in rhesus monkeys, by direct injection into the joint, and that the synoviocytes can be killed with injection of a specific prodrug. In our laboratory previous experiments have shown the efficacy of the infection and destroying of synoviocytes and fibroblasts from interface tissue by HAdV-5-Ntr (CTL102) and CB1954.[30] This study aims to destroy the interface tissue (which causes the loosening), by sensitising the cells to the prodrug CB1954, by means of HAdV-5-Ntr (CTL102). Successful destruction and removal of the interface tissue is expected to create a periprosthetic space accessible to the injection of bone cement. To stabilise the prosthesis and re-anchor it to the bone, bone cement is injected in the periprosthetic space. This method for stabilising loosened femoral stems and acetabula could be especially valuable in patients with high risk for complications due to surgical intervention, who are thus defined inoperable. Patients are admitted to the hospital for 11 days and there is no need for rehabilitation afterwards. This is intended to increase the mobility of the patient and decrease pain, without the risk of surgical complications.

Objectives

Primary endpoint

To assess the safety of intra-articular injection of the HAdV-5 vector CTL102 and the prodrug CB1954 and the percutaneous peri-prosthetic injection of bone cement.

Secondary endpoints

- To histologically investigate a biopsy of interface tissue after the procedure to make an assessment of the alterations induced by the intervention.
- To establish that this procedure does not cause shedding of the recombinant virus into the environment.
- To investigate the possibility to stabilise the loosened prosthesis by means of bone cement.
- To investigate clinical results, i.e. pain relief and improvement in ADL

Subjects

Inclusion criteria

- aseptic loosened femoral stem implant, as proved by arthrography
- debilitating pain causing ADL dependency
- arthrography volume ≤ 45 ml, but injection of a volume of ≤ 30 ml will give sufficient exposure of the interface tissue to the contrast medium
- significant co-morbidity (ASA 2 and more) causing high mortality risks (>2.5%) during or after surgery
- ability to give informed consent and express a willingness to meet all the expected requirements of the protocol
- patients must meet the following baseline laboratory value guidelines at the screening and on day 0

a	Haemoglobin	> 6 mmol/l
b	Leukocytes	4.0 – 20.0 x 10^9/l
c	Platelets	> 100 x 10^9/l
d	AST	< 50 U/l
e	ALT	< 56 U/l
f	AF	< 150 U/l
g	Bilirubin	< 21 μmol/l
h	Creatinin	< 166 mmol/l
I	PT	< 14 sec

Exclusion criteria

- patients who fail to meet the inclusion criteria
- infection of endoprosthesis
- patients with obvious adenoviral infection (eye, nose/throat)
- patients with a history of hepatitis A, B or C or HIV infection
- patients with a history of alcohol or drug abuse
- previous gene therapy of any kind
- patients who underwent chemotherapy, radiotherapy or immune therapy in the previous 28 days
- patients with known immunodeficiency
- patients with known allergy to *E coli* proteins
- patients with life expectancy of <6 months
- non-cooperating patients
- patients not able to read and understand Dutch language

Ethical considerations

Regulatory statement

The study will be conducted according to the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice and Hong Kong, Somerset West and Edinburgh), and in accordance with the Guidelines for Good Clinical Practice (CPMP/ICH/135/95-17th July 1996). The trial will be conducted in compliance with the protocol.

Recruitment and consent

The protocol of this study and any subsequent amendments will be submitted to the Medical Ethics Committee (CME) of Leiden University Medical Centre and the CCMO (Central Committee on Research Involving Human Subjects). The study will not commence before formal approval has been granted.

Patients will be recruited from the outpatients' clinic of the department of Orthopedic Surgery in Leiden. Patients who meet inclusion and exclusion criteria will be informed that they are a potential study candidate, by their own orthopaedic surgeon. They will be referred to the researcher, who will inform the patient about the study. Information will be given both orally and written. The researcher will answer any questions the patient may have about the study at that time or later, and offer the patient the opportunity to participate in the study. She will also be responsible for obtaining informed consent from the patient. Patients will be given as much time as they wish to decide on participation in the study. At least 1 week should pass between the supplying of information and a positive decision to participate in the study. After approval by the subjects, their general practitioners will be notified. Although the subjects will be told they are free to leave the study at any time, it will be attempted to recruit subjects who are likely to continue the study to completion.

Justification for the burden of the patients

A loosened prosthesis needs to be stabilised to regain function and decrease pain. The current treatment to stabilise the prosthesis is a revision arthroplasty in which the loosened prosthesis is removed and a new one is placed. Patients who undergo revision surgery will have spinal anaesthesia (or general anaesthesia). The mean surgery time for revision THA (total hip arthroplasty) is two to four hours. During surgery 1-2 litres of blood may be lost, after surgery drainage systems will collect an additional 500-1000 ml. Complications like cardiopulmonary problems may occur during or after surgery. One major risk after revision surgery is the likelihood for deep prosthetic infection (3-5%), which will

indicate removal of the prosthesis. Rehabilitation will take about 2-3 months. Patients accept this burden as a price they pay for better function of the hip and less pain.

In this study, patients with a loosened prosthesis, who cannot be operated upon, are included in the study. Currently, no treatment is available for these patients. Therefore patients included are not restrained from adequate therapy.

To adequately refix the prosthesis the interface tissue (which is responsible for the loosening) should be removed. In revision surgery this is performed manually. *In vitro* studies in our laboratory have shown that interface tissue can be killed by CTL102/CB1954 administration. To optimise local dose concentration and minimise systemic effects, CTL102 and CB1954 need to be administered as locally as possible. Intra-articular injection is an adequate method for local administration of fluids and is well tolerated. To assure free access of fluids to the periprosthetic space only patients are included with an arthrogram that shows contrast medium around the prosthesis. Therefore patients need to undergo three arthrographies (one to assure access of contrast medium, one to inject the viral vector, and one to inject the CB1954 prodrug).

When the interface tissue is successfully diminished the prosthesis needs to be refixated. To re-anchor the prosthesis to the bone, cement is injected in the periprosthetic space.

Although the primary objective of the study is to assess toxicity of local administration of CTL102/CB1954, it would be unethical to deny a patient a potentially effective therapy. For the injection of the cement, holes have to be made through the bone into the periprosthetic space. As the bone biopsies are rather painful and the bone could not be anaesthetised locally, the patients undergo spinal anaesthesia, with small risk of side effects.

To assure safety for the environment, patients will be isolated until viral shedding by the patient is excluded. Therefore patient's excreta (nose and throat swabs, urine, and faeces) are collected and analysed until negative results are obtained. Then isolation is discontinued.

To investigate safety for the patient, history, physical examination and laboratory parameter measurements are done. These examinations are necessary to detect and treat side effects as soon as possible.

Compensation for injury

The chance of injury as a result of the study is small. However the LUMC has insured this risk by taking liability insurance that is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Temporary Measure regarding Compulsory Insurance for Clinical Research in Humans of 5th July 1999).

This insurance provides cover for damage to research subjects through injury or death caused by the study. Conditions for benefit of insurance amounts are noted in the WMO-declaration.

Trial medication and investigational products

Name and description of investigational products

- The proposed treatment involves intra-articular injection of a replication deficient adenoviral delivery vector (CTL102), engineered to deliver the gene for the enzyme E. coli *nitroreductase* (Ntr). Cells infected with the vector will synthesise the enzyme under control of the CMV promoter. The drug product CTL102 is supplied by ML Laboratories plc as a sterile, clear, colourless, isotonic aqueous solution at a nominal mean potency of between 5 x 10^8 and 1 x 10^{12} particles per ml, buffered to pH 7.4, in single use polypropylene vials. CTL102 is manufactured by Cobra Biomanufacturing plc, Keele, UK, ST5 5SP and formulated into the finished dosage form by Q-One Biotech Ltd., Glasgow, G20 0XA.
- The drug product CB1954 is supplied by ML Laboratories plc as a sterile solution of CB1954 17.8 mg/ml in solvent (N-methyl-pyrrolidone 22.2% $^{v}/_{v}$, polyethylene glycol 300 77.8% $^{v}/_{v}$). Just prior to use this prodrug in solvent is diluted using sterile saline to a maximum final concentration of 2 mg/ml.
- To stabilise the prosthesis low viscosity PMMA bone cement is used.
- For arthrography iodide contrast medium is used.
- Ethylene diamine tetra-acetate (EDTA; Sigma Chemical, St Louis, MO, USA) is used in a concentration of 2mM in a saline solution to rinse the joint before injection with the vector and prodrug.

Summary of findings from non-clinical studies

Pharmacological characteristics

Genetic material delivered by adenovirus is not integrated in the genome of the target cells, because neither wild type nor recombinant adenoviruses are capable of integrating their genomes into infected host cells. The CTL102 virus genome remains extra chromosomal and does not replicate in the transduced cells. In all cell-lines and animals tested, CTL102 has the same tropism and infectivity as the wild type HAdV-5 from which it is derived. The primary effect of CTL102 infection of a cell will be the delivery of the Ntr gene expression cassette. After cell entry and unpackaging from the viral capsid, the CTL102 genome will exist as an extra chromosomal DNA element from

which the machinery of the infected cell will transcribe Ntr mRNA. This in turn will be translated into active nitroreductase, which will accumulate in the cytosol. Expression of the Ntr and its persistence is transient both in animal models and cell lines *in vitro*. This is presumed to be due to a combination of protein turnover within the cell, the spontaneous loss of CTL102 DNA in infected cells and cell turnover within the population.

Intracellular Ntr activity is responsible for the activation of prodrug CB1954 to a toxic bifunctional alkylating agent inside the cell. Cells able to bioactivate CB1954 are cytotoxically affected by crosslink formation at very high frequency. As a consequence, cells activating CB1954 are destroyed by death.

The intended outcome of this study is that infected cells expressing Ntr, which take up CB1954, will be killed by the activated prodrug.

The potential utility of the GDEPT (Gene-directed enzyme prodrug therapy) approach is augmented by the so-called "bystander-effect" in which neighbouring untransduced interface cells are killed during the prodrug therapy.[127] The Ntr/CB1954 system offers high selectivity because no natural human enzyme can activate the prodrug sufficiently to cause significant biological activity to kill cells *in vivo*.

Summary of animal experiments

In mice the peak expression level of Ntr is achieved 48 h after injection of CTL102. Continued expression ensures adequate levels of the enzyme for prodrug activation over several days. In intravenous administration of CTL102 in mice adenovirus DNA is not seen in germ cells. In the clinic CTL102 will be administered intra-articularly with low risk of systemic exposure to the vector. The safety of CTL102 has been demonstrated in single dose and in repeat dose studies in the mouse and in local tolerance studies in the rat. Administered intravenously in single doses up to 8.5×10^{10} particles/ml at a dose volume of 200 μL per animal, CTL102 was well tolerated and at higher doses resulted in some liver damage, reflected by increased liver weight, enzymes and histological effects.

In monkeys CB1954 was injected intravenously. The elimination half-lives (1.5 h) indicate there is rapid clearance of the prodrug from the plasma. Clearance is probably both hepatic and renal. In general, doses given to mice intravenously represent a 1-2 x 10^3 fold safety margin over the same dose given in humans.

To assess local tolerance CB1954 was injected subcutaneously in male CD-1 mice. High doses were injected (1.5, 6, and 24 mg/kg). As a controls group either the vehicle (5% N-methyl pyrrolidone, 17.5% PEG300 and 77.5% physiological saline) or physiological saline alone was injected. No local responses considered to be related to treatment with CB1954 were evident at macroscopic or microscopic examination of the

injection site or local lymph nodes. It was concluded from this study that a single subcutaneous administration of CB1954 to male CD-1 mice at dosages up to 24 mg/kg, was well tolerated, and was without local effect in the subcutis at the injection site or local lymph nodes.

Pharmacokinetic characteristics

Pharmacokinetic results indicate that CB1954 has a mean volume of distribution of 14.2 litres/m^2 (range 12.8-18.5 liters/m^2), a half-life of about 18 minutes (range 4-35 minutes) and clearance values (range 0.7-1.5 liters/min), which approximate hepatic blood flow.

Summary of findings from clinical studies with adenovirus gene transfer vectors and CB1954

In 1999 18-year-old Jesse Gelsinger died after a direct infusion of an Ad-vector in the right hepatic artery. He received 3.8 x 10^{13} Ad-vector particles, the highest dose ever injected in humans. After reviewing clinical and post-mortem findings as well as other related studies, members of the Working Group concluded that the research participant's death most likely resulted from a systemic, Ad vector-induced shock syndrome. A cytokine cascade led to disseminated intravascular coagulation, acute respiratory distress, and multi-organ failure. Post-mortem bone marrow biopsy revealed red cell aplasia. The data suggested that the high dose of Ad-vector delivered by infusion directly to the liver, quickly saturated available receptors for the vector within that organ and then spilled into the circulatory and other organ systems, including the bone marrow, thus inducing a systemic response.[8]

After the tragical death of Jesse Gelsinger doses given to patients by intravenous route were limited to 10^{11} Ad-vector particles. Besides, safety criteria for clinical grade adenovirus vector preparations were established, including the criteria of being free of endotoxin and infectious agents and containing $\leq$1 replication-competent adenovirus (RCA) for the total dose to be delivered.

In 2002 Harvey and Crystal brought out two lengthy reports concerning the safety and toxicity of local delivery of low ($<10^9$ particles)- and intermediate (10^9-10^{11} particles)-dose adenovirus gene transfer vectors in humans. The studies analyse the adverse events (AE), abnormal laboratory parameters, and deaths observed in 8 trials involving a total of 140 local administrations of low and intermediate doses of adenovirus gene transfer vectors to 6 sites in 90 individuals. The total experiences from the 8 trials were grouped to determine whether 10 putative risk factors could be predictive of the safety of trials. The risk factors were structured in 3 groups, associated

with the individual (age, sex, comorbid index[21], and pretherapy anti-Ad antibody titer), adenovirus vector (dose, route of administration, transgene in the expression cassette, and number of vector administrations), and the gene transfer trial (trial design and involvement of major surgery in the trial). It was found that only the comorbid index was a predictor of death. For major adverse events other than death, age and whether surgery was part of the trial were determined to be predictors. As no significant association of vector-related factors with major adverse events is seen, it is suggested that local administration of these doses of HAdV-5 vectors appear to be well tolerated. For all studies combined, the most common abnormal laboratory parameters were a low haemoglobin (13.7%) and elevated WBC (12.8%), both occurring mostly in association with surgery and returning to normal values within 30 days. Abnormal laboratory values were mostly seen in participants over 50 years of age and the number of adverse events increased with age. Anti-HAdV-5 neutralising antibody did not appear to be associated with any aspect of AE or abnormal laboratory parameter. The frequency of AEs and abnormal laboratory results slightly increased with HAdV-5 vector dose.[26,55]

There are several differences from the experimental animal studies discussed earlier and the human Ad5 vector trials by Crystal *et al.*[26] in which no association could be found between vector dose and major adverse events. First, most studies demonstrating major 'toxicity' of HAdV-5 vectors in experimental animals use doses 10^2-to 10^3-fold greater per kilogram body weight than the doses used in the human studies. Second, all the routes used in the human study were local, whereas most experimental animal studies use systemic administration. Third, in all human studies HAdV-5 vector preparations were thoroughly controlled before injection. Finally, HAdV-5 vectors may interact differently with blood elements of humans from that of animals.

25 patients with operable liver, head/neck or prostate tumours have been injected intra-tumourally with CTL102 at doses ranging from 10^8 to $5x10^{11}$ particles. No treatment-related serious adverse events have been observed. ELISAs to detect viral proteins have been conducted on urine, stools and plasma samples 24 h after injection, and the results showed no detectable shed virus at any dose. Following surgical resection of tumours in these patients Ntr expression has been monitored by immunohistochemistry on tumour sections. Dose-dependent Ntr expression has been observed, and at the higher doses Ntr expression observed in >30% of all sections through the tumours from several patients.

Toxicity of CB1954 prodrug was tested[23] in humans receiving intraperitoneal doses of 3, 6, 12, 18, and 24 mg/m^2 and intravenous doses of 3, 6, 12, 18, 24, 30, and 37.5 mg/m^2. There was mild transient treatment-related toxicity due to CB1954 observed in local (i.p.) and systemic (i.v.) administration of doses up to 24 mg/m^2. In

intravenous administration dose limiting toxicity occurred at a dose of 37.5 mg/m^2. 24 mg/m^2 is the CB1954 dose selected for the CTL102/CB1954 clinical studies as giving adequate plasma levels without toxicity.

In this clinical study CB1954 will be injected intra-articularly ensuring optimal exposure to the target tissue. Due to the small particle size of CB1954, the particles will probably leak out of the joint soon. However, when a dose of 24 mg/m^2 is injected in the relatively small joint the concentration will probably, for a short duration, be so high that it is toxic on its own. However, for efficient killing of the interface cells, a sufficiently high concentration is needed for a sufficiently long period. Therefore we take the advantage of good exposure to the interface cells over the possible toxicity. Patients will be given up to the maximum systemic dose, which is 24 mg/m^2. The dose will be limited by the volume of the periprosthetic space and the solubility of the CB1954 (2 mg/ml).

After five patients the dose was lowered to 16 mg/m^2 in conformity with protocol amendment 2 (03 Feb 2005) due to gastro-intestinal side effects and rise in liver transaminases.

Summary of known and potential risks and benefits

Safety observations found in human studies with other HAdV-5 viral vectors are confirmed with animal studies on CTL102:

- HAdV-5 vector leakage into the systemic circulation from the site of intratumoural administration gives rise to transgene delivery to the liver (mainly), as well as the spleen, kidney and lung.
- Leakage from the administration site increases with the speed of injection, as well as with the dose of the vector.
- The presence of anti-HAdV-5 neutralising antibodies tends to be ubiquitous in human adults and can be induced in animal models by simple exposure to the wild-type virus (e.g. intranasally). Such neutralising antibodies severely reduce accidental/incidental liver transduction by up to 1000-fold while peak transgene-expression was only reduced by 2.4-fold in the tumours of immune animals in intratumoural viral vector injection.[15] Varnavski *et al.*[123] performed a study to assess the impact of pre-existing immunity to HAdV-5 on the activation of innate immune responses to systemically administered HAdV-5 vector. They found that levels of IL-6 in serum after vector administration were higher in pre-immunised rhesus monkeys, relative to those in naïve animals. They concluded that the pre-existing immunity increased the activation of innate immunity. However, the HAdV-5 dosage used in the study is a sub-lethal dose; it is not clear whether a lower dose would render the same

results. Furthermore, only two monkeys are used in both groups, and the question is whether the same results would be found in larger groups.
- Possible local toxicity of high concentration of CB1954. Possible local toxicity will not be monitored, because invasive interventions are needed to evaluate this. However, we don't expect local toxicity as subcutaneous injection of CB1954 into mice at a dose of up to 24 mg/kg did not result in toxic effects at the injection site judged macroscopically.

Expected adverse events during the study
Intra-articular injection in the hip may cause moderate pain in the joint.

Rare events during the study
As with any therapy, rare side effects cannot be excluded beforehand. Occasional reports of the following adverse events have been made:
- When using strict aseptic techniques there is a risk of approximately 0.01% that an infection will occur after intra-articular injection. If infection occurs, this complication will be treated with antibiotics and drainage.
- Allergic reactions to contrast medium can occur
- In the case of systemic exposure to significant amounts of CTL102 healthy tissues infected by the viral vector will also express Ntr and could sustain damage following CB1954 exposure. The liver is in principal at greatest risk of damage as the liver takes up the majority of blood borne virus.
- From studies it cannot be fully excluded that transgene products can be integrated in germ cells, although animal studies show no integration. Therefore, patients will be expected to practice contraception for 80 days. This is the period of time in which CTL102 can be detected in gonads after administration of the viral vector.

Potential benefits
If gene therapy is successful and interface tissue is destroyed, patients can experience benefits from participation in the study. Bone cement injected into the periprosthetic space can then probably stabilise the endoprosthesis, thereby improving ADL-function (ADL = activities in daily living) and reducing pain from the loosened prosthesis.

Description and justification of route of administration and dosage
To optimise local delivery of the vector and prodrug to the interface tissue, these products will be injected intra-articularly in the diseased hip. Because of the large differences in the size of virus and prodrug, these agents will act differently. The virus has a

large particle size and when the HAdV-5 vector is administered in the joint space, most of the viral vector will probably stay there. As the joint space is a more or less closed system, shedding of the virus to other body compartments will be minimised. Therefore, local administration will give less systemic adverse effects than systemic administration. Moreover, in local administration the same dosages (compared to systemic administration) will give better therapeutic effects. The periprosthetic space is accessible to joint fluid (especially in aseptic loosening) and cells injected intra-articularly can thus probably reach target cells (interface cells). During screening period, only patients are included with an arthrogram confirming that the contrast medium is easily distributed in the total periprosthetic space. As in these patients the contrast medium is distributed through the entire periprosthetic space we expect that the viral vector and prodrug will also be distributed throughout this area. To establish a sufficiently high concentration of CTL102 we will only include patients in the study in which a small to medium arthrography volume ($\leq$ 30 ml) will give sufficient exposure of the interface tissue to the vector. Although arthrography is a rather invasive procedure in the patient group to be included, we consider it the only possible way to prove accessibility of the entire periprosthetic space.

The distribution of CB1954 is a rather different story. CB1954 is a very small molecule and will thus easily diffuse from the injection site. To assure optimal exposure of the prodrug to the transduced interface cells the maximal systemic CB1954 dosage without treatment-related toxic side effects is used. This dose (24 mg/m^2) will be administered in one injection in the hip joint*. During a short period a very high concentration of CB1954 will be present in the joint. This high dose is possibly toxic on its own (in non-infected cells) and may cause death of some non-infected cells. However the highest possible dose is needed to assure a sufficiently high concentration for a sufficiently long period.

As this is a phase I study treatment will start at a low dose of virus. Earlier studies showed no serious adverse events when using local administration of $<10^{11}$ HAdV-5 vector particles in humans. In this study no dosages of $>10^{11}$ particles will be administered. First three patients will be injected with 3 x 10^9 particles. When no dose-limiting toxicity (DLT) occurs for at least 14 days, the next three patients will be injected with a dosage of 1 x 10^{10} particles. Dose limiting toxicity is defined as any grade 3 or 4 adverse event except for nausea and vomiting. In this way dosage increment will continue to a dose of 3 x 10^{10} particles and finally 1 x 10^{11} particles will be given to the last three patients. When DLT occurs in 1 patient of three, three more patients will be included in

* After five patients the dose was lowered to 16 mg/m^2 in conformity with amendment 2 (03 Feb 2005).

the same dose. When no other DLT occurs in these patients, the dose will be increased. When DLT occurs in 2 or more of the six patients, the given dose will be the maximal administered dose and three more patients will be included in the previous dose group. When DLT occurs in 2 or more patients of three, no more patients will be included in this dose group.

CB1954 prodrug will be injected intra-articularly 48 h after administration of the viral vector. Previous studies have been done in ML Laboratories to observe optimal transduction duration of CTL102. It was shown in animal studies that peak expression level of nitroreductase (Ntr) was obtained 48 h after intravascular or intratumoural injection of CTL102.

In earlier studies optimal CB1954 dosage without treatment-related toxic side effects was found to be 24 mg/m^2. In our *in vitro* studies we pointed out that a CB1954 concentration of 50 μM for 24 h gives cell viability of <20% in vector concentrations of 200 pfu/cell. Chung-Faye *et al.*[23] studied toxicity of CB1954 in humans using intravenous and intra-peritoneal injections. In intravenous injection, a dose of 24 mg/m^2 gave a peak serum concentration of 6.3 μM. After 2 h the concentration had decreased to 1 μM. In intra-peritoneal injection, the same dose resulted in a peak concentration of 70 μM. After 18 h the concentration had dropped to 1 μM. As intra-articular injection has a small distribution area, we expect that a dose of 24 mg/m^2 will give a sufficiently high concentration (50 μM) during a sufficiently long period (24 h) to destroy interface cells. In order to maximise the likelihood of achieving adequate exposure of CTL102-infected interface cells to CB1954 the prodrug will be injected intra-articularly at a maximum dose of 24 mg/m^2. The dose will be limited by the joint capacity and the maximum solubility of the prodrug (2 mg/ml), and will have a maximum of 24 mg/m^2*. Local toxicity is not expected to occur as the prodrug will leak out of the joint space rapidly following injection. Indeed, because of this rapid efflux it will be essential to inject as much prodrug as possible. Consistent with this, subcutaneous injection of CB1954 into mice at a dose of up to 24 mg/kg did not result in toxic effects at the injection site judged macroscopically and microscopically. Systemic toxicity will not occur as this dose has been shown to be well tolerated when administered by intravenous and intraperitoneal routes.

Preparation of treatments

Treatments will be prepared in a clean room within the section IGFL (Interdivisional GMP facility LUMC) (GMP (=good manufacturing practice) facility), being part of the department of Clinical Pharmacy and Toxicology, by a specially trained and authorised

* After five patients the dose was lowered to 16 mg/m^2 in conformity with amendment 2 (03 feb 2005).

person. Preparation, transport and other actions done with the vector will be performed according to the safety regulation appended.

It is not possible to blind the results. Patients have to be injected in increasing dosages to assess treatment-related toxicity. It is therefore not necessary to label the treatments in a manner in which dosage is blinded from investigators.

Procedures for monitoring subject compliance

Study medication (HAdV-5 vector and CB1954) will be administered under direct supervision. Two investigators will check the medication label for agreement of the contents with the study number and the subject's identity. After intra-articular injection, both investigators will sign the drug deposition form.

Methods

Trial design and sample size

The study will be a Phase I, open-labeled, non-randomised trial. Patients who meet the inclusion- and exclusion-criteria will be asked to participate in the study. Informed consent will then be obtained from the study participants. Power estimations are not possible because there are no results from relevant earlier studies. The study scheme is outlined in table 1.

A total of 12 patients will be enrolled in the study. The first three patients will receive 3 x 10^9 HAdV-5 vector particles, the second three will receive 1 x 10^{10} particles, the next three 3 x 10^{10} particles and the last three 1 x 10^{11} particles. The particles will be added to saline in a volume 10% less than the volume used at the inclusion arthrogram. This to prevent lymphatic drainage of the vector due to a high pressure, while creating a pressure high enough to allow the fluid to reach the periprosthetic space. Two days after injection of the viral vector (day 2) patients will receive CB1954 by intra-articular injection at a concentration of 2mg/ml. The total dose will depend on the arthrogram volume of the treated patient and will thus vary from patient to patient. The dose will, however, not exceed 24 mg/m^2*. To minimise influence of synovial fluid on the vector and prodrug the joint will be extensively rinsed with 2 mM EDTA solution before administration of vector and prodrug. The EDTA will improve efficiency of gene transfer, whereas viability of the cells is not influenced. In a study by de Roos *et al*.[31] infection of liver cells increased from 21% to 54% after administration of EDTA. The

* After four patients the dose was lowered to 16 mg/m^2 in conformity with amendment 2 (03 Feb 2005)

exact mechanism of the EDTA on the improvement of gene transfer is unknown, but it is assumed that the EDTA causes dissociation of the cells or widening of the fenestrae in the cell by binding of Ca^{2+}. After saturation of the EDTA by Ca^{2+} (from the interface cells and from the bone) and other metal ions, the effect on the cells is extinguished and EDTA is excreted by the kidneys. On day 9 interface biopsies will be taken to investigate efficacy of the interface destruction. The patient will undergo spinal anaesthesia and a thick needle is introduced in the bone through which the biopsy is taken (at three sites around the femoral stem and at one site in the acetabulum). The needle will then be used as an introduction site for the injection of bone cement (figure 1). Bone cement is injected in the periprosthetic space under fluoroscopic guidance to prevent leakage of the cement into the joint space.

Figure 1. Schematic outline of study

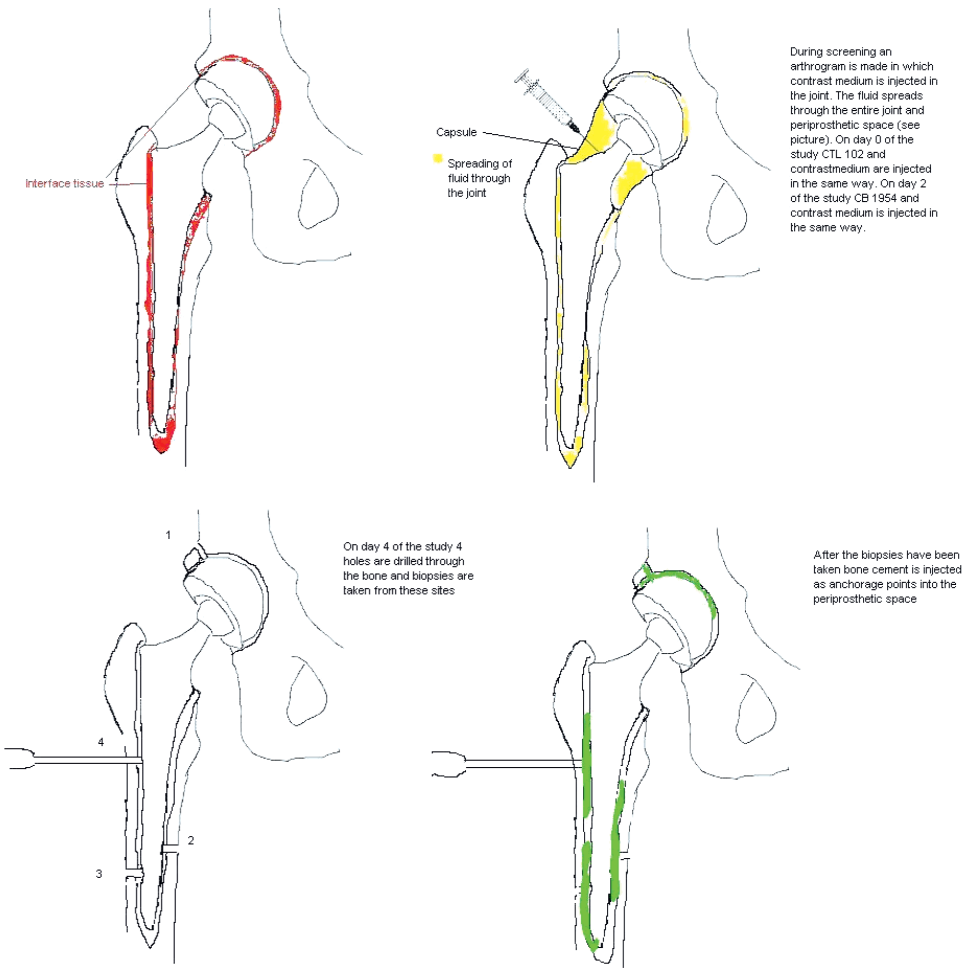


Table 1. Schematic presentation of frequency investigations

	Inclusion	0 d	1 d	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	10 d	4 w	6 w	3 m	6 m	1 y	every year
History, physical examination, AEs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
RR and Temp	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Ad5.Ntr injection		x																
Arthrography	x	x		x														
Xrays	x											x				x	x	x
CB-1954 injection				x														
Blood sampling	x	x		x			x		x				x	x	x		x	
Biopsies, cement stabilisation											x							
Collection excreta			x	x[a]	x[a]	x[a]	x[a]	x[a]	x[a]	x[a]	x[a]							
VAS for pain	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
VAS walking distance + independency, HHS	x											x	x	x	x	x	x	x

Abbreviations: d: day; w: week; m: month; y: year; HHS: Harris Hip Score; RR: blood pressure
Blood test: HB, HT, WBC and differentiation, platelets, ESR, CRP, creatinin, Na, K, Ca, Mg, albumin, amylase, glucose, AST, ALT, LDH, Alk Phos, bilirubin and PT, PTT; CB1954 concentration (on day 2 (2x))
a: whenever earlier samples were positive

Primary study endpoints

Adverse events

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the intervention. Adverse events reported by the patient or observed by the researcher or other investigators will be subscribed and classified according to intensity, duration and time of occurrence. All adverse events will be recorded on the adverse event data collection form.

For each event the relationship to drug (definite, probable, possible, unknown, definitely not) as judged by the investigator, as well as eventual actions taken, will be recorded. The occurrence of an adverse event that is fatal, life-threatening, disabling or requires or prolongs in-patient hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly of birth defect will be described according to Clinical Trials Directive 2001/20/EC as 'serious adverse event (SAE)'.

The responsible investigator will report SAEs as follows:

- All SAEs will be reported to ML Labs. plc. by telephone and in writing as soon as practical, but at least within 24 h of recognition;
- All SAEs will be reported by the investigator to the Medical Ethics Review Board and the CCMO that approved the study, by telephone and in writing as soon as practical, but at least within 15 days;
- SAEs with a suspected (probable or definite) relationship to trial medication (as indicated by the responsible investigator) will be reported to the Drug Safety Unit of the Healthcare Inspectorate ('Hoofdinspectie voor de Farmacie en de Medische technologie, Inspectie voor de Gezondheidszorg').

Reports to the Health Authorities are submitted in conjunction with ML Labs. plc. ML Labs can prepare additional reports for other authorities.

Safety parameters

Blood pressure, temperature and 22 laboratory parameters (see below) are measured. We specifically chose these parameters because they are relevant to laboratory abnormalities observed in experimental animals receiving high doses of HAdV-5 vectors. Abnormality of laboratory parameters will be classified according to WHO recommendations for grading of acute and sub-acute toxic effects.

- *General safety measurements*

  After HAdV-5 vector administration the researcher will visit the patient at least according to the following scheme and whenever it is clinically indicated. In each visit medical history and physical examination (inspection of injection-area and auscultation of heart and lungs) will be done. Furthermore, vital signs will be recorded.

Visits to the patient will be made directly before and after viral vector injection, ½ hourly to 4 h, hourly to 8 h and 4-hourly to 24 h. After 24 h, visits will be made at 6 hourly intervals until CB1954 administration. Vital signs will then be recorded daily until discharge from the hospital, and at each assessment point throughout the study.

- *Safety laboratory measurements*
  Hematology*:* The following assessments will be performed: Haemoglobin, hematocrit, ESR, white cell count (WBC), leukocyte differential count and platelet count.
  Blood biochemistry**:** The following assessments will be performed: lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, glucose, amylase, bilirubin, creatinin, Na, K, Ca, Mg, albumin, CRP, partial thromboplastin time (PTT) and prothrombin time (PT). CB1954 concentration in the blood will be measured 5 minutes and 1 and 2 hours after administration.
  Urine: In the patient's room a urinestick will be dipped in the urine to assess proteinuria and haematuria.

Secondary study endpoints

- *Investigation of a biopsy of interface tissue after the procedure*
  On day 9 of the study patients will undergo an interface tissue biopsy under spinal anaesthesia. Interface tissue biopsies will be done using Jamshidi needles. From biopsy holes interface tissue will be taken. The Pathology laboratory will describe the histological appearance of the interface tissue and compare it to interface tissue derived during revision surgery.
- *Endpoints to exclude shedding of the virus and prodrug*
  To assess whether any of the vector was shed from the target organ systemically or in the environment patients will be monitored on blood, urine, pharyngeal, nasal, and rectal samples. Samples will be collected for PCR analysis 24 h after CTL102 injection, and daily until negative results are obtained. CB1954 concentration is measured in the blood 5 minutes and 1 and 2 h after administration.
- *Endpoints to investigate stabilisation of the prosthesis in the femur*
  X-rays will be made to estimate periprosthetic space before and after the procedure. X-rays will be made at screening and one day after cement injection, after 6 months and then every year.
- *Endpoints to investigate clinical results of the study*
  Pain, independency and walking distance
  Pain experienced by the patient will be measured by using a Visual Analogue Scale (VAS). In this, patients are asked to score the pain they experience on a gliding

scale. The scale is a 10 cm long line, at the left beginning with 'no pain at all' and at the right ending with 'unbearable pain'. Patients can score their pain by placing a mark on the gliding scale. In the same manner a visual analogue scale will also be used to quantify independency and walking distance.

Ability to walk/ improvements in ADL

Improvements of ADL-function will be measured using the Harris Hip Score. In this score performance is measured on the following items: pain, limp, support, walking distance, stair climbing, putting on socks and shoes, sitting, and ability to use public transport.

Withdrawal of individual subjects

Subjects can leave the study at any time and for any reason if they wish to do so without consequences. The responsible investigator can also withdraw a subject if any of the following events occur:

- Significant protocol violation or non-compliance, either on the part of the patient or the investigator
- Refusal of the patient to continue treatment and/or observations
- Any infection after vector administration, not intended in the study
- Unacceptable or dose limiting toxicity
- Decision by the investigator that termination is in the patient's best medical interest
- Unrelated medical illness or complication
- Loss to follow-up
- Death of patient (will be reported to the researcher by the general practitioner)

Replacement of individual subjects after withdrawal

Patients who withdraw from the study will not be replaced by other patients.

Follow up of subjects withdrawn from treatment

Patients withdrawn from treatment will preferably be followed up in the same manner as other patients in the study. When patients withdrawn from the study don't consent to further follow up a last analysis is done to investigate whether there are any toxic effects at that moment (laboratory parameters and adverse events) and whether there is any viral shedding to the environment (urine, faeces, blood, and nose and throat swabs). In abnormality of parameters necessary actions will be taken to secure safety for the patient and the environment.

Discontinuation criteria for the study

The study will be discontinued when an unacceptable adverse event occurs, that is definitely or probably related to the procedures in the study.

Data analysis

Data handling and record keeping

Data will be recorded first on a paper Case Record Form (CRF) and then on a spreadsheet in which all patient's data are listed. After validation data will be entered in a computer system for subsequent tabulation and statistical analysis. The data will be handled confidentially and anonymously. All patients who enter the trial will be analysed, the patients who complete the trial as well as patients who withdraw from the study after injection of the virus and prodrug.

Statistical analysis

Statistical analysis will be performed at the Department of Orthopaedic Surgery in the LUMC.

Means and standard deviations will be used to describe outcome parameters, supplemented by calculation of confidence intervals wherever this aids interpretation. A p - value of <0.05 will be considered significant. Graphical presentations will be made in which patterns of adverse events are displayed.

Laboratory parameters will be subjected to linear regression in which 1.25 x upper normal value is taken as upper limit of normal values. Also a linear regression is done in which a 25% difference between baseline and follow-up values is taken as upper limit for normal variation.

Appendix 1. WHO-recommendations for grading of toxic side effects

	Grade 0	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Hematological					
Leukocytes ($x10^9$/L)	4.0 – 20.0	3.0 – 3.9	2.0 – 2.9	1.0 – 1.9	< 1.0
Thrombocytes ($x10^9$/L)	≥ 100	75 - 99	50 - 74	25 - 49	< 25
Hemoglobin (mmol/L)	≥7.5	6.2 – 7.4	5.0 – 6.1	4.0 – 4.9	< 4.0
Granulocytes ($x10^9$/L)	≥ 2.0	1.5 – 1.9	1.0 – 1.4	0.5 – 0.9	< 0.5
Lymphocytes ($x10^9$/L)	≥ 2.0	1.5 – 1.9	1.0 – 1.4	0.5 – 0.9	< 0.5
Hemorrhage	None	Mild, no transfusion needed	Gross, 1-2 units transfusion needed	Gross, 3-4 units transfusion needed	Massive, > 4 units transfusion needed
Coagulation					
Protrombin time (sec)	≤ 14	14.1 – 17.5	17.6 – 21	21.1 - 28	> 28
APTT (sec)	≤ 33	33.1 – 54.8	54.9 – 76.9	77 - 99	> 99
Metabolic					
Hyperglycemia (mmol/L)	< 6.4	6.4 – 8.9	9.0 – 13.9	14.0 – 27.8	> 27.8
Hypoglycemia (mmol/L)	> 3.6	3.1 – 3.6	2.2 – 3.0	1.7 – 2.1	< 1.7
Amylase (U/L)	50 – 220	221 – 330	331 – 440	441 – 1100	> 1100
Hypocalcaemia (mmol/L)	< 2.72	2.72 – 2.95	2.96 – 3.21	3.22 – 3.44	> 3.44
Hypocalcaemia (mmol/L)	> 2.15	2.00 – 2.15	1.79 – 1.99	1.56 – 1.78	< 1.56
Hypomagnesaemia (mmol/L)	> 0.57	0.49 – 0.57	0.37 – 0.48	0.25 – 0.36	< 0.25
Gastrointestinal					
Nausea	None	Reasonable intake	Decreased intake, but able to eat	No significant intake	-------
Vomiting	None	1 episode in 24 h	2 - 5 episodes in 24 h	6 - 10 episodes in 24 h	> 10 episodes in 24 h or parenteral support required
Diarrhea	None	Increase 2 - 3x/ day	Increase 4 – 6x/ day or nocturnal stools or moderate cramping	Increase 7 – 9x/ day or incontinence or severe cramping	Increase > 10x/ day or bloody diarrhea or parenteral support required
Stomatitis	None	Painless ulcers, erythema, or mild soreness	Painful erythema, edema, or ulcers, able to eat solids	Painful erythema, edema, or ulcers, and cannot eat solids	Needs parenteral support for alimentation

	Grade 0	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Liver					
Bilirubin (μmol/L)	≤ 17	-------	17 – 25	26 – 51	> 51
AST (U/L)	5 – 40	41 – 100	101 – 200	201 – 800	> 800
ALT (U/L)	5 – 45	46 – 112	113 – 224	225 – 896	> 896
Alkaline phosphatase (U/L)	40 – 120	121 – 300	301 – 600	601 – 2400	> 2400
Liver clinical	No change	-------	-------	Pre-coma	Hepatic coma
Renal					
Creatinine (μmol/L)	70 – 133	134 – 200	201 – 400	401 – 800	> 800
Proteinuria	No change	1+ or 3g/L	2+/3+ or 3 - 10g/L	4+ or >10g/L	Nephritic syndrome
Hematuria	Negative	Microscopic	Macroscopic, no clots	Macroscopic + clots	Transfusion or cystectomy required
Weight loss	< 5%	5.0 – 9.9%	10.0 – 20.0%	>20.0%	-------
Pulmonary	No change	Asymptomatic, with abnormality in PFTs	Dyspnea when exercising	Dyspnea at normal level of activities	Dyspnea at rest
Cardiac					
Cardiac arrhythmias	none	Asymptomatic, transient, no treatment needed	Recurrent or persistent, no treatment needed	Treatment required	Monitoring required, or hypotension or VT or VF
Cardiac function	No change	Asymptomatic, decline in resting EF <20% of baseline value	Asymptomatic, decline in resting EF >20% of baseline value	Mild CHF, responsive to treatment	Severe or refractionary CHF

Appendix 2. Harris Hip Score

HARRIS HIP SCORE

I PAIN (MAX 44 POINTS)

None or ignores it	44 pt
Slight, occasional, no compromise in activities	40 pt
Mild pain, no effect on average activities, rarely moderate pain with unusual activity, may take aspirin	30 pt
Moderate pain, tolerable but makes concessions to pain. Some limitations of ordinary activity or work. May require occasional pain medication stronger than aspirin	20 pt
Marked pain, serious limitation of activities	10 pt
Totally disabled, crippled, pain in bed, bedridden	0 pt

II FUNCTION (MAX 47 POINTS)

Walking (max 33 points)

Limp

None	11 pt
Slight	8 pt
Moderate	5 pt
Severe	0 pt

Support

None	11 pt
Cane for long walks	7 pt
Cane most of the time	5 pt
One crutch	3 pt
Two canes	2 pt
Two crutches	0 pt
Not able to walk (specify reason)	0 pt

Distance walked

Unlimited	11 pt
500 – 1000 m	8 pt
100 – 500 m	5 pt
Indoors only	2 pt
Not able to walk	0 pt

Activities (max 14 points)

Stairs

Normally without using a rail	4 pt
Normally using a rail	2 pt
In any matter	1 pt

Unable to do stairs		0 pt
Shoes and socks		
With ease		4 pt
With difficulty		2 pt
Unable		0 pt
Sitting		
Comfortably in ordinary chair one hour		5 pt
On a high chair for one-half hour		3 pt
Unable to sit comfortably in any chair		0 pt
Enter public transportation	(if yes)	1 pt

III ABSENCE OF DEFORMITIES (MAX 4 POINTS)
(If all are applicable 4 points, otherwise 0 points)

Less than 30° fixed flexion contracture

Less than 10° fixed adduction

Less than 10° fixed internal rotation in extension

Limb-length discrepancy less than 3.2 cm

IV RANGE OF MOTION (MAX 5 POINTS)

Appendix 3. Patient information

Patient information for a scientific study of gene therapy as an experimental treatment in a loosened hip prosthesis.

Responsible investigator: Prof. Dr. R.G.H.H. Nelissen
Department: Orthopaedic surgery
Telephone: 071-5263606

Dear Sir, Madam,

In continuation of the consult with your own orthopaedic surgeon you hereby receive information in writing about a scientific study in which we ask your cooperation.
We do not ask you to make a decision immediately. Do take some time for reflection before you decide to contribute to the study. We advise you to discuss your co-operation with your family, general practitioner or others.
Would you feel any need to put your questions to a doctor that is not directly involved in the study, you can find the name and telephone number of this independent doctor in these information pages.

Introduction
As was discussed with you, the prosthesis in your hip has loosened. Therefore, you have complaints of pain in the hip and walking difficulty. Usually the treatment for this loosening is an operation. In this operation the old prosthesis is taken out and a new one is put in. In some patients (including you) the risk for adverse events during such an operation is high. That is why we are searching for alternative treatments to refix a loosened prosthesis.
We asked your contribution in a study with a new, not yet registered, gene therapy. To gain more information about safety, tolerability and optimal dosing of this experimental treatment, studies in patients are necessary.
In animal studies with this form of gene therapy the adverse events were limited up till now. There have been limited studies in humans. About the safety and tolerability of the treatment, the following can be stated: The doses of the treatments used in patients in this study have not caused serious adverse events in other studies.

Study objectives
From previous studies we know that certain inflammatory cells, which break down the bone around the prosthesis, cause prosthesis loosening. Up till now there are no

medications that can stop these inflammatory cells. By means of gene therapy we are aiming to remove these inflammatory cells. The effect is as follows:
Gene therapy is a new experimental treatment, which is thoroughly investigated at the moment. With gene therapy you can give certain cells specific instructions. An adenovirus (which normally causes a cold) is used to deliver this instruction into the cell (like a letter). We need the virus, because the letter is not able to go to the cell on its own. As a virus normally enters the cells, it can well be used to deliver the message. Before, the virus was modified so that it is not able to multiply. All information that the virus uses to make people ill, is removed. The virus only knows how to penetrate the cell, and that it has to deliver a message.
In this study a virus is used that penetrates the inflammatory cells (which cause the loosening) efficiently. These cells get the instruction to make a certain protein in an amount as high as possible. After 2 days a drug is administered to the joint that specifically destroys the cells that have produced a high quantity of the protein. The body then automatically clears these cells away. We investigated in our laboratory that we can kill the inflammatory cells in this way. But we don't know yet whether it also works in humans. We will now investigate what happens when the modified virus and the drug are injected in a patient with a loosened hip prosthesis. We will study whether there are adverse events and whether the virus can leak to the environment. And of course we want to know whether the inflammatory cells can be destroyed by the gene therapy. We also want to know if we can put bone cement in the space where the inflammatory cells were, to refix the prosthesis.
The study will take place in the Leiden University Medical Center (LUMC) and a total of 12 patients will participate in the study.

Design of the study

The participating patients will be divided in 4 groups. Each group will receive a different amount of virus to see which dose is the best. First three patients receive the lowest dose. Then these patients are followed during 2 weeks to see whether they have adverse events. Only after this period the second group receives a higher dose, etc.
When you want to participate in the study history is taken and physical examination will be done. Also blood will be drawn and X-rays of your hip are made. These investigations have to be done to see whether you are a good candidate for the study. If you appear to be an appropriate candidate and you still want to participate, an examination will be done in which contrast fluid is injected in the hip joint and X-rays (arthrography) will be made to see how loose the prosthesis is. If you are going to participate in the study appointments will be made for the days when you are expected in the hospital for examinations.

During the study you will be admitted to the hospital for 11 days, of which the first few days in an isolated room. The first day of your admission the virus will be injected in your hip joint. On the third day the drug, that will destroy the inflammatory cells, will be injected in the same way. To see whether the therapy was successful four holes will be drilled through the bone around the prosthesis. Because the bone cannot be anaesthetised you will have spinal anaesthesia. Through the drilled holes some of the inflammatory tissue will be removed to examine whether the cells were destroyed. Furthermore, we will inject bone cement through these holes to refix the prosthesis.
During the 11 days of your admittance to the hospital several examinations will be done to investigate whether there are adverse events from the treatment. Just like in other medications the body can react to the therapy and you can have adverse effects. From the moment that the virus is administered, someone will check on you every 30 minutes, and measure blood pressure and temperature. When you are doing fine, controls will be phased out. To trace down potential adverse effects as soon as possible blood will be drawn on day 2 and 5 for laboratory analysis. To check that the virus will stay in the hip joint and does not migrate through the body and the environment extra examinations will be done. Therefore, you will be asked to cooperate to examination of urine, faeces, nose and throat swabs and blood. During the year after your admission to the hospital you will visit the out-patient clinic 7 times to check on clinical effects of the treatment.

Potential risk of the study
As this form of treatment with gene therapy is new, the experience is still limited. This study will be done to track down potential adverse effects. Up till now there are no indications that the virus will shed outside the injected joint. Still, to prevent every potential shedding of the virus, the virus will be injected in an isolated room. A sample of your blood, faeces, urine and sputum will be collected after one day and analysed for presence of virus, and every day until negative results. You are asked to stay in your room until we know for sure that there is no virus in your urine, faeces, sputum or blood.

During the study you will have two injections in your hip joint, once to administer the virus, and once to administer the prodrug. Injections in the joint can be painful. After injection there is a minimal risk to infection.

On the fifth day of the admission, 4 small holes will be drilled in the bone (under spinal anaesthesia). Through these holes through a thick needle, a piece of bone and inflammatory tissue will be removed for analysis (biopsy). Through the same needle bone

cement will be injected in the open space between prosthesis and bone. The injection from the spinal anaesthesia can be painful. There is a small chance that you experience a headache after the spinal anaesthesia. The cuts in your skin are so small that you don't need any stitches.

Potential benefits from the study

If the inflammatory cells, which are responsible for the loosening of the prosthesis, turn out to be destroyed by the gene therapy, and if the space between bone and prosthesis can be filled with bone cement, you could possibly experience a benefit from participation in the study. As this study is the first one in which gene therapy is used to treat loosening of prostheses, there are no results from previous studies available. The results, which will proceed from this study are difficult to predict. That is why you'd better anticipate that you won't experience any benefits from participation in the study.

Blood transfusion and organ donation

The Minister of VROM (environment) has stated that patients who participate in a gene therapy study are not allowed to donate blood for blood transfusion. In case of organ donation the physicians need to be informed about the gene therapy study you participated in. Although it is unlikely that the virus will still be present in an organ, they want to rule out the possibility that the virus will be transferred by blood transfusion or organ donation. You therefore have to sign a written declaration.

Voluntary participation

Your participation to the study is voluntarily. If you decide to cooperate in this study, you have the opportunity to come back at your decision at any moment. You don't have to give any argumentations, but you are asked to report your decision to your physician as soon as possible. He will discuss with you whether discontinuation has any consequences for you that make it necessary to start another treatment. Your physician can also decide to discontinue your participation to the study, when he thinks this is better for your well-being. When this is the case your physician will discuss this with you. When, during the study, new information becomes available, your physician will discuss this with you. The decision to participate or not to participate in the study won't have any influence on the understanding you have with your physician.

Financial compensation

When you make extra travelling expenses because of participation of the study, these will be compensated.

Confidentiality of data

You can be assured that all data that are accumulated under code (i.e. without report of your name and address) about you, will be handled confidentially according to the medical treatment agree (WGBO (*this is a Dutch law in which patient-physician relation is made clear*)) and that non-competent outsiders don't have access to your data. The results of this study will be used in a scientific publication, but also then the data will not be reducible to you as a person. We will inform your general practitioner about your participation in the study.

Insurance

The LUMC has effected an insurance to cover potential damage inflicted by the study. When you think you have experienced damage from participation in the study you can contact the researcher.

In conclusion

Would you have any questions regarding the information of this study you can ask your physician, the researcher and the independent physician.

For general information on participation in scientific studies we refer to the brochure "Asked for medical scientifical research" (*this is a brochure which patients can ask at the information desk*), which is available at the patients-service-bureau on the second floor of the LUMC.

When you decide to participate in this study we ask you, at the next visit with reference to the study, to sign the informed consent form. By signing this form you are not committed to anything (your signature is not binding), but you give to understand that you have received and understood the information and that you know what is expected from you with regard to the study.

Researcher	JJ de Poorter	Indep. physician	Dr. CF Allaart
Department	Orthop. Surgery	Department	Rheumatology
Telephone	071-5263606	Telephone	071-5263598

Outline of all burdening of the study

During hospital admittance:

Injections in the joint: To administer the virus and the drug as close as possible to the inflammatory cells (which cause the loosening) they are injected into the joint. This will take place in the radiology department. First, the skin will be anaesthetised and then the fluid will be injected using a thin needle. The injection can be painful. Injections will take place on the first and third day of the hospital admittance.

Bone biopsies and injection of cement: On the seventh day of the admittance four small holes are drilled through the bone to collect a little inflammatory tissue and cement is injected to refix the prosthesis. Because the bone cannot be anaesthetised locally you will have spinal anaesthesia. In total this will take about one hour. After the procedure you will be taken back to your room.

Collection of urine, faeces, nose and throat swabs and blood (These are called excreta). These investigations will be done to find out where the virus sheds after we have injected it. A sample of the urine and faeces you produce will be collected after 24 h after the injection of the virus and will be analysed for presence of virus. Also, with a piece of cotton-wool, a culture will be taken from your nose and throat and a blood sample will be taken. All samples will be evaluated as soon as possible. The next day samples will be taken again if virus was present the previous day. When all samples are free of virus, you are allowed to leave the room whenever you want.

Adverse effects: To track down potential adverse effects of the gene therapy you will be observed carefully. We do this by asking you questions about how you are feeling and by physical examinations (listening to heart, lungs and abdomen and palpation of the abdomen). Also, blood samples will be taken to investigate whether you have adverse effects you haven't mentioned. The physical examinations will take place every day, the blood samples will be taken on the second day and the fifth day.

Hospital visits: In the first part of the study you will be admitted to the hospital during 11 days. You will have a separate room. During the first days you are not allowed to come out of the room. Nevertheless you are allowed to receive visitors.

Until 1 year after the injections you will visit the researcher in the hospital a total of 7 times for this study. During the visits she will ask you whether there are any problems or complaints and she will do physical examinations. The traveling-expenses you make for visiting the hospital will be compensated. The researcher can arrange that a taxi will collect you at home and bring you back after the visit, if you like that.

Blood samples: The first three times that you visit the hospital for the study you will be asked to have 2 tubes of blood drawn. This will be done to investigate whether there are adverse effects that you haven't mentioned. Whenever possible, potential adverse effects will be treated. Three months and 1 year after the injections in the hip joint another tube of blood is taken to investigate how the immune system has responded to the gene therapy.

X-rays: To investigate whether the treatment was successful some X-rays will be made. At the last day of your admittance an X-ray will be taken from your hip and pelvic region (these pictures are the standard pictures you have had before). In the control visits after 2 weeks, after 6 months and after 1 year similar pictures are taken.

The burdening you may experience from the study is outlined in the scheme on the next page.

Scheme for the study

		>>> Hospital admittance <<<				>>>>>>>>>>>> Control visits <<<<<<<<<<<<					
	First visit	Day 1	Day 2	Day 3	Day 4-9	Day 10	After 4 weeks	After 6 weeks	After 3 months	After 6 months	After 1 year	Every year max 5
History, physical examination	x	x	x	x	x	x	x	x	x	x	x	x
Injections in joint	x	x		x								
Blood samples	x	x	x	x	x	x	x		x		x	
Collection of urine, feces, nose and throat swabs		x	x	x	x	x						
X-rays	x					x				x	x	x

Appendix 4. Patiënten-informatie

Patiënteninformatie ten behoeve van een wetenschappelijk onderzoek naar gentherapie als experimentele behandeling bij een losgelaten heupprothese.

Versie 5, 29 september 2003

Verantwoordelijk onderzoeker:	Dr R.G.H.H. Nelissen
Afdeling:	Orthopaedie
Tel.:	071-5263606

Geachte mevrouw, mijnheer,

In aansluiting op het gesprek met Uw behandelend arts ontvangt U hierbij de schriftelijke informatie met betrekking tot een wetenschappelijk onderzoek waarvoor Uw medewerking is gevraagd.
Wij vragen U niet om onmiddellijk een beslissing te nemen. Neemt U rustig enige bedenktijd voordat U beslist of U meedoet of niet. Wij raden U aan om Uw deelname te bespreken met Uw partner, familie, huisarts of anderen.
Mocht U behoefte hebben Uw vragen voor te leggen aan een arts die niet direct bij dit onderzoek betrokken is, dan kunt U de naam en het telefoonnummer van deze onafhankelijk arts vinden op de vijfde pagina van deze informatie.

Inleiding
Zoals met U besproken is, is de prothese in Uw heup los gaan zitten. Daardoor heeft U klachten als pijn in de heup en moeite met lopen. Meestal wordt als behandeling bij zo'n losse prothese een operatie gedaan. Bij deze operatie wordt de oude prothese eruit gehaald en wordt er een nieuwe ingezet. Bij sommige mensen (en ook bij u) is de kans op nadelige gevolgen van zo'n operatie echter te groot. Daarom zijn we op zoek naar andere methoden om een losse prothese vast te zetten.
Wij hebben U gevraagd medewerking te verlenen aan een onderzoek met een nieuwe, nog niet geregistreerde gentherapie. Voor het verkrijgen van meer informatie over de veiligheid, verdraagbaarheid en juiste dosering van deze experimentele behandeling zijn onderzoeken bij patiënten noodzakelijk.
Bij dierexperimenteel onderzoek met gentherapie met dit soort virus zijn de bijwerkingen tot op heden beperkt gebleken. Er is in beperkte mate onderzoek bij mensen gedaan. Wat betreft de veiligheid en verdraagbaarheid van de therapie uit dit onderzoek kan het volgende worden gezegd: De hoeveelheden van het middel dat in dit onder-

zoek bij patiënten wordt ingespoten hebben bij andere onderzoeken niet gezorgd voor ernstige bijwerkingen.

Doel van het onderzoek

Uit eerdere onderzoeken die gedaan zijn weten we dat een prothese loslaat doordat bepaalde ontstekingscellen het bot afbreken dat rond de prothese zit. Tot nu toe zijn er geen medicijnen die deze ontstekingscellen kunnen tegenhouden. Door middel van gentherapie willen we deze ontstekingscellen verwijderen. Dit werkt als volgt:

Gentherapie is een nieuwe experimentele behandeling waar momenteel veel onderzoek naar wordt gedaan. Met gentherapie kun je specifieke cellen een opdracht uit laten voeren. Er wordt een verkoudheidsvirus gebruikt om deze opdracht (als een soort brief) af te leveren in de cel. Het virus is nodig, omdat de boodschap niet vanzelf de cel in kan. Aangezien een virus normaal ook cellen binnendringt, kan hij goed gebruikt worden om de boodschap in de cel te bezorgen. Het virus is van tevoren wel zo aangepast dat het zich niet meer kan vermenigvuldigen. Daardoor is de kans dat mensen ziek worden van het virus veel kleiner. Het virus weet alleen nog hoe het in de cel moet komen en dat hij de opdracht moet afgeven.

In dit onderzoek wordt een virus gebruikt dat onder andere naar de ontstekingscellen gaat die de loslating van de prothese veroorzaken. Deze cellen krijgen dan als opdracht om zoveel mogelijk van een bepaald eiwit te maken. Na 2 dagen wordt er een medicijn ingespoten dat specifiek de cellen kapot maakt die heel veel van het eiwit hebben gemaakt. We verwachten dat we deze cellen daarna uit de ruimte kunnen wegspoelen en dat een gedeelte van de cellen vanzelf door het lichaam wordt opgeruimd. We hebben in het laboratorium onderzocht dat we op deze manier de ontstekingscellen onschadelijk kunnen maken. Maar we weten nog niet of het ook bij mensen werkt. Nu zal onderzocht worden wat er gebeurt als we het aangepaste virus en het medicijn bij patiënten met een loszittende prothese inspuiten.

We gaan onderzoeken of mensen er geen bijwerkingen van krijgen en of het virus niet in de omgeving terechtkomt. En natuurlijk willen we ook weten of de ontstekingscellen kapot gemaakt kunnen worden met de gentherapie. Tevens willen we onderzoeken of we in de ruimte waar eerst de ontstekingscellen zaten, botcement kunnen spuiten om de prothese weer vast te zetten.

Het onderzoek vindt plaats in het LUMC te Leiden en in totaal zullen 12 patiënten deelnemen.

De opzet van het onderzoek

De deelnemende patiënten zullen in 4 groepen worden verdeeld. Iedere groep krijgt een andere hoeveelheid virus ingespoten om te kijken welke hoeveelheid het beste

is. De eerste drie patiënten krijgen de laagste dosis. Dan wordt gedurende minimaal 2 weken gekeken of zij geen bijwerkingen krijgen. Pas daarna krijgt de tweede groep een hogere dosis ingespoten, enz.
Voordat het onderzoek begint worden, als U mee wilt werken aan het onderzoek, een aantal gegevens van U genoteerd, U wordt lichamelijk onderzocht, er worden 2 buisjes bloed afgenomen en er worden röntgenfoto's van Uw heup gemaakt. Als blijkt dat U een geschikte kandidaat bent voor de studie en U wilt nog steeds meedoen, wordt een onderzoek gedaan waarbij een contrastvloeistof in het heupgewricht gespoten wordt en er röntgenfoto's worden gemaakt om te kijken hoe los de prothese zit (arthrografie). Als U mee gaat doen in het onderzoek worden er afspraken met U gemaakt over dagen dat U in het ziekenhuis verwacht wordt voor het onderzoek.
Tijdens het onderzoek wordt U 11 dagen opgenomen in het ziekenhuis, waarvan ongeveer de eerste vier dagen op een eenpersoonskamer. De eerste dag krijgt U het virus in het heupgewricht gespoten. Op de derde dag krijgt U het medicijn ingespoten dat de cellen kapot moet maken. Om te kijken of de therapie succes heeft gehad worden er op de negende dag vier gaatjes geboord door het bot rond de prothese (3 in het bovenbeen en 1 in het bekken). Als verdoving krijgt U hierbij een ruggeprik. Via de geboorde gaatjes wordt wat van het ontstekingsweefsel weggehaald om te onderzoeken of de cellen kapot zijn gegaan. Bovendien gaan we via deze gaatjes nieuw botcement inspuiten om de prothese weer vast te zetten.
Tijdens de 11 dagen dat U opgenomen bent worden er verschillende onderzoeken gedaan om te kijken of er bijwerkingen zijn van de behandeling. Net zoals bij medicijnen kan het lichaam reageren op de therapie en kunt U bijwerkingen krijgen. Vanaf het moment dat het virus ingespoten is komt er ieder half uur iemand bij U langs om te informeren hoe het met U gaat en om bloeddruk en temperatuur te meten. Als het goed met U gaat zullen de controles wat minder vaak gedaan worden. Om eventuele bijwerkingen zo snel mogelijk op te sporen wordt ook een aantal keer bloed afgenomen voor laboratoriumonderzoek. Dit gebeurt op dag 2 en 5. Om te controleren dat het virus alleen in de heup blijft en dat er niets 'lekt' naar de rest van het lichaam en de omgeving worden extra onderzoeken gedaan. Daarom wordt U gedurende de studie regelmatig gevraagd mee te werken aan lichamelijk onderzoek en aan de controle van bloed, urine, ontlasting en een keel- en neusuitstrijk. U zult in het jaar na de opname nog zeven keer gecontroleerd worden op eventuele bijwerkingen van de behandeling.

De eventuele risico's van het onderzoek
Omdat deze vorm van behandeling met gentherapie nieuw is, is de ervaring nog beperkt. Deze studie is bedoeld om eventuele bijwerkingen op te sporen.
Tot nu toe zijn er geen aanwijzingen dat het virus zich buiten het ingespoten gewricht

zal verspreiden. Toch zal, om elke mogelijke verspreiding van het virus te voorkomen, het virus ingespoten worden op een eenpersoonskamer op de afdeling reumatologie. Uw ontlasting, urine en speeksel zullen opgevangen worden en onderzocht worden op aanwezigheid van het ingespoten virus. Het is de bedoeling dat U Uw kamer niet verlaat totdat wij zeker weten dat er geen virus in Uw urine, ontlasting of speeksel zit.

Tijdens deze studie zult U 2 maal in Uw heupgewricht geprikt worden, eenmaal om het virus in te spuiten en eenmaal om het medicijn in te spuiten. Injecties in het gewricht kunnen pijnlijk zijn. Na injectie in een gewricht bestaat een minimaal risico op een infectie.

Op de tiende dag van de opname worden 4 gaatjes in de huid gemaakt (verdoofd met een ruggeprik). Via deze gaatjes wordt via een dikke naald een stukje bot en ontstekingsweefsel weggehaald (biopsie). Via dezelfde naald wordt daarna botcement in de open ruimte tussen prothese en bot gespoten. De ruggeprik kan pijnlijk zijn. Er is een kleine kans dat U hoofdpijn krijgt na de ruggeprik. De gaatjes in de huid zijn zo klein dat ze niet gehecht hoeven te worden.

Mogelijke voordelen van het onderzoek

Als zou blijken dat de ontstekingscellen die verantwoordelijk zijn voor het loslaten van de prothese kapot gemaakt kunnen worden door de gentherapie en als de ruimte tussen de prothese en het bot opgevuld kan worden met botcement, zou U mogelijk een gunstig effect kunnen ondervinden van deelname aan het onderzoek. Omdat dit onderzoek het eerste is waarbij gentherapie gebruikt wordt om loslating van protheses te behandelen zijn er nog geen resultaten uit andere onderzoeken beschikbaar. De resultaten die uit deze studie zullen voortkomen zijn moeilijk te voorspellen. Daarom kunt U er beter vanuit gaan dat U geen voordelen zult ondervinden van deelname aan de studie.

Bloedtransfusie en orgaandonatie

De minister van VROM heeft bepaald dat patiënten die aan een gentherapie studie deelnemen geen bloed voor bloedtransfusie mogen afstaan. In geval van orgaandonatie voor transplantatiedoeleinden dienen de artsen geïnformeerd te worden over de gentherapie. Alhoewel het onwaarschijnlijk is dat het virus nog in een orgaan aanwezig is, wil men uitsluiten dat het virus door bloedtransfusie of orgaantransplantatie wordt overgedragen. U dient hiervoor een schriftelijke verklaring te ondertekenen.

Vrijwilligheid van deelname

Uw medewerking aan dit onderzoek is vrijwillig. Als U toestemming geeft om aan dit

onderzoek mee te doen, heeft U te allen tijde de vrijheid om op die beslissing terug te komen. U hoeft hiervoor geen reden op te geven, wel wordt U gevraagd dit direct aan Uw behandelend arts te melden. Hij zal dan met U bespreken of het stoppen van het onderzoek consequenties voor U heeft die het eventueel nodig maken een andere behandeling te starten.
De eerste dagen na injectie in het gewricht zult U verzocht worden op Uw kamer te blijven. Uw ontlasting, urine en speeksel worden in deze dagen onderzocht op uitscheiding van het virus om te onderzoeken of U mogelijk mensen in Uw omgeving zou kunnen besmetten met het virus. Het is belangrijk voor de mensen in Uw omgeving dat U zich niet uit de studie terugtrekt gedurende deze periode. Na deze periode staat het U vrij op elk moment te stoppen met deelname aan het onderzoek.
Ook Uw behandelend arts kan Uw deelname aan het onderzoek stopzetten als deze vindt dat dit ten aanzien van Uw gezondheid beter is. Uw arts bespreekt dit dan met u. Wanneer tijdens het onderzoek nieuwe informatie bekend wordt, zal Uw behandelend arts dit eveneens met U bespreken.
Het wel of niet meedoen heeft op geen enkele wijze gevolgen voor de verstandhouding met Uw arts.

Vergoeding
Wanneer U in het kader van deelname aan het onderzoek extra reiskosten maakt, worden deze vergoed.

Vertrouwelijkheid van de gegevens
U kunt ervan verzekerd zijn dat alle gegevens die tijdens dit onderzoek onder code (d.w.z. zonder vermelding van Uw naam en adres) over U verzameld worden, vertrouwelijk behandeld worden volgens de wet geneeskundige behandelingsovereenkomst (WGBO) en dat niet-bevoegde buitenstaanders geen inzage hebben in Uw gegevens. De resultaten van dit onderzoek worden gebruikt in een wetenschappelijke publicatie, maar ook dan zijn de gegevens niet tot U als persoon herleidbaar.
De stukjes ontstekingsweefsel die via de boorgaten worden verwijderd zullen bewaard worden. Met dit weefsel kan later eventueel nog onderzoek gedaan worden. De arts-onderzoeker zal aan U toestemming vragen om de stukjes ontstekingsweefsel te bewaren. Ook vragen wij U toestemming om Uw huisarts op de hoogte te stellen van Uw deelname aan het onderzoek en andere informatie die wij van belang vinden. Ook vragen wij U of wij Uw huisarts informatie mogen vragen over Uw vroegere en huidige ziekten.

Verzekering
Er is door het LUMC een verzekering afgesloten waaruit eventuele schade als gevolg

van het onderzoek betaald kan worden. Wanneer U vindt dat U schade heeft ondervonden als gevolg van deelname aan het onderzoek kunt U contact opnemen met de arts-onderzoeker. Informatie over de afgesloten verzekering treft U aan in de bijlage.

Tot slot

Mocht U naar aanleiding van deze informatie nog vragen hebben met betrekking tot dit onderzoek dan kunt U daarmee terecht bij Uw behandelend arts, de arts-onderzoeker of de onafhankelijk arts.

Voor algemene informatie over deelname aan wetenschappelijk onderzoek verwijzen wij U naar de brochure "Gevraagd voor medisch-wetenschappelijk onderzoek", die verkrijgbaar is bij het patiëntenservicebureau op de tweede etage van het LUMC.

Wanneer U besluit aan dit onderzoek deel te nemen vragen we U bij het eerstvolgende bezoek aan het ziekenhuis in het kader van dit onderzoek een handtekening te zetten (het informed consent). Met de ondertekening verplicht U zich nergens toe (Uw handtekening is niet 'bindend'), maar geeft U te kennen dat U deze informatie ontvangen en begrepen heeft en weet wat er van U verwacht wordt met betrekking tot het onderzoek.

Artsonderzoeker:	JJ de Poorter	Onafhankelijk arts:	Dr CF Allaart
Afdeling:	Orthopedie	Afdeling:	Reumatologie
Telefoonnummer:	071-5263606	Telefoonnummer:	071-5263598

Verantwoordelijk onderzoeker:	Dr R.G.H.H. Nelissen
Afdeling:	Orthopaedie
Tel.:	071-5263606

De belasting van het onderzoek op een rijtje

Tijdens de ziekenhuisopname:

Injecties in het gewricht: Om het virus en het medicijn zo dicht mogelijk bij de ontstekingscellen (die zorgen voor de loslating van de prothese) te brengen worden ze in het gewricht ingespoten. Dit gebeurt op de röntgenafdeling. Eerst wordt de huid verdoofd en daarna wordt met een dun naaldje de vloeistof ingespoten. De injectie kan pijnlijk zijn. De injecties vinden plaats op de eerste en derde dag van de ziekenhuisopname.

Botbioptieën en inspuiten van cement: Op de negende dag van de ziekenhuisopname worden vier gaatjes door het bot geboord om een beetje ontstekingsweefsel weg te halen en wordt cement ingespoten om de prothese vast te zetten. Omdat het bot niet verdoofd kan worden met een lokale verdoving krijgt U een ruggeprik. Om zo hygiënisch mogelijk te werken zal dit alles op de operatiekamer gebeuren. In totaal zal dit ongeveer een uur duren. Daarna wordt U terug naar Uw kamer gebracht.

Verzamelen van urine, ontlasting, keel- en neuskweken en bloedonderzoek (Dit worden excreta genoemd). Deze onderzoeken worden gedaan om te onderzoeken waar het virus blijft nadat we het hebben ingespoten. De urine en ontlasting die U produceert worden gedurende de eerste 24 uur verzameld en er wordt onderzocht of er virus in zit. Verder wordt met een wattenstokje een kweek genomen uit de keel en uit de neus en wordt een keer bloed afgenomen. Al deze onderzoeken worden zo snel mogelijk beoordeeld. De volgende dag worden alleen de excreta verzameld waar de vorige dag nog virus zat. (Bij voorbeeld als in Uw bloed en ontlasting geen virus was aangetoond hoeft U dit niet meer in te leveren). Als nergens meer virus in zit mag U de kamer weer verlaten wanneer U dat wilt.

Bijwerkingen: Om eventuele bijwerkingen van de gentherapie op te sporen wordt U zorgvuldig geobserveerd. Dit gebeurt door aan U te vragen of U klachten heeft en door lichamelijk onderzoek (luisteren naar het hart, de longen en de buik en voelen aan de buik). Daarnaast wordt ook bloed geprikt om te onderzoeken of er bijwerkingen zijn waar U zelf nog niets van merkt. Het lichamelijk onderzoek gebeurt iedere dag, de bloedafnames gebeuren op de tweede dag (2 keer) en op de vijfde dag (1 keer).

Bezoeken aan het ziekenhuis: In het begin van het onderzoek wordt U gedurende 11 dagen opgenomen in het ziekenhuis. U ligt dan op een eenpersoonskamer. Gedurende de eerste dagen mag U Uw kamer niet verlaten. U mag natuurlijk wel bezoek ontvangen. Tot 1 jaar na het onderzoek wordt U in totaal 7 keer op de polikliniek orthopedie verwacht bij de arts-onderzoeker. Hierna komt U een

keer per jaar op controle bij Uw behandelend orthopaed. Tijdens de poliklinische bezoeken informeert de arts-onderzoeker hoe het met U gaat en of er problemen zijn. Ook wordt gevraagd of U mee wilt werken aan een kort lichamelijk onderzoek. De reiskosten die U maakt om naar het ziekenhuis te komen worden vergoed. Als U dat wilt kan de arts-onderzoeker een taxi voor U regelen die U thuis ophaalt en na het polibezoek weer naar huis brengt.

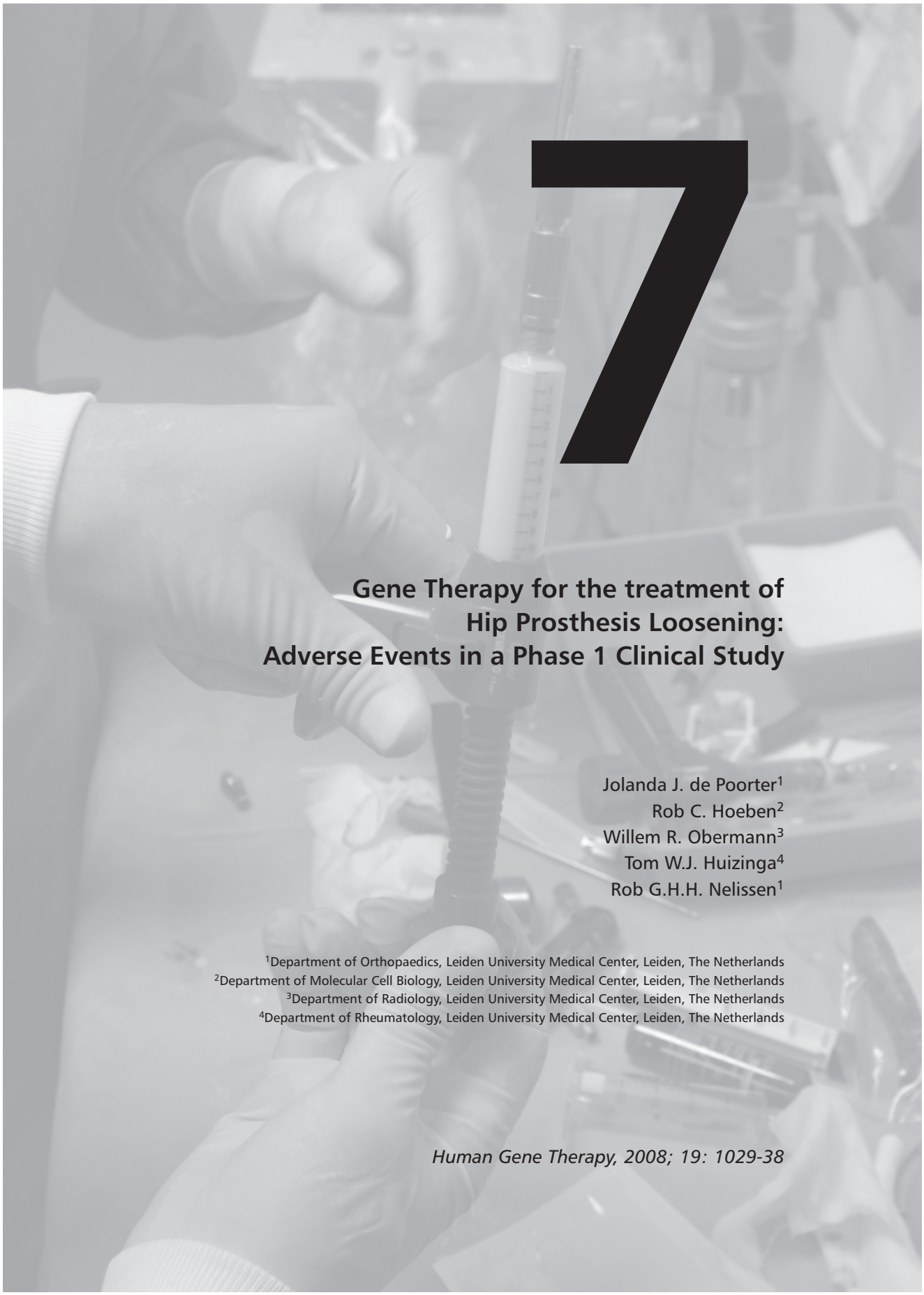
Bloedafnames: De eerste 3 keer dat U op de polikliniek komt wordt U gevraagd om na het polibezoek bloed te prikken (2 buisjes). Dit wordt gedaan om te kijken of er bijwerkingen zijn van de behandeling waar U zelf niets van merkt. De arts-onderzoeker belt U een paar dagen later thuis op om de uitslag van het bloedonderzoek te bespreken. Als het mogelijk is worden eventuele bijwerkingen behandeld. 3 Maanden en 1 jaar na de ziekenhuisopname wordt nog 1 buisje bloed afgenomen om te onderzoeken hoe het immuunsysteem heeft gereageerd op de gentherapie.

Röntgenfoto's: Om te onderzoeken of de behandeling succes heeft gehad worden een aantal röntgenfoto's gemaakt. Op de laatste dag van de ziekenhuisopname worden een foto van het bekken en van de heup gemaakt (zoals U die wel eerder hebt laten maken bij polikliniekbezoeken). Bij de nacontroles na 2 weken, na 6 maanden en na 1 jaar worden nogmaals dezelfde foto's gemaakt.

De belasting die U zult ondervinden van het onderzoek is samengevat in het schema op de volgende pagina.

Schema voor het onderzoek

		>>>>>>>>>>> Ziekenhuisopname <<<<<<<<<<<<<										>>>>>>>> Poli-bezoeken <<<<<<<<						
	Eerste onder-zoek	Dag 1	Dag 2	Dag 3	Dag 4	Dag 5	Dag 6	Dag 7	Dag 8	Dag 9	Dag 10	Na 2 weken	Na 4 weken	Na 6 weken	Na 3 mnd	Na 6 mnd	Na 1 jaar	Ieder jaar
Lichamelijk onderzoek, vragenlijst	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Injectie in gewricht	x	x		x														
Bloedafnames	x	x	x	x	x			x				x	x		x		x	
Verzamelen urine, ontlasting, keel- en neuskweken		x	x	x	x													
Röntgenfoto's	x										x	x				x	x	x



7

Gene Therapy for the treatment of Hip Prosthesis Loosening: Adverse Events in a Phase 1 Clinical Study

Jolanda J. de Poorter[1]
Rob C. Hoeben[2]
Willem R. Obermann[3]
Tom W.J. Huizinga[4]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
[4]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

Human Gene Therapy, 2008; 19: 1029-38

Abstract

Revision surgery for loosened hip prostheses is a heavy burden for elderly patients with comorbidity. As an alternative to surgery we performed a study to stabilise the prosthesis by percutaneous cement injection after removing inflammatory tissue with an intra-articular virus-directed enzyme prodrug approach. Twelve elderly patients with debilitating pain from a loosened hip prosthesis were included in a phase 1 dose-escalating clinical study. The patients were admitted to the hospital for 10 days for an intra-articular vector and prodrug injection, and subsequently for a percutaneous bone cement injection. This paper reports the adverse and serious adverse events of the study. After prodrug injection 9 of 12 patients had gastrointestinal adverse events (nausea, vomiting, and diarrhoea), and 8 patients had hepatic adverse events (rise in aspartate aminotransferase and alanine aminotransferase). Five patients developed anaemia (World Health Organisation grade 1 or 2) from hematomas after cement injection. There were four serious adverse events in the first 6 months after vector injection, but these were not related to gene therapy as judged by an independent safety committee. There was no dose-limiting toxicity. However, extensive comorbidity in these patients makes it difficult to fully establish the safety of this approach in this small and heterogeneous patient population.

Introduction

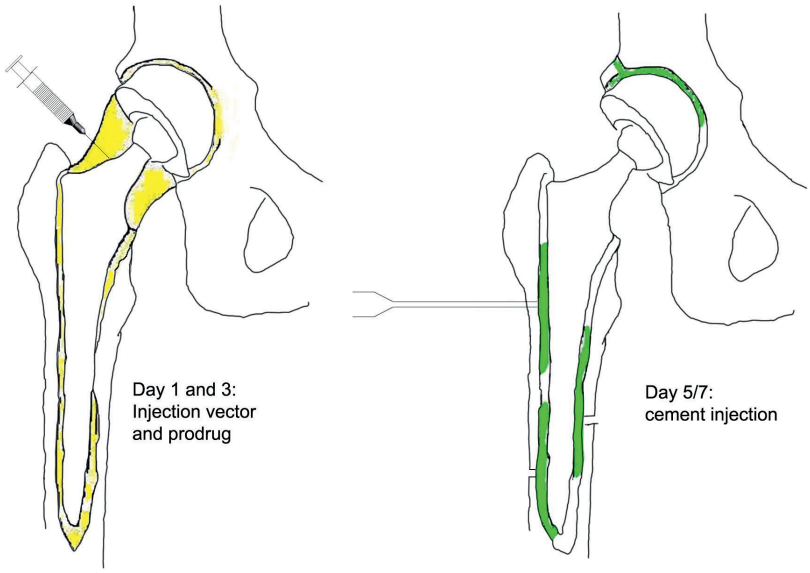
A major complication in total hip arthroplasties is loosening of the prosthesis, resulting not only in pain and walking difficulties but also leading to a higher risk for dislocations and pathological fractures.[54] Revision surgery has a high morbidity and mortality rate, especially in elderly patients with comorbidity. At present there are no alternative treatments for prosthesis loosening other than extensive surgical revision of the prosthesis.

Aseptic loosening by particulate-induced osteolysis is the most common cause of implant failure. Wear particles, produced by any artificial joint, such as particles of polyethylene and metal, are phagocytosed by macrophages, leading to secretion of inflammatory cytokines.[43] The resulting chronic inflammation eventually produces interface tissue, a pseudomembrane of synovium-like interface tissue with activated macrophages, fibroblasts, giant cells and osteoclasts.

The prodrug CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] is a weak monofunctional alkylating agent, which is converted by the *Escherichia coli* enzyme *nitroreductase* (Ntr) to a cytotoxic derivative.[68] Cells containing Ntr convert CB1954 into a bifunctional alkylating agent that is capable of forming DNA interstrand cross-links, resulting in apoptosis or cell death.[18,34,102] Because there is no human homolog to Ntr[23], only cells expressing the nitroreductase gene are killed when exposed to CB1954. This makes the Ntr-CB1954 combination exploitable for a virus-directed enzyme prodrug therapy (VDEPT) approach. To this end, CTL102 was constructed, an E1, E3-deleted replication-deficient human adenovirus serotype 5 vector, engineered to contain the *E. coli nfsB* gene under the control of the immediate-early (IE) promoter of the human cytomegalovirus.[34,125] In our laboratory, previous experiments have shown the efficacy of the transduction and destruction of synoviocytes and fibroblasts from interface tissue by HAdV-5-Ntr (CTL102) and CB1954.[30] On the basis of promising preclinical findings we performed a phase 1 clinical trial to test the safety and feasibility of an intra-articular VDEPT approach to destroy periprosthetic interface tissue and refix loosened prostheses with percutaneous cement injection into the periprosthetic space. The clinical results of this phase 1 clinical trial were previously published.[28]

The study has four potential sources of toxicity: the vector, the prodrug, the vector-prodrug combination, and the percutaneous cement injection. The safety of the prodrug CB1954 in patients was shown by Chung-Faye and coworkers;[23] the safety of the vector CTL102 was shown by Palmer and coworkers.[91] This paper describes the safety of the vector-prodrug combination and the cement injection.

Figure 1. Schematic overview of study protocol



Materials and methods

Trial design

For a description of the trial design, patient selection, preparation and administration of vector and prodrug, and cementing technique (Figure 1), the reader is referred to de Poorter *et al.*,[28] where clinical results are shown.

Safety measurements

Safety for the patient was the primary objective in this study. Adverse events reporting is based on the World Health Organisation (WHO, Geneva, Switzerland) recommendations for grading of acute and subacute toxic side effects (World Health Organisation), using a five-point scale (0, within normal limits; 1, minimal; 2, moderate; 3, severe; 4, intolerable). Adverse events are defined as pre-existent (before gene therapy), early (0-7 days after vector administration), intermediate (8-30 days after vector administration), and late (> 30 days after vector administration). Adverse events were recorded up to 6 weeks after vector administration. Vital signs (blood pressure, pulse rate, temperature, and breathing frequency), medical history, and a visual analogue scale (VAS) for pain were measured after vector injection every 30

minutes for the first 4 h, then hourly to 8 h after vector injection, and then every 4 h to 24 h. After 24 h these controls were done every 6 h until prodrug injection, and subsequently every day. In addition, vital signs were controlled 30 min and 1, 2, 4, 6, and 12 h after prodrug injection. Blood samples for haematological and biochemical analysis and urine samples were taken 1, 3, 6 and 8 days after vector injection. After discharge from the hospital adverse events were monitored at time points 2, 4, and 6 weeks after vector injection. In some patients who lived far from the hospital these controls were done after 3 and 6 weeks. For each adverse events the following characteristics were recorded: patient number, description of adverse event, WHO grading, date of first occurrence, date of last occurrence, relation with study, outcome of the event, relation with gene therapy, and whether the adverse event required treatment.

Results

Patient characteristics

Twelve patients between 72 and 91 years (mean, 82 years) were treated. Relevant medical histories of the patients are outlined in Table 1.

Table 1. Demographic characteristics and relevant medical history per patient.

Pt nr	Age (years)	Sex (M/F)	ASA	Relevant medical history
1	82	F	4	Myocardial infarction; mamma-carcinoma; congestive cardiac failure; hyperthyreoidism
2	72	F	2	CVA; meningitis
3	78	F	4	Rheumatoid arthritis; COPD; myocardial infarction; spondylodiscitis; congestive cardiac failure; diverticulosis; osteoporosis; BPPV
4	91	M	3	Prostatectomy; amaurosis fugax; Multiple myeloma stadium II
5	75	F	3	Pneumonia; M Parkinson
6	86	F	3	CABG; aorta valve prosthesis
7	85	F	3	TIA; myocardial infarction; macula degeneration
8	86	F	4	Myocardial infarction; rheumatoid arthritis
9	81	F	2	Recurrent urinary tract infections
10	82	F	2	Uterus extirpation; mamma-carcinoma
11	76	F	4	Rheumatoid arthritis; myocardial infarction
12	89	F	2	Dementia

Abbreviations: ASA, category according to the American Society of Anesthesiologists; CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease; BPPV, benign paroxysmal position vertigo; CABG, coronary artery bypass grafting; TIA, transient ischemic attack.

Table 2. Demographic characteristics, and information on vector and prodrug dose.

Pt nr	Age (years)	Sex (M/F)	ASA-cat	Under-lying Disease	Body surface (m^2)	Vector injection		Prodrug injection		
						Planned dose (particles)	Actual dose (particles)	Planned dose (per m^2 / in mg)	Actual dose (per m^2 / in mg)	Injected volume (mL)
1	82	F	4	OA	2.13	$3x10^9$	$3x10^9$	24/ 51.1	24/ 51.1	27
2	72	F	2	OA	1.64	$3x10^9$	$3x10^9$	24/ 39.4	**20/ 32.0**	16
3	78	F	4	RA	1.58	$3x10^9$	$3x10^9$	24/ 37.9	24/ 37.9	30
4	91	M	3	OA	2.04	$1x10^{10}$	**$8.3x10^9$**	24/ 49.0	24/ 49.0	30
5	75	F	3	OA	1.83	$1x10^{10}$	$1x10^{10}$	24/ 43.9	**8.2/ 15.0**	7.5
6	86	F	3	OA	1.48	$1x10^{10}$	$1x10^{10}$	16/ 23.7	16/ 23.7	22
7	85	F	3	OA	1.87	$3x10^{10}$	**$2.6x10^{10}$**	16/ 29.9	**15/ 27.9**	14
8	86	F	4	RA	1.77	$3x10^{10}$	$3x10^{10}$	16/ 28.3	**11/ 20.0**	10
9	81	F	2	OA	1.66	$3x10^{10}$	$3x10^{10}$	16/ 26.6	16/ 26.6	27
10	82	F	2	OA	1.73	$1x10^{11}$	$1x10^{11}$	16/ 27.7	16/ 27.7	30
11	76	F	4	RA	1.68	$1x10^{11}$	$1x10^{11}$	16/ 26.9	**11/ 18.0**	9
12	89	F	2	OA	1.6	$1x10^{11}$	$1x10^{11}$	16/ 25.6	16/ 25.6	18

Abbreviations: F, female; M, male; OA, osteoarthritis; RA, Rheumatoid Arthritis.

The dose of CTL102 vector ranged from 3 x 10^9 particles to 1 x 10^{11} particles. One patient in the second dose group (1 x 10^{10} particles) and one patient in the third dose group (3 x 10^{10} particles) actually received a lower vector dose (8.3 x 10^9 and 2.6 x 10^{10} particles, respectively) than was planned, because the contents of the syringe could not be injected entirely.

All patients were intended to receive a prodrug dose of 24 mg/m^2 with a maximal concentration of 2mg/ml in the joint space, but after gastrointestinal and hepatic adverse events in the first four patients this dose was lowered to 16 mg/m^2. Five of the patients received a lower dose than planned because of a small joint capacity. Patient characteristics and actual vector and prodrug doses are shown in table 2 and have been previously published.[28]

Adverse events

Overall there were 100 adverse events observed in the 12 patients (Table 3). Fifteen adverse events were hematologic, 12 were metabolic, 17 gastro-intestinal, 19 hepatic, 15 renal and 22 were in the category 'other'. There were no adverse events in the coagulation, pulmonary, cardiovascular and neurological categories. The number of adverse events per patient ranged from three for patient 6 to 19 for patient 4. Thirty-two

Table 3. Overview of adverse events

Study individual	Safety parameters[a,b]																												
	Heme				Coag	Metabolic				GI					Hepatic				Renal				Pulmonary	CV	Neuro	Other			
1	1[c]	0	0	0	--	--			0	1[d]	1[k]	0	0	1[e]	0	0	1[f]	1[g]	0	0	--	--	--	4[h,i]	0	0	0
2	0	0	1[j]	0	--	--			0	3[d]	1[k]	0	0	2[e]	1[l]	0		--			--	--	--	4[h,i]	0	0	0
3	1[c]	0	2[j]	0	--	--			0	1[d]	0	0	0	2[e]	0	0		--			--	--	--	4[h,i]	0	0	0
4	1[c]	2[m]	0	0	--	1[n]	2[n]	1[o]	0	0	3[d,p]	0	0	0	1[e]	2[q]	0	1[r]	2[s]	0	0	--	--	--	4[h]	1[i]	0	0
5	0	0	1[j]	0	--	0	0	1[t]	0		--			0	1[e]	0	0	0	0	1[u]	0	--	--	--	4[h,i]	0	0	0
6		--			--		--				--				--			0	1[v]	0	0	--	--	--	4[h,i]	0	0	0
7	0	1[w]	0	0	--	0	2[x]	0	0	0	1[d]	0	0	0	1[e]	0	0	0	0	1[y]	0	--	--	--	4[h,i]	0	0	0
8	0	1[z]	2[j]	0	--	1[aa]	2[aa]	0	0		--				--			0	1[bb]	0	0	--	--	--	4[h]	0	0	0
9		--			--		--			0	1[d]	0	0		--				--			--	--	--	4[h i]	2	0	0
10		--			--	0	1[x]	0	0	0	1[d]	0	0	1[dd]	1[dd]	0	0		--			--	--	--	4[h]	1[i]	0	0
11	1[c]	1[ee]	0	0	--	0	1[x,ff]	0	0	1[gg]	0	0	0	0	2[e]	0	0	0	1[v]	0	0	--	--	--	4[h,i]	0	0	0
12	0	0	1[j]	0	--	0	1[x]	0	0	0	2[d]	0	0	1[hh]	0	0	0	0	1[v]	0	0	--	--	--	4[h]	0	0	0

[a] The adverse events reporting is based on the WHO Recommendations for grading of acute and subacute toxic side effects, using a 5-point scale (0 = within normal limits; 1 = minimal; 2 = moderate; 3 = severe; 4 = intolerable). Each adverse event is defined in a footnote. The adverse events are defined as pre-existent (before gene therapy); early (0-7 days after vector administration); intermediate (8-30 days after vector administration); and late (> 30 days after vector administration). Adverse events were recorded up to 6 weeks after vector administration. The numbers in the table indicate the severity and time period of occurrence of the adverse event. E.g. 0-1-2-0 means an adverse event grade 1 in the early time period and occurrence of a grade 2 adverse event in the intermediate time period. [b] Abbreviations: Heme, hematologic; Coag, coagulation; GI, gastrointestinal; CV, cardiovascular; Neuro, neurologic. [c] Pre-existent anaemia. [d] Nausea and/or vomiting after prodrug injection. [e] Increase in AST and/or ALT after prodrug administration. [f] Hematuria in a patient with uterine carcinoma. [g] Proteinuria from 5 days after vector administration. [h] In all patients grade 4 pain due to prosthesis loosening, which is one of the inclusion criteria. [i] Temperature of higher than 37.0 degrees Celsius. [j] Occurrence of anaemia or aggravation of pre-existent anaemia following percutaneous cement-injection (hematomas). [k] Diarrhoea one week after prodrug injection. [l] Increase of alkaline phosphatase after percutaneous cement injection with large hematoma. [m] Aggravation of pre-existent anaemia not explained by hematomas from cement injection. [n] Patient treated for M Kahler (multiple myeloma) had a hypocalcaemia at inclusion. The calcium levels decreased during hospital admission to grade 2 levels when corrected for low albumin. [o] Low magnesium levels in a patient with kidney failure and diarrhoea. [p] Diarrhoea after prodrug injection. [q] Increase of AST and alkaline phosphatase after feeding with nasogastric tube. [r] High creatinin level and hematuria in a patient with multiple myeloma and kidney failure. [s] Pre-existing proteinuria in a patient with multiple myeloma and kidney failure aggravating 6 days after vector administration. [t] Hypocalcaemia occurring one day after percutaneous cement injection. [u] Transient hematuria and proteinuria 9 days after vector administration in a patient with a cystitis. [v] Transient hematuria (and in patient 11 also proteinuria) starting 1 day after vector administration. [w] Low leukocyte count one day after vector administration. [x] Transient period of hyperglycemia occurring 1 to 3 days after vector injection in patients without pre-existent diabetes. [y] Transient period of proteinuria. [z] Anaemia at the time of hospital-admittance not present at the time of inclusion. [aa] Hyperglycemia in a patient with pre-existing diabetes and transient aggravation of hyperglycaemia 3 days after vector injection. [bb] Transient period of hematuria starting 6 days after vector administration. [cc] Temperature of 38.1 degrees Celcius in one measurement. [dd] Pre-existent high level of AST, further increasing after prodrug administration, also ALT increase after prodrug administration. [ee] Low leukocyte and thrombocyte count one day after vector administration. [ff] Transient increase in amylase level 1 day after prodrug injection. [gg] Pre-existent nausea and vomiting, aggravated after prodrug administration. [hh] Pre-existing high level of alkaline phosphatase

of the findings that had to be marked as adverse event according to the WHO criteria were present during the inclusion procedure, but were not a reason to exclude the patient from the study. Fifty-one adverse events occurred in the first week after vector injection. Seventeen adverse events occurred between the second and sixth weeks. The events are discussed per organ system.

Hematologic adverse events

Four patients had pre-existent anaemia grade 1 (patient 1, Hb 6.7 mmol/litre; patient 3, Hb 6.5 mmol/litre; patient 4, Hb 6.4 mmol/litre; patient 11, Hb 6.3 mmol/litre). One patient had a low haemoglobin level at hospital admittance, but not during the inclusion procedure (Patient 8: at inclusion Hb 7.8 mmol/litre, at hospital admittance 7.3 mmol/litre), and one patient with a grade 1 anaemia at inclusion had grade 2 anaemia at hospital admittance (patient 4, Hb 6.0 mmol/litre at hospital admittance). Three patients without previous anaemia developed anaemia grade 1 after cement injection (patient 2, Hb at inclusion 8.7 mmol/litre, Hb after cement injection 7.2 mmol/litre; patient 5, Hb 7.9 and 7.2 mmol/litre at inclusion and after cement injection; Patient 12, Hb 8.2 and 7.0 mmol/litre at inclusion and after cement injection), and in two patients with pre-existent anaemia, the anaemia worsened to grade 2 (patient 3, Hb 6.1 mmol/litre; patient 8, Hb 6.1 mmol/litre) after cement injection. One patient in the third vector dose group had a low (grade 1) leukocyte count (patient 7, 3.4 x 10^9 leukocytes/litre) 1 day after vector injection with normal values 3 days after vector injection, and a patient in the highest vector dose group had a low leukocyte and thrombocyte count (patient 11, 3.5 x 10^9 leukocytes/litre 1 day after vector injection, recovering to 4.6 x 10^9 leukocytes/litre 5 days after vector injection; and thrombocytes 99 x 10^9/litre 1 day after vector injection and still less than 100 x 10^9/litre at end of follow-up).

Metabolic adverse events

One patient had diabetes mellitus and had grade 1 hyperglycaemia (Patient 8, glucose 7.3 mmol/litre) at inclusion. Four patients without diabetes had grade 1 or 2 hyperglycaemia 1 to 3 days after vector injection once (Patient 10, glucose 8.4 mmol/litre 1 day after vector injection; patient 11, glucose 7.1 - 7.4 mmol/litre 1 to 3 days after vector injection; patient 12, glucose 7.3 mmol/litre 1 day after vector injection; patient 7, glucose 9.2 mmol/litre 3 days after vector injection), and the patient with diabetes had grade 2 hyperglycaemia (Patient 8, glucose 9.2 mmol/litre) on day 3 after vector injection. One patient with multiple myeloma, treated with prednisolone, had grade 1 hypocalcaemia (patient 4, calcium 2.15 mmol/litre) at inclusion, worsening to grade 2 (calcium 1.80 mmol/litre) during hospital stay. This patient also had a low magnesium level (grade 1) (magnesium 0.57 mmol/litre) 3 weeks after vector injection. Patient 5

Table 4. Gastrointestinal adverse events.

Pt nr	Prodrug dose (mg/m^2)	Total prodrug dose (mg)	Nausea (grade)	Vomiting (grade)	Diarrhea (grade)
1	24	51.1	1	-	1
2	20	32	1	3	1
3	24	37.9	1	1	-
4	24	49	3	3	1
5	8.2	15	-	-	-
6	16	23.7	-	-	-
7	15	27.9	1	1	-
8	11	20	-	-	-
9	16	26.6	1	-	-
10	16	27.7	1	-	-
11	11	18.0	1	1	-
12	16	25.6	1	2	-

To stress a possible relationship with the prodrug, prodrug concentration is also shown. Nausea, vomiting and diarrhoea are graded according to WHO criteria. Nausea: grade 1, reasonable intake; grade 2, decreased intake; grade 3, no meaningful intake. Vomiting: grade 1, 1 episode in 24 h; grade 2, 2-5 episodes in 24 h; grade 3, 6-10 episodes in 24 h. Diarrhoea: grade 1, increase of defecation two or three times per day

had transient low calcium (grade 1), 1 day after cement injection (2.13 mmol/litre), and one patient had a transient increase in amylase level (patient 11, amylase 266 U/litre) 1 day after prodrug injection. All metabolic adverse events were asymptomatic.

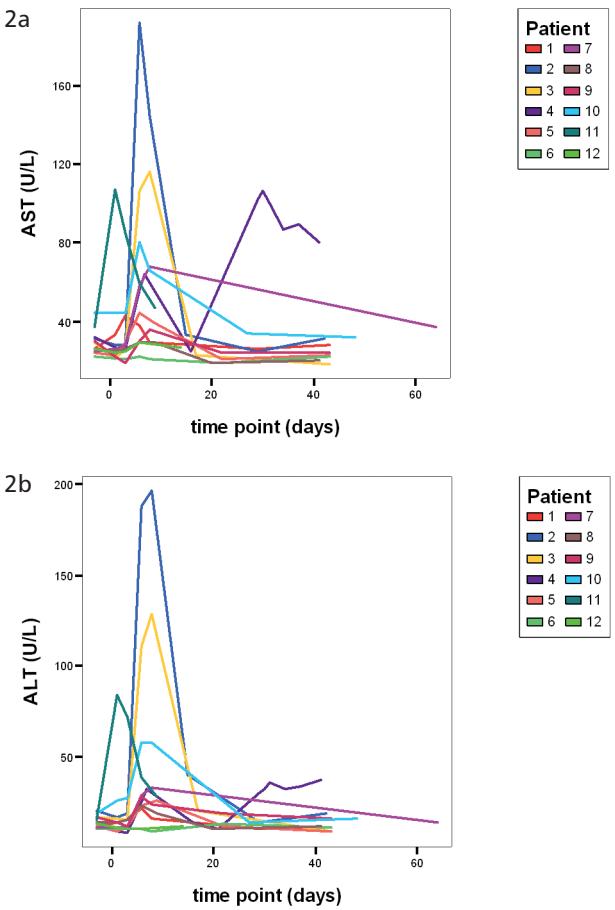
Gastro-intestinal adverse events

One patient had pre-existent nausea and vomiting. Approximately 6 h after prodrug injection 9 of 12 patients developed nausea and vomiting (Table 4). Patient 2 had vomiting grade 3 (8 episodes in 24 h). Patient 4 had nausea grade 3 (insufficient intake) and vomiting grade 3, and needed parenteral fluid administration because of dehydration. Three of the first four patients had diarrhoea grade 1 (increase of defecation frequency of two to three times per day) up to 2 weeks after prodrug injection. After the fourth patient the prodrug dose was lowered to 16 mg/m^2.

Hepatic adverse events

Figure 2 shows aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels over time per patient. In patients with adverse events regarding AST and ALT the patient number is placed near the corresponding line in the Figure 2. Patient 11 had high AST and ALT at hospital admittance, returning to normal in 1 week, whereas she had normal levels at inclusion. Eight patients had a rise in AST 4 to 6 days after vector

Figure 2. AST and ALT levels after gene therapy.



(A) AST levels per patient over time; (B) ALT levels per patient over time. Time point -1 is the inclusion day. Time point 0 is the day of vector injection. In patients with adverse events regarding transaminases, the patient number is noted near the corresponding line, and is further discussed in the Results and Discussion sections.

injection (2 to 4 days after prodrug injection), and four of these patients also had a rise in ALT. In patients 2 and 3 these were grade 2. Patient 4 had a second rise in AST 30 days after vector injection, and a second rise in ALT with a maximum at 7 weeks follow-up.

Patient 2 had a temporary rise in alkaline phosphatase (157 U/litre) 3 weeks after vector injection. Patient 12 had a pre-existent high level of alkaline phosphatase (201 U/litre at inclusion, decreasing to 156 U/litre at the end of follow-up).

Renal adverse events

Patient 1 had pre-existent grade 1 hematuria (Hb: 2+/ 25 Ery/μl) at inclusion and developed proteinuria (protein 1+/ 30mg/dl) 5 days after vector injection and continuing until after the end of follow-up. Patient 4 had high creatinine levels (creatinine, 156 μmol/litre), proteinuria (protein 1+/ 30mg/dL) and grade 1 hematuria (Hb: 2+/ 25 Ery/μl) at inclusion; the proteinuria worsened to grade 2 (protein 2+/ 100mg/dl) on day 6 after vector injection. Three patients had a transient hematuria 1 day after vector injection (patient 6: Hb: 1+/ 10 Ery/μl; patient 11, Hb 2+/ 25 Ery/μl; Patient 12, Hb 2+/ 25 Ery/μl), and one of these patients also had transient proteinuria (patient 11, protein 1+/ 30mg/dl). Two more patients had transient hematuria, one after 6 days (patient 8, Hb: 2+/ 25 Ery/μl), and one 9 days after vector injection (patient 5, Hb: 1+/ 10 Ery/μl). One patient had a transient period of proteinuria starting 9 days after vector injection (patient 5, Protein 1+/ 30mg/dl).

Other adverse events

All patients had pre-existing pain in the hip (WHO-grade 4; needing pain killers), which was an inclusion criterion. Ten of 12 patients had at any time point a body temperature between 37 and 38°C. One of these patients had a body temperature of 38.1°C 0.5 h after prodrug injection, which is an adverse event grade 2.

Serious adverse events

Four serious adverse events were registered during 6 months follow-up; none was directly related to the VDEPT approach. Patient 1 was diagnosed with uterine carcinoma 8 weeks after vector injection. The patient died of the carcinoma 4 months after vector injection.

Patient 4 was a 91-year old patient with multiple myeloma who died of progressive kidney failure 3 months after vector injection. The exact cause of the kidney failure could not be determined, but it was assumed to have its origin in a kidney localisation of the multiple myeloma. Consent for autopsy was not obtained.

Patient 6 was admitted to the hospital 6 months after vector injection for dehydration and was discharged after a few days.

Patient 8 was admitted to the hospital 3 months after vector injection for hyponatremia and hypotension and was discharged after a few days.

Discussion

In this phase 1 clinical gene therapy study no dose-limiting toxicity occurred. Vector administration could be continued until the proposed highest dose of 1 x 10^{11} particles.

However, prodrug dose was lowered because of inconvenient adverse events in the first four patients (i.e., nausea and vomiting, and hepatic adverse events). At 6 weeks follow-up, 8 of 12 patients had clinical benefit from the gene therapy and cement injection treatment.[28]

Harvey *et al*.[55] summarise experiences on adverse events in local administration of HAdV-5 vectors in 90 individuals. The most common adverse events they reported were transient fever and leucocytosis. In our study on hip prosthesis loosening 10 of 12 patients had grade 1 fever at any time (mostly already at inclusion), and 1 patient had a temperature of 38.1°C (grade 2) 30 min after prodrug injection. Occurrence of fever was not related to the time of vector injection. As temperature exceeding 37°C were already present mostly at inclusion, and it did not increase much further during the study, it is not likely that it was caused by any of the study products. In a phase 1 study for safety of the CTL102 vector alone, the vector was injected directly into hepatic tumours up to doses of 5 x 10^{11} particles (Palmer *et al.*)[91] and asymptomatic fever (≤38.5°C) was seen in 4 of 18 patients 4 to 8 h after vector injection; leukocytosis was not seen in any of the patients. As a joint is a more or less closed compartment, and inclusion of the patients was limited to those who had neutralising antibodies against HAdV-5, thereby inactivating the viral vector outside the joints, exposure of the vector particles to the immunologic system may be minimal, which explains the lack of leukocytosis. Two patients, one in the third and one in the fourth dose group, had leukopenia (grade 1) 1 day after vector injection. This has been described previously in a suicide gene therapy trial, and was explained by bone marrow depression by the prodrug, whereas in our study the leukopenia occurred before the prodrug was injected. The cause of leukopenia in our study is unknown.

One patient in the highest vector dose group, who already had a platelet count of 112 x 10^9 at inclusion, showed an asymptomatic decrease in platelet level grade 1. This feature of thrombopenia was shown previously in a toxicity-study with baboons,[85] and was also shown in one study with human subjects,[110] and in these studies was attributed to probable cytokine induction.

Besides a rise in creatinine levels no renal adverse events after local vector injection are recorded in the literature.[55] In our study 8 of 12 patients had hematuria or proteinuria. Patient 1 had hematuria at inclusion and developed proteinuria during follow-up. This patient was diagnosed with uterine carcinoma, which could be an explanation for her hematuria and proteinuria. Patient 4 had pre-existent kidney failure with hematuria, proteinuria and high levels of creatinine. Patient 11 had a catheter à *demeure* at hospital admittance, which could explain her hematuria and proteinuria. Two patients (patient 5 and 12) developed cystitis during hospital stay, explaining the hematuria and proteinuria. Hematuria among the other three patients might be explained by antico-

agulants, which are given to all surgical patients in the department during hospital stay for thrombosis prophylaxis.

Nine of 12 patients experienced nausea and vomiting starting 6 h after prodrug injection, with patient 4 needing parenteral fluid administration because of dehydration. Nausea and vomiting, and other flulike symptoms, were reported by previous studies as side effects from Ad-vectors.[65,110,113] However, in the present study the nausea and vomiting are more likely to be caused by side effects from the prodrug, as the occurrence decreased after lowering the prodrug dose and did not increase with the 30-fold increase in HAdV-5 vector. Besides nausea and vomiting eight patients had a rise in AST levels, with a maximum 4 days after prodrug injection, and four of these patients also had a rise in ALT levels. These were asymptomatic and completely reversible. Two patients in the high prodrug dose group (and lowest adenoviral vector group) had grade 2 rises in AST and ALT levels, and three had diarrhoea. In the prodrug toxicity study of Chung-Faye *et al*.[23] these adverse events were also reported. In their study patients received a prodrug dose of 24, 30 or 37.5 mg/m^2 intravenously, without previous vector administration. In the 24 mg/m^2 group three of four patients experienced nausea and vomiting (up to grade 3), but no biochemical liver abnormalities occurred. In the higher dose groups five of eight patients did have a transaminase elevation, after which the authors concluded that 24 mg/m^2 would be the recommended dose. One patient received 24 mg/m^2 in a peritoneal injection and experienced grade 1 diarrhoea and grade 1 transaminitis. The pathophysiology of the gastrointestinal adverse events of the prodrug is unknown; two possible explanations are considered. First, the most likely explanation is indirect gastrointestinal epithelial toxicity. Another explanation might be conversion of CB1954 to the activated form by Ntr from *E. coli* present in colonic flora. This pathophysiologic mechanism is impossible to prove because the activated form of CB1954 is highly reactive and has a halftime (i.e., 18 min) that is too short to be detected. In the study by Chung-Faye *et al.*[23] less than 5% of the drug was detected in the urine and the drug clearance was 184 to 958 ml/min, indicating that the CB1954 metabolism is predominantly hepatic. The latter underscores that the rise in transaminase levels can be explained by liver cell damage by CB1954 metabolites during drug clearance. Although liver toxicity by CB1954 would be a feasible explanation for the rise in transaminases, previous studies with intravenous HAdV-5 injections have also shown a role for HAdV-5 vectors in transaminitis.[110] Morral *et al.*[85] gave a very high dose of adenoviral vector (1.2 x 10^{13} particles/kg) to a baboon and observed severe liver damage, with AST levels rising from 37 to 7440 U/litre after 48 h and ALT levels rising from 30 to 1520 U/litre. The platelet count decreased from 558,000 to 18,000/ mm^3 with this high vector dose. Liver toxicity caused by local administration of adenoviral vector was not reported in the meta-analysis by Harvey *et al.*[55] The CTL102

safety study performed by Palmer *et al.*[91] also showed, throughout the study period, no changes in biochemical estimates of liver function after local injection, giving an additional argument that the transaminitis found in our study is caused by the prodrug rather than the (intra-articularly administered) vector. The dose and delivery routes used in the Palmer study and ours may minimise potential adenovirus-mediated liver toxicity. Patient 4 had a second rise of AST and ALT 1 month after vector injection, most probably due to feeding by stomach tube.

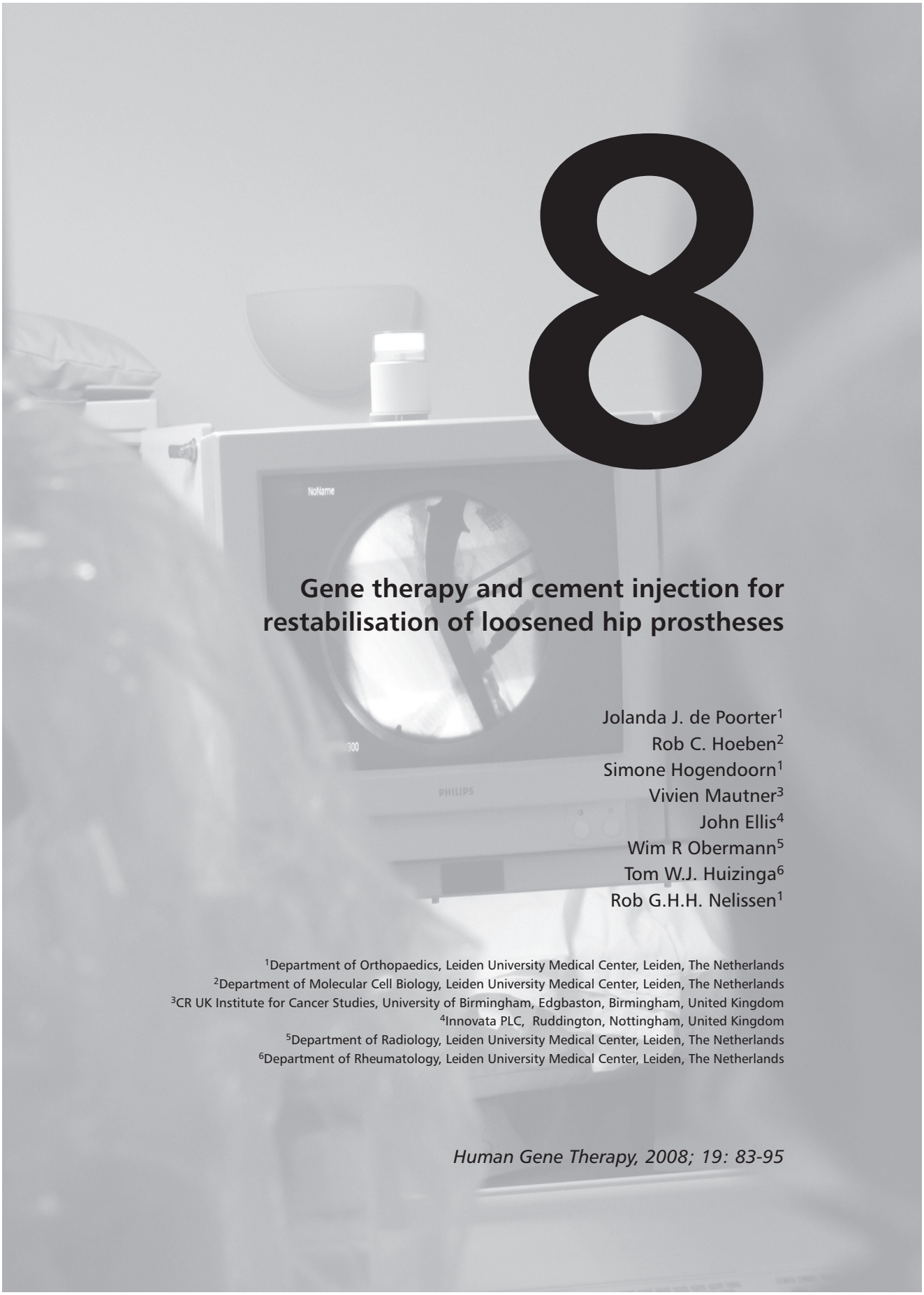
None of the patients had adverse events from the spinal anaesthesia. Besides local pain, the percutaneous cement injection caused hematomas and anaemia in five patients. One patient had a large hematoma and developed a transient rise in alkaline phosphatase levels. These adverse events due to cement injection were inconvenient, but relatively mild compared with local complications in revision surgery. Complications caused by the cement itself are rare, as described in the literature, and are associated mostly with hypotension directly after injection of cement or with fat embolisation.[86,111] Furthermore, these complications will probably not occur with percutaneous cement injection, in which only small amounts of cement are injected. To our knowledge no hematological or biochemical changes have been reported after cement injection.

Four patients not previously diagnosed with diabetes had grade 1 hyperglycaemia 1 to 3 days after vector-injection and one patient with diabetes had grade 2 hyperglycaemia. Hyperglycaemia has been reported as an adverse event in another suicide gene therapy approach by Freytag *et al.*.[40] A definite explanation for the hyperglycaemia could not be found, but the authors concluded that it may be related to the fact that many elderly patients are known to be borderline diabetics and/ or experiencing treatment-related stress.[40]

All four serious adverse events were reported to the Medical Ethics Review Board and the Central Committee on Research Involving Human Subjects by telephone and in writing within 24 h after the SAE was known to the investigators.

In all cases the study's independent safety committee judged the relationship of the SAE with the study. In all four adverse events the safety committee could not find a causal relation between the gene therapy and the occurrence of the serious adverse event.

In conclusion, an intra-articular VDEPT approach with an adenoviral vector and CB1954 as prodrug and percutaneous transosseous bone cement injection is a viable option in the treatment patients with hip prosthesis loosening. No dose limiting toxicity occurred and all adverse effects could be treated. However, the extensive comorbidity in these patients makes it difficult to fully establish the safety of this approach in this small and heterogeneous patient population.



8

Gene therapy and cement injection for restabilisation of loosened hip prostheses

Jolanda J. de Poorter[1]
Rob C. Hoeben[2]
Simone Hogendoorn[1]
Vivien Mautner[3]
John Ellis[4]
Wim R Obermann[5]
Tom W.J. Huizinga[6]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands
[3]CR UK Institute for Cancer Studies, University of Birmingham, Edgbaston, Birmingham, United Kingdom
[4]Innovata PLC, Ruddington, Nottingham, United Kingdom
[5]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
[6]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

Human Gene Therapy, 2008; 19: 83-95

Abstract

Loosening of orthopaedic hip prostheses is an increasing health problem. In elderly patients with comorbidity, revision surgery may lead to high mortality rates. A less invasive surgical technique is therefore required to reduce these patient risks. To this end a percutaneous gene therapy approach was designed to destroy the periprosthetic loosening membrane, and enable refixing of the hip prosthesis with percutaneous bone cement injections under radiological guidance. In this phase 1/2 dose-escalating gene therapy clinical trial, 12 patients were treated. Toxicity and hip function variables were monitored up to 6 months posttreatment. All patients completed the study and no dose-limiting toxicity was observed. Improvement in walking distance, independence, and pain was demonstrated particularly in patients receiving 3×10^{10} and 1×10^{11} viral particles. Taken together, these data show that this gene therapy approach targeted at the interface membrane around a loosened hip prosthesis is a feasible treatment option for elderly patients for whom surgical intervention is not appropriate.

Introduction

Annually, approximately 1 million total hip replacement operations are performed worldwide. This number is likely to increase considerably in the next decade because of two effects: (1) the longer life expectancy of populations in Western society and (2) the relative success of hip replacements at long-term follow-up, and the tendency to insert prostheses at a younger age (i.e., patients younger than 55 years). However, within 10 years of follow-up after primary hip replacement 7-13% of patients will require revision surgery because of loosening of their implant.[78]

The major cause of loosening in the longer term is aseptic, mechanical loosening due to wear-related particles of the articulating artificial joint. These wear particles, such as particles of polyethylene and metal, are phagocytosed by macrophages, initiating the secretion of inflammatory cytokines.[43] This in turn activates a cascade of inflammatory processes resulting in periprosthetic bone resorption and the formation of a loosening membrane, the interface tissue. This tissue constitutes a pseudo-membrane of synovium-like tissue with activated macrophages, fibroblasts, giant cells and osteoclasts.

The induced bone resorption process causes mechanical loosening of the prosthesis. Because a loosened prosthesis migrates into the endosteal medullary canal of the femur, pain is caused by any movement of the hip. At first pain is brought on by walking, but ultimately any movement (i.e., turning in bed) may cause incapacitating pain. The only treatment for this inevitable prosthesis-loosening process is revision surgery, during which the loosened prosthesis and the interface tissue are removed and a new prosthesis is implanted. This is an extensive procedure with subsequent blood loss and an infection risk that is twice as high as during primary surgery. Increasing age (the patients are at least 10 years older than during the primary hip prosthesis implantation), hypovolemia during surgery, and administration of large amounts of fluid intra- and post-operatively are risk factors for multiorgan failure.[57] Consequently, revision surgery has a high morbidity and mortality rate, especially in elderly patients with comorbidity. In the United States Medicare Population 5.3% of 3165 patients who received revision surgery at age 80 and older died within 90 days of surgery.[77] Strehle *et al.*[114] registered complications and social outcome in a cohort of 53 patients older than 80 years of age and undergoing revision total hip arthroplasty. Eleven patients (21%) were admitted postoperatively to the intensive care unit, and 3 patients died during their hospital stay. Despite the invalidating pain, these high morbidity and mortality rates are the major determinants for the eligibility for surgery of elderly patients. At present there are no alternative treatments for prosthesis loosening.

Hip prostheses can either be fixed to the bone with bone cement or designed to

have bone ongrowth properties such as a calcium phosphate coating or a rough titanium surface. Bone cement, acting as a filler for the periprosthetic cavities next to the metal prosthesis, has also been used to refix loosened hip prostheses during revision surgery. In these cases the old cement of the primary hip arthroplasty is left in place and a new prosthesis is inserted in this cement canal using new cement. A prerequisite for this recementation technique is that a good integration of new and old bone cement is possible.[49,73] These data led to the idea of a percutaneous technique for removal of the loosening membrane (i.e., interface tissue) and subsequent percutaneous cement injection in the periprosthetic cavity. Neither part of this technique has been described so far in the literature. As was shown by our group, the interface tissue can be transduced and killed *in vitro*[30] and *in vivo* in animals[46] by the viral vector HAdV-5-Ntr in combination with the prodrug 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB1954). To evaluate this concept of removal of interface tissue by a gene-directed therapy and cement refixation of the prosthesis, a phase 1/2 clinical trial was performed in 12 elderly patients with considerable comorbidity. Because these patients had an increased surgery risk, revision surgery is no longer feasible and these patients are wheelchair bound. This paper describes the feasibility and 6-months follow-up of the clinical results.

Materials and methods

Trial design

In this phase I dose escalation study of the replication-deficient adenoviral vector CTL102 and the prodrug CB1954, safety is the primary objective. Adverse events were defined according to World Health Organisation (WHO, Geneva, Switzerland) recommendations for grading of acute and sub-acute toxic effects. Dose-limiting toxicity was defined as any WHO grade 3 or 4 adverse event except for pain, nausea, and vomiting. Secondary end points were to assess virus distribution, efficiency of cell killing by the gene therapy approach, and clinical outcome.

Patients eligible for inclusion were informed orally and in writing about the study and were given at least 1 week for reflection before informed consent was obtained. For inclusion an arthrogram of the hip with aspiration of joint fluid was performed to exclude bacterial infection, to confirm loosening, and to determine containment (i.e., leakage of contrast medium) and the volume of the hip joint. Patients who satisfied the inclusion and exclusion criteria and who were willing to participate in the study were admitted to the hospital for 11 days. The study had a dose-escalating design, with four patients in each group. On day 1 the vector was injected directly into the hip joint

and 2 days later the prodrug was injected in the same way. Five to 7 days after vector injection, cement was injected around the prosthesis under spinal anaesthesia. Patients were ambulated the day after cement injection. After discharge from the hospital patients were assessed after 3 and 6 weeks, 3 and 6 month and every year.

Patient selection

Patients eligible for inclusion were elderly patients with debilitating pain from a loosened hip prosthesis causing ADL (activities of daily living) dependency, and with significant comorbidity making them ineligible for normal revision surgery. Exclusion criteria included infection of the prosthesis, absence of neutralising antibodies against human adenovirus subtype 5, obvious adenoviral infection at the time of vector injection, history of hepatitis, human immunodeficiency virus (HIV) infection, alcohol or drug abuse, and known immunodeficiency (including chemotherapy, radiotherapy or immunotherapy within the previous 28 days). The study protocol was approved by the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands); the Ministry of Housing, Spatial Planning and the Environment (VROM, The Hague, The Netherlands); and the local ethics committee and was conducted according to the principles of the Declaration of Helsinki (as amended in Tokyo, Venice and Hong Kong, Somerset West and Edinburgh), and in accordance with the Guidelines for Good Clinical Practice (CPMP/ICH/135/95- July 17, 1996).

Vector and prodrug preparation

The adenoviral vector CTL102 was constructed by homologous recombination in PER.C6 helper cells,[38] as described in previous studies.[34] CTL102 is an E1, E3-deleted replication-deficient human adenovirus serotype 5 (HAdV-5) vector, engineered to contain the *Escherichia coli nfsB* gene under the control of the cytomegalusvirus immediate-early (CMV-IE) promoter.[34,125] The complete *Escherichia coli* nitroreductase (Ntr) expression cassette was cloned into an E1-deleted HAdV-5 adenovirus transfer vector. Virus batches of 4.2×10^{10} and 4×10^{11} particles/ml with a particle : infectivity ratio of 18 and 20, respectively, were used in the study. The batches were free of replication-competent adenovirus (RCA) at a detection limit of 1 in 10^{10} plaque-forming units, and free of mycoplasma and other impurities, thereby meeting the specifications agreed upon with the Dutch regulatory authorities. The drug product CB1954 was supplied by ML Laboratories plc (Liverpool, UK) as a sterile solution of CB1954 17.8 mg/ml in solvent (N-methyl-pyrrolidone 22.2% $^{v}/_{v}$, polyethylene glycol 300 77.8% $^{v}/_{v}$). The prodrug CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] is a weak monofunctional alkyl-

ating agent, which is converted by Ntr to a cytotoxic derivative.[68] Cells containing Ntr convert CB1954 into a bifunctional alkylating agent that is capable of forming DNA interstrand cross-links, resulting in cell death.[18,34,102] Because there is no human homolog to Ntr,[23] cells expressing the nitroreductase gene are killed when exposed to CB1954, as are neighbouring cells by means of bystander effect. This makes the Ntr-CB1954 combination exploitable for a virus-directed enzyme prodrug therapy (VDEPT) approach. Just before use this prodrug in solvent was diluted in sterile saline to a maximum final concentration of 2 mg/ml.

Administration of vector and prodrug

The vector CTL102 was diluted in an isotonic buffer (Tris [25 mmol/litre, pH 7.4]; 0.14 M NaCl; KCl [5 mmol/litre]; Na_2HPO_4 [0.6 mmol/litre]; $CaCl_2$ [0.9 mmol/litre]; $MgCl_2$ [0.5 mmol/litre]; 5% sucrose) in a volume representing 90% of the volume injected during the inclusion arthrogram. The initial dose was 3 x 10^9 particles (Table 1). Dose escalation proceeded in 3-fold increments up to 1 x 10^{11} particles. The vector was administered by direct intra-articular injection. Therefore, a spinal needle directed at the femoral neck was positioned in the hip joint under fluoroscopic guidance and synovial fluid was aspirated. Before injection of the vector, the joint was rinsed with ethylenediaminetetraacetic acid (EDTA; Sigma Chemical, St Louis, MO, USA) at a concentration of 2 mM in a sodium salt solution to remove as much neutralising antibody from the joint cavity as possible.

The prodrug CB1954 was diluted in sterile saline to the same volume as the vector and was also injected intra-articularly. Before injection of the prodrug, synovial fluid was aspirated for quantitative ploymerase chain reaction (PCR) analysis of adenoviral DNA. All patients were intended to have a prodrug dose of 24 mg/m^2 with a maximal concentration of 2 mg/ml injected in the joint space, but after gastrointestinal and hepatic adverse events in the first four patients this dose was lowered to 16 mg/m^2.

Cementing technique

Two techniques for cementation of the prosthesis were used in the study: fluoroscopy-controlled and computer tomography (CT)-controlled combined with fluoroscopy. For both techniques, areas of the periprosthetic space most suitable for cement injection were identified on the basis of previously performed X-rays, that is, the region where the greatest amount of periprosthetic radiolucency was present was targeted. All patients underwent spinal anaesthesia. Patients were positioned supine with the hip and groin region sterilely covered. In the CT-controlled group a planning scan was performed to exactly define the positions for introduction of the needles. Three to five ver-

Table 1. Patient characteristics

						Vector injection		Prodrug injection		
Pt nr	**Age (years)**	**Sex (M/F)**	**ASA-cat**	**Under-lying Disease**	**Body surface (m^2)**	**Planned dose (particles)**	**Actual dose (particles)**	**Planned dose (per m^2 / in mg)**	**Actual dose (per m^2 / in mg)**	**Injected volume (mL)**
1	82	F	4	OA	2.13	$3x10^9$	$3x10^9$	24/ 51.1	24/ 51.1	27
2	72	F	2	OA	1.64	$3x10^9$	$3x10^9$	24/ 39.4	**20/ 32.0**	16
3	78	F	4	RA	1.58	$3x10^9$	$3x10^9$	24/ 37.9	24/ 37.9	30
4	91	M	3	OA	2.04	$1x10^{10}$	**$8.3x10^9$**	24/ 49.0	24/ 49.0	30
5	75	F	3	OA	1.83	$1x10^{10}$	$1x10^{10}$	24/ 43.9	**8.2/ 15.0**	7.5
6	86	F	3	OA	1.48	$1x10^{10}$	$1x10^{10}$	16/ 23.7	16/ 23.7	22
7	85	F	3	OA	1.87	$3x10^{10}$	**$2.6x10^{10}$**	16/ 29.9	**15/ 27.9**	14
8	86	F	4	RA	1.77	$3x10^{10}$	$3x10^{10}$	16/ 28.3	**11/ 20.0**	10
9	81	F	2	OA	1.66	$3x10^{10}$	$3x10^{10}$	16/ 26.6	16/ 26.6	27
10	82	F	2	OA	1.73	$1x10^{11}$	$1x10^{11}$	16/ 27.7	16/ 27.7	30
11	76	F	4	RA	1.68	$1x10^{11}$	$1x10^{11}$	16/ 26.9	**11/ 18.0**	9
12	89	F	2	OA	1.6	$1x10^{11}$	$1x10^{11}$	16/ 25.6	16/ 25.6	18

Shown are demographic characteristics and information about vector and prodrug dose.
Actual prodrug dose: milligrams per square meter/ total milligrams
Abbreviations: F, female; M, male; ASA-cat, category according to American Society of Anesthesiologists; OA, osteo- arthritis; RA, Rheumatoid Arthritis.

tebroplasty needles of 3.2 x 100 mm (Biomet, Dordrecht, The Netherlands) were introduced into the periprosthetic space using a hammer. The position of the needles was controlled by CT guidance. After placement of the needles the C-arm was positioned over the patient. The polymethylmethacrylate (PMMA) cement (Osteopal, Biomet; Disc-O-Tech, Disc-O-Tech Medical technologies , Herzeliya, Israel) was injected into the periprosthetic space under high pressure with a Cementoset (Biome). During injection the flow of the PMMA cement was continuously monitored by fluoroscopy. Injection was continued until the periprosthetic space was filled and was discontinued when the cement threatened to leak into the joint space or when there was leakage of cement into the soft tissues (i.e., extraosseous). Before removal, the needles were turned in a clockwise or counterclockwise manner several times to ensure easy removal.

Objectives

Safety measurements

Safety for the patient was the primary objective in this study. Adverse events were monitored according to WHO Recommendations for Grading of Acute and Subacute

Toxic Effects. Vital signs (blood pressure, pulse rate, temperature, and breathing frequency), medical history, and a Visual Analogue Scale (VAS) for pain were done after vector injection every 30 min for the first 4 h, and then hourly to 8 h after vector injection, and every 4 h to 24 h. After 24 h these measurements were done every 6 h until prodrug injection, and subsequently every day. In addition, vital signs were monitored 30 min and 1, 2, 4, 6, and 12 h after prodrug injection. Blood samples for hematological and biochemical analysis and urine samples were taken 1, 3, 6 and 8 days after vector injection. After discharge from the hospital adverse events were monitored at time points 3 and 6 weeks after vector injection.

Virus shedding

After vector injection, patients were kept in isolation until virus shedding was negative. Plasma, urine, stool, and nose and throat swabs were analysed for the presence of adenoviral DNA as previously described[35] by real-time PCR. Samples were taken 24 h after vector injection and every 24 h until negative results. The detection limit of this assay was determined to be 500 copies/ml.

Biopsies

Whenever possible, during cement injection procedure, biopsies from the periprosthetic interface area were taken. These biopsies were investigated by the Department of Pathology (Leiden University Medical Center, Leiden, The Netherlands) for the presence of apoptotic and necrotic tissue.

X-ray measurements

Standard anteroposterior (AP) and lateral radiographs of the hip were performed in all patients during the inclusion period and at 1 day, 6 weeks, 6 months and every year after cement injection. On the radiographic images the perisprosthetic space was divided in 14 zones (Gruen zones) according to Gruen, McNeice, and Amstutz.[50] In the AP image zone 1 to 7 could be identified, with zone 1 being located on the lateral proximal side, zone 4 on the distal side, and zone 7 on the medial proximal side. In the lateral image zone 8 to 14 could be identified, with zone 8 being the proximal posterior zone, zone 11 the distal zone, and zone 14 being the proximal anterior zone. The maximal cement layer thickness and the minimal and maximal wideness of radiolucency were measured using Ortho-CMS software (Medis, Leiden, The Netherlands). These measurements were performed for X-rays made during the inclusion period and 1 day and 6 months after cement injection, and the differences between these measurements were analysed.

Patient satisfaction

For clinical evaluation, the Harris Hip Score[53], and Visual Analogue Scales (VASs) for pain, walking distance and dependency in activities of daily living were done pretreatment and 3 and 6 weeks, 3 and 6 months and every year after treatment. The HHS was used to evaluate outcome of hip prostheses. The score (maximum, 100 points) is composed of outcome measurements for pain (maximum, 44 points), activities (maximum, 14 points), walking (maximum, 33 points), absence of deformities (maximum, 4 points), and range of motion (maximum, 5 points). Each VAS was administered by asking the patient to place a mark on a 10 cm line at an appropriate distance between two end points. For purposes of, 0 cm was always a bad result and 10 cm a good result. To compare the VAS with the HHS , the HHS results were multiplied to obtain a maximum score of 100. For comparison with pain by VAS the pain score from HHS was used (maximum, 44 points). For walking distances the HHS measurement of walking distance (maximum, 11 points) was compared with the VAS.

Statistical analysis

Means and standard deviations for all variables, and correlations between HHS and VAS scores, were calculated using SPSS 12.0 for Windows (SPSS, Chicago, IL, USA).

Results

Patient characteristics

Twelve patients ranging in age from 72 to 91 years (mean, 82 years) were treated; 1 male, and 11 females. Comorbidity of the patients was graded according to the criteria of the American Society of Anesthesiologists (ASA categories).[121] Four patients were in ASA category 2 (mild systemic disease); four patients were in ASA category 3 (severe systemic disease), and four patients were in ASA category 4 (severe systemic disease that is a constant threat to life). Three patients were diagnosed with rheumatoid arthritis; the other patients had osteoarthritis. Neutralisation tests using Ad5 β-galactosidase showed that all patients had specific activity against Ad5 before treatment. Two patients had a somewhat lower level of neutralising antibodies than the other patients. There was no relationship between level of neutralising antibodies and the occurrence of adverse events, shedding of the virus, or therapeutic benefits. The dose of the CTL102 vector ranged from 3×10^9 particles to 1×10^{11} particles (Table 1). All patients were intended to have a prodrug dose of 24 mg/m^2 with a maximal concentration of 2mg/ml injected in the joint space, but after moderate gastrointestinal

and hepatic toxicity in the first four patients this dose was lowered to 16 mg/m^2. Two patients had a slightly lower vector dose and five patients had a lower prodrug dose than was planned, because the contents of the syringe could not be injected entirely (Table 1).

Toxicity

Nine of 12 patients experienced nausea and vomiting starting 6 h after prodrug injection, with 1 patient needing parenteral fluid administration for dehydration. Besides nausea and vomiting, eight patients had a rise in aspartate aminotransferase (AST) levels, with a maximum 4 days after prodrug injection, and four of these patients also had a rise in alanine aminotransferase (ALT) levels. These were asymptomatic and completely reversed. Two patients in the high prodrug dose group had grade 2 rises in AST and ALT levels, and three had diarrhoea.

Four serious adverse events occurred. One patient was diagnosed with a uterine carcinoma, and died of the carcinoma at 4 months follow-up. One patient developed kidney failure, probably from a previously diagnosed multiple myeloma, and died at 3 months follow-up. One patient was admitted to the hospital for dehydration at 6 months follow-up, and one patient was admitted to the hospital for hyponatremia and hypotension at 3 months follow-up. A detailed description of all adverse events is provided elsewhere.

Virus Shedding

Plasma, urine, and stool samples, and nose and throat swabs were analysed for the presence of adenoviral DNA by real-time PCR as previously described.[35] All patients in the lowest three vector dose groups (3 x 10^9, 1 x 10^{10}, 3 x 10^{10} particles) had negative shedding results (guaranteed detection limit of 500 copies/ml) at the first measurement (after 24 h). Two patients in the highest dose group (1 x 10^{11} particles) tested positive. One patient had 250 copies/ml in her blood plasma after 24 h. Urine and stool samples, and nose- and throat-swabs were negative. All samples taken at 48 h showed no presence of adenoviral DNA. Another patient in the highest dose group showed a plasma concentration of vector DNA equivalent to 480 adenoviral particles per millilitre at 24 h. All other samples were negative. The next day samples were taken again; these all tested negative.

To investigate whether the adenoviral DNA was successfully introduced into the interface tissue and joint space, joint fluid was tested for adenoviral DNA by PCR. Before rinsing the joint and injecting the prodrug at day 2, joint fluid was aspirated whenever pos-

Table 2. Adenoviral DNA in the joint fluid.

Patient number	Joint capacity	Particles injected	Particles 2 days post-injection	Additional measurements
1	27	$3x10^9$	NS	
2	16	$3x10^9$	5.24×10^5	
3	30	$3x10^9$	5.07×10^7	+10 days: 2.60×10^5 particles
4	30	$8.3x10^9$	3.21×10^7	
5	7.5	$1x10^{10}$	1.45×10^7	
6	22	$1x10^{10}$	1.23×10^7	
7	14	$2.6x10^{10}$	NS	
8	10	$3x10^{10}$	NS	
9	27	$3x10^{10}$	NS	
10	30	$1x10^{11}$	1.00×10^7	
11	9	$1x10^{11}$	NS	+11 months: 1.88×10^4 particles
12	18	$1x10^{11}$	positive	

Whenever possible before prodrug injection, joint fluid was aspirated for quantitative analysis of adenoviral DNA by PCR. In five patients aspiration was not successful, or the amount of aspirated fluid was too small for analysis (NS, not successful). In one patient only qualitative analysis was done showing positive results. In the remaining patients number of particles in the joint fluid is shown in the table as the number of particles per ml multiplied by the joint capacity. In two patients an additional measurement was done.

sible. In five patients aspiration was unsuccessful or the amount of aspirated fluid was too small for analysis. In one patient only qualitative analysis was performed, showing adenoviral DNA in the joint fluid. In the other six patients quantitative analysis showed the presence of a considerable number of adenoviral particles in the joint (Table 2). Plaque assays of the adenoviral DNA from the joint fluid showed no infectious virus. In two patients the hip joint was punctured at another time point, giving the opportunity for collecting joint fluid. These samples both showed the presence of adenoviral DNA, one at 10 days after vector injection and the other at 11 months after vector injection.

Biopsies

Whenever possible during injection of cement, needle biopsies from the interface tissue were taken for histological investigation. Biopsy of interface tissue was unsuccessful in three patients. The specimens were analysed for apoptotic cells or necrosis. In two patients the amount of material obtained was insufficient to analyse. In the specimens from the remaining seven patients, necrotic tissue was found in the biopsies of four patients. The other materials showed partly vital and partly degenerative fibrous tissue, bone marrow, and blood.

Outcome measured by imaging: Radiographic analysis

To objectively measure the changes on X-rays, for each patient X-rays taken at inclusion period, 1 day after cement injection and at 6 months of follow-up were compared. At each of these time points anteroposterior and lateral X-ray views of the hip were taken. In each Gruen zone the maximal cement thickness and maximum and minimum widths of radiolucency were measured. Figure 1 shows the mean increase in cement mantle thickness per Gruen zone and per dose group, after cement injection compared with the inclusion period. In Figure 1 the periprosthetic bone is divided in 14 areas according to Gruen and co-workers.[50] For each zone the maximal thickness of cement was measured before and 1 day after cement injection. The increase in cement thickness was calculated and in Figure 1 the mean increase is shown per dose group. The exact numbers of these calculations and the standard deviations are shown in Table 3. Table 3 shows that the largest amount of cement could be injected in Gruen zone 1 (3.98 +/- 5.4mm). In the higher dose groups more cement could be injected (mean in group 1 was 0.44; in group 2 it was 0.77; in group 3 it was 1.74; in group 4 it was 2.05). This difference in increase in cement thickness was significant only in the third group (Group 1, $p = 0.836$; group 2, $p = 0.450$; group 3, $p = 0.024$; group 4, $p = 0.418$).

Figure 2 shows the filling of the maximum radiolucent zone by cement per dose group. In Figure 2 the maximal radiolucency width before cement injection is shown, along with the part of the zone that was filled by cement after cement injection.

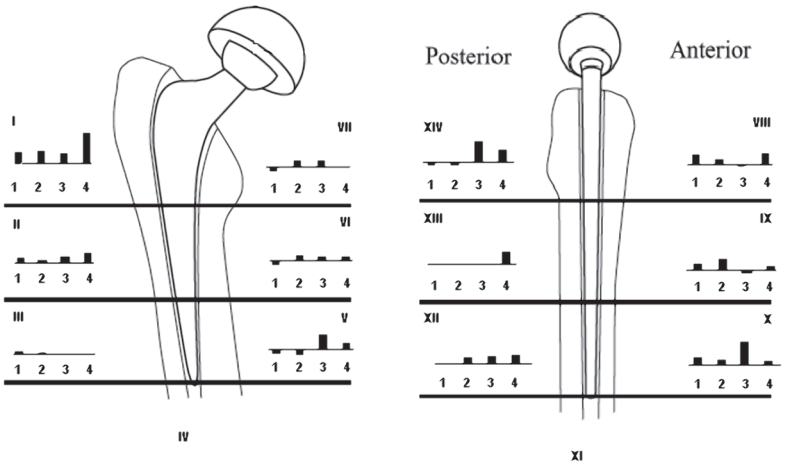
Maximal cement thickness and maximal radiolucency width were also measured at 6 months of follow-up. Compared with 1 day after cement injection the maximal cement thickness at 6 months follow-up had decreased by 0.02 mm ($p = 0.91$). The correlation between maximal cement thickness at 1 day and at 6 months after cement injection was 0.94 ($p < 0.01$). After 6 months the maximal radiolucency width had increased with 0.19 mm ($p = 0.11$). Correlation between maximal radiolucency width at 1 day and at 6 months after cement injection was 0.820 ($p < 0.01$).

Figure 3 shows an example of radiographic examination before and after gene therapy and cement injection. Figure 3 shows that cement that is injected can easily spread through the periprosthetic space to provide more strength and stability.

Clinical outcome: Patient satisfaction

At inclusion and at 3 and 6 weeks and 3 and 6 months after cement injection Visual Analogue Scales (VASs) for pain, walking distance and activities in daily living (ADL) and the Harris Hip Score[53] were performed. Figure 4a and 4b shows means and standard deviations for, respectively, decrease in pain and increase in walking distance by VAS

Figure 1. Mean and standard deviations for the increase in cement mantle thickness per Gruen zone, per dose group after cement injection compared to the situation in the inclusion period.

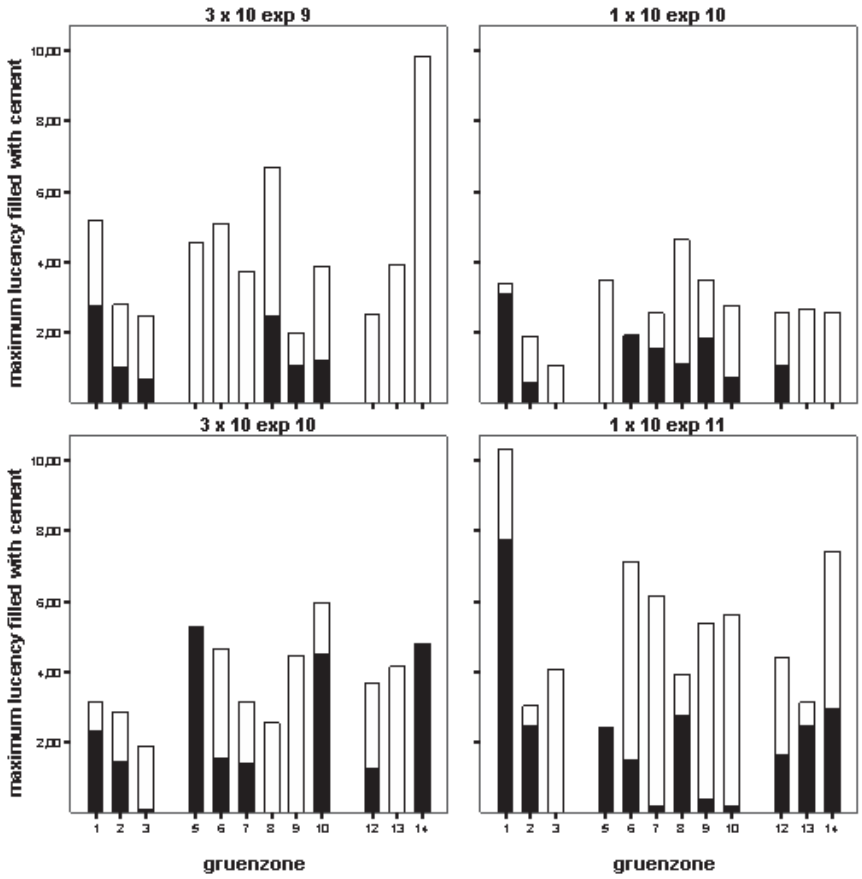


The figure shows division of the periprosthetic space in 14 zones according to Gruen. Per zone the maximum cement thickness is measured before and after cement injection. The increase in cement thickness is represented graphically per Gruen zone and per dose group. Table 3 shows the exact numbers.

Table 3. Mean and standard deviations for the increase in cement mantle thickness per Gruen zone, per dose group after cement injection compared to the situation in the inclusion period.

	Increase in cement thickness (mm) mean and sd, per dose-group (particles)				
Gruen-zone	3 x 10^9 particles	1 x 10^{10} particles	3 x 10^{10} particles	1 x 10^{11} particles	Total of all groups
1	2.75 (sd 5.7)	3.09 (sd 1.9)	2.32 (sd 5.0)	7.75 (sd 8.4)	3.98 (sd 5.4)
2	1.00 (sd 1.2)	0.57 (sd 5.2)	1.41 (sd 0.3)	2.43 (sd 20.)	1.35 (sd 2.5)
3	0.68 (sd 1.0)	-0.29 (sd 1.3)	0.06 (sd 1.6)	-0.02 (sd 1.1)	0.11 (sd 1.2)
5	-0.83 (sd 0.3)	-1.30 (sd 1.3)	5.27 (sd 2.4)	2.42 (sd 2.7)	1.39 (sd 3.2)
6	-0.68 (sd 0.3)	1.90 (sd 2.6)	1.54 (sd 2.3)	1.46 (sd 1.3)	1.06 (sd 1.9)
7	-0.73 (sd 0.6)	1.54 (sd 1.2)	1.39 (sd 1.8)	0.17 (sd 0.6)	0.59 (sd 1.4)
8	2.46 (sd 0.5)	1.08 (sd 0.8)	-0.69 (sd 0.9)	2.73 (sd 1.6)	1.30 (sd 1.7)
9	1.04 (sd 0.2)	1.81 (sd 1.8)	-0.85 (sd 1.8)	0.37 (sd 5.1)	0.55 (sd 2.8)
10	1.18 (sd 1.8)	0.69 (sd 2.5)	4.49 (sd 2.0)	0.16 (sd 4.2)	1.67 (sd 3.0)
12	-0.43 (sd 1.1)	1.05 (sd 0.9)	1.22 (sd 1.0)	1.64 (sd 2.3)	0.99 (sd 1.5)
13	-0.36 (sd 0.7)	-0.42 (sd 0.6)	-0.07 (sd 2.4)	2.48 (sd 2.7)	0.48 (sd 2.1)
14	-0.61 (sd 7.4)	-0.48 (sd 0.4)	4.79 (sd 2.0)	2.95 (sd 2.2)	1.87 (sd 3.6)
Total	0.43 (sd 2.5)	0.77 (sd 2.1)	1.74 (sd 2.8)	2.05 (sd 3.6)	1.28 (sd 2.8)

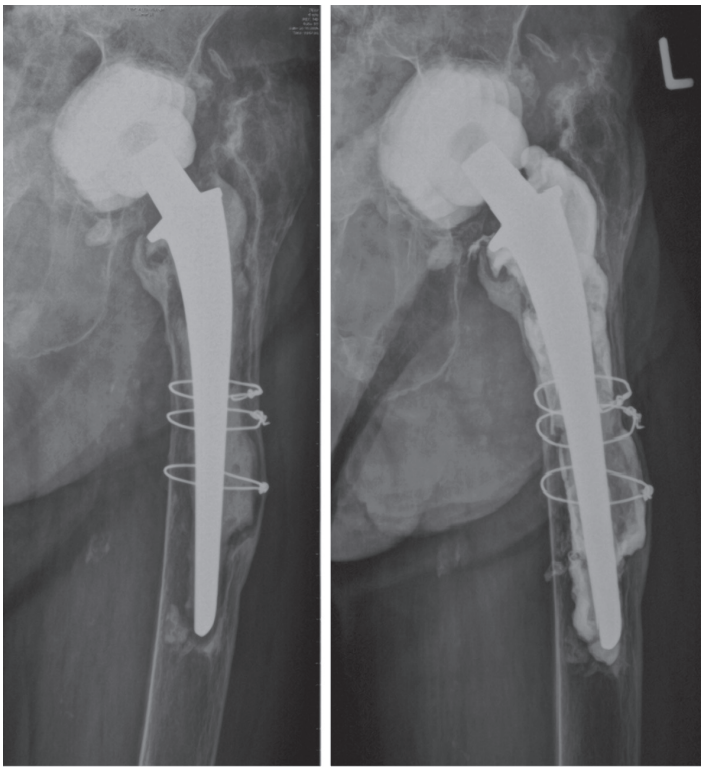
Figure 2. Maximal width of the radiolucent area per Gruen zone and per dose group.



Also shown is the filling of this area with cement after gene therapy and cement injection. Per Gruen zone, the filling of cement in the widest radiolucent area is represented. Open portions of the columns show preoperative maximal radiolucency zones. Solid portions of the columns show filling of these radiolucent zones after cement injection.

measurement and from the Harris Hip Score at various time points and per dose group. Figure 4a shows that when pain is measured with VAS, maximum pain reduction is at 3 to 6 weeks follow-up and declined further over time, except for the second dose group. In the second dose group one patient died after 6 weeks and one patient was lost to follow-up after 3 months. Final follow-up results of these patients were extrapolated for the remaining of the follow-up period. Correlation between the two types of pain measurement was 0.6 ($p < 0.01$), showing that the two methods indicated the same outcome, but an HHS of 100 points did not correspond to a VAS score of 100 points.

Figure 3. Example of radiographic results after cement injection.



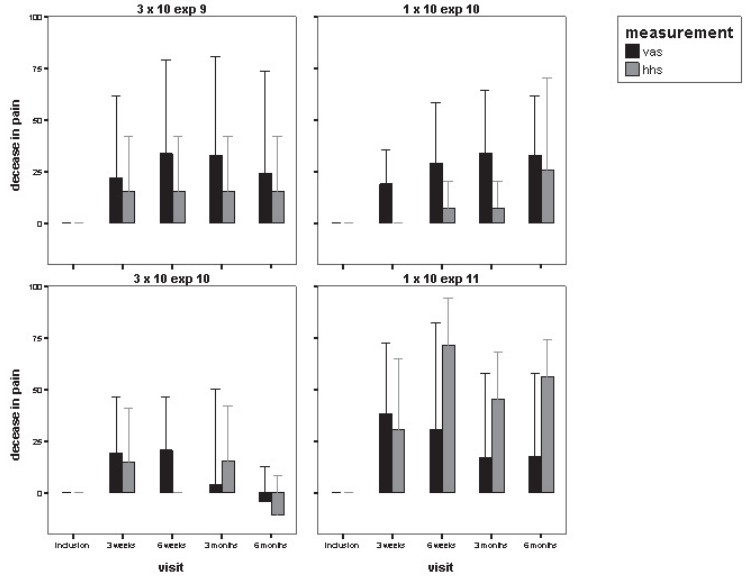
Shown are X-rays from patient 12 before *(left)* and after *(right)* gene therapy and cement injection. Note that the newly injected cement is radio-opaque and therefore has a whiter appearance than the older cement. The radiolucent zone has a dark appearance on the pretreatment X-ray.

Figure 4b shows increase in walking distance as measured by Visual Analogue Scale and Harris Hip Score (walking distance). Walking distance increased after cement injection, especially indose groups 3 and 4. In both measurements (VAS and HHS) the best improvement seems to be at 3 to 6 weeks of follow-up, after which walking distance declined to a level that was higher than preoperatively. Correlation between HHS and VAS for measurement of walking distance was 0.746 ($p < 0.01$).

Figure 5a shows means and standard deviations for improvement in dependency for activities in daily living at various time points per dose group. Best improvement is achieved in the highest dose group. Figure 5b shows increase in total Harris Hip Score at various time points per dose group. Scores increased after cement injection, especially in the highest dose group.

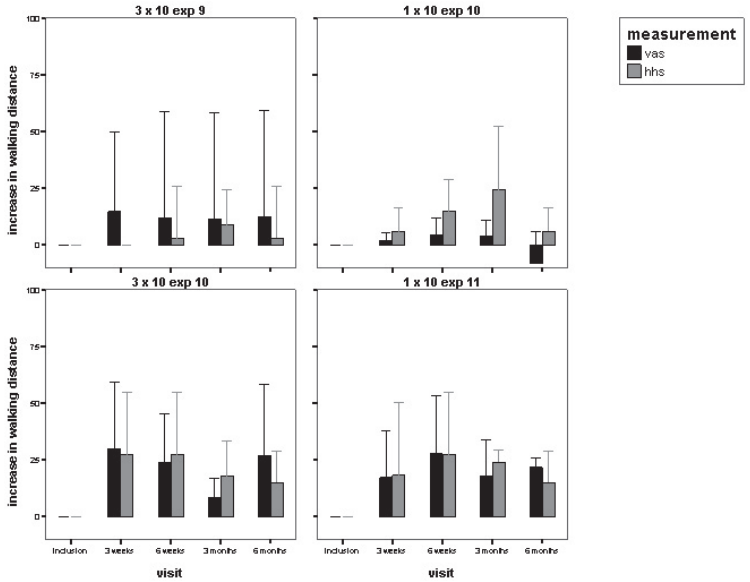
Figure 4. Mean and standard deviations for pain (a) and for walking distance (b) at various time points per dose group (group 1, 3 x 10^9 particles; group 2, 1 x 10^{10} particles; group 3, 3 x 10^{10} particles; group 4, 1 x 10^{11} particles) .

4a



Solid columns represent VAS-measurements and shaded columns represent HHS-measurements for the same outcome variable. A score of 0 means unbearable pain; 100 means no pain;

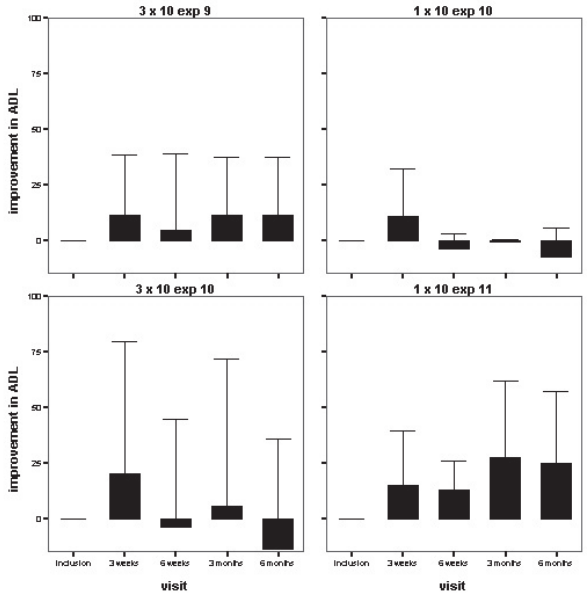
4b



Solid columns represent VAS-measurements and shaded columns represent HHS-measurements for the same outcome variable. A score of 0 means not able to walk; 100 means unlimited walking distance.

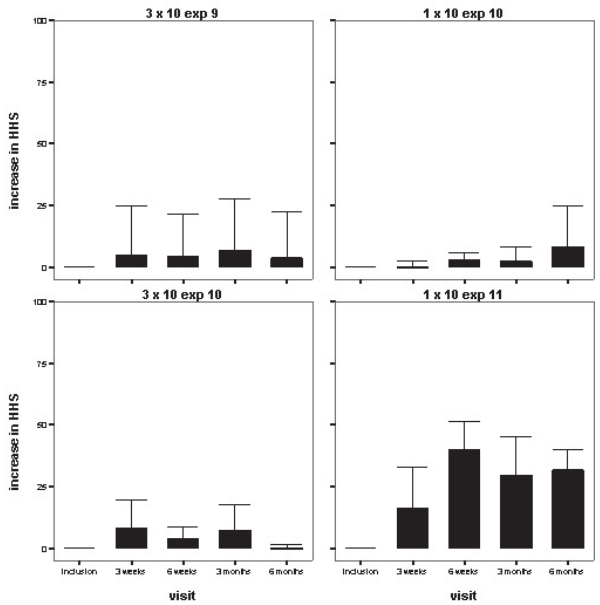
Figure 5. Means and standard deviations for Activities of Daily Living (ADL) dependency (a) total Harris Hip Score (b) per dose group (group 1, 3 x 10^9 particles; group 2, 1 x 10^{10} particles; group 3, 3 x 10^{10} particles; group 4, 1 x 10^{11} particles).

5a



A score of 0 means totally dependent on others; a score of 100 means totally independent for ADL.

5b



This score represents function after total hip prosthesis, with a maximum score of 100 points.

Discussion

This study provides the first description of the use of gene therapy and cement injection to stabilise loosened hip prostheses. The study was designed as an alternative to revision surgery for elderly patients with serious comorbidity and thereby a high morbidity and mortality risk perioperatively.

The primary objective of the study was to determine safety and tolerability for the vector CTL102, the prodrug CB1954 and the cement injection. Secondary objectives included measurement of shedding of the virus, histological analysis of interface biopsies, and clinical outcome. Up to the maximal vector dose of 1 x 10^{11} particles no dose-limiting toxicity was observed, and no adverse events were reported. A prodrug dose of 24 mg/m^2 resulted in nausea and vomiting and rises in AST and ALT, despite the fact that prodrug concentrations measured in plasma in this study were substantially lower than in earlier toxicity studies.[23] Therefore the prodrug dose was lowered to 16 mg/m^2; this did not result in dose-limiting toxicity. Cement injection resulted in pain at the site of injection or hematoma in some patients, but these subsided over the following days. Four serious adverse events occurred, and these were all reported to and investigated by the local medical ethics review board (MEC) and the Central Committee on Research Involving Human Subjects (CCMO). In all four patients the safety committee, in agreement with the MEC and CCMO, could not find a connection between the study and the occurrence of the serious adverse event.

Shedding of the virus in blood plasma, stool, and urine samples, and nose and throat swabs, was measured by quantitative PCR. Two patients in the highest dose group (1 x 10^{11} particles) showed virus load in the blood plasma after 24 h. These results were both below 500 particles/ ml. Because of the positive results the patients were kept in isolation and new samples were taken the next day, showing no virus load in either of these two patients. Previous studies with CTL102 as a vector showed no shedding of viral particles in blood plasma, urine, or stool samples, or nose and throat swabs after 24 h.[91] An explanation for the presence of viral particles in the blood plasma after 24 h in this study could be that the joint is a more or less closed compartment from which particles will slowly release. Although adenoviral DNA could not be detected outside the joint, puncture of joint fluid after 48 h tested positive for adenovirus in all patients. In one of the patients in whom joint fluid was collected 11 months after gene therapy the sample showed adenoviral DNA at a concentration of 1.88 x 10^4 DNA particles per millilitre. In seven patients needle biopsies provided a sufficient amount of material for histological analysis. The samples were analysed for apoptosis and necrosis. None of the samples showed apoptotic cells; however four samples showed necrosis. Lipinski *et al.*[76] studied the mode of action of CTL102-CB1954 suicide gene

therapy and concluded that cells appeared to die by pathways that suggest necrosis more than that of classical apoptosis. This finding supports the presence of necrosis without apoptosis in the interface samples.

In this study the systemic presence of neutralising antibodies was desirable, although neutralising antibodies in synovial fluid inhibit HAdV-5 gene transfer.[47] Therefore, before local injection of the vector each joint was rinsed to remove as many neutralising antibodies as possible. This was done to ensure maximal transduction of interface cells in the more or less closed periprosthetic space while protecting the patient against adverse events, as the HAdV-5 particles that leak from the joint are immediately recognised and attacked by the systemically present neutralising antibodies. The presence of anti-HAdV-5 neutralising antibodies tends to be ubiquitous in human adults,[15] but in this group of vulnerable elderly patients with comorbidity this presence was investigated before viral injection to ensure adequate immunologic response to possible systemic leakage of HAdV-5 particles. Two patients had a somewhat lower level of antibodies compared with the other patients, but there was no relationship with shedding, adverse events, or therapeutic benefits.

This study is the first to describe percutaneous cement injection periprosthetically to stabilise loosened hip prostheses. Additional injection of PMMA-cement has been used to refix prostheses,[73] and is shown to have good biomechanical properties.[49] Results of this study show that part of the radiolucent zone can be filled with cement, but not all. However, the stability of each prosthesis in the bone has increased. The question that remains unanswered in this short follow-up concerns whether stabilisation of the prosthesis leads to an increase in bone stock over time, due to reduction of stress shielding of the bone, and consequent bone loading after fixation of the prosthesis. This principle has been proven by the Wagner SL revision stem, which is used to achieve a successful revision total hip replacement in patients with severe bone loss in the proximal part of the femur (Gruen zones 1, 7, 8 and 14). Distal fixation of the prosthesis in the bone with only minor prosthesis-bone contact, in a study by Böhm *et al.*,[14] led to restoration of the proximal bone stock of the femur in 88% of the patients after a follow-up of 5 years. Whether the principle of the Wagner revision stem also applies for stabilisation of the prosthesis with percutaneous cement injection remains to be seen.

Analysis of clinical outcome by Harris Hip Score and Visual Analogue Scale for pain, walking and Activities of Daily Living showed improvement of function and pain after gene therapy and cement injection, especially in the two highest dose groups. Whether this difference between dose groups is caused by the higher dose of adenoviral vector or by an increasing learning curve for percutaneous cement injection cannot be differentiated. However, this is inevitable when implementing a new technique. According

to both VAS and HHS results, patients benefited from the treatment. In this study, patient's opinion on clinical outcome was considered more valid than physician's opinion, as physicians tend to assign better rankings to walking distance, pain and dependency in ADL.[72,82]

In conclusion, gene therapy and cement injection as an alternative for revision surgery in elderly patients with comorbidity represent a safe and potentially feasible approach. Clinical outcome improves according to patient's opinion and radiographs show filling of radiolucent zones by cement. Whether stabilisation of the prosthesis leads to increase of bone stock on the longer term remains to be seen.

9

Percutaneous periprosthetic cement injection as an alternative treatment for aseptic hip prosthesis loosening in elderly patients with significant comorbidity. A report of seven cases.

Jolanda J. de Poorter[1]
Arian R. van Erkel[2]
Rob G.H.H. Nelissen[1]

[1]Department of Orthopaedics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

Abstract

Background

Elderly patients are sometimes not eligible for revision arthroplasty due to high mortality risks. Previously, a minimal invasive approach was developed to percutaneously inject bone cement periprosthetically after removal of interface tissue using a suicide gene therapy approach. This case series was performed to investigate the possibility of injecting bone cement around the loosened prosthesis without previous removal of the interface.

Methods

Seven patients with high morbidity risks due to high age, serious comorbidity or low bone stock were treated with percutaneous, periprosthetic cement injection. Patients had spinal anaesthesia and bone cement was injected after CT-guided placement of vertebroplasty needles. The amount of cement that could be injected and the distribution in the periprosthetic space was recorded. Patients were followed up in the outpatient clinic.

Results

A mean volume of 16 ml of cement could be injected around the stem and 5.5 ml around the cup. All seven patients reported improvement in walking distance and pain.

Interpretation

This small case series shows that bone cement can be injected periprosthetically in patients with loosened hip prostheses, as an alternative for revision surgery in high risk patients. Due to small patient numbers no conclusions can be drawn on the necessity to remove interface tissue before injecting bone cement to stabilise aseptically loosened prostheses.

Introduction

Revision surgery for hip prosthesis loosening has a high complication rate in elderly patients with comorbidity.[6,92,99,114] Revision hip arthroplasty is associated with a higher number of complications and less improvement in social outcome compared to primary hip arthroplasty.[103] Due to the tendency to insert orthopaedic implants in younger patients and their longer life expectancy, the number of revision surgeries in elderly patients is likely to increase considerably in the next decades. Kessler *et al*.[64] concluded that duration of surgery, which is about 3 times longer in revision than in primary surgery, is the main indicator for the severity of the operation. Elderly patients with ASA-category 3 and more (American Society of Anaesthesiologists)[121] are at higher risk for developing major and moderate complications after revision hip arthroplasty.[6]

One of the major difficulties in revision surgery is the removal of cement from the femoral shaft, without fracturing the femur. Biomechanical studies showed that recementing can give a good interface strength with the old cement.[49] Lieberman *et al.*[73] showed this in practice in 19 patients where a new prosthesis was cemented in an old cement mantle. In a previous study we showed that bone cement can be injected percutaneously in the periprosthetic space of loosened hip prostheses after the interface tissue was removed with a suicide gene-directed enzyme-prodrug therapy (GDEPT).[28] Some of these patients had to be recemented after 6 months, with successful relief of symptoms. Therefore, and to bypass the more complicated GDEPT-approach, we refixated prostheses in patients that were at high risk for perioperative morbidity. In a small case series of percutaneous peri-prosthetic cement injections, the results for this increase of stabilisation of loosened hip prostheses were evaluated.

Materials and methods

Patients

Goal was the evaluation of CT-guided periprosthetic percutaneous cement injection to increase stabilisation of aseptically loosened total hip prostheses. Patients with debilitating pain from a loosened hip prosthesis and a high perioperative morbidity and mortality risk if revision total hip arthroplasty was performed, were included. Risks were advanced age, serious comorbidity, and low bone stock. Before the procedure, laboratory tests (ESR, CRP) were done, and a routinely performed arthrogram was made with aspiration of joint fluid to exclude the presence of a (low-grade) infection and to verify loosening of the prosthesis. Furthermore, marcainisation of the hip joint was performed to confirm that the pain experienced by the patient was due to

a loosend hip prosthesis. Patients who had undergone previous peri-prosthetic percutaneous cement injections combined with gene therapy[28] were excluded from the case series. Patients undergoing the cement injection were admitted to the hospital for 1 or 2 days. On the first day bone cement was injected peri-prosthetically in a predefined space as described below. Depending on post-procedural pain and / or travelling distance patients stayed overnight before returning home. Patients were then once seen for follow-up in the outpatient's clinic, or at a later time point whenever patients were capable.

Cementing technique

On previously performed routine radiographs and a CT-scan of the hip and pelvis the areas most suitable for cement injection were identified. These were the areas with the widest periprosthetic radiolucency. All patients were screened by the anaesthesiologist prior to the procedure and underwent spinal anaesthesia preferably. Patients were positioned supine on the CT-table with a marker grid fixed to the patient's hip region and a planning CT-scan was performed. The markers and planning CT were used to exactly define the positions for introduction of the needles through the skin and the angle by which the needle needed to be introduced to reach the designated periprosthetic radiolucent zone. In this way 3 to 5 points were marked on the skin for introduction of the needles. All patients received prophylactic antibiotics (cephalosporin) intravenously as is routinely used for any arthroplasty procedure. The hip and groin region were then disinfected and sterilely covered, and 3 to 5 vertebroplasty needles of 1.8 or 3.2 x 100 mm (Biomet, Dordrecht, The Netherlands) were introduced with soft blows into the periprosthetic space where the cortex was thinnest (usually <2mm), using a hammer, advancing 1-2 mm per blow and controlling the position with CT-scan (Figure 1). If the cortex was too thick (i.e. >3mm) a needle with a drill bit was used first, after which the cement needle was used. The areas where the radiolucent zones were widest were preferrably chosen as entrance points. The position of the needles and the depth of the insertion were then controlled by CT guidance (Figure 2). The needles were turned in such a way that the opening faced the prosthesis to minimise leakage of the cement. After placement of the needles the fluoroscopy C-arm was positioned over the patient (Figure 3). Polymethylmethacrylate (PMMA) cement (Osteopal [Biomet] or Disc-OTech [Disc-O-Tech Medical Technologies, Herzeliya, Israel]) was injected into the periprosthetic space under high pressure with a Cementoset (Biomet). During injection, the flow of the PMMA cement was continuously monitored by fluoroscopy, and intermittantly with CT-scan. Injection was continued until the periprosthetic space was filled or until the cement

Figure 1. CT-guided placement of vertebroplasty needles in the periprosthetic space around stem and cup a) and cement injection under fluoroscopic guidance (b).

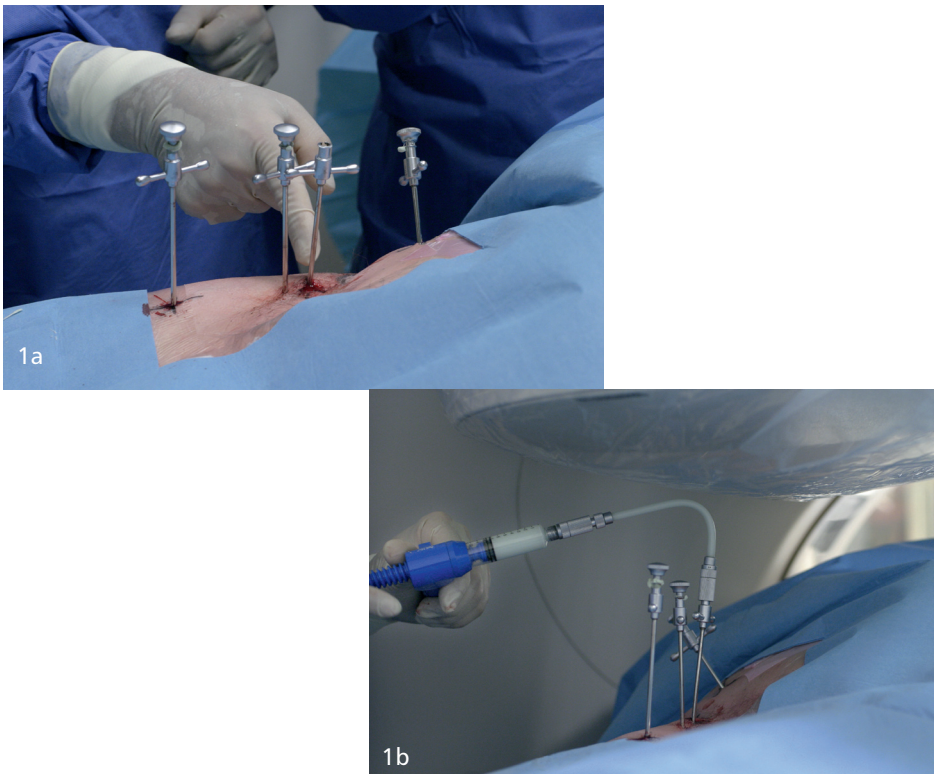


Figure 2. CT-guided verification of correct needle tip position. Needle tip is facing the prosthesis

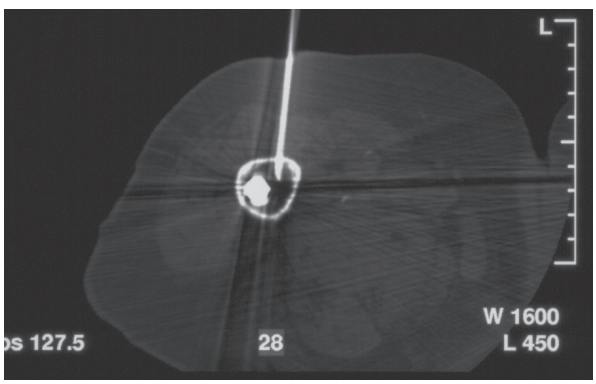


Figure 3. Positioning of the fluoroscopy C-arm over the patient (a) and position of the fluoroscopy C-arm over the patient (b).

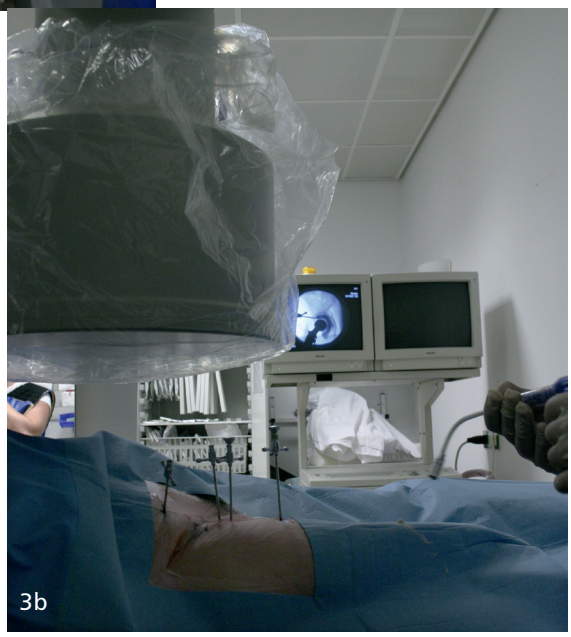
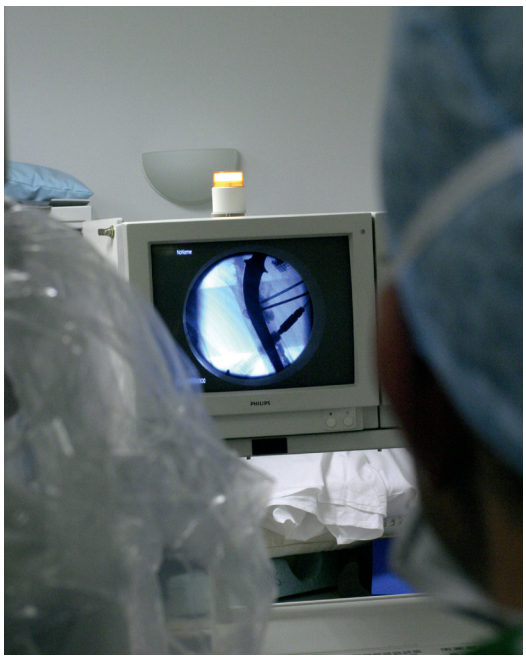


Figure 4. Fluoroscopy-guided injection of bone cement



threatened to leak into the joint space or when there was leakage of cement into the soft tissues (i.e., extraosseous) (Figure 4). Before removal, the needles were turned in a clockwise or counterclockwise manner several times to ensure easy removal.

Follow-up

Standard anteroposterior (AP) and lateral radiographs of the hip were performed in all patients pre- and post-operatively (before discharge) and whenever patients came to visit the outpatient's clinic. On the radiographic images the periprosthetic space was divided into 14 zones according to Gruen, McNeice, and Amstutz.[50] The maximal cement layer thickness was measured with Ortho-CMS software (Medis, Leiden, The Netherlands). These measurements were performed for X-rays made pre-operatively and the first post-procedural X-ray. The differences between these measurements were analysed.

The objective was to measure functional outcome in this small, diverse patient group the patients were asked about the differences in pain before and after the procedure, changes in walking distance and changes in walking aids. No statistical analyses were performed.

Table 1. Patient characteristics

Patient	Sex	Age (years)	ASA-category	Side	Age of prosthesis (years)	Volume of cement injected
1	F	88	2	R	14	Stem: 18 mL
2	F	84	3	R	20	Stem: 25 mL; cup: 12 mL
3	M	91	2	R	16	Stem: 23 mL; cup: 2 mL
4	F	83	2	R	7	Not registered
5	M	81	3	R	11	Cup: 4.5 mL
6	F	87	3	R	25	Stem: 10 mL; cup: 7 mL
7	F	76	2	R + L	17 and 16	L: stem: 10 mL; cup: 3 mL R: stem: 8 mL; cup: 4.5 mL
Mean		85	2.4		16	Stem: 16 mL; cup: 5.5 mL

Results

5 Female and 2 male patients, with a mean age of 85 years (range 76-91 years) were treated. One patient had cement injections around both hips. Table 1 shows patient characteristics and the volumes of cement that could be injected. One patient could be discharged to her home on the same day of treatment; 3 patients could be discharged the next day. The patient with injections in both hips was admitted for 3 days, and the other 2 patients were admitted for 5 and 11 days. In the patient with bilateral cement injections a periprosthetic fissure was initiated in the right femur. This patient had to mobilise with partial weight bearing for 3 months. X-rays after this period showed good consolidation of the fracture and full weight bearing was allowed. No other adverse events occurred.

Figure 5 shows an example of radiographic examination before and after cement injection in patient 2. The figure shows that the injected cement can easily spread through the periprosthetic space to provide more strength and stability. To objectively measure the changes on anteroposterior and lateral X-rays taken before and after cement injection, in each Gruen zone (except for zones IV and XI) the maximal cement thickness was digitally measured for each patient. In one patient accidentally no post-procedural X-ray was made, and consequently this patient was excluded from X-ray analysis. Figure 6 shows the mean (with standard deviation and range) increase in cement mantle thickness per Gruen zone after cement injection compared to the cement mantle before the procedure. The largest increase in cement thickness were in Gruen zones I (mean 3.8 mm, range -0.5 – 7.9 mm), zone VIII (mean 6.5 mm, range 2.1 – 13.8 mm) and zone XIII (mean 4.0, range 1.2 – 8.6 mm).

Figure 5. X-rays of right hip in anteroposterior and lateral view before percutaneous cement injection (a) and after percutaneous cement injection (b).

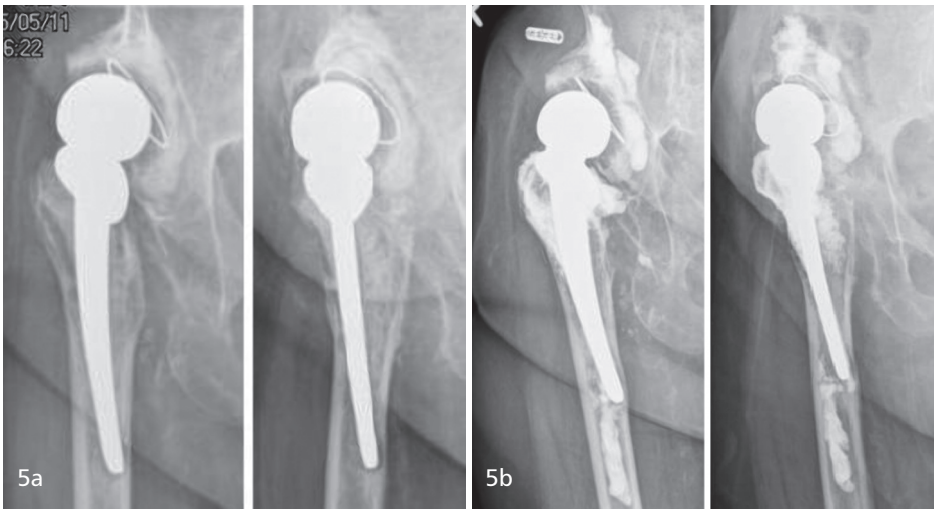
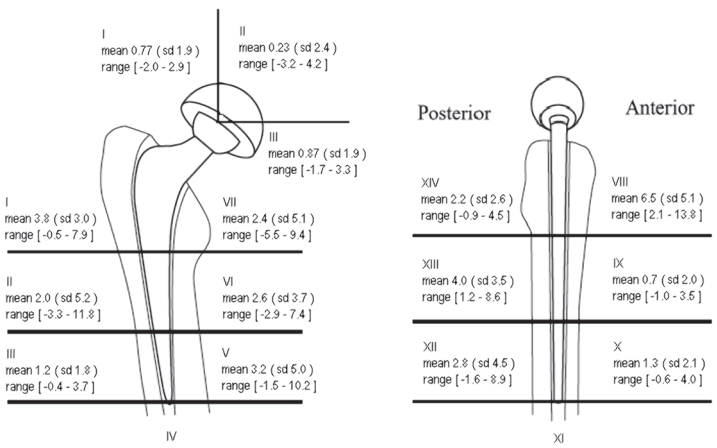


Figure 6. Increase in cement mantle thickness after percutaneous periprosthetic cement injection.



Number show mean increase in millimetres, with standard deviations in brackets and the range in square brackets.

Before the cement injection, patients were asked about the walking aids they needed and the maximal distance they could walk. After cement injection patients were asked again about the walking aids and distance, and differences in pain and performance.

Patient 1 could walk less than 500 meters using a walker and had pain in the hip before the cement injection. Acetaminophen had no effect on the pain. 14 Months after the procedure she could walk for 800 meters with a walker, she reported only mild pain in the groin, and better movement of the hip. Putting on her shoes was easier than before the cement injection.

Patient 2 could walk 50 meters with a cane in-house and used a scoot-mobile for mobilisation outside the house before the procedure. She had pain in her right hip. 10 Months after percutanous cement injection she reported no pain and she did not need home care anymore. Walking had improved.

Patient 3 had complaints of the right hip with nocturnal pain and problems with raising the right leg. His left hip was revised three times, even more both his knees showed severe osteoarthritis. He walked with 2 canes before the procedure. In order to distinguish between right joint pain and pain in the other lower extremity joints marcainisation of the right hip was performed. This completely resolved the pain in his right leg and improved his walking capability despite the instability complaints of his left leg. 12 Months after cement injection the patient reported only mild pain in the groin, he still walked with 2 canes (due to complaints of the knees and left leg), but could now do his own grocery shopping.

Patient 4 had pain in her right leg and groin for which she used Indometacin 3 times a day, Acetaminophen, and morphine. She could only walk for 40 meters with a walker in-house. 2 Years after cement injection she had no pain and did not use any painkillers. She still used a walker, but could now walk for 300 meters, and she could do her own grocery shopping.

Patient 5 had pain in his right hip and could walk 100-200 meters without any walking aids. 9 months after cement injection the patient could walk for 1 kilometre without any walking aids, and he had no pain.

Patient 6 had debilitating pain in the right hip and could only walk in-house with a walker. She used Acetaminophen as a painkiller. 7 weeks after cement injection she reported no pain and an improvement in walking.

Patient 7 had pain in both hips with nocturnal pain and pain during walking. Her walking distance was 100 meters maximum. She used Acetaminophen and tramadol as painkillers. During the procedure a fissure occurred in the right femur. Directly after the procedure she was told to minimise weight bearing of the right leg. After 6 weeks weight bearing of the right leg could be increased to 50%, and six weeks later full weight bearing was allowed. Consecutive X-rays showed increasing consolidation of

the fissure. At 10 months follow-up she still used Acetaminophen as a painkiller for pain in the left hip. She could walk 1 kilometre using 2 crutches. Pain recurred after 12 months, due to the severe bone loss and the varus position of the hips.

Discussion

This small case series shows that it is possible to inject cement around a loose total hip prosthesis percutaneously without previously removing the interface layer. However, the effect of this extra cement was different among patients. In the 7 patients a mean volume of 16 ml could be injected around the stem and 5.5 ml around the cup. All patients reported an increase in walking distance and a decrease in pain. Although peri-prosthetic cement injection is possible without removing the interface layer it seems logical that a better fixation of the prosthesis is achieved when the interface is removed. In a previous study we performed percutaneous periprosthetic cement injection in elderly patients with aseptic loosening of the hip after gene directed enzyme prodrug therapy to remove interface tissue. In this study patients also reported increase in walking distance and decrease in pain.[28] However, the gene therapy itself required at least one week hospital admittance and some of the patients suffered from systemic adverse events by the prodrug.[29] Due to the low numbers of patients in both studies no conclusions can be drawn on the best method to refix the prosthesis. Furthermore, no predictions can be made for follow-up results.

Although the percutaneous cement injection is a relatively small procedure with probably low risks for the patient, the procedure should only be performed in patients with very high risks for peri-operative morbidity and mortality during normal revision surgery. This is because normal revision surgery in low risk patients has proven to be effective and has better functional results at long time follow-up compared to the percutaneous cement injection technique. On the other hand, after percutaneous cement injection, regular revision surgery can always be considered as a last resort option. Furthermore, if a high risk patient of 80+ years has less or no nocturnal pain and can walk better for more ADL indepency with a small procedure, quality of life will be improved. Percutaneous cement injection can only be performed when fixation in the present prosthesis position will relieve the complaints, i.e., not in patients with complaints due to polyethylene wear or recurrent dislocations.

In conclusion, this small series shows that percutaneous cement injection around an aseptically loosened prosthesis without previously removing the interface tissue is a feasible approach, but based on the current data no conclusions can be made on the preference to remove the interface tissue before injecting cement. However, it seems

logical that removal of the interface tissue will give an improved stabilisation compared to a situation where this soft tissue is left in place. The patient with the best clinical results had a radiolucency of <1mm, indicating presence of little soft interface tissue.

10

Summary and discussion

Approximately one million total hip replacement operations are performed worldwide annually, mostly for osteoarthritis and rheumatoid arthritis. A major complication in total hip arthroplasties is loosening of the prosthesis leading to pain and walking difficulties and a higher risk for dislocations and pathological fractures.[54] Within ten years of primary hip replacement 7-13 percent of patients need revision surgery due to loosening of the implant.[79] The number of revision surgeries in elderly patients is likely to increase considerably in the next decades, due to the tendency to insert orthopaedic implants at younger ages and the longer life expectancy of patients. Revision surgery has a high complication rate in elderly patients[6,92,99,114] and is associated with less improvement in social outcome, compared to primary hip arthroplasty in patients of all ages.[103] Many studies have focused on the relatively good technical outcome of revision hip arthroplasty in patients of all ages. However, the impact on the patient's life, especially in elderly patients has been underemphasised.

This thesis was set up to develop and analyse an alternative treatment for hip prosthesis loosening. We used a gene-directed enzyme-prodrug therapy to kill and remove interface tissue, after which we injected bone cement around the prosthesis percutaneously under CT- and fluoroscopy guidance. The first chapter describes the social and medical impact of revision hip arthroplasty in patients 80 years and older, and thereby the reason we searched for an alternative treatment. The next three chapters describe preclinical studies, in chapter 3 and 4 cell studies are described that show that interface cells can be killed by the gene therapy. Chapter 5 shows the importance of a pre-treatment arthrogram. In chapters six to eight the clinical protocol is outlined, and results are shown. Finally, chapter nine describes a short case series of patients who had percutaneous cement injections without previous interface removal.

Revision surgery in octogenarians

In **Chapter 2** we retrospectively reviewed all patients 80 years and older undergoing revision total hip arthroplasty in two hospitals in the Netherlands between 1994 and 2007. Primary objective was social outcome after hospital admittance for revision hip surgery. Secondary objectives were occurrence of complications during hospital stay, patient survival, and use of walking aids before and after revision surgery. After hospital admittance for revision surgery eventually 75% of patients could return to their previous social situation, in 12% the social situation worsened, and in 4% the situation improved. For the patients living independently before the revision surgery, we used a logistic regression analysis to predict which patients could return home, and which patients had to go to a nursing facility for a longer period of time. The regression analysis revealed that the sole predictor for returning home was the presence of a spouse. This

feature was also mentioned in the study by Strehle *et al.*,[114] who found that 95% of patients living with a spouse could return to home, compared to 70% of patients not living with a spouse. Although revision surgery of the hip prosthesis was intended to improve the ADL (Activities in Daily Living) of the patients, our study shows that the social situation worsened more than improved. There can be several reasons for this. One explanation can be that although function of the hip is improved by revision surgery, the patient's condition is deteriorated by the hospital stay. Although there was no correlation between hospital stay and the number of complications occurred, the duration of hospital stay was long (mean of 34 days), and the amount of complications was high, with a mean of 1.3 complications per patient, and a statistically significant correlation between ASA - category (American Society of Anesthesiologists)[121] and number and seriousness of complications. 26% of the patients had a delirium, implicating that the revision surgery had a high impact on physical and mental status. Beside deterioration of the physical and mental status of the patient during hospital stay, another explanation for worsening in social situation is that the situation at home was already unacceptable, and the hospital admittance was the trigger to change it. Another feature that involves elderly patients is that the risk for peri-prosthetic fractures is higher than in younger patients.[92] This is explained by the fact that elderly patients usually have a poorer bone stock. Although we only studied octogenarians, we found that 13% of patients in ASA-3 had a periprosthetic fracture, compared to 5.4% of patients in ASA-2 ($p = 0.10$). In conclusion, chapter 2 shows that revision hip surgery has a high impact on the physical, mental and social status of elderly patients. Indications for revision surgery should be extensively considered, and consequently there remains a group of elderly patients with serious comorbidity and/or a low bone stock, who are not eligible for revision surgery. For these patients the alternative treatment described in this thesis is developed.

Preclinical studies

For the removal of the interface tissue between prosthesis and bone a gene-directed enzyme prodrug therapy (the vector CTL102 in combination with the prodrug CB1954) is used. The primary effect of CTL102 infection of a cell is the delivery of the Ntr gene expression cassette. After cell entry and unpackaging from the viral capsid, the CTL102 genome exists as an extra chromosomal DNA element from which the machinery of the infected cell transcribes Ntr mRNA. This in turn will be translated into active nitroreductase, which will accumulate in the cytosol. Intracellular Ntr activity is responsible for the activation of prodrug CB1954 to a toxic bifunctional alkylating agent inside the cell.[68] Cells able to bioactivate CB1954 are cytotoxically affected by crosslink formation

at very high frequency. As a consequence, cells activating CB1954 are destroyed.[18,34] The intended outcome is that infected cells expressing Ntr, which take up CB1954, will be killed by the activated prodrug. Synovial tissue has the histological and histochemical characteristics of interface tissue[43] and in a previous study in our lab by Goossens *et al.*[46] it was demonstrated that genes can be transferred to synovial tissue *in vivo* in rhesus monkeys, by direct injection into the joint, and that the synoviocytes can be killed with injection of a specific prodrug.

In **Chapter 3** the efficient killing of interface cells by gene-directed enzyme prodrug therapy is shown. To test the susceptibility of interface cells to HAdV-5 vectors, primary cultures of interface cells were exposed to the HAdV-5 vector Ad.CMV.LacZ. The transduction efficiency increased with vector concentration and at 400 pfu/ cell 88% of cells expressed the reporter gene. Separately, the vector CTL102 and the prodrug CB1954 were not toxic to the interface cells at low to intermediate doses. However, CTL102-transduction of interface cells resulted in a 60-fold sensitisation to the prodrug CB1954. Before intra-articular injection of a therapeutic ingredient the position of the needle in the joint space is usually confirmed by injection of a small amount of contrast medium into the cavity, while making fluoroscopic images. For the use in a clinical study, a possible effect of contrast medium on the efficiency of transduction and killing of interface cells should be tested. Results in chapter 3 show that the contrast medium has no effect on the viability of interface cells but, in the presence of iodide-containing contrast medium, transduction of the cells by an adenoviral vector is almost negligible. The mechanism for this remains unclear, however we showed that the effect is not caused by a change in the cells themselves as transient exposure does not lead to inactivation of the vector. Furthermore, the effect is independent on the receptor, and is not caused by the iodide itself. In conclusion, chapter 3 shows that interface cells can be killed by the Ntr/CB1954 enzyme prodrug approach. However, the currently employed contrast medium cannot be used to verify the position of the needle during intra-articular injection of the vector and prodrug, given the effect on the transduction of cells.

Chapter 4 describes two methods we tested to optimise transgene expression. When the gene expression can be made more predictable and efficient, the vector dose can be decreased. This has several advantages, including less evocation of an immune response and a smaller demand for the production of clinical grade adenovirus.[66] One of the methods to increase gene expression is to facilitate interaction of the promoter with the DNA sequences. Normally, the chromatin of DNA is wrapped around a complex of histone proteins. Sodium butyrate (NaB) causes hyperacetylation of histones, which alters the chromatin structure and increases gene expression. Furhtermore, NaB probably upregulates transcription factors which also increases reporter-gene expres-

sion.[11,17,27,42] We showed that NaB at a concentration of 6mM increases reporter gene expression *in vitro* with a factor 7 to 16 compared to a control condition without NaB. NaB has two main disadvantages that could withhold use in a clinical study. The first disadvantage is that NaB is rapidly metabolised *in vivo* (half-life of 6.1 min), making it difficult to maintain at an effective therapeutic level. To overcome this problem esterified butyrate derivatives with longer half-lives have been developed.[88,96,97] Pobably, when a high concentration of NaB can be achieved locally (when injected in a closed compartment, like a joint), NaB can be advantageous to increase transgene expression *in vivo*. The second disadvantage is that the effect of NaB will not only be on the transgene intended, but also on other genes that are being transcribed. Additional studies are needed to examine the long-term effects of NaB before it can be used in a clinical gene therapy study.

In an attempt to increase local effect on transgene expression while keeping systemic effects at a minimum, a vector was developed with a ubiquitous chromatin opening element (UCOE) inserted in the vector DNA. The mode of action of this UCOE is that the promoter is packed in a DNA sequence containing methylation-free CpG-islands, making the promoter resistant to heterochromatin-mediated silencing of these genes.[74,126] This will prevent that transgene expression decreases over time. In our study we found no difference in expression between the vector with and without UCOE. A possible explanation can be that the promoter is not silenced in the first days after infection, rendering no effect of an anti-silencer insert. In a clinical study where transgene expression is needed for a longer period of time, insertion of a UCOE in the vector DNA could probably be a good option.

In all studies a good exposure of the target tissue is essential, and for clinical studies in which the active ingredient is injected intra-articularly, the active ingredient must remain in the joint for a sufficiently long period. Beside size of the therapeutic particle[46,90], the integrity of the surrounding joint capsule (containment) is important in retaining the active particles within the joint space. Containment of the joint space can be visualised by arthrography in which the joint space is filled with radio-opaque contrast medium and this procedure is visualised with fluoroscopy. Efficacy of intra-articular treatment is also dependent on the joint volume. In large joint spaces the active ingredient will be more diluted than in small joint spaces or a higher dose will be required with the potential for increased toxicity. In a clinical study where intra-articular injection is used as the method of delivery of the therapeutic ingredient, it is important to perform an arthrogram in the inclusion period. This ensures that patients who have a non-contained joint can be excluded from the study. Furthermore, the volume of the joint space is known and this is important for the preparation of the therapeutic solution. **Chapter 5** shows a retrospective study of 221 hip arthrograms performed

for diagnosis of prosthesis loosening. In 74% of the arthrograms the joint space was contained, and these patients would have been suitable for intra-articular therapy. The mean volume of the contained joints was 31 mL. This chapter shows that, as 26% of the patients have a non-contained joint and the volume differs considerably among patietns, it is important to perform an arthrogram before intra-articular therapy to know which patients should be excluded from treatment and what volume can be injected.

Clinical studies

After completion of the pre-clinical studies a study protocol for a phase -1 clinical trial was developed. In this study 12 patients were included. Treatment involved intra-articular injection of the human adenoviral-5 vector CTL102 and, 2 days later, intra-articular injection of the prodrug CB1954. 7 Days later the loosened hip prosthesis was refixed by percutaneous peri-prosthetic bone cement injection. The study protocol was approved by the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands); The Ministry of Housing, Spatial Planning, and the Environment (VROM, The Hague, The Netherlands), and the local ethics committee and was conducted according to the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, Somerset West, and Edingburgh), and in accordance with the Guidelines for Good Clinical Practice (CPMP/ICH/135/95-July 17, 1996). **Chapter 6** describes the clinical protocol in detail.

As the clinical study is a phase-1 trial, safety is the primary objective. Recommendations for grading of acute and subacute toxic side effects (World Health Organisation, 1979) were used to report adverse events. Adverse events were recorded up to 6 weeks after vector injection. **Chapter 7** describes all adverse events that occurred during the first 6 weeks after gene therapy. No dose limiting toxicity occurred. Vector administration could be continued until the proposed highest dose of 1×10^{11} particles. Nine of 12 patients experienced nausea and vomiting starting 6 h after prodrug injection, with one patient needing parenteral fluid administration because of dehydration. These side effects are likely to be caused by the prodrug (and not the vector), as their occurrence decreased after lowering the prodrug dose and did not increase with the 30-fold increase in HAdV-5 vector. Besides nausea and vomiting eight patients had a rise in aspartate aminotransferase (AST), with a maximum 4 days after prodrug injection, and four of these patients also had a rise in alanine aminotransferase (ALT). These hepatic side effects were asymptomatic and completely reversible. However, as 2 of the patients had grade 2 rises in AST and ALT, and one patient needed parenteral feeding due to dehydration, prodrug dose was lowered to 16 mg/m^2 after 4 patients were treated. Pathophysiology of the gastrointestinal adverse events of the prodrug is

unknown; two possible explanations are considered. First, the most likely explanation is indirect gastrointestinal epithelial toxicity. Another explanation might be conversion of CB1954 to the activated form by Ntr from *E.coli* present in colonic flora. CB1954 metabolism is predominantly hepatic.[23] This underscores that the rise in transaminase levels can be explained by liver cell damage by CB1954 metabolites during drug clearance. In conclusion, no dose-limiting toxicity occurred with Ntr-CB1954 gene therapy. However, prodrug dose was lowered to 16 mg/m^2 due to inconvenient gastrointestinal and hepatic side effects.

Secondary objectives in the study were (1) to establish that this procedure does not cause shedding of the virus into the environment (2) histological investigation of biopsies of interface tissue (3) to investigate the possibility to stabilise the loosened prosthesis by means of bone cement and (4) to investigate clinical results, i.e., pain relief and improvement in ADL. Secondary objectives are discussed in **Chapter 8**. Shedding of the virus in blood plasma, urine, and stool samples, and in nose and throat swabs, was measured by quantitative polymerase chain reaction (PCR) analysis. Two patients in the highest vector dose group (1 x 10^{11} particles) showed virus load in the blood plasma after 24 h. Therefore, these patients were kept in isolation and new samples were taken the next day, which showed negative. In previous studies with CTL102 as a vector no shedding was shown after 24 h.[91] An explanation for the presence of vector in our study can be that the vector is injected in the joint, which is a more or less closed space from which particles will slowly release. Although adenoviral vector DNA could not be detected outside the joint after 48 h, puncture of joint fluid showed positive for adenoviral DNA even after 11 months in one of the patients. In seven patients needle biopsies provided a sufficient amount of tissue for histological analysis. These samples mostly showed tissue necrosis without apoptosis. This is in concordance with the study by Lipinksi *et al.,*[76] who concluded that in CTL102-CB1954 suicide gene therapy, cells appeared to die by pathways that suggest necrosis more than classical apoptosis. Percutaneous cement injection was used to stabilise the prosthesis after killing of the interface tissue. Chapter 8 shows that part of the radiolucent zone can be filled with cement, but not all. The question remains whether stabilisation of the prosthesis with cement injection will lead to increase of bone stock over time, due to reduction of stress shielding of the bone, and consequent bone loading after fixation of the prosthesis. Finally, clinical outcome was assessed by Harris Hip Score and by Visual Analogue Scales (VASs) for pain, walking distance, and activities of daily living. All these features of clinical outcome improved after cement injection, especially in the two highest dose groups. Whether this difference in dose groups is caused by a higher vector dose or by an increasing learning curve for the percutaneous cement injection cannot be differentiated. However, this is inevitable when implementing a new tech-

nique. In clonclusion, gene therapy to remove interface tissue, followed by percutaneous cement injection to stabilise the prosthesis is a safe and feasible approach. Clinical function improves according to the patient's opinion and radiographs show partial filling of the radiolucent zone with cement. However, it remains to be seen whether stabilisation of the prosthesis leads to increase in bone stock over the longer term.

A drawback of gene therapy to remove interface tissue is that patients need to be admitted to the hospital for at least one week. Furthermore, most of the patients suffered from systemic side effects by the prodrug. To investigate whether percutaneous periprosthetic cement injection was possible without previous removal of interface tissue, we performed a small case series on 7 patients. This case series and the results are discussed in **Chapter 10**. Seven elderly patients not eligible for regular revision hip arthroplasty due to serious comorbidity or poor bone stock were entered in the case series. These patients received CT- and fluoroscopy guided periprosthetic cement injection, like the patients in the gene therapy trial, but without the gene therapy. A mean volume of 16 ml of bone cement could be injected around the stem, and 5.5 ml around the cup of the prosthesis. All patients reported clinical improvement. Due to the low numbers of patients in both studies no conclusions can be drawn on the best method to refix the prosthesis. Furthermore, no predictions can be made for results at the longer term follow up. Although the percutaneous cement injection is a relatively small procedure, we recommend only to use it in patients who are ineligible for revision surgery, as regular revision surgery has proven to be effective although complications may occur. Furthermore, long term results of the percutaneous injections are yet unknown. On the other hand, after percutaneous cement injection, the option for revision surgery is still open. A drawback of percutaneous cement injection is that it can only be performed when the position of the prosthesis is good and the patient's complaints are not caused by excessive polyethylene wear or recurrent dislocations. Especially in patients with high risks, it is very important to verify that the pain is caused by loosening of the prosthesis, e.g., by marcainisation, and not by other causes (e.g. lower back pathology), before considering revision surgery. In conclusion, chapter 10 shows that percutaneous cement injection around an aseptically loosened prosthesis without previously removing the interface tissue is possible, but since the interface tissue is not removed, it will probably be limited to rare indications. Furthermore, based on the current data no conclusions can be made on the preference to remove the interface tissue before injecting cement. However, it seems logical that removal of the interface tissue will give a better stabilisation compared to a situation where the interface tissue is left in place.

11

Samenvatting en discussie

Samenvatting en discussie

Jaarlijks worden wereldwijd ongeveer 1 miljoen totale heupprothesen geplaatst, meestal bij patiënten met reumatoïde artritis (5%) of artrose (95%). Een belangrijke complicatie bij totale heupprothesen is mechanische loslating van de prothese, wat kan leiden tot pijn en problemen met lopen en een groter risico op luxaties en pathologische fracturen.[54] Binnen 10 jaar na plaatsing van de totale heupprothese heeft 7-13% van de patiënten een revisie nodig, omdat er loslating van de prothese is opgetreden.[79] Het aantal revisie operaties van een heupprothese gaat waarschijnlijk de komende jaren flink toenemen, omdat we geneigd zijn protheses op steeds jongere leeftijd te plaatsen en omdat de patiënten steeds ouder worden. Revisie chirurgie van de heup geeft veel complicaties bij oudere patiënten[6,92,99,114] en is geassocieerd met minder verbetering in sociale uitkomst, vergeleken met primaire totale heup artroplastiek bij patiënten van alle leeftijden.[103] Veel studies hebben zich geconcentreerd op de relatief goede technische resultaten van revisie heupartroplastiek bij patiënten van alle leeftijden. De impact op de kwaliteit van het leven van de patiënt, vooral bij oudere patiënten, wordt echter onderbelicht.

Dit proefschrift is opgezet om een alternatieve behandeling voor heup prothese loslating te ontwikkelen en te analyseren. We gebruikten een gene-directed enzyme prodrug therapie (GDEPT) om interface weefsel te doden en te verwijderen, waarna we botcement rond de prothese spoten onder doorlichting en CT-geleid. Het eerste hoofdstuk beschrijft de sociale en medische impact van een revisie heup artroplastiek bij patiënten van 80 jaar en ouder, en daarmee de reden waarom we deze alternatieve behandeling ontwikkelden. De volgende drie hoofdstukken beschrijven pre-klinische studies; in hoofdstuk 3 en 4 worden celstudies beschreven die laten zien dat interface cellen gedood kunnen worden door gentherapie. Hoofdstuk 5 laat zien hoe belangrijk het is om voor de start van de behandeling een artrogram te verrichten. In de hoofdstukken 6 t/m 8 wordt het klinische protocol samengevat en worden de resultaten van de klinische studie getoond. Uiteindelijk wordt in hoofdstuk 9 een korte case-serie beschreven van patiënten die percutane cement injectie hebben ondergaan zonder dat van tevoren het interface weefsel is verwijderd.

Revisie chirurgie bij 80-plussers

In **Hoofdstuk 2** hebben we alle patiënten van 80 jaar en ouder die een revisie totale heupprothese operatie ondergingen tussen 1994 en 2007 in 2 ziekenhuizen in Nederland, retrospectief nagekeken. De primaire onderzoeksvraag was de sociale uitkomst na de ziekenhuisopname voor revisie heup artroplastiek. Secundaire onderzoeksvragen

waren het optreden van complicaties tijdens het verblijf in het ziekenhuis, overleving van de patiënten en het gebruik van loop hulpmiddelen voor en na de operatie. Na de ziekenhuisopname voor de revisie-operatie kon uiteindelijk 75% van de patiënten terug naar hun eerdere sociale situatie, in 12% van de gevallen verslechterde de situatie en in 4% verbeterde de situatie. Om te kunnen voorspellen welke patiënten terug zouden kunnen naar hun eigen huis en welke patiënten voor langere tijd in een verpleeghuis zouden moeten verblijven, hebben we een logistische regressie analyse gedaan voor de patiënten die voor de opname zelfstandig in een huis of appartement woonden. De regressie analyse liet zien dat de enige voorspeller voor het terugkeren naar het eigen huis de aanwezigheid van een partner was. Dit gegeven was ook al beschreven in de studie van Strehle *et al.*[114] die zagen dat 95% van de patiënten die met een partner zelfstandig woonden naar huis terug konden na revisie totale heup artroplastiek, vergeleken met 70% van de patiënten die zonder partner zelfstandig woonden. Hoewel een van de doelen van revisie chirurgie van de heupprothese was om de activiteiten van het dagelijks leven (ADL) van de patiënt te verbeteren, laat onze studie zien dat de sociale situatie vaker verslechterde dan verbeterde. Hiervoor kunnen verschillende verklaringen zijn. Eén verklaring kan zijn dat hoewel de functie van de heup verbetert door de revisie chirurgie, de conditie van de patiënt achteruit gaat door de ziekenhuisopname. Hoewel we geen correlatie konden vinden tussen duur van de ziekenhuisopname en het aantal complicaties dat optrad, was de opname duur lang (gemiddeld 34 dagen) en het aantal complicaties hoog, met een gemiddelde van 1,3 complicaties per patiënt, en een statistisch significante correlatie tussen ASA-categorie (American Society of Anesthesiologists)[121] en het aantal en de ernst van de complicaties. 26% van de patiënten had een delier, wat aangeeft dat de revisie chirurgie een grote impact had op de fysieke en mentale status van de patiënt. Naast achteruitgang in fysieke en mentale status van de patiënt tijdens de ziekenhuisopname, zou een andere verklaring voor de verslechtering in sociale situatie kunnen zijn dat de situatie thuis eigenlijk al onacceptabel was en dat de ziekenhuisopname een aanleiding was om die situatie te veranderen. Een ander belangrijk probleem bij oudere patiënten is dat het risico voor periprosthetische fracturen hoger is dan bij jongere patienten.[92] Dit wordt verklaard door het feit dat oudere patiënten meestal een slechtere bone stock hebben. Hoewel we alleen 80-plussers hebben onderzocht, vonden we dat 13% van de ASA-3 patiënten een periprosthetische fractuur had, vergeleken met 5,4% van de ASA-2 patiënten ($p = 0{,}10$). Concluderend laat hoofdstuk 2 zien dat revisie chirurgie een hoge impact heeft op fysieke, mentale en sociale status van oudere patiënten. Indicaties voor revisie chirurgie moeten daarom uitgebreid gewogen worden in het licht van potentiële voordelen, maar zeker ook nadelen voor de patiënt. Er zal hierdoor altijd een groep patiënten overblijven met ernstige comorbiditeit of een slechte botkwantiteit, die niet

in aanmerking komt voor revisie chirurgie. Voor deze patiënten is de alternatieve behandeling ontwikkeld die in dit proefschrift beschreven wordt.

Preklinische studies

Voor het verwijderen van het interface weefsel tussen de prothese en het bot wordt een gene-directed enzyme prodrug therapie (de vector CTL102 in combinatie met de prodrug CB1954) gebruikt. Het primaire effect van de CTL102 infectie van de cellen is de bezorging van de Ntr gen expressie cassette. Na binnenkomst in de cel en uitpakken uit het virale kapsel, bestaat het CTL102 genoom als extrachromosomaal DNA element waarvan mRNA transcriptie optreedt door de transcriptie-machinerie van de geïnfecteerde cel. Dit wordt vervolgens weer getransleerd in actief nitroreductase, wat zich ophoopt in het cytosol. De intracellulaire Ntr activiteit is verantwoordelijk voor de activatie van de prodrug naar een toxische bifunctioneel alkylerend middel binnen in de cel.[68] In cellen die in staat zijn tot bioactivatie van CB1954 ontstaat vorming van een grote hoeveelheid crosslinks, wat toxisch is voor de cel. Cellen die CB1954 activeren worden daarom vernietigd.[18,34] De beoogde uitkomst is dat geïnfecteerde cellen die Ntr tot expressie brengen en CB1954 opnemen, gedood worden door activatie van de prodrug. Synoviaal weefsel heeft dezelfde histochemische en histologische kenmerken als interface weefsel[43] en in een eerdere studie in ons lab door Goossens *et al.*[46] is al aangetoond dat genen getransfereerd kunnen worden naar synoviaal weefsel *in vivo* in rhesus apen door directe injectie in het gewricht, en dat de synoviocyten gedood kunnen worden door injectie van een specifieke prodrug.

In **Hoofdstuk 3** wordt het efficiënt doden van de interface cellen door gene-directed enzyme prodrug therapie getoond. Om de gevoeligheid van interface cellen voor HAdV-5 vectoren te testen werden primaire kweken van interface cellen blootgesteld aan de HAdV-5 vector Ad.CMV.LacZ. De effectiviteit van transductie nam toe met de vector concentratie en bij 400 pfu/cel bracht 88% van de cellen het reporter-gen tot expressie. Los van elkaar waren de vector CTL102 en de prodrug CB1954 niet toxisch voor interface cellen in lage tot gemiddelde concentraties. Echter, CTL102-transductie van interface cellen zorgde voor een 60-voudige sensitisatie voor de prodrug CB1954. Voor intra-artriculaire injectie van een therapeutisch middel wordt de positie van de naald in de gewrichtsholte meestal gecontroleerd door injectie van een kleine hoeveelheid contrastmiddel, terwijl doorlichtingsbeelden worden gemaakt. Voordat het in een klinische studie gebruikt kan worden, moet een mogelijk effect van het contrastmiddel op de effectiviteit van transductie en doden van de interface cellen getest worden. De resultaten in hoofdstuk 3 laten zien dat het contrastmiddel geen effect heeft op de vitaliteit van de interface cellen, maar in de aanwezigheid van jodide-bevattend con-

trastmiddel is de transductie van de cellen door een adenovirale vector bijna verwaarloosbaar. Het mechanisme hiervan is onduidelijk, maar we hebben wel aangetoond dat het effect niet veroorzaakt wordt door een verandering in de cellen zelf, omdat tijdelijke blootstelling van de cellen aan het contrastmiddel niet leidde tot een inactivatie van de vector. Daarnaast is het effect onafhankelijk van de receptor en wordt het niet veroorzaakt door het jodide zelf. Concluderend laat hoofdstuk 3 zien dat interface cellen gedood kunnen worden door de Ntr/CB1954 enzym prodrug benadering. Echter, het op dit moment gebruikte contrastmiddel kan niet gebruikt worden om de positie van de naald tijdens intra-articulaire injectie te controleren, gezien het effect daarvan op de transductie van de cellen.

Hoofdstuk 4 beschrijft 2 methoden die we hebben getest om transgen-expressie te optimaliseren. Als we de genexpressie voorspelbaarder en efficiënter kunnen maken, kan de vectordosis verlaagd worden. Dit heeft verschillende voordelen, onder andere dat de immuunrespons die optreedt minder groot zal zijn en dat er minder vector voor klinische toepassingen geproduceerd hoeft te worden.[66] Eén van de methoden om de genexpressie te vergroten is door de interactie van de promoter met de DNA-sequenties te vereenvoudigen. Normaal is het chromatine van DNA gevouwen om een complex van histon eiwitten. Natrium butyraat (NaB) veroorzaakt hyperacetylatie van de histonen, waardoor de chromatine structuur verandert en de genexpressie toeneemt. Daarnaast zorgt NaB voor up-regulatie van transcriptiefactoren, waardoor de reportergen expressie nog meer toeneemt.[11,17,27,42] We hebben aangetoond dat NaB in een concentratie van 6 mM de reporter-gen expressie *in vitro* vergroot met een factor 7-16 vergeleken met een controle groep zonder NaB. NaB heeft 2 nadelen die gebruik in een klinische studie kunnen tegenhouden. Het eerste nadeel is dat NaB snel gemetaboliseerd wordt *in vivo* (half-waarde tijd 6,1 min), waardoor het moeilijk wordt om een effectief therapeutisch niveau te behouden. Om dit probleem op te lossen is een geesterde afgeleide van butyraat ontwikkeld die een langere half-waarde tijd heeft.[88,96,97] Als een hoge concentratie van NaB lokaal kan worden verkregen (bijvoorbeeld als het in een afgesloten ruimte wordt ingespoten, zoals in een gewricht) kan de toevoeging van NaB waarschijnlijk voordelig zijn om de transgen-expressie *in vivo* te laten toenemen. Het tweede nadeel is dat het effect van NaB waarschijnlijk niet alleen zal optreden bij het beoogde transgen, maar dat ook de expressie van andere genen zal toenemen. Aanvullende studies zijn nodig om de lange termijn effecten van NaB te onderzoeken voordat het gebruikt kan worden in een klinische gentherapie studie.

In een poging het lokale effect van transgen-expressie te vergroten terwijl de systemische effecten tot een minimum worden beperkt, is een vector ontwikkeld met een ubiquitous chromatin opening element (UCOE) ingevoegd in het DNA van de vector. Het werkingsmechanisme van deze UCOE is dat de promoter wordt ingepakt in een

DNA-sequentie die methylering-vrije CpG-eilanden bevat, waardoor de promoter resistent is tegen heterochromatine-gemedieerde silencing van de genen.[74,126] Dit zal voorkomen dat de transgen-expressie afneemt in de tijd. In onze studie vonden we geen verschil in expressie tussen de vector met en zonder UCOE. Een mogelijke verklaring kan zijn dat de promoter niet wordt gesilenced in de eerste dagen na de infectie, waardoor er geen effect is van een anti-silencer invoeging. In een klinische studie waar transgen-expressie voor een langere tijd nodig is zou insertie van een UCOE in het vector DNA wel een goede optie kunnen zijn.

In alle studies is een goede blootstelling van het beoogde weefsel essentieel en voor klinische studies waarbij het actieve middel in het gewricht wordt gespoten is het belangrijk dat het middel lang genoeg in het gewricht blijft. Naast grootte van de therapeutische deeltjes,[46,90] is de integriteit van het omgevende gewrichtskapsel (containment) belangrijk in het behouden van de actieve deeltjes in het gewricht. Containment van de gewrichtsholte kan gevisualiseerd worden door artrografie, waarbij de gewrichtsholte wordt gevuld met radio-opaak contrastmiddel onder doorlichting. Effectiviteit van de intra-articulaire behandeling is ook afhankelijk van het gewrichtsvolume. In grote gewrichten zal het actieve middel meer verdund worden dan in kleine gewrichten of een hogere dosis zal nodig zijn met een grotere kans op toxiciteit. In een klinische studie waar intra-articulaire injectie wordt gebruikt als methode om het therapeutische middel op de juiste plaats te brengen is het belangrijk om van tevoren een artrogram te doen in de inclusie-periode. Dit garandeert dat patiënten met een non-contained gewricht ge-excludeerd kunnen. Verder is dan het volume van het gewricht bekend en dit kan belangrijk zijn voor de bereiding van de studie medicatie. In **Hoofdstuk 5** wordt een retrospectieve studie getoond van 221 heup-artrogrammen die verricht zijn voor de diagnose van prothese loslating. In 74% van de artrogrammen was het gewricht afgesloten, en deze patiënten zouden in aanmerking kunnen komen voor intra-articulaire therapie. Het gemiddelde volume dat in de gewricht was ingespoten was 31 ml. Dit hoofdstuk laat zien dat, omdat toch 26% van de patiënten een niet-afgesloten gewricht hebben en het volume tussen de patiënten behoorlijk varieert, het verrichten van een artrogram belangrijk is alvorens intra-articulaire therapie te starten.

Klinische studies

Nadat de pre-klinische studies waren afgerond werd een studie protocol voor een klinische fase 1 studie ontworpen. In deze studie werden 12 patiënten geïncludeerd. De behandeling bestond uit intra-articulaire injectie van de humane adenovirale-5 vector CTL102 en 2 dagen later intra-articulaire injectie van de prodrug CB1954. 7 Dagen later werd de losse heupprothese weer vastgezet door middel van percutane peri-prostheti-

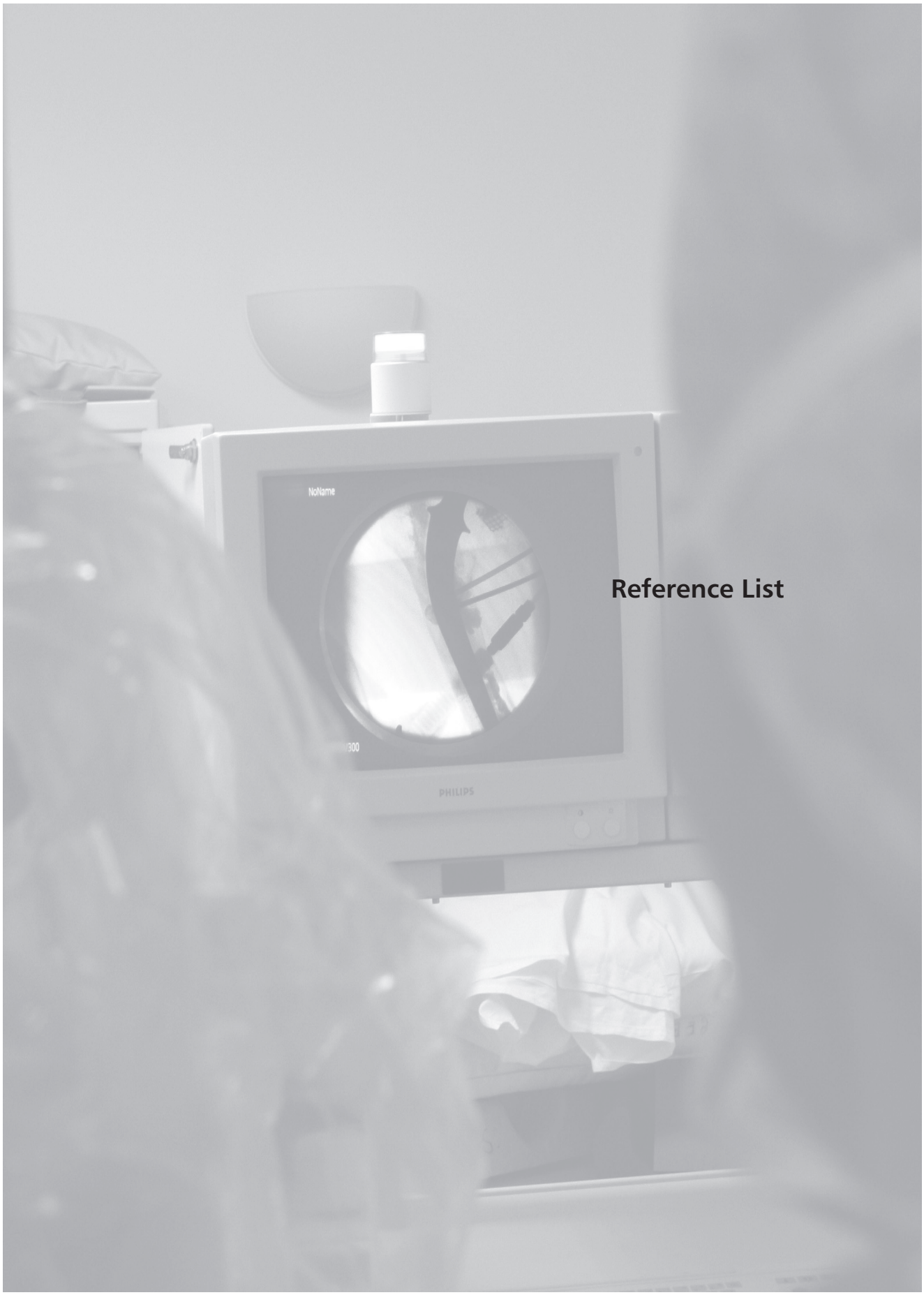
sche bot-cement injectie. Het studieprotocol werd goedgekeurd door de Centrale Commissie voor Mensgebonden Onderzoek (CCMO), het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieu (VROM) en de locale medisch ethische commissie; en werd uitgevoerd volgens de principes van de Verklaring van Helsinki (zoals aangepast in Tokyo, Venetië, Hong Kong, Somerset West en Edinburgh) en volgens de Richtlijnen voor Good Clinical Practice (CPMP/ICH/135/95-July 17.1996). **Hoofdstuk 6** beschrijft het klinische protocol in detail.

Omdat de klinische studie een fase 1 onderzoek is, is veiligheid de primaire onderzoeksvraag. Aanbevelingen voor het graderen van acute en subacute toxische bijwerkingen (World Health Organisation, 1979) werden gebruikt om bijwerkingen te rapporteren. De bijwerkingen werden bijgehouden tot 6 weken na vector injectie. **Hoofdstuk 7** beschrijft alle bijwerkingen die optraden in de eerste 6 weken na de gentherapie. Er is geen dose-limiting toxicity opgetreden. De vector toediening kon gecontinueerd worden tot de voorgestelde hoogste dosis van 1 x 10^{11} deeltjes. Negen van de 12 patiënten hadden last van misselijkheid en braken, beginnend 6 uur na de prodrug injectie, waarbij één patiënt parenterale voeding nodig had in verband met dehydratie. Deze bijwerkingen worden waarschijnlijk veroorzaakt door de prodrug (en niet door de vector), omdat het voorkomen van de bijwerkingen verminderde nadat de prodrug dosis verlaagd was en niet toenam na een 30-voudige toename in HAdV-5 vector dosis. Naast misselijkheid en braken hadden 8 patiënten een stijging in aspartaat aminotransferase (ASAT), met een piek 4 dagen na prodrug injectie en 4 van deze patiënten hadden ook een stijging in alanine aminotransferase (ALAT). Deze hepatische bijwerkingen waren asymptomatisch en volledig reversibel. Echter, omdat 2 patiënten een graad 2 stijging in ASAT en ALAT hadden en omdat één van de patiënten parenterale voeding nodig had in verband met dehydratie, werd de prodrug dosis na 4 patiënten verlaagd naar 16 mg/ m^2. De pathofysiologie van de gastrointestinale bijwerkingen van de prodrug is onbekend; twee mogelijke verklaringen worden overwogen. Ten eerste is de meest waarschijnlijke verklaring een indirecte toxiciteit op het gastrointestinale epitheel. Een andere verklaring kan zijn dat het CB1954 omgezet wordt naar de geactiveerde vorm door nitroreductase van *E. coli* die aanwezig is in de darmflora. Het CB1954 metabolisme is voornamelijk hepatisch.[23] Dit benadrukt dat de stijging in transaminases verklaard kan worden door levercel schade door CB1954 metabolieten tijdens klaring van het middel. Concluderend trad er geen dose-limiting toxicity op tijdens de Ntr-CB1954 gentherapie. De prodrug dosis werd echter verlaagd naar 16 mg/m^2 in verband met nadelige gastrointestinale en hepatische bijwerkingen. Secundaire onderzoeksvragen in de studie waren (1) beoordelen of de procedure niet zorgt voor vrijkomen van het virus in het milieu (2) histologisch onderzoek van biopten van interface weefsel (3) de mogelijkheden onderzoeken om de losse prothese

weer vast te zetten door middel van botcement en (4) de klinische uitkomsten van het onderzoek bekijken, zoals vermindering van pijn en verbetering in ADL. De secundaire onderzoeksvragen worden besproken in **Hoofdstuk 8**. Vrijkomen van het virus in bloedplasma, urine, feces en neus- en keeluitstrijken werd gemeten met kwantitatieve polymerase ketting reactie (PCR) analyse. Twee patiënten in de hoogste vector dosis groep (1 x 10^{11} deeltjes) hadden nog een aantoonbare virus hoeveelheid in hun bloedplasma na 24 uur. Daarom werden deze patiënten in isolatie gehouden en werden de dag erna opnieuw monsters afgenomen, die negatief bleken. In eerdere studies met CTL102 werd geen vrijkomen van virus meer gemeten na 24 uur.[91] Een verklaring voor aanwezigheid van vector na 24 uur in onze studie kan zijn dat de vector is geinjecteerd in een gewricht, wat een min of meer afgesloten ruimte is waaruit de vector maar langzaam zal vrijkomen. Hoewel de vector na 48 uur niet meer buiten het gewricht kon worden waargenomen, liet punctie van gewrichtsvloeistof uit de heup zien dat zelfs na 11 maanden bij één patiënt nog adenoviraal DNA aanwezig was. Bij 7 patiënten kon bij biopten van het interface weefsel voldoende weefsel worden verkregen voor histologische analyse. Deze monsters lieten met name necrose zien zonder apoptose. Dit is in overeenstemming met de studie van Lipinski *et al.*[76], die concludeerde dat in CTL102-CB1954 gentherapie, de cellen meer blijken te sterven door necrose dan door de klassieke apoptose. Percutane cement injectie werd gebruikt om de prothese te stabiliseren na het doden van het interface weefsel. Hoofdstuk 8 laat zien dat een gedeelte van de radiolucente zone gevuld kan worden met cement maar niet alles. De vraag blijft of stabilisatie van de prothese door cement injectie leidt tot toename van bot kwantiteit over de tijd door afname van stress shielding van het bot en toename van belasting na fixatie van de prothese. Uiteindelijk werd de klinische uitkomst bekeken door de Harris Hip Score en door Visual Analogue Scales (VASsen) voor pijn, loopafstand en activiteiten van het dagelijks leven (ADL). Al deze onderdelen van de klinische uitkomst verbeterden na de cement injectie, vooral in de twee hoogste vector dosis groepen. Of dit verschil veroorzaakt wordt door de hogere vector dosis of door een toenemende leercurve voor de percutane cementinjectie kan niet worden beoordeeld. Dit is echter onvermijdbaar als een nieuwe techniek wordt geïmplementeerd. Concluderend kan gezegd worden dat gentherapie om interface weefsel te verwijderen, gevolgd door percutane cement injectie om de prothese te stabiliseren, een veilige en haalbare benadering is. De klinische functie verbetert volgens de mening van de patiënt en röntgenfoto's laten zien dat de radiolucente zone gedeeltelijk gevuld kan worden met cement. Het blijft echter de vraag of stabilisatie van de prothese op de langere termijn leidt tot vergroting van de bone stock.

Een nadeel van de gentherapie om het interface weefsel te verwijderen is dat de patiënten minimaal een week in het ziekenhuis moeten worden opgenomen. Daarnaast

hadden de meeste patiënten last van systemische bijwerkingen van de prodrug. Om te onderzoeken of percutane periprosthetische cementinjectie mogelijk was zonder vooraf het interface weefsel te verwijderen, hebben we een kleine patiëntenserie van 7 patiënten verricht. Deze patiëntenserie en de resultaten hiervan worden bediscussieerd in **Hoofdstuk 10**. Zeven patiënten die niet in aanmerking kwamen voor normale revisie-chirurgie omdat ze ernstige comorbiditeit hadden of een slechte bot kwantiteit, deden mee in de serie. De patiënten ondergingen CT-geleide periprosthetische cement injectie onder doorlichting, zoals de patiënten in de gentherapie-studie, maar zonder de gentherapie. Gemiddeld kon 16 ml cement rond de steel geïnjecteerd worden en 5,5 ml rond de cup. Alle patiënten meldden een klinische verbetering. Door het geringe aantal patiënten in beide studies kunnen geen conclusies getrokken worden over wat de beste methode is om de prothese te fixeren. Daarnaast kunnen geen voorspellingen gedaan worden voor de langere termijn. Ondanks dat de percutane cementinjectie een relatief kleine procedure is, adviseren we het alleen te gebruiken bij patiënten die niet in aanmerking komen voor revisie chirurgie, omdat reguliere revisie chirurgie bewezen effectief is en de langere termijn effecten van percutane cementinjectie nog onbekend zijn. Aan de andere kant blijft de optie voor revisie chirurgie na percutane cementinjectie gewoon open. Een nadeel van de percutane cementinjectie is dat het alleen uitgevoerd kan worden als de prothese in een goede stand staat en als de klachten van de patiënt niet veroorzaakt worden door polyethyleen slijtage of recidiverende luxaties. In ieder geval, en zeker bij patiënten met hoge risico's, is het belangrijk te verifiëren of de pijn veroorzaakt wordt door loslating van de prothese, bv door marcainisatie, en niet door een andere oorzaak, voordat revisie chirurgie overwogen wordt. Concluderend laat hoofdstuk 10 zien dat percutane cementinjectie rond een aseptisch losgelaten prothese mogelijk is zonder dat eerst het interface weefsel verwijderd is. Met de huidige data kunnen geen conclusies getrokken worden over de voorkeur om het interface weefsel te verwijderen voor de cementinjectie. Het lijkt echter logisch dat verwijdering van het interface weefsel een betere stabilisatie geeft vergeleken met de situatie waarbij het interface weefsel nog in situ is.



Reference List

1. Pressure ulcers prevalence, cost and risk assessment: consensus development conference statement--The National Pressure Ulcer Advisory Panel. *Decubitus.* **2**, 24-28 (1989).
2. Abdullah,S. *et al.* Ubiquitous Chromatin Opening Element (UCOE) Extends Duration of Airway Gene Expression by Naked DNA. *Mol. Ther.* **7**, S73 (2003).
3. Aliabadi,P., Baker,N.D. & Jaramillo,D. Hip arthrography, aspiration, block, and bursography. *Radiol. Clin. North Am.* **36**, 673-690 (1998).
4. Antoniou,M. *et al.* Transgenes encompassing dual-promoter CpG islands from the human TBP and HNRPA2B1 loci are resistant to heterochromatin-mediated silencing. *Genomics* **82**, 269-279 (2003).
5. Archibeck,M.J., Jacobs,J.J., Roebuck,K.A. & Glant,T.T. The basic science of periprosthetic osteolysis. *Instr. Course Lect.* **50**, 185-195 (2001).
6. Ballard,W.T., Callaghan,J.J. & Johnston,R.C. Revision of total hip arthroplasty in octogenarians. *J. Bone Joint Surg. Am.* **77**, 585-589 (1995).
7. Barentsz,J.O., Lemmens,J.M. & Slooff,T.J. The use of subtraction arthrography in total hip arthroplasties. *ROFO Fortschr. Geb. Rontgenstr. Nuklearmed.* **144**, 440-446 (1986).
8. Beardsley,T. Gene therapy setback. *Sci. Am.* **282**, 36-37 (2000).
9. Benton,T. *et al.* The use of UCOE vectors in combination with a preadapted serum free, suspension cell line allows for rapid production of large quantities of protein. *Cytotechnology* **38**, 43-46 (2002).
10. Berend,M.E. *et al.* Total joint arthroplasty in the extremely elderly: hip and knee arthroplasty after entering the 89th year of life. *J. Arthroplasty* **18**, 817-821 (2003).
11. Berger,S.L. Gene activation by histone and factor acetyltransferases. *Curr. Opin. Cell Biol.* **11**, 336-341 (1999).
12. Berquist,T.H., Bender,C.E., Maus,T.P., Ward,E.M. & Rand,J.A. Pseudobursae: a useful finding in patients with painful hip arthroplasty. *AJR Am. J. Roentgenol.* **148**, 103-106 (1987).
13. Bilsland,A.E. *et al.* Selective ablation of human cancer cells by telomerase-specific adenoviral suicide gene therapy vectors expressing bacterial nitroreductase. *Oncogene* **22**, 370-380 (2003).
14. Bohm,P. & Bischel,O. Femoral revision with the Wagner SL revision stem : evaluation of one hundred and twenty-nine revisions followed for a mean of 4.8 years. *J. Bone Joint Surg. Am.* **83-A**, 1023-1031 (2001).
15. Bramson,J.L., Hitt,M., Gauldie,J. & Graham,F.L. Pre-existing immunity to adenovirus does not prevent tumor regression following intratumoral administration of a vector expressing IL-12 but inhibits virus dissemination. *Gene Ther.* **4**, 1069-1076 (1997).
16. Brander,V.A., Malhotra,S., Jet,J., Heinemann,A.W. & Stulberg,S.D. Outcome of hip and knee arthroplasty in persons aged 80 years and older. *Clin. Orthop. Relat Res.* 67-78 (1997).
17. Braun,H., Koop,R., Ertmer,A., Nacht,S. & Suske,G. Transcription factor Sp3 is regulated by acetylation. *Nucleic Acids Res.* **29**, 4994-5000 (2001).
18. Bridgewater,J.A. *et al.* Expression of the bacterial nitroreductase enzyme in mammalian cells renders them selectively sensitive to killing by the prodrug CB1954. *Eur. J. Cancer* **31A**, 2362-2370 (1995).

19. Carmody,E.E., Schwarz,E.M., Puzas,J.E., Rosier,R.N. & O'Keefe,R.J. Viral interleukin-10 gene inhibition of inflammation, osteoclastogenesis, and bone resorption in response to titanium particles. *Arthritis Rheum.* **46**, 1298-1308 (2002).
20. Chapchal,G.J., Slooff,T.J. & Nollen,A.D. Results of total hip replacement. A clinical follow-up study. *Clin. Orthop. Relat Res.* 111-117 (1973).
21. Charlson,M.E., Pompei,P., Ales,K.L. & MacKenzie,C.R. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J. Chronic. Dis.* **40**, 373-383 (1987).
22. Charnley,J. Principles and practice in hip replacement. *Proc. R. Soc. Lond B Biol. Sci.* **192**, 191-198 (1976).
23. Chung-Faye,G. *et al.* Virus-directed, enzyme prodrug therapy with nitroimidazole reductase: a phase I and pharmacokinetic study of its prodrug, CB1954. *Clin. Cancer Res.* **7**, 2662-2668 (2001).
24. Clohisy,J.C. & Harris,W.H. The Harris-Galante uncemented femoral component in primary total hip replacement at 10 years. *J. Arthroplasty* **14**, 915-917 (1999).
25. Cruz-Esteban,C. & Wilke,W.S. Innovative treatment approaches for rheumatoid arthritis. Non-surgical synovectomy. *Baillieres Clin. Rheumatol.* **9**, 787-801 (1995).
26. Crystal,R.G. *et al.* Analysis of risk factors for local delivery of low- and intermediate-dose adenovirus gene transfer vectors to individuals with a spectrum of comorbid conditions. *Hum. Gene Ther.* **13**, 65-100 (2002).
27. Davie,J.R. Inhibition of histone deacetylase activity by butyrate. *J. Nutr.* **133**, 2485S-2493S (2003).
28. de Poorter,J.J. *et al.* Gene therapy and cement injection for restabilization of loosened hip prostheses. *Hum. Gene Ther.* **19**, 83-95 (2008).
29. de Poorter,J.J., Hoeben,R.C., Obermann,W.R., Huizinga,T.W. & Nelissen,R.G. Gene therapy for the treatment of hip prosthesis loosening: adverse events in a phase 1 clinical study. *Hum. Gene Ther.* **19**, 1029-1038 (2008).
30. de Poorter,J.J. *et al.* Towards gene therapy in prosthesis loosening: efficient killing of interface cells by gene-directed enzyme prodrug therapy with nitroreductase and the prodrug CB1954. *J. Gene Med.* **7**, 1421-1428 (2005).
31. de Roos,W.K. *et al.* Isolated-organ perfusion for local gene delivery: efficient adenovirus-mediated gene transfer into the liver. *Gene Ther.* **4**, 55-62 (1997).
32. Dion,L.D. *et al.* Amplification of recombinant adenoviral transgene products occurs by inhibition of histone deacetylase. *Virology* **231**, 201-209 (1997).
33. Djeha,A.H. *et al.* Expression of Escherichia coli B nitroreductase in established human tumor xenografts in mice results in potent antitumoral and bystander effects upon systemic administration of the prodrug CB1954. *Cancer Gene Ther.* **7**, 721-731 (2000).
34. Djeha,A.H. *et al.* Combined adenovirus-mediated nitroreductase gene delivery and CB1954 treatment: a well-tolerated therapy for established solid tumors. *Mol. Ther.* **3**, 233-240 (2001).

35. Echavarria,M., Forman,M., Ticehurst,J., Dumler,J.S. & Charache,P. PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *J. Clin. Microbiol.* **36**, 3323-3326 (1998).
36. Espehaug,B., Havelin,L.I., Engesaeter,L.B., Langeland,N. & Vollset,S.E. Patient satisfaction and function after primary and revision total hip replacement. *Clin. Orthop.* 135-148 (1998).
37. Evans,C.H., Ghivizzani,S.C., Oligino,T.A. & Robbins,P.D. Future of adenoviruses in the gene therapy of arthritis. *Arthritis Res.* **3**, 142-146 (2001).
38. Fallaux,F.J. *et al.* New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Hum. Gene Ther.* **9**, 1909-1917 (1998).
39. Flaherty,D.M. *et al.* Adenovirus vectors activate survival pathways in lung epithelial cells. *Am. J. Physiol Lung Cell Mol. Physiol* **287**, L393-L401 (2004).
40. Freytag,S.O. *et al.* Phase I trial of replication-competent adenovirus-mediated suicide gene therapy combined with IMRT for prostate cancer. *Mol. Ther.* **15**, 1016-1023 (2007).
41. Fry,C.J. & Peterson,C.L. Transcription. Unlocking the gates to gene expression. *Science* **295**, 1847-1848 (2002).
42. Gaetano,C. *et al.* Transcriptionally active drugs improve adenovirus vector performance in vitro and in vivo. *Gene Ther.* **7**, 1624-1630 (2000).
43. Goldring,S.R. *et al.* The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. *J. Bone Joint Surg. Am.* **65**, 575-584 (1983).
44. Goossens,P.H. *et al.* Infection efficiency of type 5 adenoviral vectors in synovial tissue can be enhanced with a type 16 fiber. *Arthritis Rheum.* **44**, 570-577 (2001).
45. Goossens,P.H. & Huizinga,T.W. Adenoviral-mediated gene transfer to the synovial tissue. *Clin. Exp. Rheumatol.* **20**, 415-419 (2002).
46. Goossens,P.H. *et al.* Feasibility of adenovirus-mediated nonsurgical synovectomy in collagen-induced arthritis-affected rhesus monkeys. *Hum. Gene Ther.* **10**, 1139-1149 (1999).
47. Goossens,P.H. *et al.* The influence of synovial fluid on adenovirus-mediated gene transfer to the synovial tissue. *Arthritis Rheum.* **44**, 48-52 (2001).
48. Green,N.K. *et al.* Immune enhancement of nitroreductase-induced cytotoxicity: studies using a bicistronic adenovirus vector. *Int. J. Cancer* **104**, 104-112 (2003).
49. Greenwald,A.S., Narten,N.C. & Wilde,A.H. Points in the technique of recementing in the revision of an implant arthroplasty. *J. Bone Joint Surg. Br.* **60**, 107-110 (1978).
50. Gruen,T.A., McNeice,G.M. & Amstutz,H.C. "Modes of failure" of cemented stem-type femoral components: a radiographic analysis of loosening. *Clin. Orthop.* 17-27 (1979).
51. Haddad,F.S. *et al.* The expectations of patients undergoing revision hip arthroplasty. *J. Arthroplasty* **16**, 87-91 (2001).
52. Hannallah,D., Peterson,B., Lieberman,J.R., Fu,F.H. & Huard,J. Gene therapy in orthopaedic surgery. *Instr. Course Lect.* **52**, 753-768 (2003).

53. Harris,W.H. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation. *J. Bone Joint Surg. Am.* **51**, 737-755 (1969).
54. Harris,W.H. Wear and periprosthetic osteolysis: the problem. *Clin. Orthop.* 66-70 (2001).
55. Harvey,B.G. *et al.* Safety of local delivery of low- and intermediate-dose adenovirus gene transfer vectors to individuals with a spectrum of morbid conditions. *Hum. Gene Ther.* **13**, 15-63 (2002).
56. Hellman,E.J., Capello,W.N. & Feinberg,J.R. Omnifit cementless total hip arthroplasty. A 10-year average followup. *Clin. Orthop. Relat Res.* 164-174 (1999).
57. Henao,F.J., Daes,J.E. & Dennis,R.J. Risk factors for multiorgan failure: a case-control study. *J. Trauma* **31**, 74-80 (1991).
58. Hendrix,R.W., Wixson,R.L., Rana,N.A. & Rogers,L.F. Arthrography after total hip arthroplasty: a modified technique used in the diagnosis of pain. *Radiology* **148**, 647-652 (1983).
59. Heuft-Dorenbosch,L.L., de Vet,H.C. & van der Linden,S. Yttrium radiosynoviorthesis in the treatment of knee arthritis in rheumatoid arthritis: a systematic review. *Ann. Rheum. Dis.* **59**, 583-586 (2000).
60. Hide,I.G. & Gangi,A. Percutaneous vertebroplasty: history, technique and current perspectives. *Clin. Radiol.* **59**, 461-467 (2004).
61. Jacobs,J.J., Roebuck,K.A., Archibeck,M., Hallab,N.J. & Glant,T.T. Osteolysis: basic science. *Clin. Orthop.* 71-77 (2001).
62. Jones,L.C., Frondoza,C. & Hungerford,D.S. Immunohistochemical evaluation of interface membranes from failed cemented and uncemented acetabular components. *J. Biomed. Mater. Res.* **48**, 889-898 (1999).
63. Katz,S., Downs,T.D., Cash,H.R. & Grotz,R.C. Progress in development of the index of ADL. *Gerontologist* **10**, 20-30 (1970).
64. Kessler,S., Kinkel,S., Kafer,W., Puhl,W. & Schochat,T. Influence of operation duration on perioperative morbidity in revision total hip arthroplasty. *Acta Orthop. Belg.* **69**, 328-333 (2003).
65. Khuri,F.R. *et al.* a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* **6**, 879-885 (2000).
66. Kibbe,M.R. *et al.* Optimizing cardiovascular gene therapy: increased vascular gene transfer with modified adenoviral vectors. *Arch. Surg.* **135**, 191-197 (2000).
67. Knowles,B.B., Howe,C.C. & Aden,D.P. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* **209**, 497-499 (1980).
68. Knox,R.J. *et al.* Bioactivation of 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) by human NAD(P)H quinone oxidoreductase 2: a novel co-substrate-mediated antitumor prodrug therapy. *Cancer Res.* **60**, 4179-4186 (2000).
69. Kormano,M. & Frey,H. Toxicity of X-ray contrast media in cell cultures. *Invest Radiol.* **15**, 68-71 (1980).

70. Lee,C.T. *et al.* Differential effects of adenovirus-p16 on bladder cancer cell lines can be overcome by the addition of butyrate. *Clin. Cancer Res.* **7**, 210-214 (2001).
71. Leung,J.M. & Dzankic,S. Relative importance of preoperative health status versus intraoperative factors in predicting postoperative adverse outcomes in geriatric surgical patients. *J. Am. Geriatr. Soc.* **49**, 1080-1085 (2001).
72. Lieberman,J.R. *et al.* Differences between patients' and physicians' evaluations of outcome after total hip arthroplasty. *J. Bone Joint Surg. Am.* **78**, 835-838 (1996).
73. Lieberman,J.R., Moeckel,B.H., Evans,B.G., Salvati,E.A. & Ranawat,C.S. Cement-within-cement revision hip arthroplasty. *J. Bone Joint Surg. Br.* **75**, 869-871 (1993).
74. Lipinski,K.S. *et al.* Ubiquitous Chromatin Opening Elements (UCOEs) prevent promoter silencing in retroviral vectors and boost transgene expression from adenoviral vectors. J.Gene Med. S11-S12. 1-6-2006.
75. Lipinski,K.S. *et al.* Tumour-specific therapeutic adenovirus vectors: repression of transgene expression in healthy cells by endogenous p53. *Gene Ther.* **8**, 274-281 (2001).
76. Lipinski,K.S. *et al.* Nitroreductase-based therapy of prostate cancer, enhanced by raising expression of heat shock protein 70, acts through increased anti-tumour immunity. *Cancer Immunol. Immunother.* **55**, 347-354 (2006).
77. Mahomed,N.N. *et al.* Rates and outcomes of primary and revision total hip replacement in the United States medicare population. *J. Bone Joint Surg. Am.* **85-A**, 27-32 (2003).
78. Malchau H,H.P.S.P.O.A. Prognosis of total hip replacement. Update and validation of results from the Swedish national hip arthroplasty registry 1979-1998. *Presented at 67th annual meeting of the American Academy of Orthopedic surgeons, March 15-19, 2000, Orlando, USA.* (2000).
79. Malchau,H., Herberts,P., Söderman,P. & Odén,A. Prognosis of total hip replacement. Update and validation of results from the Swedish national hip arthroplasty registry 1979-1998. *Presented at 67th annual meeting of the American Academy of Orthopedic surgeons, March 15-19, 2000, Orlando, USA.* (2000).
80. Marcantonio,E.R., Flacker,J.M., Wright,R.J. & Resnick,N.M. Reducing delirium after hip fracture: a randomized trial. *J. Am. Geriatr. Soc.* **49**, 516-522 (2001).
81. Maus,T.P., Berquist,T.H., Bender,C.E. & Rand,J.A. Arthrographic study of painful total hip arthroplasty: refined criteria. *Radiology* **162**, 721-727 (1987).
82. McGee,M.A., Howie,D.W., Ryan,P., Moss,J.R. & Holubowycz,O.T. Comparison of patient and doctor responses to a total hip arthroplasty clinical evaluation questionnaire. *J. Bone Joint Surg. Am.* **84-A**, 1745-1752 (2002).
83. McNeish,I.A. *et al.* Virus directed enzyme prodrug therapy for ovarian and pancreatic cancer using retrovirally delivered E. coli nitroreductase and CB1954. *Gene Ther.* **5**, 1061-1069 (1998).
84. Miller,A.A., Kurschel,E., Osieka,R. & Schmidt,C.G. Clinical pharmacology of sodium butyrate in patients with acute leukemia. *Eur. J. Cancer Clin. Oncol.* **23**, 1283-1287 (1987).

85. Morral,N. *et al.* Lethal toxicity, severe endothelial injury, and a threshold effect with high doses of an adenoviral vector in baboons. *Hum. Gene Ther.* **13**, 143-154 (2002).
86. Nicholson,M.J. Cardiac arrest following acrylic-cement implants. *Anesth. Analg.* **52**, 298-302 (1973).
87. Nita,I. *et al.* Direct gene delivery to synovium. An evaluation of potential vectors in vitro and in vivo. *Arthritis Rheum.* **39**, 820-828 (1996).
88. Nudelman,A. *et al.* Novel anticancer prodrugs of butyric acid. 2. *J. Med. Chem.* **35**, 687-694 (1992).
89. Okegawa,T., Nutahara,K., Pong,R.C., Higashihara,E. & Hsieh,J.T. Enhanced transgene expression in urothelial cancer gene therapy with histone deacetylase inhibitor. *J. Urol.* **174**, 747-752 (2005).
90. Oligino,T.J., Yao,Q., Ghivizzani,S.C. & Robbins,P. Vector systems for gene transfer to joints. *Clin. Orthop.* S17-S30 (2000).
91. Palmer,D.H. *et al.* Virus-directed enzyme prodrug therapy: intratumoral administration of a replication-deficient adenovirus encoding nitroreductase to patients with resectable liver cancer. *J. Clin. Oncol.* **22**, 1546-1552 (2004).
92. Parvizi,J. *et al.* Revision total hip arthroplasty in octogenarians. A case-control study. *J. Bone Joint Surg. Am.* **89**, 2612-2618 (2007).
93. Perlman,H. *et al.* Modifications in adenoviral coat fiber proteins and transcriptional regulatory sequences enhance transgene expression. *J. Rheumatol.* **29**, 1593-1600 (2002).
94. Phillips,C.B. *et al.* Incidence rates of dislocation, pulmonary embolism, and deep infection during the first six months after elective total hip replacement. *J. Bone Joint Surg. Am.* **85-A**, 20-26 (2003).
95. Phillips,W.C. & Kattapuram,S.V. Prosthetic hip replacements: plain films and arthrography for component loosening. *AJR Am. J. Roentgenol.* **138**, 677-682 (1982).
96. Planchon,P. *et al.* New stable butyrate derivatives alter proliferation and differentiation in human mammary cells. *Int. J. Cancer* **48**, 443-449 (1991).
97. Pouillart,P., Cerutti,I., Ronco,G., Villa,P. & Chany,C. Enhancement by stable butyrate derivatives of antitumor and antiviral actions of interferon. *Int. J. Cancer* **51**, 596-601 (1992).
98. Prause,G. *et al.* Can ASA grade or Goldman's cardiac risk index predict peri-operative mortality? A study of 16,227 patients. *Anaesthesia* **52**, 203-206 (1997).
99. Raut,V.V., Wroblewski,B.M. & Siney,P.D. Revision hip arthroplasty. Can the octogenarian take it? *J. Arthroplasty* **8**, 401-403 (1993).
100. Rea,D. *et al.* Highly efficient transduction of human monocyte-derived dendritic cells with subgroup B fiber-modified adenovirus vectors enhances transgene-encoded antigen presentation to cytotoxic T cells. *J. Immunol.* **166**, 5236-5244 (2001).
101. Reid,T., Warren,R. & Kirn,D. Intravascular adenoviral agents in cancer patients: lessons from clinical trials. *Cancer Gene Ther.* **9**, 979-986 (2002).
102. Roberts,J.J., Friedlos,F. & Knox,R.J. CB 1954 (2,4-dinitro-5-aziridinyl benzamide) becomes a

DNA interstrand crosslinking agent in Walker tumour cells. *Biochem. Biophys. Res. Commun.* **140**, 1073-1078 (1986).

103. Robinson,A.H., Palmer,C.R. & Villar,R.N. Is revision as good as primary hip replacement? A comparison of quality of life. *J. Bone Joint Surg. Br.* **81**, 42-45 (1999).
104. ROGERS,S. Induction of arginase in rabbit epithelium by the Shope rabbit papilloma virus. *Nature* **183**, 1815-1816 (1959).
105. ROGERS,S., Lowenthal,A., Terheggen,H.G. & Columbo,J.P. Induction of arginase activity with the Shope papilloma virus in tissue culture cells from an argininemic patient. *J. Exp. Med.* **137**, 1091-1096 (1973).
106. Schroeder,T.M. & Westendorf,J.J. Histone deacetylase inhibitors promote osteoblast maturation. *J. Bone Miner. Res.* **20**, 2254-2263 (2005).
107. Seelen,J.L., Bruijn,J.D., Kingma,L.M., Bernoski,F.P. & Bloem,J.L. Radiographic loosening of cementless threaded acetabular cups. No additional diagnostic value of arthrography in 30 patients. *Acta Orthop. Scand.* **66**, 415-417 (1995).
108. Shanbhag,A.S., Hasselman,C.T. & Rubash,H.E. The John Charnley Award. Inhibition of wear debris mediated osteolysis in a canine total hip arthroplasty model. *Clin. Orthop.* 33-43 (1997).
109. Shibata,T., Giaccia,A.J. & Brown,J.M. Hypoxia-inducible regulation of a prodrug-activating enzyme for tumor-specific gene therapy. *Neoplasia.* **4**, 40-48 (2002).
110. Small,E.J. *et al.* A phase I trial of intravenous CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer. *Mol. Ther.* **14**, 107-117 (2006).
111. Smith,R.E. & Turner,R.J. Total hip replacement using methylmethacrylate cement. An analysis of data from 3,482 cases. *Clin. Orthop. Relat Res.* 231-238 (1973).
112. Soderman,P. *et al.* Outcome after total hip arthroplasty: Part II. Disease-specific follow-up and the Swedish National Total Hip Arthroplasty Register. *Acta Orthop. Scand.* **72**, 113-119 (2001).
113. Stewart,A.K. *et al.* Adenovector-mediated gene delivery of interleukin-2 in metastatic breast cancer and melanoma: results of a phase 1 clinical trial. *Gene Ther.* **6**, 350-363 (1999).
114. Strehle,J., DelNotaro,C., Orler,R. & Isler,B. The outcome of revision hip arthroplasty in patients older than age 80 years: complications and social outcome of different risk groups. *J. Arthroplasty* **15**, 690-697 (2000).
115. Sud,S., Yang,S.Y., Evans,C.H., Robbins,P.D. & Wooley,P.H. Effects of cytokine gene therapy on particulate-induced inflammation in the murine air pouch. *Inflammation* **25**, 361-372 (2001).
116. Tang,D.C., Johnston,S.A. & Carbone,D.P. Butyrate-inducible and tumor-restricted gene expression by adenovirus vectors. *Cancer Gene Ther.* **1**, 15-20 (1994).
117. Taura,K. *et al.* Impact of novel histone deacetylase inhibitors, CHAP31 and FR901228 (FK228), on adenovirus-mediated transgene expression. *J. Gene Med.* **6**, 526-536 (2004).

118. Terheggen,H.G., Lowenthal,A., Lavinha,F., Colombo,J.P. & ROGERS,S. Unsuccessful trial of gene replacement in arginase deficiency. *Z. Kinderheilkd.* **119**, 1-3 (1975).
119. Terheggen,H.G., Schwenk,A., Lowenthal,A., Van,S.M. & Colombo,J.P. [Hyperargininemia wityh arginase deficiency. A new familial metabolic disease. I. Clinical studies]. *Z. Kinderheilkd.* **107**, 298-312 (1970).
120. Ulrich-Vinther,M. *et al.* Recombinant adeno-associated virus-mediated osteoprotegerin gene therapy inhibits wear debris-induced osteolysis. *J. Bone Joint Surg. Am.* **84-A**, 1405-1412 (2002).
121. Vacanti,C.J., VanHouten,R.J. & Hill,R.C. A statistical analysis of the relationship of physical status to postoperative mortality in 68,388 cases. *Anesth. Analg.* **49**, 564-566 (1970).
122. van der Eb,M.M. *et al.* Gene therapy with apoptin induces regression of xenografted human hepatomas. *Cancer Gene Ther.* **9**, 53-61 (2002).
123. Varnavski,A.N. *et al.* Preexisting immunity to adenovirus in rhesus monkeys fails to prevent vector-induced toxicity. *J. Virol.* **76**, 5711-5719 (2002).
124. Vernia,P. *et al.* Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: results of a multicentre trial. *Eur. J. Clin. Invest* **33**, 244-248 (2003).
125. Weedon,S.J. *et al.* Sensitisation of human carcinoma cells to the prodrug CB1954 by adenovirus vector-mediated expression of E. coli nitroreductase. *Int. J. Cancer* **86**, 848-854 (2000).
126. Williams,S. *et al.* CpG-island fragments from the HNRPA2B1/CBX3 genomic locus reduce silencing and enhance transgene expression from the hCMV promoter/enhancer in mammalian cells. *BMC. Biotechnol.* **5**, 17 (2005).
127. Wilson,W.R. *et al.* Quantitation of bystander effects in nitroreductase suicide gene therapy using three-dimensional cell cultures. *Cancer Res.* **62**, 1425-1432 (2002).
128. Wooley,P.H. & Schwarz,E.M. Aseptic loosening. *Gene Ther.* **11**, 402-407 (2004).
129. Yang,S.Y. *et al.* Adeno-associated virus-mediated osteoprotegerin gene transfer protects against particulate polyethylene-induced osteolysis in a murine model. *Arthritis Rheum.* **46**, 2514-2523 (2002).
130. Yang,Y., Li,Q., Ertl,H.C. & Wilson,J.M. Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *J. Virol.* **69**, 2004-2015 (1995).
131. Zhang,H., Holt,C.M., Malik,N., Shepherd,L. & Morcos,S.K. Effects of radiographic contrast media on proliferation and apoptosis of human vascular endothelial cells. *Br. J. Radiol.* **73**, 1034-1041 (2000).
132. Zimmermann,T. *et al.* Isolation and characterization of rheumatoid arthritis synovial fibroblasts from primary culture--primary culture cells markedly differ from fourth-passage cells. *Arthritis Res.* **3**, 72-76 (2001).

List of publications

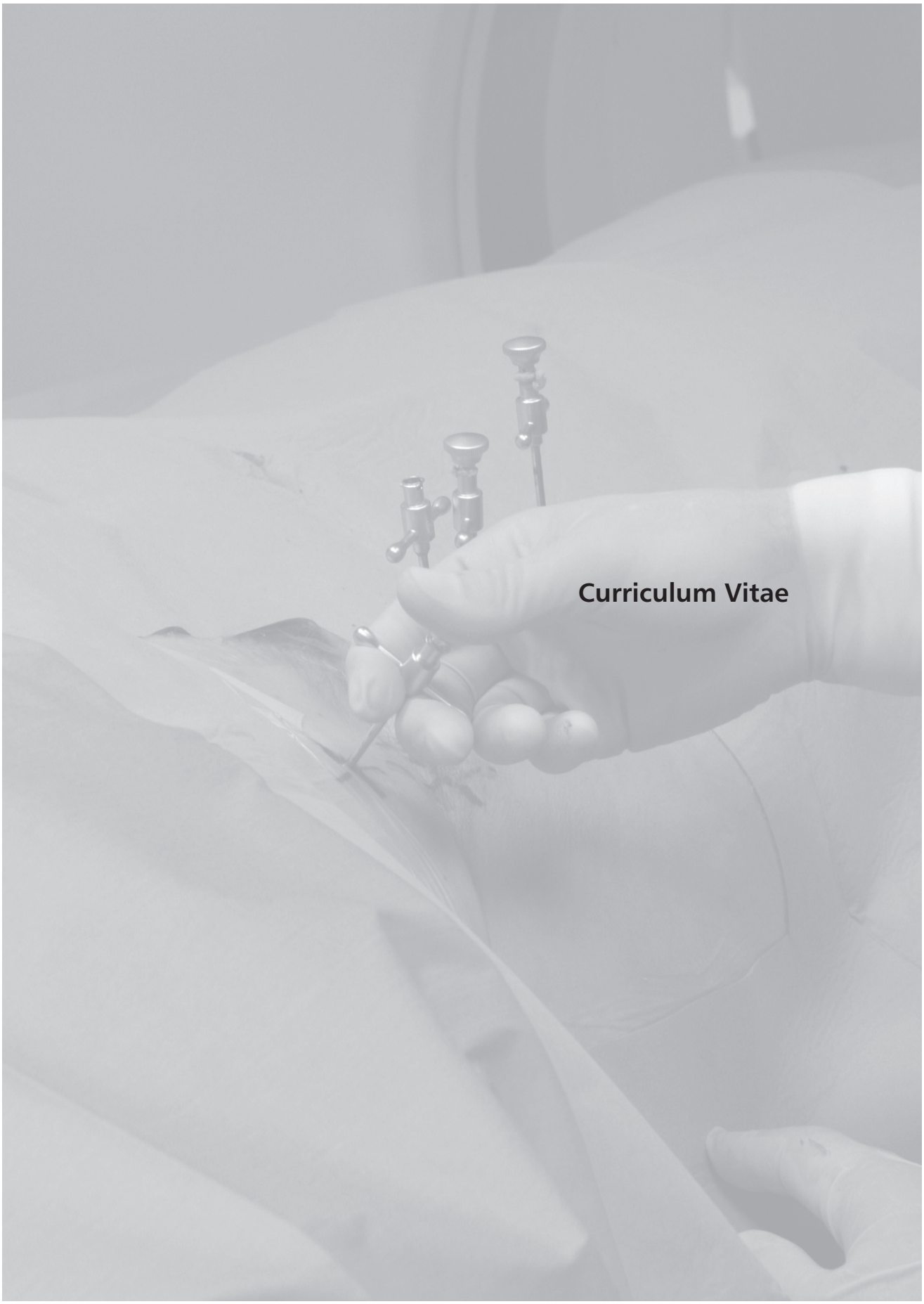
de Poorter,J.J. *et al.* Towards gene therapy in prosthesis loosening: efficient killing of interface cells by gene-directed enzyme prodrug therapy with nitroreductase and the prodrug CB1954. *J. Gene Med.* **7**, 1421-1428 (2005).

de Poorter,J.J., Obermann,W.R., Huizinga,T.W. & Nelissen,R.G. Arthrography in loosened hip prostheses. Assessment of possibilities for intra-articular therapy. *Joint Bone Spine* **73**, 684-690 (2006).

de Poorter,J.J., Lipinski,K.S., Nelissen,R.G., Huizinga,T.W. & Hoeben,R.C. Optimization of short-term transgene expression by sodium butyrate and ubiquitous chromatin opening elements (UCOEs). *J. Gene Med.* **9**, 639-648 (2007).

de Poorter,J.J. *et al.* Gene therapy and cement injection for restabilization of loosened hip prostheses. *Hum. Gene Ther.* **19**, 83-95 (2008).

de Poorter,J.J., Hoeben,R.C., Obermann,W.R., Huizinga,T.W. & Nelissen,R.G. Gene therapy for the treatment of hip prosthesis loosening: adverse events in a phase 1 clinical study. *Hum. Gene Ther.* **19**, 1029-1038 (2008).



Curriculum Vitae

Jolanda Johanna de Poorter werd geboren op 7 januari 1976 in Eindhoven. In 1994 deed ze eindexamen VWO aan het Hertog Jan College in Valkenswaard.
Omdat ze werd uitgeloot voor geneeskunde ging ze in 1994 eerst Gezondheidswetenschappen studeren aan de Universiteit Maastricht. Daar volgde ze de afstudeerrichting bewegingswetenschappen en kwam ze in aanraking met de orthopaedie. In 1996 mocht ze alsnog geneeskunde gaan studeren. Tijdens haar studie was ze enthousiast lid van de Maastrichtse Studenten Roeivereniging Saurus, waar ze in 1996-1997 in het bestuur zat als materiaalcommissaris. Tijdens de studie deed ze ook onderzoek bij de afdeling orthopaedie van het Academisch Ziekenhuis Maastricht onder begeleiding van Prof. Dr. S.K. Bulstra naar de betrouwbaarheid en validiteit van het Dynaport Knee systeem. In 2000 haalde ze haar doctoraaldiploma Gezondheidswetenschappen (afstudeerrichting bewegingswetenschappen) met de afstudeerstage en scriptie "Walking pattern in knee OA" onder begeleiding van Prof. Dr. S.K. Bulstra. In 2000 haalde ze haar doctoraaldiploma en in 2002 haar artsexamen geneeskunde (beide cum laude).

In 2002 begon Jolanda als onderzoeker aan haar promotie-onderzoek in het Leids Universitair Medisch Centrum onder begeleiding van Prof. Dr. R.G.H.H. Nelissen. De uitvoering van het onderzoek werd gedeeltelijk gefinancierd door ML Laboratories plc, Verenigd Koninkrijk (nu Vectura plc). Haar werk werd gepresenteerd op nationale en internationale congressen en werd onderscheiden met verschillende prijzen.

Na drie jaar onderzoek begon Jolanda met de tweejarige vooropleiding chirurgie in het Rijnland Ziekenhuis te Leiderdorp onder supervisie van Dr. S.A. da Costa. In 2008 begon ze met de opleiding orthopaedie in het Leids Universitair Medisch Centrum onder supervisie van Prof. Dr. R.G.H.H. Nelissen. Ze werkt nu als assistent orthopaedie in het HAGA-ziekenhuis te Den Haag onder begeleiding van Dr. R.L.M. Deijkers.

Jolanda woont samen met Robin de Vries in Voorhout. In november 2008 werd hun dochter Karlijn geboren.