Bone and joint disorders: screening and early clinical drug development

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CHAPTER 7

Administration of an adeno-associated viral vector expressing interferon-β in patients with inflammatory hand arthritis, results of a phase I/II study

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ABSTRACT

OBJECTIVE Inflammatory hand arthritis (IHA) results in impaired function. Local gene therapy with ART-IO2, a recombinant adeno-associated viral (AAV) serotype 5 vector expressing interferon (IFN)-β, under the transcriptional control of nuclear factor κ-B responsive promoter, was preclinically shown to have favorable effects. This study aimed to investigate the safety and tolerability of local gene therapy with ART-IO2 in patients with IHA.

METHODS In this first-in-human, dose-escalating, cohort study, 12 IHA patients were to receive a single intra-articular (IA) injection of ART-IO2 ranging 0.3×10^{12}-1.2×10^{13} genome copies in an affected hand joint. Adverse events (AEs), routine safety laboratory and the clinical course of disease were periodically evaluated. Baseline- and follow-up contrast enhanced magnetic resonance images (MRIs), shedding of viral vectors in bodily fluids, and AAV5 and IFN-β immune responses were evaluated. A data review committee provided safety recommendations.

RESULTS Four patients were enrolled. Long-lasting local AEs were observed in 3 patients upon IA injection of ART-IO2. The AEs were moderate and could be treated conservative. Given the duration of the AEs and their possible or probable relation to ART-IO2, no additional patients were enrolled. No systemic treatment emergent AEs were observed. The MRIS reflected the AEs by (peri)arthritis. No T-cell response against AAV5 or IFN-β, nor IFN-β antibodies could be detected. Neutralizing antibody titers against AAV5 raised post-dose.

CONCLUSIONS Single IA doses of 0.6×10^{12} or 1.2×10^{12} ART-IO2 vector genomes were administered without systemic side effects or serious AEs. However, local tolerability was insufficient for continuation.

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Introduction

Osteoarthritis (OA) and rheumatoid arthritis (RA) can both manifest with inflammatory hand arthritis (IHA) and cause considerable disability.[1,2] Although pathophysiology of OA and RA differ, inflammation of the synovium plays a pivotal role in both.[3–6] This inflammation occurs in exacerbations and leads to destruction of joint tissues, joint pain and impaired function.[7] Currently, no registered therapy halts the deterioration of joints caused by OA.[8] For RA, systemic disease modifying anti-rheumatic drugs are available, but some patients respond poorly to these.[9] The lack of full efficacy of pharmacological interventions may be due to ineffective interference with pathophysiological pathways, poor penetration into the synovium, or timing of the drug-availability in relation to the inflammatory status within the joint.[10] Hence, inflammation driven, intra-articular (IA) treatment may take preference over systemic treatment in mono- or oligo-arthritis.

Interferon (IFN)-β has anti-inflammatory properties, such as the inhibition of tumor necrosis factor and interleukin-1β production by macrophages in the inflamed synovium.[11,12] IFN-β has been administered in multiple clinical trials, confirming its safety after intra-muscular, and subcutaneous injection.[12–14] Efficacy studies with subcutaneously administered IFN-β in arthritic patients showed ambiguous results: In a small study, patient reported- and histological efficacy was shown.[13] In a larger study, these effects could not be confirmed, which was hypothesized to be due to low local exposure of the inflamed joint and the short half-life of IFN-β.[12] Novel approaches, enabling local and inducible expression of IFN-β in case of an exacerbation may provide efficacy.

Such an approach, a recombinant adeno-associated virus (AAV) vector expressing IFN-β under the transcriptional control of a promoter responsive to the pro-inflammatory nuclear factor κB (NF-κB), ART-IO2, was investigated in this study. The *IFN*-β expression cassette of ART-IO2 is flanked by two AAV2 derived inverted terminal repeats and is packaged in the capsid of AAV5. NF-κB has been described to be upregulated in both RA and OA.[15,16] Recombinant AAV vectors are replication-deficient vectors which have been shown to be safe in multiple clinical trials.[17–20] The capsid of AAV5 was chosen because of its efficient transduction in synovial tissue and the low incidence of pre-existing neutralizing antibodies for AAV5.[21–24] As such, ART-IO2 was designed to produce an anti-inflammatory compound (IFN-β) locally in the joint in periods of inflammation. In *in vitro* studies with

fibroblast-like synoviocytes from RA- and OA patients, decreased synovial inflammation was observed. Pre-clinical studies for biodistribution, safety and initial efficacy in animal models for arthritis in rats and rhesus monkeys showed that ART-IO2 was well tolerated and decreased synovial inflammation due to expression of IFN-β.[25–27] Altogether, these results warranted evaluation of ART-IO2 in RA and OA patients. Here we describe the first-in-human study in which the safety and tolerability of a single IA administration of ART-IO2 was investigated in patients with IHA.

Methods

STUDY DESIGN

This was a single center, open label, first-in-human, dose escalating study to investigate the safety and tolerability of a single, IA injection of ART-IO2 in up to 12 IHA patients. The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands. Patient enrollment was in 3 cohorts (3:3:6 patients). The IA doses for cohort I (patients 1-3) were 1.2×10^{12}, 0.6×10^{12}, or 0.3×10^{12} vector genomes (VG) for the carpometacarpal (CMC) and metacarpophalangeal (MCP)-, proximal interphalangeal (PIP)- and distal interphalangeal (DIP)- joints, respectively. A ten-fold increase in dose was planned for cohort II (patients 4-6). Cohort III (patients 7-12) was planned to receive the highest tolerated dose, as determined from the safety data from the previous cohorts. The injection volumes were 500µL, 250µL and 125µL for the CMC/MCP, PIP and DIP joints, respectively. The injections were performed in a sterile environment, under ultrasound-guidance, by board-certified musculoskeletal radiologists (MR or ANC with respectively 24 and 13 years of experience). Patients were followed for 24 weeks after study drug administration; long-term safety follow-up is conducted by yearly telephone calls up to 5 years.

The study was approved by the Central Committee on Research Involving Human Subjects (CCMO), The Hague, The Netherlands, and was registered in the clinicaltrials.gov registry (NCT02727764). All patients provided written informed consent prior to participation. Study related procedures were conducted in accordance with the Declaration of Helsinki and the Dutch Act regarding Medical Research Involving Human Subjects. An environmental permit on 'deliberate release into the environment' (according to the directive 2001/18/EC of the European Parliament and of the Council) had been granted prior to the study (License: GGOIM-MV16-001). An independent data review committee (DRC) was installed to review the safety data after each cohort and to give recommendations for dose escalation and stopping decisions (supplementary methods 1).

INVESTIGATIONAL PRODUCT

Construction of the ART-IO2 vector has been described previously.[26] ART-IO2 was produced using polyethylenimine (PEIPRO™) mediated transient transfection of HEK293T/17 cells with pART-IO2 vector plasmid and PDP5-KAN3 helper/packaging plasmid, a derivative of PDP5 with the ampicillin resistance gene replaced by the kanamycin resistance gene. ART-IO2 was purified in steps including affinity chromatography, ion exchange chromatography and filtration.[28,29] ART-IO2 was manufactured in accordance with Good Manufacturing Practices. QC testing was performed according to Ph.Eur.chapter "5.14". The ratio of vector genomes:AAV-particles of ART-IO2 was 1:6.6. Starting doses were selected based on pre-clinical results of ART-IO2 effectivity- and toxicity studies,[21,26] and RAAV vectors in other clinical trials.[30,31] Injection volumes were based on current clinical practice with IA injections.

PARTICIPANTS

Patients with an inflammatory arthritis of the CMC, MCP, PIP or DIP joints and an indication to undergo surgical intervention of the target joint, were eligible. The indication for surgical intervention and diagnosis of OA or RA had to be established by a treating physician and inflammation was confirmed on Magnetic Resonance Imaging (MRI). At screening, baseline characteristics and medical history were collected, physical examination, routine safety laboratory and urinalysis were performed, and further in- and exclusion criteria were assessed. Exclusion criteria included presence of neutralizing antibodies against AAV5 and/or IFN-β, previous treatment with an AAV5 and a poor functional status. Patients could remain on their current medication and stop or start medication as appropriate. Full in- and exclusion criteria are provided in supplementary methods 2.

SAFETY

Patients remained in the clinic for at least 4 hours to observe the initial reaction to the ART-IO2 administration. Clinical follow-up visits took place at 24 hours, and 1, 2, 4, 8, 12, 16, 20 and 24 weeks after administration. Safety was assessed by physical examination, vital signs, 12-lead electrocardiography, safety laboratory evaluation, urinalysis, and the presence of- and changes

in adverse events (AEs) according to the Rheumatology Common Toxicity Criteria (R-CTCAE).[32]

After 24 weeks, patients proceeded into a 5-year follow-up with annual phone calls to monitor long-term safety, consisting of a standardized questionnaire including the occurrence of hospitalization or surgical intervention, potentially treatment related events and relevant oncologic, infectious, neurological, hematological or immunological events.

FUNCTIONAL ASSESSMENTS

The functionality of the injected joint was monitored by assessment according to the Composite Change Index (CCI) at each follow-up visit.[33,34] The CCI score is calculated from six outcomes: a physician completed part, including assessment of function, joint tenderness, swelling and efficacy, all on a 4-point scale, and a patient completed part including a visual analogue score for pain (0-10) and efficacy (4-point scale). Based on changes from baseline, a score between 0 and 10 was calculated at follow-up. Scores <5 were defined as no effect or deterioration, scores ≥5 were defined as successful treatment. The CCI scoring and calculation methods are given in supplementary methods 3. In addition, flexion and extension range of motion were measured in degrees, for the MCP, PIP and DIP joints, using a goniometer.

MRI

The level of arthritis of the target joint was evaluated using MRI scans at screening, 12, and 24 weeks after study drug administration. Images were obtained by static and dynamic, contrast enhanced MRIs from the CMC joints to the fingers distally, using a 3T MR scanner (Philips, Eindhoven, The Netherlands), and dedicated small extremity MR coil. All MRI scans were made in the Leiden University Medical Center (CHDR), Leiden, The Netherlands. The following sequences were acquired before contrast injection: coronal and axial T1-weighted Turbo Spin Echo (TSE) sequence (repetition time/echo time TR/TE 623/18ms) and coronal T2 Dixon (TR/TE 2500/60ms) and axial T2 Dixon (3286/60ms). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1mmol/kg), a dynamic contrast-enhanced (DCE-MRI) sequence was performed, using 8 slices.

After DCE-MRI sequence the following sequences were obtained: T1-weighted TSE sequence with frequency selective fat saturation in the coronal and axial plane (TR/TE 727/18ms). The field-of-view was 130mm. Coronal sequences had 20 slices with a slice thickness of 2mm, no slice gap. The axial sequences had 50 slices, with thickness of 2.5mm, no slice gap.

The target joints were assessed qualitatively and semi-quantitatively. A musculoskeletal radiologist (MR or ANC) assessed the scans for quality and performed a qualitative assessment of the target joint, in a narrative report. Semi-quantitative scoring was done by experienced readers in these assessments (YD and FK). Reported outcomes were based on categories of validated MRI scoring systems for hand OA and RA, and included synovitis, bone marrow edema (RA) or bone marrow lesions (OA) and bone erosions (RA) or subchondral bone defects/erosive damage (OA).[35–37]

IMMUNOLOGY

The humoral and cellular immune responses against AAV5 and IFN-β, as well as the presence of IFN-β, were measured at set time points during the study using validated assays (Table 1).

The presence of AAV5-neutralizing antibodies (titers >15) was measured using an inhibition of transduction assay. In this assay, the residual expression of luciferase was measured in HEK293T cells after transduction with an AAV5 vector pre-incubated with the test serum. Luciferase was quantified using a VictorX microplate reader, PerkinElmer (Waltham, MA, USA) (undiluted to 1:405 diluted). Binding antibodies against AAV5 were determined by ELISA using the BioTek PowerWaveXS spectrophotometer (Winooski, VT, USA) (dilutions of 1:100 to 1:24300).

Binding antibodies against IFN-β were measured using a bridging assay format (MesoScale Discovery platform, Rockville, MD, USA). Serum samples were pre-incubated with biotin labeled and SULFO-TAG™ labeled IFN-β, and subsequently transferred to a microtiter plate coated with Streptavidin and incubated for 1hr at room temperature. After washing, the plates were stained with 2× Read buffer T and quantified using the MESO QuickPlex SQ120 imager. Samples were tested 1:10 diluted in the screening assay, and in case they were positive, further two-fold serial dilutions were made to determine the titer. Neutralizing antibodies against IFN-β were analyzed in the iLite IFN-β neutralizing antibody assay (SVAR, Malmö, Sweden), but only if binding antibodies were positive.

T-cell responses against IFN-β and AAV5 were tested using peripheral blood mononuclear cells (PBMCs), in Interferon-γ ELISpot assays (ImmunoSpot® S6 CORE, Shaker heights, OH, USA), using three peptide pools of overlapping 15-mer peptides of IFN-β and AAV5.

The plasma protein IFN-β concentrations were measured, using a human IFN-β serum ELISA assay with a lower limit of quantification of 2.3-18.8 pg/mL (VeriKine-HS™, PBL Assay science, Piscataway, NJ, USA).

VIRAL SHEDDING

Shedding of ART-I02 was measured using quantitative polymerase chain reaction (QPCR) in blood, saliva, urine and feces (QuantStudio 7 real-time PCR, applied Biosystems, Foster City, CA, USA). The Limit of detection was 15-67 copies/µg DNA in blood, 86 copies/mL in saliva and urine, and 15 copies/µg DNA in feces. For each bodily fluid of each patient the viral shedding was analyzed up to three consecutive negative samples. The synovial fluid and tissue would be analyzed for transduction of ART-I02 in case tissue samples were available.

STATISTICS

As this was an exploratory phase I-II study, there was no formal power calculation; outcomes are presented in a descriptive manner.

Results

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Figure 1 contains a flow diagram for the patients in the study. Four patients were included in the trial, their baseline characteristics are presented in Table 2. The three patients of cohort I received the starting dose, i.e. 0.6×10^{12} VG/PIP joint (patient 1) and 1.2×10^{12} VG/CMC joint (patients 2 and 3). Upon review of the safety data of cohort I by the investigator and the DRC, and approval by the ethics committee, cohort II was started with the same (low) dose. Thus, patient 4 received 0.6×10^{12} VG in the PIP joint. An additional DRC meeting was scheduled as the fourth patient developed injection site symptoms. The DRC advised against further study drug administrations and therefore no additional patients were enrolled. All four included participants have completed the clinical follow-up, and the follow-up phone calls up to 2 years post-dose.

SAFETY

During- and immediately after administration, one patient experienced injection site pain. This AE resolved immediately and spontaneously. At the visit 24hrs after injection, no AEs were observed. Three of the four patients (patients 2,3 and 4) developed injection site reactions 4 to 12 days after study drug administration. The symptoms included increased joint tenderness, diminished grip strength and swelling, and were clinically diagnosed as tenosynovitis. These symptoms lasted for 5 weeks to 4 months and had a fluctuating course (patient 2), or gradually decreased over time (patients 3 and 4). The symptoms were treated with over-the-counter analgesics and instructions to restrict movement with- or without orthoses. These AEs were assessed to be R-CTCAE grade 2 (moderate) in severity and considered possibly or probably related to the injection of ART-I02. None of the patients experienced treatment-emergent events apart from these local AEs. Although the injection site reaction related to the administration of ART-I02 persisted for 5 weeks to 4 months, it posed no major health problem nor chronic impairment. There were no abnormalities, in safety measures, or signs of infection. At the one-year follow-up, all patients reported their status to be similar (patients 2, 3 and 4) to the status at baseline or better (patient 1). No treatment emergent AEs were reported by the patients at the telephone follow-up.

FUNCTIONAL ASSESSMENTS

In patient 1, improvement compared to baseline, reflected by a calculated CCI score ≥5, was demonstrated at all visits except for the visit in weeks 4 and 8. The calculated CCI scores of the other three patients were mostly <5, indicating no improvement compared to baseline, throughout the study. The range of motion was not affected by the injection of ART-I02 (data not shown).

MRI

A full overview of the quantitative outcomes is given in Table 3. In the qualitative assessments, the synovitis and peri-arthritis of the target joint in patient 1 slightly improved after ART-I02 injection compared to baseline, this was not reflected in the quantitative scores: Synovitis remained stable and bone marrow edema and bone erosions remained absent from baseline throughout the study.

In the qualitative assessment of patient 2, peri-arthritis was reported at week 12, which had recovered by week 24. The synovitis scores remained unchanged during the study. New bone marrow lesions had developed in week 12, which had not fully recovered at 24-weeks. Subchondral bone defects remained stable.

The MRI of patient 3 showed increased synovitis (baseline grade 1, week 12 grade 3) and new bone marrow lesions in week 12 (baseline grade 0, week 12 grade 3). Neither had fully recovered at week 24. These outcomes were reflected in the qualitative assessment: peri-arthritis was present at week 12 and had slightly improved at week 24.

Patient 4 had an additional MRI in week 4, because of the observed AEs. The MRI after 4 weeks (Figure 2B), showed a strong increase of the synovitis (baseline grade 1, week 4 grade 3) and peri-arthritis (qualitative assessment) bone marrow lesions and erosive damage remained unchanged compared to baseline (grade 1). The synovitis had returned to the baseline situation at week 24 (Figure 2D).

IMMUNOLOGY AND SYSTEMIC IFN-β PROTEIN

A full overview of the immunology outcomes is given in Table 4. Before injection, serum sample titers for patients 1, 2, and 3 were negative (<1:100) for total binding antibodies against AAV5 as measured by ELISA, and low for patient 4 (1:193). For all four patients, titers raised after injection of ART-102 to >1:17,000. Neutralizing antibodies were analyzed at screening using an AAV5 inhibition of transduction assay to exclude patients with titers >1:15 to avoid inhibition of ART-102 transduction. The neutralizing antibody titers in the four patients enrolled in this study ranged between 1:1 to <1:8 before injection. The titers increased to >1:405 at week 4 after injection.

Local administration of ART-102 did not result in a detectable increase of IFN-β in the circulation. Plasma samples from all four patients collected before and after injection of ART-102 were negative, i.e. below the lower limit of detection of 2.3-18.8 pg/mL (Table 4).

Serum samples from all four patients collected before and after injection of ART-102 were negative for IFN-β binding antibodies. Therefore, IFN-β neutralizing antibody assays were not performed. T-cell responses against IFN-β and AAV5 did not show a change from baseline.

SHEDDING

Peak levels of ART-102 vector DNA in blood were observed at one day after injection and subsequently decreased. All blood samples were negative at four weeks after injection. A full overview is included in Table 4. Vector DNA was detected in saliva of three patients one day after injection, and all saliva samples were negative from one week after administration onwards. No vector DNA was detectable in urine or feces at any time. None of the patients opted for surgical intervention during the clinical follow-up period, hence no synovial fluid- or tissue was available for examination.

Discussion

In this phase I-II study, ART-102 (rAAV2/5-hIFN-β) was administered intra-articularly in four patients with an inflammatory hand joint mono-arthritis. No significant systemic abnormalities were observed and no serious adverse events occurred. Despite systemic safety, late-onset (4-10 days post-dose) injection site reactions manifested in three patients. Peri-arthritis, the inflammation of the tissues surrounding the joint including tendons (teno-synovitis) and subcutaneous tissue, was seen in three patients. None of the patients opted for a surgical intervention. Although the symptom state of the target joints has reverted to the baseline level, the duration of the symptoms at the injection site and the possible or probable relation to ART-102, precluded the enrollment of additional patients in the study.

The exact etiology of the observed AEs is currently unclear; drug might have leaked into the soft tissues after ultrasound guided administration and may have caused the periarticular reaction, but a direct association with the injection procedure seems unlikely because of the late onset of the AEs and their long duration. It seems to be more plausible that the experimental gene product was causative, although this cannot be fully proven with the data of the current study.

A cause of the observed events could be an immune response against the viral vector. We observed an increase in the AAV5 binding- and neutralizing antibodies at 4- and 24 weeks. The plasma T-cell responses against AAV5 did not show a relevant change over time, but a local response cannot be excluded. The observed pattern of immune responses was comparable among patients, regardless of the baseline titers of neutralizing antibodies and adverse events. The observed changes in the AAV5 antibodies were also identified in a non-human primate arthritis model of ART-102, in combination with a T-cell response against AAV5. These immune responses did not lead to local or systemic adverse events.[26] In a clinical study, in which an AAV2 vector encoding for tumor necrosis factor immunoglobulin Fc (rAAV2-TNFR:Fc) was injected IA in the (knee- ankle, wrist, MCP and elbow) joints of arthritis patients, administration site reactions occurred more commonly after administration of rAAV2-TNFR:Fc than after administration of placebo. These AEs were dose dependent, but no relation was found between the

AEs and pre-existing- or developing antibody titers against AAV2.[30] In total, 24 administration site reaction were observed after 191 administrations (12.6%), of which 4 (2.1%) were severe and the investigators chose to treat the patients with steroids in 3 cases (1.6%).[30] In two other clinical studies, the increased AAV-antibodies may have caused the observed transient alanine aminotransferase levels increase that were observed after intravenous administration. These signs of hepatocellular toxicity resolved upon administration of a tapering dose of prednisolone.[20,38,39] Finally, ART-102 dose is based on VG, but the amount of viral particles administered was higher (ratio 1:6.6). If future studies would prove AAV particles to be causative of adverse events, these might be prevented by improved separation of empty- and full particles in production, the development of optimized AAV vectors allowing for lower doses potentially in combination with interventions to reduce immune responses.

Another explanation for the locoregional adverse events, may be IA IFN-β expression. Although IFN-β was chosen for its favorable anti-inflammatory properties in arthritis, it also has pro-inflammatory effects, which may have manifested in this study.[40] Studies that investigated the effect of IFN-β in arthritic patients, reported an anti-inflammatory effect or no effect at all.[12,13] However, the different administration routes (subcutaneous vs. IA), formulation and the injection in an inflamed site, might have created an environment in which IFN-β has pro-inflammatory properties.[12,13]

As synovial samples from the injected joints were not obtained, a correlation between locoregional AEs and IFN-β expression or other local biochemical changes could not be assessed. Although we cannot be certain of the local IFN-β expression in this study, AAV5 induced transgene expression was previously confirmed as of 3 days after IA vector administration in pre-clinical non-human primate studies, without the occurrence of local AEs.[25,26] In these monkeys, local expression of IFN-β was confirmed, but did not result in elevated systemic IFN-β levels. Thus, the fact that elevated serum IFN-β levels, antibody responses, and T-cell responses against IFN-β were not observed in the patients in this study, does not preclude that IFN-β was expressed in the injected joints.

IA administration of AAV may be the preferred route to establish prolonged local exposure, while avoiding systemic exposure and toxicity. Although samples were lacking to measure the local levels of vector in synovium, the analysis of body fluids confirmed that the vector remained predominantly local. Vector DNA levels were within the limits of quantification only in blood and feces and solely 1 day after study drug administration. A similar pattern in blood was observed upon IA administration of rAAV2-TNFR:Fc in two other studies.[30,31] Systemic vector concentrations decreased below the limit of quantification between 4-8 weeks upon administration. These studies observed sustained presence of rAAV2-TNFR:Fc in synovium after IA administration up to 49 weeks after administration in a subset of patients. However, in none of the synovial fluid- or tissue samples, the TNFR protein nor mRNA specific to rAAV2-TNFR:Fc were detected.[30,31] Thus it may be argued that the efficiency of transduction was insufficient to result in detectable TNFR protein expression in the latter studies. This process could not be confirmed in our study either.

A limitation of this study was the small number of patients that was studied. Furthermore, interpretation of the results is hampered by the erratic course of IHA. We performed regular clinical assessments including MRIs of the target joint and blood sampling up to 24 weeks after study drug administration, as per protocol. It may be considered to further extend the observation period in similar studies, particularly because some of the patients mentioned subjective improvement of the injected joint at the telephone follow-up.

Further research in gene-and cell-therapy approaches is required to find an effective vector-based therapy for IHA. Two AAVs (AAV2 encoding TNFR:Fc and AAV5 expressing IFN-β) have now independently shown to cause (dose dependent) administration site reactions upon IA injection, which should be taken into account with further research in this field. One approach could be to combine IA injection of an AAV based vector with a short-acting anti-inflammatory compound.[41] This approach is successfully applied in AEs seen in systemic AAV therapy.[20,38,39] Its multifactorial aspect and hiatus in knowledge of OA pathophysiology complicates drug development. Cell-based therapy, based on TGF-β enhancement, has been investigated in phase III trials, but currently, the heterogeneity in cell preparation leads to concerns and a recommendation against their application.[42,43]

For the first time in humans, we administered RAAV2/5-HIFN-β IA in IHA patients. The vector remained predominantly local, systemic exposure and shedding were negligible. We report adverse reactions at the injection site of which the mechanism is currently not understood. The nature and duration of these reactions ask for further modifications and improvements to AAV based gene therapy approaches to explore its potential to treat inflamed joints in arthritis, while minimizing side effects.

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Table 1 Study design Shaded cells areas indicated which assessments were done at which time point in the study.

Study day	**-28 to -1**	**0**	**1**
Event Description	**Screening** - AAV5/IFN-β antibody analysis - In/exclusion criteria - Medical history - Baseline MRI	**ART-102 administration** - Ultrasound-guided injection of the target joint with ART-102 - Safety assessments	**First follow-up visit** Measurements for: - Safety (AEs, lab, vitals) - Shedding

Measurement **Study week**	**1**	**2**	**4**	**8**	**12**	**16**	**20**	**24**	**year 1-5**
Safety (AEs, vital signs, safety lab)	■	■	■	■	■	■	■	■	
IFN-β *protein*	■	■	■	■	■	■	■	■	
AAV5/IFN-β *Antibodies*			■					■	
T-cell response			■	■	■	■		■	
Shedding	■	■	■	■	■	■	■	■	
Functional assessment	■	■	■	■	■	■	■	■	
MRI					■			■	
Yearly questionnaire									■

Table 2 Patient demographic and baseline characteristics

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Sex	Female	Female	Female	Female
Age at enrollment (years)	51	59	58	65
Weight (kg)	68.7	85.6	63.3	56.3
BMI (kg/m²)	24.8	28.7	22.8	20.8
Diagnosis	RA	OA	OA	OA
Target joint	PIP III	CMC	CMC	PIP II
MRI synovitis score (0-3)	1	2	1	1
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian

BMI, body mass index; RA, Rheumatoid Arthritis; OA, Osteoarthritis; PIP, proximal interphalangeal joint; CMC, carpometacarpal joint; MRI, magnetic resonance imaging.

Table 3 Quantitative MRI outcomes Quantitative MRI scores of the target joints. Synovitis was graded 0 to 3, with 0 no synovitis and 3 extensive synovitis. Bone marrow edema (RA) or bone marrow lesions (OA) were graded 0 to 3, with affectedness in increments of 33%. Bone erosions were graded 0 to 10 for the RA patient, with increments of 1, with 0 no bone erosions, and 10 100% of the articular surface affected. Subchondral bone defects (CMC) or erosive damage (PIP) were graded 0-3 for the OA patients (0: none, 1: ≤25%, 2: 25-50%, 3: >50% of bone volume or joint surface affected).

	Subject 1			**Subject 2**			**Subject 3**			**Subject 4**			
	BL	w.12	w.24	BL	w.12	w.24	BL	w.12	w.24	BL	w.4	w.12	w.24
Synovitis (0-3)	1	1	1	2	2	2	1	3	2	1	3	3	1
Bone erosions (0-10)	0	0	0										
Subchondral bone defects/ erosive damage (0-3)				1	1	1	1	1	1	1	1	1	1
Bone marrow edema (0-3)	0	0	0										
Bone marrow lesions (0-3)				1	2	2	0	3	2	1	1	0	0

BL Baseline, w. week.

Table 4 Immuno-assay outcomes outcomes

	Patient	**pre-dose**	**d.1**	**w.1**	**w.2**	**w.4**	**w.8**	**w.12**	**w.16**	**w.24**
AAV5-bAb titer Method: ELISA LOQ: 100, ULOQ: 24300	**1**	<100	-	-	-	658	-	-	-	18235
	2	<100	-	-	-	19526	-	-	-	>24300
	3	<100	-	-	-	12181	-	-	-	>24300
	4	193	-	-	-	3718	-	-	-	17158
AAV5-nAb titer Method: ELISA LOQ: 1, ULOQ: 405	**1**	<1	-	-	-	>405	-	-	-	>405
	2	<1	-	-	-	>405	-	-	-	>405
	3	<1	-	-	-	>405	-	-	-	>405
	4w	8	-	-	-	>405	-	-	-	>405
IFN protein Method: ELISA LOQ 2.3-18.8 pg/mL	**1-4**	All samples were below the limit of quantification.								
IFN bAb Method: electrochemiluminescence, bridging assay format Screening cut-point: ≥ 1.4 relative electrochemiluminescence, and confirmatory cut-point: ≥13% displacement.	**1-4**	<	-	-	-	<	-	-	-	<

(Continuation Table 4)

	Patient	pre-dose	d.1	w.1	w.2	w.4	w.8	w.12	w.16	w.24
IFN nAb	**1-4**	As bAb were negative, IFN nAb were not measured.								
T-cell response to AAV5 Method: ELISpot (number of spots/0.3×106 PBMC for the 3 peptide pools)	**1**	19/35/62	-	-	-	19/46/65	38/48/70	44/48/80	28/49/69	15/70/42
	2	21/30/26	-	-	-	3/5/5	14/23/10	4/4/7	1/19/2	8/10/7
	3	6/7/17	-	-	-	3/2/4	14/21/29	8/26/34	6/40/3	8/18/16
	4	31/48/55	-	-	-	33/27/27	54/56/42	2/6/2	40/38/25	36/30/24
T-cell response to IFN-β Method: ELISpot (number of spots/0.3×106 PBMC for the 3 peptide pools)	**1**	27/16/26	-	-	-	62/39/38	76/38/41	60/36/57	51/27/30	50/25/29
	2	37/23/26	-	-	-	4/1/2	8/7/8	2/3/6	0/0/0	8/9/24
	3	5/2/10	-	-	-	1/1/0	10/9/7	7/13/7	7/2/8	3/15/1
	4	35/52/56	-	-	-	16/17/8	28/14/16	0/1/8	21/25/22	48/42/39
Shedding - blood (copies/µg DNA. Method: qPCR LOQ, 50 copies/µgDNA, LOD: 15 copies/µg DNA LOQ and LOD applicable when 400ng DNA were tested	**1**	< 15	9.9 × 101	< 15	< 15	< 15	-	-	-	-
	2	< 15	7.3 × 102	< 15	< 15	< 15	-	-	-	-
	3	< 15	1.9 × 102	< 67	< 67	< 15	< 15	< 15	-	-
	4	< 15	1.2 × 102	< 50	< 15	< 15	< 15	-	-	-
Shedding - saliva (copies/ml) Method: qPCR LOQ, 290 copies/ml, LOD: 86 copies/ml	**1**	<	< LOQ	<	<	<	-	-	-	-
	2	<	< LOQ	<	<	<	-	-	-	-
	3	<	<	<	<	<	-	-	-	-
	4	<	7.8 × 103	<	<	<	-	-	-	-
Shedding - feces and urine Method: qPCR **Feces:** LOQ: 50 copies/µg DNA, LOD: 15 copies/µg DNA, **Urine:** LOQ, 290 copies/ml, LOD: 86 copies/ml	**1-4**	All samples were below the limit of detection.								

d, day; w, week; bAb, binding antibody; nAb, neutralizing antibody; LOQ, Lower limit of quantification; LOD, lower limit of detection; ULOQ, upper limit of quantification; ELISA, enzyme-linked immune sorbent assay; ELISPOT, Enzyme-linked immune absorbent spot; <, below lower limit of detection, or quantification whichever is the lowest indicated; <LOQ, below lower limit of quantification and higher than lower limit of detection; -, not measured, as planned per protocol.

Figure 1 CONSORT-based flow diagram for the enrollment, follow-up and analysis of patients

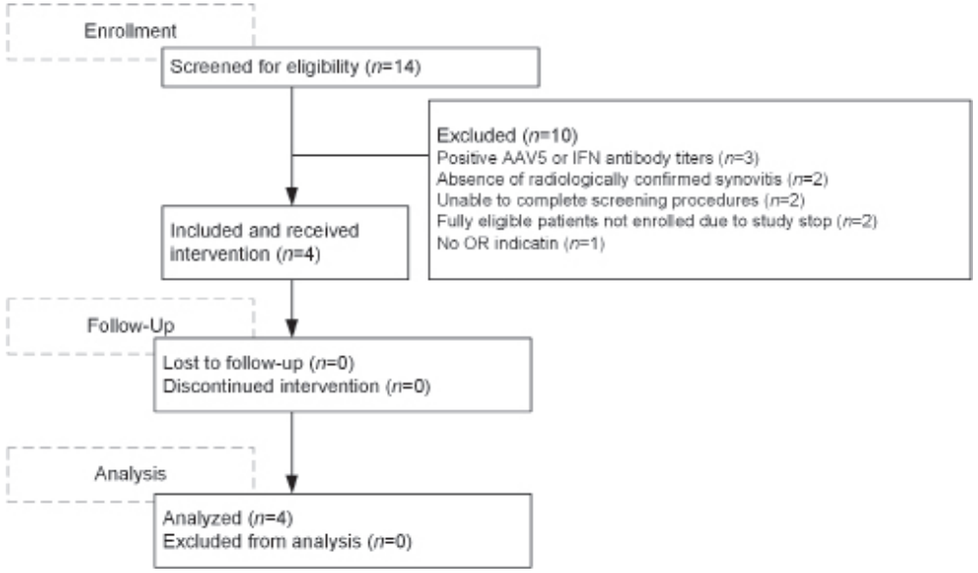

Figure 2 MRIs of patient 4 MRI of the target joint, the proximal interphalangeal joint of the second digit (PIP dig. II) of the left hand, of patient 4, at four different time points during the study. T1-weighted, fat suppressed, Gadolinium-enhanced images, in corresponding coronal- and axial planes shown vertically. Panel A. Baseline scan, showing definite osteoarthritis of PIP II, with minimal synovitis (circle) and an enhancing synovial cyst in the ulnar base of the middle phalanx. The synovitis and cysts are also reflected in the axial pane (bottom pane). Panel B. Scan at 4 weeks after study drug administration, showing substantial increase of swelling of the finger with synovitis in the PIP II joint and peri-arthritis with increased swelling around the extensor tendon and of the soft tissues along the proximal interphalangeal phalanx to the second metacarpophalangeal (MCP II) joint (arrows). The extensive peri-arthritis is also well recognized in the axial plane (bottom pane, arrow). Panel C. Scan at 12 weeks after study drug administration: minimally remaining synovitis, reduction of the peri-arthritis. Panel D. Scan at 24 weeks after study drug administration, image similar compared to baseline (Vitamin capsule as marker in situ).