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## Gene therapy and cement injection for restabilisation of loosened hip prostheses

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## 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  $3 \times 10^{10}$  and  $1 \times 10^{11}$  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.<sup>78</sup>

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.<sup>43</sup> 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.<sup>57</sup> 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.<sup>77</sup> Strehle *et al.*<sup>114</sup> 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.<sup>49,73</sup> 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*<sup>30</sup> and *in vivo* in animals<sup>46</sup> 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 months 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,<sup>38</sup> as described in previous studies.<sup>34</sup> 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 cytomegalovirus immediate-early (CMV-IE) promoter.<sup>34,125</sup> The complete *Escherichia coli* nitroreductase (Ntr) expression cassette was cloned into an E1-deleted HAdV-5 adenovirus transfer vector. Virus batches of  $4.2 \times 10^{10}$  and  $4 \times 10^{11}$  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.<sup>68</sup> Cells containing Ntr convert CB1954 into a bifunctional alkylating agent that is capable of forming DNA interstrand cross-links, resulting in cell death.<sup>18,34,102</sup> Because there is no human homolog to Ntr,<sup>23</sup> 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 (VD-EPT) 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<sub>2</sub>HPO<sub>4</sub> [0.6 mmol/litre]; CaCl<sub>2</sub> [0.9 mmol/litre]; MgCl<sub>2</sub> [0.5 mmol/litre]; 5% sucrose) in a volume representing 90% of the volume injected during the inclusion arthrogram. The initial dose was  $3 \times 10^9$  particles (Table 1). Dose escalation proceeded in 3-fold increments up to  $1 \times 10^{11}$  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 polymerase chain reaction (PCR) analysis of adenoviral DNA. All patients were intended to have a prodrug dose of 24 mg/m<sup>2</sup> 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<sup>2</sup>.

#### 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

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

tibroplasty 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<sup>35</sup> 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.<sup>50</sup> 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<sup>53</sup>, 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).<sup>121</sup> 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  $\beta$ -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  $3 \times 10^9$  particles to  $1 \times 10^{11}$  particles (Table 1). All patients were intended to have a prodrug dose of 24 mg/m<sup>2</sup> 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<sup>2</sup>. 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.<sup>35</sup> All patients in the lowest three vector dose groups ( $3 \times 10^9$ ,  $1 \times 10^{10}$ ,  $3 \times 10^{10}$  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 ( $1 \times 10^{11}$  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	$3 \times 10^9$	NS	
2	16	$3 \times 10^9$	$5.24 \times 10^5$	
3	30	$3 \times 10^9$	$5.07 \times 10^7$	+ 10 days: $2.60 \times 10^5$ particles
4	30	$8.3 \times 10^9$	$3.21 \times 10^7$	
5	7.5	$1 \times 10^{10}$	$1.45 \times 10^7$	
6	22	$1 \times 10^{10}$	$1.23 \times 10^7$	
7	14	$2.6 \times 10^{10}$	NS	
8	10	$3 \times 10^{10}$	NS	
9	27	$3 \times 10^{10}$	NS	
10	30	$1 \times 10^{11}$	$1.00 \times 10^7$	
11	9	$1 \times 10^{11}$	NS	+ 11 months: $1.88 \times 10^4$ particles
12	18	$1 \times 10^{11}$	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.<sup>50</sup> 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.

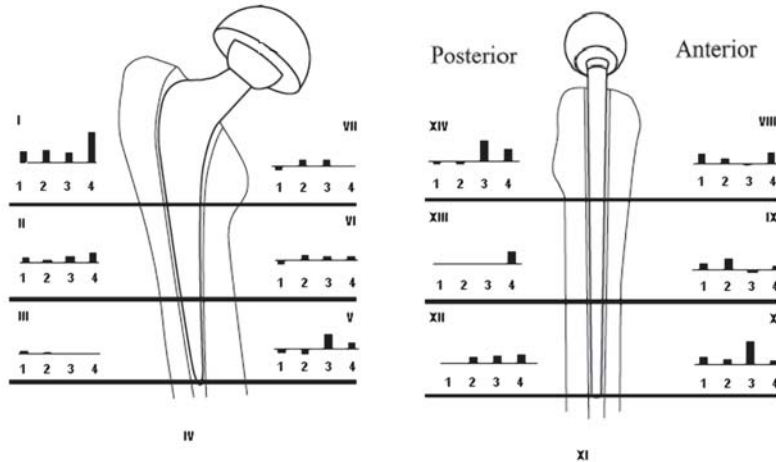
Maximal cement thickness and maximal radiolucency width were also measured at 6 months of follow-up. Compared with 1 day after cement injection the maximal cement thickness at 6 months follow-up had decreased by 0.02 mm ( $p = 0.91$ ). The correlation between maximal cement thickness at 1 day and at 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<sup>53</sup> 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.

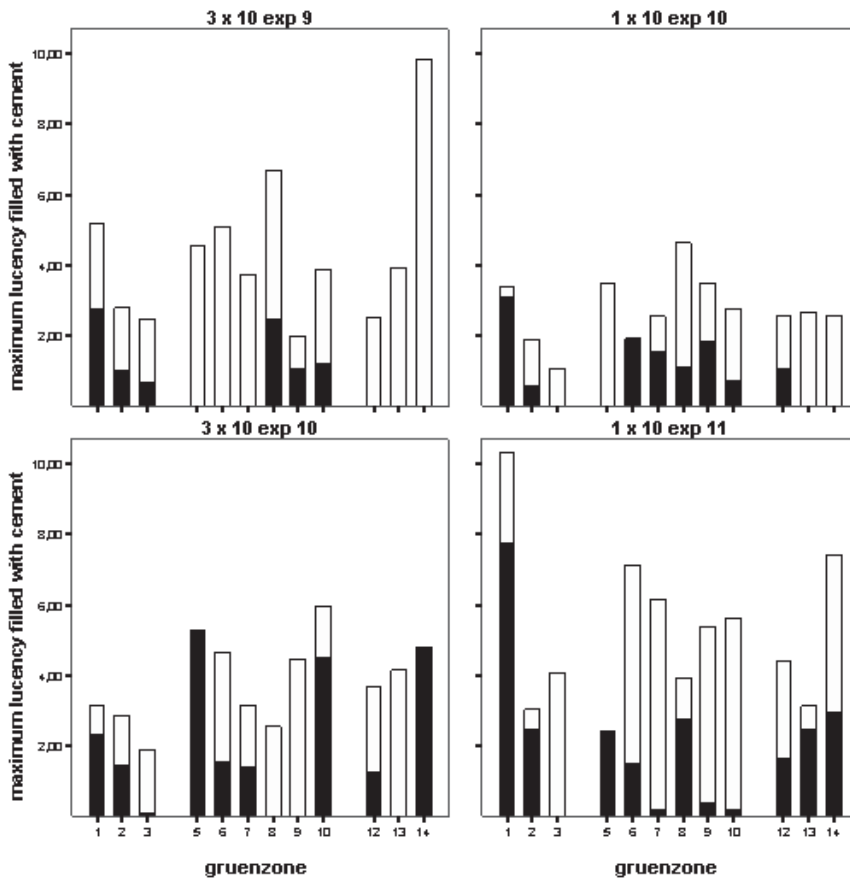


The figure shows division of the periprosthetic space in 14 zones according to Gruen. Per zone the maximum cement thickness is measured before and after cement injection. The increase in cement thickness is represented graphically per Gruen zone and per dose group. Table 3 shows the exact numbers.

**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.

Gruen-zone	Increase in cement thickness (mm) mean and sd, per dose-group (particles)				Total of all groups
	$3 \times 10^9$ particles	$1 \times 10^{10}$ particles	$3 \times 10^{10}$ particles	$1 \times 10^{11}$ particles	
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.



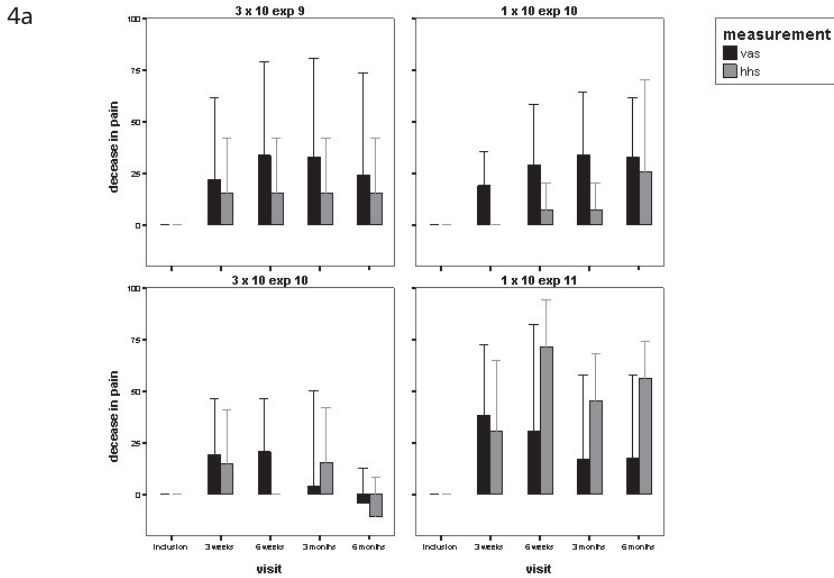
Shown are X-rays from patient 12 before (*left*) and after (*right*) gene therapy and cement injection. Note that the newly injected cement is radio-opaque and therefore has a whiter appearance than the older cement. The radiolucent zone has a dark appearance on the pretreatment X-ray.

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 in dose 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 ( $p < 0.01$ ).

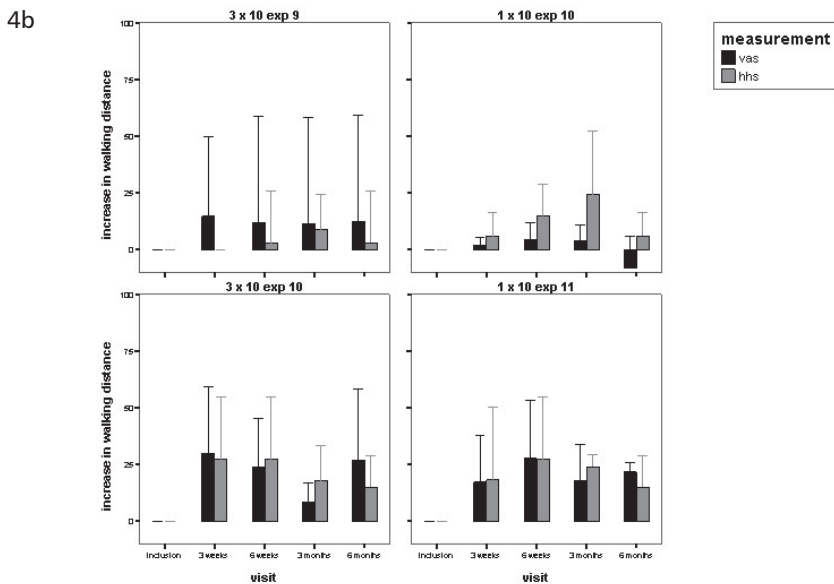
Figure 5a shows means and standard deviations for improvement in dependency for activities in daily living at various time points per dose group. Best improvement is achieved in the highest dose group. Figure 5b shows increase in total Harris Hip Score at various time points per dose group. Scores increased 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,  $3 \times 10^9$  particles; group 2,  $1 \times 10^{10}$  particles; group 3,  $3 \times 10^{10}$  particles; group 4,  $1 \times 10^{11}$  particles) .



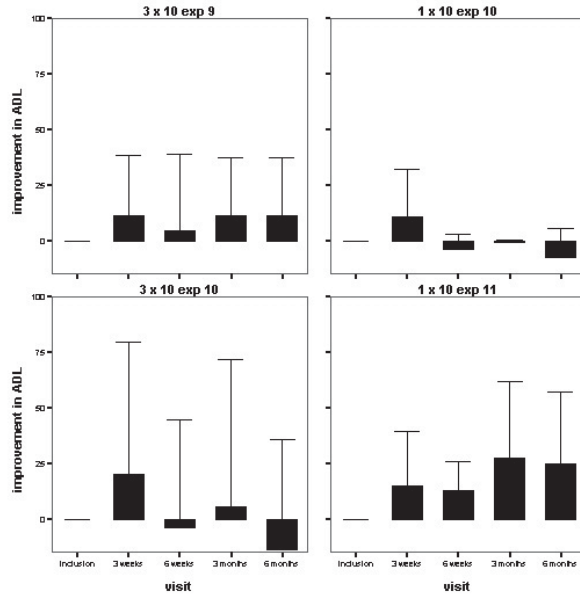
Solid columns represent VAS-measurements and shaded columns represent HHS-measurements for the same outcome variable. A score of 0 means unbearable pain; 100 means no pain;



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.

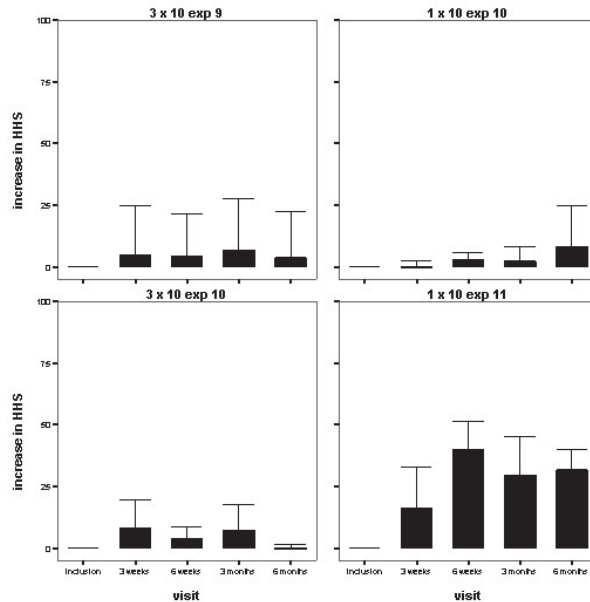
**Figure 5.** Means and standard deviations for Activities of Daily Living (ADL) dependency (a) total Harris Hip Score (b) per dose group (group 1,  $3 \times 10^9$  particles; group 2,  $1 \times 10^{10}$  particles; group 3,  $3 \times 10^{10}$  particles; group 4,  $1 \times 10^{11}$  particles).

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  $1 \times 10^{11}$  particles no dose-limiting toxicity was observed, and no adverse events were reported. A prodrug dose of  $24 \text{ mg/m}^2$  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.<sup>23</sup> Therefore the prodrug dose was lowered to  $16 \text{ mg/m}^2$ ; 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 ( $1 \times 10^{11}$  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.<sup>91</sup> 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  $1.88 \times 10^4$  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.*<sup>76</sup> 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.<sup>47</sup> 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,<sup>15</sup> 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,<sup>73</sup> and is shown to have good biomechanical properties.<sup>49</sup> 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.*,<sup>14</sup> 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.<sup>72,82</sup>

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.