Safety, Tolerability, and Clinical Outcomes after Intraarticular Injection of a Recombinant Adeno-associated Vector Containing a Tumor Necrosis Factor Antagonist Gene: Results of a Phase 1/2 Study

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ABSTRACT. ***Objective.*** To assess safety and clinical outcomes in patients with inflammatory arthritis after intraarticular (IA) injection of rAAV2-TNFR:Fc, a recombinant adeno-associated viral vector containing the human tumor necrosis factor (TNF) receptor-immunoglobulin (IgG1) Fc fusion (TNFR:Fc) gene.

Methods. In this phase 1/2 randomized study, adults with persistent moderate or severe inflammation in a target joint, being treated with or without systemic anti-TNF therapy, received a single IA injection of either rAAV2-TNFR:Fc (1×10^{11}, 1×10^{12}, or 1×10^{13} DNase-resistant particles/ml joint volume) or placebo, followed by open-label rAAV2-TNFR:Fc 12–30 weeks later, depending on when the target joint met predetermined criteria for reinjection.

Results. 127 subjects received the first injection of blinded study drug; 95 subjects received open-label rAAV2-TNFR:Fc. Administration site reactions, consisting of transient mild to moderate increases in tenderness and swelling of the injected joint, occurred after 23/191 (12%) rAAV2-TNFR:Fc injections and were dose-dependent. Rates of other adverse events were not dose-dependent. Notable serious adverse events (SAE) included culture-negative septic arthritis in a subject receiving leflunomide and fatal disseminated histoplasmosis considered unrelated to rAAV2-TNFR:Fc in a subject receiving adalimumab. In the phase 2 portion of the study, a 30% decrease in target joint global visual analog scale was observed in 21/50 (42%) rAAV2-TNFR:Fc subjects and 3/16 (19%) placebo subjects 12 weeks after first injection ($p = 0.14$).

Conclusion. IA rAAV2-TNFR:Fc resulted in administration site reactions after 12% of injections. A fatal SAE, disseminated histoplasmosis, was considered not related to study agent. Patient-reported outcome measures of clinical response showed greater improvement in treated patients than placebo patients. (First Release Dec 23 2009; J Rheumatol 2010;37:692–703; doi:10.3899/jrheum.090817)

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Treatment of inflammatory arthritides such as rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS) has undergone remarkable improvements with the advent of biologic therapies[1,2]. With the continued success of these new therapies, the goal of treatment has shifted to achieving tighter disease control to gain the result of low disease activity or remission, as well as to reduce structural damage[3]. While debate has arisen as to the optimal timing and sequencing of these therapies to achieve this goal[4,5], there is also increased recognition of the potential side effects of these potent therapies, particularly infection[6-8].

Local treatment of affected joints with tumor necrosis factor (TNF) antagonists may provide a means to supplement the partial benefit achieved with systemic TNF antagonists, or to provide local TNF antagonism to patients not receiving systemic TNF antagonists while minimizing systemic side effects. Small studies of intraarticular (IA) administration of etanercept[9-13] and infliximab[14,15] have been conducted, with variable success.

An alternative to IA therapeutic proteins is the injection of DNA coding for therapeutic protein. This process, called gene transfer, offers the advantage of potentially providing sustained concentrations of the therapeutic protein within the joint, thus reducing the need for frequent IA injections. Use of a vector allows for uptake of the DNA by cells within the joint, which are then able to produce the therapeutic protein. A recombinant adeno-associated virus (rAAV) serotype 2-based vector containing the human TNF-immunoglobulin Fc fusion gene (rAAV2-TNFR:Fc or tgAAC94) was developed for clinical use. rAAV2-TNFR:Fc is based on AAV, a naturally occurring, nonpathogenic, nonintegrating, and nonreplicating virus that depends on a helper virus for replication[16,17]. The dose of rAAV2-TNFR:Fc is represented in units of DNase-resistant particles (DRP), as a measure of particles containing the TNFR:Fc fusion gene. A study conducted in a rat model of streptococcal cell wall-induced experimental arthritis demonstrated that a single dose of ~1 $\times$ 10^{12} DRP/ml of joint volume AAV2-ratTNFR:Fc administered by IA injection resulted in suppression of arthritis in the injected joint as well as the contralateral joint[18].

A phase 1 study of rAAV2-TNFR:Fc recently demonstrated the safety and tolerability of rAAV2-TNFR:Fc at doses up to 1 $\times$ 10^{11} DRP/ml joint volume in patients with inflammatory arthritis not receiving systemic TNF antagonists[19]. This phase 1/2 study was undertaken to determine the safety and tolerability of repeat injections of higher doses of rAAV2-TNFR:Fc in subjects being treated with or without systemic TNF antagonists, and to determine the clinical effect on the injected joint.

MATERIALS AND METHODS

Study design. In this double-blind, placebo-controlled phase 1/2 study, subjects (dosing goal ≥ 120) were enrolled in 6 cohorts of ~20 subjects each and randomized in a 3:1 ratio of rAAV2-TNFR:Fc to placebo. In the phase 1 dose-escalation portion, 3 cohorts received escalating dose concentrations of rAAV2-TNFR:Fc, ranging from 1 $\times$ 10^{11} to 1 $\times$ 10^{13} DRP/ml or matching placebo, followed by open-label rAAV2-TNFR:Fc at the same concentrations after 12 to 30 weeks, depending on when swelling in the target joint met criteria for reinjection. In the phase 2 expansion, 3 additional cohorts received the same 3 concentrations of rAAV2:TNFR:Fc or placebo in the same schedule, but were enrolled simultaneously. The volume injected depended on the joint, and was determined based on clinical experience with IA steroid in patients with inflammatory arthritis. Knees were injected with 5 ml, ankles 2 ml, elbows 1.5 ml, wrists 1 ml and metacarpophalangeals with 0.5 ml.

Study population. The study was conducted in compliance with the Helsinki Declaration. Study subjects were recruited from 21 rheumatology practices in the United States. The study was approved by the institutional review board and institutional biosafety committee at each site. Written informed consent was obtained prior to conduct of any study procedures. The study was overseen by an independent Data Monitoring Committee.

Entry criteria included a confirmed diagnosis of an inflammatory arthritis with peripheral joint involvement (RA, PsA, or AS) according to published criteria[20-22] and persistent moderate (grade 2) or severe (grade 3) joint swelling in at least 1 peripheral joint eligible for injection. Other entry criteria included age > 18 years, and for subjects with RA, failure or inadequate response to at least 1 disease-modifying antirheumatic drug (DMARD), which could include a biologic DMARD, prior to screening. Subjects taking DMARD were required to be on the same regimen for the previous 3 months, with no changes in dose in the 4 weeks prior to screening. Exclusion criteria included disease severe enough to warrant a change in the regimen for inflammatory arthritis in the next 3 months and discontinuation of etanercept in the past because of safety concerns.

Study agent. The active study agent was rAAV2-TNFR:Fc (tgAAC94), a recombinant AAV serotype 2 vector genetically engineered to contain the cDNA for the TNFR:Fc fusion gene, which consists of the cDNA of the human TNF extracellular domain fused in-frame to the cDNA of the human immunoglobulin (IgG1) Fc domain. The DNA sequence of TNFR:Fc in rAAV2-TNFR:Fc is identical to that used in the production of etanercept. rAAV2-TNFR:Fc was produced under current good manufacturing practice (cGMP) guidelines at Targeted Genetics Corporation, Seattle, WA. rAAV2-TNFR:Fc was formulated in a sterile isotonic buffered salt solution, which also served as placebo. rAAV2-TNFR:Fc and placebo were vialed at Molecular Medicine, San Diego, CA, under cGMP.

Study procedures. After completion of screening procedures, qualified subjects were randomized to receive a single IA injection of placebo or rAAV2-TNFR:Fc at the dose level for the cohort. Subjects were evaluated prior to and 0.5, 1, 4, 8, 12, 18, 24, and 30 weeks after the first dose or until second injection. If the swelling in the target joint was at baseline or worse at a study visit on or after 12 weeks, the subject was scheduled for a dose of open-label rAAV2-TNFR:Fc within 2 weeks. If the subject did not meet the criteria for reinjection by 30 weeks after initial dosing, an injection of open-label rAAV2-TNFR:Fc was given at 30 weeks. Subjects were evaluated prior to and 0.5, 1, 4, 8, 12, 18, 24, and 30 weeks after the second dose. Safety was evaluated by serial medical history and physical examination, complete blood count with white blood cell differential, blood chemistries, and urinalysis.

Gene transfer. The presence of rAAV2-TNFR:Fc genomic DNA in peripheral blood, synovial fluid, and tissues was assessed using a qualified TaqMan-based DNA-polymerase chain reaction (PCR) assay with TNFR:Fc-specific primers and probes. Since no systemic biodistribution was observed in the 1 $\times$ 10^{11} DRP/ml dose level in a previous study[19], systemic biodistribution of rAAV2-TNFR:Fc was assessed in peripheral blood collected prior to and 1, 4, 8, and 12 weeks after first dosing in the first 10

subjects enrolled in the second (1×10^{12} DRP/ml or placebo) and third (1×10^{13} DRP/ml or placebo) cohorts (Targeted Genetics Corporation; limit of detection 15 copies/μg DNA). Local distribution of rAAV2-TNFR:Fc into synovial fluid was assessed in subjects who had joint effusions prior to and 4 and 12 weeks after dosing and at unscheduled visits (Althea Technologies, San Diego, CA; limit of detection 22 copies/20 μl synovial fluid). Local distribution of rAAV2-TNFR:Fc into tissue samples was also assessed (Althea Technologies; limit of detection 22 copies/μg DNA).

Gene expression. Presence of circulating TNFR:Fc expressed from rAAV2-TNFR:Fc in serum and synovial fluid was assessed by measuring TNF binding activity using a quantitative radioimmunoassay (Biomonitor A/S, Copenhagen, Denmark). The amount of functional TNF-α antagonist activity was quantified using an etanercept (TNFR:Fc) standard curve for subjects not receiving TNF antagonists (limit of detection 0.012 μg/ml), and the corresponding standard curve for subjects receiving TNF antagonists (etanercept standard curve, 0.15 to 2.0 ng/ml; adalimimab standard curve, 2.5 to 20 ng/ml; infliximab standard curve, 1.5 to 15 ng/ml). In addition, the Human sTNF RII Immunoassay Kit (R&D Systems, Minneapolis, MN, USA) was adapted for quantitation of TNFR:Fc in serum and synovial fluid. An etanercept standard curve (dynamic range 0.0625–4.0 ng/ml) replaced the sTNF RII standard supplied in the kit. This assay allowed quantitation of TNFR:Fc without interference from adalimumab or infliximab.

Presence of rAAV2-TNFR:Fc RNA in tissues was assessed using a qualified one-step RT-PCR assay using oligonucleotide primers and probes designed to detect and amplicon-specific to the expression of rAAV2-TNFR:Fc (Althea Technologies; limit of detection 10 pg template RNA).

Immune response to AAV2 capsid. Humoral immune response to AAV2 capsid was assessed by measuring preexisting and induced anti-AAV2 capsid neutralizing titers (NT) in serum prior to dosing and 4, 12, 24, and 30 weeks after each dose using a microtiter neutralization assay that determined the serial dilution at which expression of AAV2-green fluorescence protein was inhibited.

Cellular immune response to AAV2 capsid was measured prior to and 4 and 12 weeks after the initial dose in subjects in the first 3 cohorts using a modified interferon-γ (IFN-γ) enzyme-linked immunosorbent spot (ELISPOT) assay (Cellular Technologies, Ltd., Shaker Heights, OH, USA), designed to detect the number of T cells releasing IFN-γ from frozen peripheral blood mononuclear cells after exposure to 4 synthetic peptide pools (Genemed Synthesis Inc., San Antonio, TX, USA), consisting of 10 mers overlapping by 7 amino acids each spanning the entire AAV2 capsid sequence.

Clinical outcomes. Clinical response was evaluated by examination of the target joint for tenderness and swelling separately on a scale ranging from 0 (none) to 3 (severe), based on guidelines published in the *Dictionary of Rheumatic Diseases*[23]. Although subjects were randomized within rather than between cohorts in the dose-escalation portion of the study, the placebo subjects were combined and compared with active recipients in the analysis. Subjects in the phase 2 expansion, who were randomized simultaneously across cohort, were compared by treatment assignment.

Additional evaluations piloted in the phase 2 expansion were (1) patient assessment of target joint, consisting of three 10-cm visual analog scales (VAS) addressing global symptoms, function, and satisfaction with response to study drug injection; and (2) a functional assessment of the target joint, using the Disabilities of the Arm, Shoulder and Hand scale[24,25] for subjects whose target joint was in the upper extremity, and a modification of the Rheumatoid Arthritis Outcome Score[26] for subjects whose target joint was in the lower extremity.

Prior to analysis of data from the phase 2 expansion, outcome measures considered to be most clinically relevant were prospectively selected as follows: (1) the proportion of subjects with a 30% improvement in target joint global VAS; (2) the proportion of subjects with a 30% improvement in target joint function VAS; and (3) the proportion of subjects with a 2-point decrease in target joint pain, a question included in the functional assessment questionnaires for both upper and lower extremities.

Systemic efficacy measures included tender and swollen joint counts (28 joints), patient's self assessment of pain (10 cm VAS), patient and physician global assessment of disease activity (10 cm VAS), the Health Assessment Questionnaire[27], erythrocyte sedimentation rate, and C-reactive protein. In addition, subjects with AS completed the Bath Ankylosing Spondylitis Functional Index and the Bath Ankylosing Spondylitis Disease Activity Index[28,29]. For subjects with RA or PsA, clinical response was determined using the American College of Rheumatology-20 response criteria[30]. Disease activity was assessed using the modified Disease Activity Score[31,32].

RESULTS

Study enrollment and disposition. A total of 142 subjects were screened for the study. Thirteen subjects did not qualify, and 2 withdrew consent prior to randomization. A total of 127 subjects were randomized and received the first dose of blinded study agent (rAAV2-TNFR:Fc or placebo), 61 in the 3 dose-escalation cohorts, and 66 in the phase 2 expansion cohorts. There were no statistically significant differences between treatment groups, except more elbow target joints were enrolled in the high-dose group in the dose escalation cohorts (Table 1), reflecting a protocol amendment implemented after the second dose escalation cohort was enrolled, which allowed enrollment of subjects with the elbow as a target joint.

In July 2007, after the study was fully enrolled and 74 subjects had received a second dose of study agent (open-label rAAV2-TNFR:Fc), the study was put on clinical hold because of an unexpected serious adverse event (SAE) described below. The protocol was amended to rescreen subjects prior to administering the second dose of study agent and to provide for additional safety monitoring. The study was resumed, and 21 of 37 subjects eligible for a second injection at the time of the clinical hold received a second dose of study agent 2 to 7 months later than indicated in the protocol. The remaining 16 subjects were withdrawn early from the study, in most cases (n = 12) because they withdrew consent.

Safety. The rates of adverse events were similar among treatment groups after first and second injections of study drug (Table 2). Some categories of adverse events (gastrointestinal disorders, nervous system disorders) were more common after receipt of rAAV2-TNFR:Fc, but did not appear to be dose-dependent. The rate of infections was similar across all treatment groups. Six serious infections were reported, 3 after administration of rAAV2-TNFR:Fc 1×10^{11} DRP/ml, 2 after 1×10^{12} DRP/ml, and 1 after 1×10^{13} DRP/ml. All 6 of these subjects were taking systemic DMARD; 3 of 6 were receiving a TNF antagonist. The rate of laboratory abnormalities was similar across all treatment groups.

Administration site reactions, consisting of transient increases in injected joint tenderness and swelling, sometimes accompanied by warmth, redness, or itching that occurred within 2 weeks of study agent administration, were more common after receipt of rAAV2-TNFR:Fc (Table 2). The rate of administration site reactions was dose-depend-

Table 1. Demographics and baseline characteristics of study subjects.

	Dose Escalation				Phase 2 Expansion			
	Placebo, N = 15	1×10^{11}, N = 16	1×10^{12}, N = 15	1×10^{13}, N = 15	Placebo, N = 16	1×10^{11}, N = 17	1×10^{12}, N = 17	1×10^{13}, N = 16
Female, n (%)	12 (80)	13 (81)	12 (80)	9 (60)	13 (81)	13 (76)	12 (71)	13 (81)
Age, yrs, mean ± SD	54.2 ± 14.3	52.4 ± 12.5	51.5 ± 12.8	55.2 ± 12.4	56.6 ± 13.7	52.4 ± 14.3	51.6 ± 13.7	52.7 ± 13.7
Caucasian, n (%)	14 (93)	15 (94)	14 (93)	12 (80)	13 (81)	14 (82)	15 (88)	13 (81)
Type of arthritis, n (%)								
Rheumatoid arthritis	13 (87)	13 (81)	9 (60)	11 (73)	12 (75)	15 (88)	13 (76)	15 (94)
Psoriatic spondylitis	2 (13)	2 (13)	4 (27)	4 (27)	3 (19)	1 (6)	4 (24)	1 (6)
Ankylosing spondylitis	0	1 (6)	2 (13)	0	1 (6)	1 (6)	0	0
Target joint, n (%)								
Knee	7 (47)	9 (56)	8 (53)	6 (40)	6 (38)	4 (24)	7 (41)	3 (19)
Ankle	2 (13)	3 (19)	4 (27)	1 (7)	3 (19)	2 (12)	4 (24)	5 (31)
Wrist	5 (33)	2 (13)	2 (13)	2 (13)	5 (31)	4 (24)	3 (18)	6 (38)
Metacarpophalangeal	1 (7)	2 (13)	1 (7)	2 (13)	2 (13)	4 (24)	2 (12)	1 (6)
Elbow	0	0	0	4 (27)	0	3 (18)	1 (6)	1 (6)
Currently taking TNF-α antagonist, n (%)	9 (60)	8 (50)	10 (67)	7 (47)	8 (50)	12 (71)	7 (41)	10 (63)
Etanercept/infliximab/adalimumab	6/1/2	4/1/3	6/2/2	4/1/2	5/0/3	7/1/4	3/2/2	4/3/3
Currently taking methotrexate, n (%)	12 (80)	13 (81)	8 (53)	12 (80)	13 (81)	10 (59)	12 (71)	11 (69)
Currently taking prednisone, n (%)	5 (33)	11 (69)	5 (33)	6 (40)	5 (31)	4 (24)	5 (29)	4 (25)
Disease status, mean ± SD								
Swollen joints (28 count)	4.2 ± 4.2	9.4 ± 7.5	7.4 ± 7.9	5.6 ± 7.5	7.9 ± 6.4	5.7 ± 4.9	5.9 ± 7.3	5.3 ± 6.1
Tender joints (28 count)	5.8 ± 5.8	9.7 ± 6.9	5.8 ± 6.3	3.8 ± 3.6	8.9 ± 8.2	7.4 ± 6.8	6.6 ± 8.7	5.1 ± 6.1
Patient global assessment (cm)	4.3 ± 2.3	6.0 ± 1.4	4.9 ± 2.0	4.8 ± 2.1	5.1 ± 2.0	4.6 ± 2.5	5.9 ± 2.7	4.9 ± 2.6
Patient assessment of pain (cm)	3.7 ± 2.3	5.5 ± 2.1	4.5 ± 2.3	4.3 ± 2.2	5.1 ± 2.4	4.0 ± 2.5	5.7 ± 2.8	4.7 ± 2.7
Health assessment questionnaire	1.0 ± 0.5	1.4 ± 0.7	1.3 ± 0.5	0.9 ± 0.5	1.1 ± 0.7	1.2 ± 0.6	1.2 ± 0.7	1.0 ± 0.7
Physician global assessment (cm)	3.7 ± 1.5	4.5 ± 1.7	3.8 ± 1.9	3.5 ± 1.9	4.0 ± 1.9	3.6 ± 2.2	5.0 ± 1.8	3.7 ± 1.7
Erythrocyte sedimentation rate, mm/h	26 ± 18	18 ± 14	20 ± 11	22 ± 13	34 ± 36	24 ± 21	31 ± 29	30 ± 29
Disease Activity Score	3.8 ± 1.1	4.2 ± 1.4	4.0 ± 1.3	3.6 ± 0.9	4.3 ± 1.6	4.0 ± 1.3	3.9 ± 1.3	3.7 ± 1.3

Table 2. Incidence of adverse events after first and second injections of study drug.

	1st Injection — Blinded Study Drug (Placebo or rAAV2–TNFR:Fc)					2nd Injection — Open-Label rAAV2-TNFR:Fc			
	Placebo, N = 31	1×10^{11}, N = 33	1×10^{12}, N = 32	1×10^{13}, N = 31	All Active, N = 96	1×10^{11}, N = 32	1×10^{12}, N = 29	1×10^{13}, N = 34	All Active, N = 95
Any adverse event	23 (74)	28 (85)	24 (75)	25 (81)	77 (80)	27 (84)	26 (90)	29 (85)	82 (86)
Gastrointestinal disorders	1 (3)	9 (27)	4 (13)	4 (13)	17 (18)	13 (41)	6 (21)	5 (15)	24 (25)
General and administration site disorders	4 (13)	8 (24)	6 (19)	8 (26)	22 (23)	3 (9)	9 (31)	11 (32)	23 (24)
Infections and infestations	13 (42)	15 (45)	12 (38)	7 (23)	34 (35)	13 (41)	11 (38)	15 (44)	39 (41)
Injury, poisoning, procedural complications	3 (10)	3 (9)	6 (19)	5 (16)	14 (15)	6 (19)	4 (14)	4 (12)	14 (15)
Investigations	1 (3)	2 (6)	3 (9)	3 (10)	8 (8)	2 (6)	3 (10)	1 (3)	6 (0)
Metabolism and nutrition disorders	2 (6)	6 (18)	1 (3)	0	7 (7)	2 (6)	1 (3)	0	3 (3)
Musculoskeletal and connective tissue disorders	13 (42)	14 (42)	12 (38)	9 (29)	35 (36)	15 (47)	12 (41)	14 (41)	41 (43)
Nervous system disorders	1 (3)	6 (18)	3 (9)	3 (10)	12 (13)	5 (16)	2 (7)	5 (15)	12 (13)
Respiratory, thoracic, mediastinal disorders	7 (23)	4 (12)	4 (13)	0	8 (8)	4 (13)	9 (31)	1 (3)	14 (15)
Skin and subcutaneous tissue disorders	2 (6)	4 (12)	3 (9)	1 (3)	8 (8)	5 (16)	2 (7)	2 (6)	9 (9)
Serious adverse event	1 (3)	2 (6)	1 (3)	1 (3)	4 (4)	2 (6)	3 (10)	1 (3)	6 (6)
Serious infections*	0	1 (3)	1 (3)	0	2 (2)	2 (6)	1 (3)	1 (3)	4 (4)
Administration site reaction	1 (3)	3 (9)	0	7 (23)	10 (10)	1 (3)	6 (21)	6 (18)	13 (14)

* Serious infection is an infection that was also considered a serious adverse event because it required hospitalization or resulted in death.

ent, occurring after 1/31 (3%), 4/65 (6%), 6/61 (10%), and 13/65 (20%) injections of placebo, 1×10^{11} DRP/ml, 1×10^{12} DRP/ml, and 1×10^{13} DRP/ml, respectively. The rate of administration site reactions was similar among subjects receiving TNF antagonists and those who were not. Most administration site reactions were mild to moderate and were treated with analgesics and/or ice, but 3 of 24 were severe enough that the investigator chose to treat the subject with IA steroids or oral prednisone. The geometric mean anti-AAV2 antibody titer prior to injection was 1/35 (range

< 1/4 to 1/4096) in 7 mild administration site reactions; 1/414 (range < 1/4 to 1/32,768) in 13 moderate administration site reactions; and 1/256 (range < 1/4 to 1/4096) in 4 severe administration site reactions. The anti-AAV2 antibody titers of the 3 subjects who were treated with oral or IA steroids were 1/512, 1/2048, and 1/4096 prior to injection, respectively.

Ten subjects experienced 12 SAE (Table 3). One SAE, culture-negative septic arthritis, was considered probably related to study agent. A 56-year-old woman with RA receiving leflunomide developed acute left knee pain and swelling approximately 15 weeks after injection of rAAV2-TNFR:Fc 5×10^{12} DRP. Synovial fluid revealed 135,000 white blood cells/μl and negative crystal examination. Synovial fluid and blood cultures were negative. The joint improved promptly with open irrigation and debridement and intravenous antibiotics. Since she experienced a grade 3 or greater adverse event probably related to study agent, she was not eligible for the second dose of study agent, and was withdrawn from the study.

Two SAE, fatal disseminated histoplasmosis and retroperitoneal hematoma, occurred in a 36-year-old woman with RA receiving adalimumab, methotrexate (MTX), and prednisone. The subject developed a febrile illness contemporaneously with her second injection of rAAV2-TNFR:Fc 5×10^{13} DRP into the right knee, 18 weeks after her first injection. She developed progressive fever, nausea, and abdominal pain associated with an elevated white blood cell count and liver enzymes, prompting hospitalization 10 days into her illness. Five days later, an acute retroperitoneal bleed caused marked hypotension and respiratory distress, requiring inotropic support and mechanical ventilation. Continued retroperitoneal bleeding resulted in an abdominal compartment syndrome. She developed multiorgan failure and died 23 days after first becoming ill. Blood cultures drawn on the day of death subsequently grew *Histoplasma capsulatum*.

Autopsy revealed disseminated histoplasmosis and a large retroperitoneal hematoma. *Histoplasma* organisms were identified in the liver, lung, kidney, bone marrow, spleen, kidney, lymph nodes, and brain, but not in the injected knee, and no granulomas were noted. The large retroperitoneal hematoma encased the left kidney and displaced the abdominal contents to the right and the diaphragm upward. No anatomical source of bleeding was identified. Of note, the patient lived in an area endemic for histoplasmosis. Her chest radiograph was normal prior to her hospitalization.

To evaluate the potential contribution of IA rAAV2-TNFR:Fc to the level of systemic TNF antagonism from adalimumab, the subject's archived serum samples were tested for the amount of functional TNF-α antagonist using an adalimumab standard curve. The amount of TNF-α binding activity ranged from 5.4 to 8.6 μg/ml during the study, within the range expected for someone receiving adalimumab and MTX, and dropped as expected after those medications were discontinued. Serum levels of TNFR:Fc as determined using the ELISA that detects TNFR:Fc and does not cross-react with adalimumab were not higher than the expected endogenous levels (data not shown).

Table 3. Serious adverse events.

Dose Level, DRP/ml	Age, yrs, Sex	Type of Arthritis	Arthritis Medications	Target Joint	Most Recent Injection	Days Since Last Dose	Symptoms/Diagnosis	Toxicity Grade	Relationship to Study Agent
Placebo	77 F	RA	ADA, MTX	R MCP2	First	14	Degenerative joint disease requiring L knee replacement	Severe	Not related
1×10^{11}	57 M	RA	AZA	R elbow	First	34	Infected incision after repair of traumatic ankle fracture	Severe	Not related
	64 F	AS	LEF, PRED	R wrist	First	97	Abdominal pain from constipation	Severe	Unlikely
					Second	7	Cellulitis and ulcer leg calf	Severe	Unlikely
	61 F	RA	ETAN, MTX, PRED	L knee	Second	90	Acute pyelonephritis	Severe	Unlikely
1×10^{12}	56 F	RA	LEF	L knee	First	102	Septic arthritis of injected joint	Severe	Probable
	33 F	RA	HCQ, MTX, PRED	R MCP2	Second	58	Myocardial infarction	Life-threatening	Unlikely
	67 F	PsA	ETAN, MTX	L ankle	Second	30	Massive pulmonary emboli	Fatal	Unlikely
	75 M	RA	ETAN, MTX, PRED	L wrist	Second	163	Pneumonia	Severe	Unlikely
1×10^{13}	64 M	RA	MTX, PRED	R wrist	First	70	Syncope from coronary artery disease	Moderate	Not related
	36 F	RA	ADA, MTX, PRED	R knee	Second	1 15	Disseminated histoplasmosis Retroperitoneal hemorrhage	Fatal	Unlikely

ADA: adalimumab; AS: ankylosing spondylitis; AZA: azathioprine; DRP: DNase-resistant particles; ETAN: etanercept; HCQ: hydroxychloroquine; LEF: leflunomide; MCP: metacarpophalangeal; MTX: methotrexate; PRED: prednisone; PsA: psoriatic arthritis.

Extraarticular tissues including blood, liver, lung, spleen, adrenal, tonsil, lymph node, porta hepatis lymph node, heart, small bowel, costal muscle, trachea, bladder, kidney, brain, and noninjected knee obtained at autopsy 22 days after administration of rAAV2-TNFR:Fc had either no detectable vector DNA or very low levels (< 22–29 copies/μg DNA).

Systemic biodistribution. rAAV2-TNFR:Fc DNA was not detected in peripheral blood after administration of placebo and 1×10^{11} DRP/ml, but was detected at very low levels after administration of 1×10^{12} DRP/ml and 1×10^{13} DRP/ml in 11/24 (46%) and 14/22 (61%) subjects, respectively. rAAV2-TNFR:Fc DNA was quantifiable in 8/23 (35%) subjects 7 days after administration of 1×10^{13} DRP/ml at levels ranging from 35 to 150 copies/μg DNA, and became detectable but not quantifiable by 4 to 8 weeks after administration and undetectable 12 to 18 weeks after administration.

Local administration of rAAV2-TNFR:Fc into joints did not result in systemic circulation of TNFR:Fc protein. TNF binding activity was not detected in the serum of 17 subjects not taking systemic TNF antagonists tested after administration of the highest dose of rAAV2-TNFR:Fc, 1×10^{13} DRP/ml. Serum TNF binding activity and TNFR:Fc levels were not higher than expected in subjects receiving etanercept, adalimumab, and infliximab (data not shown).

Local gene transfer and expression. Gene transfer was assessed in either synovial fluid or synovial tissue of 10 subjects who received rAAV2-TNFR:Fc at doses of 1×10^{12} or 1×10^{13} DRP/ml (Table 4). rAAV2-TNFR:Fc DNA was detected in the synovial fluid of 5 of 6 subjects at 8 of 9 timepoints 0.1 to 13.7 weeks after administration of 1×10^{13} DRP/ml. Synovial fluid DNA levels were highest immediately after administration of rAAV2-TNFR:Fc and gradually decreased with time. rAAV2-TNFR:Fc DNA was detected in synovial tissue in 3 subjects who underwent wrist synovectomy, knee replacement, or knee sampling at autopsy 3.3 to 48.6 weeks after administration of 1×10^{13} DRP/ml, but not in 1 subject who underwent knee replacement 19.7 weeks after administration of a lower dose, 1×10^{12} DRP/ml.

TNF binding activity was not detected in the synovial fluid samples tested from 9 subjects not receiving TNF antagonists who received 1×10^{11} DRP/ml (n = 3), 1×10^{12} DRP/ml (n = 4), or 1×10^{13} DRP/ml (n = 2) at 14 timepoints 2 to 30 weeks after dosing. TNF binding activity was detected in the synovial fluid of 21/21 subjects receiving systemic TNF antagonists at 31 timepoints before and after dosing with rAAV2-TNFR:Fc. mRNA specific to rAAV2-TNFR:Fc was not detected in 3 samples of synovial tissue obtained at synovectomy, joint replacement, or autopsy, despite the presence of rAAV2-TNFR:Fc DNA (Table 4).

Immune response to AAV2 capsid. Subjects exposed to rAAV2-TNFR:FC experienced a dose-dependent humoral response to AAV2 capsid. Prior to administration of the first dose of rAAV2-TNFR:Fc, anti-AAV2 capsid-neutralizing titers ranged from < 1/4 to 1/8192 (geometric mean 1/33), with 45% of subjects having titers ≤ 1/4. After administration of rAAV2-TNFR:Fc, anti-AAV2-neutralizing titers rose in a dose-dependent fashion (Figure 1). As expected, anti-AAV2-neutralizing titers did not rise after receipt of placebo. Immediately prior to administration of open-label rAAV2-TNFR:Fc, anti-AAV2 capsid-neutralizing titers ranged from < 1/4 to 1/1024 (geometric mean 1/21) for placebo recipients and from < 1/4 to 1/32,768 (geometric

Table 4. Local gene transfer and expression.

Dose	Publication ID	Systemic TNF Antagonist	Sample Type	Sample Source	Most Recent Injection	Time Since Injection, weeks	DNA-PCR, copies/μg DNA	mRNA by RT-PCR, copies/μg	Anti-TNF Binding Activity, μg/ml
1×10^{12}	P01	None	Synovium, injected knee	Total knee replacement	Second	19.7	< 22	Neg	< 0.010
1×10^{13}	P02	ETAN	Synovial fluid, knee	Joint aspiration	First	14.1	NT	NT	0.75
			Synovial fluid, knee	Joint aspiration	Second	0.1	> 2,200,000	NT	NT
	P03	ETAN	Synovial fluid, knee	Joint aspiration	Second*	1.4	14,348	NT	NT
			Synovial fluid, knee	Joint aspiration	Second*	12.0	68	NT	NT
	P04	None	Synovial fluid, knee	Joint aspiration	First	2.0	11,989	NT	< 0.010
	P05	ETAN	Synovial fluid, knee	Joint aspiration	First	13.7	30	NT	4.6
			Synovial fluid, knee	Joint aspiration	Second	4.0	109	NT	1.8
			Synovial fluid, knee	Joint aspiration	Second	12.1	54	NT	2.6
	P06	None	Synovial fluid, knee	Joint aspiration	Second*	4.4	2,578	NT	< 0.012
	P07	ADA	Synovial fluid, knee	Joint aspiration	First	13.0	< 22	NT	256
	P08	ADA	Articular scraping, knee	Autopsy	Second	3.3	454,000	Neg	NT
			Synovium and tendon, knee	Autopsy	Second	3.3	36,100	Neg	NT
	P09	None	Synovium, wrist	Synovectomy	First	21.1	422,185	Neg	NT
	P10	ADA	Synovium, injected knee	Total knee replacement	Second*	48.6	1721	Neg	NT
			Synovium, contralateral knee	Total knee replacement	Second*	48.6	27	Neg	NT

* First injection of study drug was placebo; second injection was rAAV2-TNFR: Fc. NT: not tested; ETAN: etanercept; ADA: adalimumab.

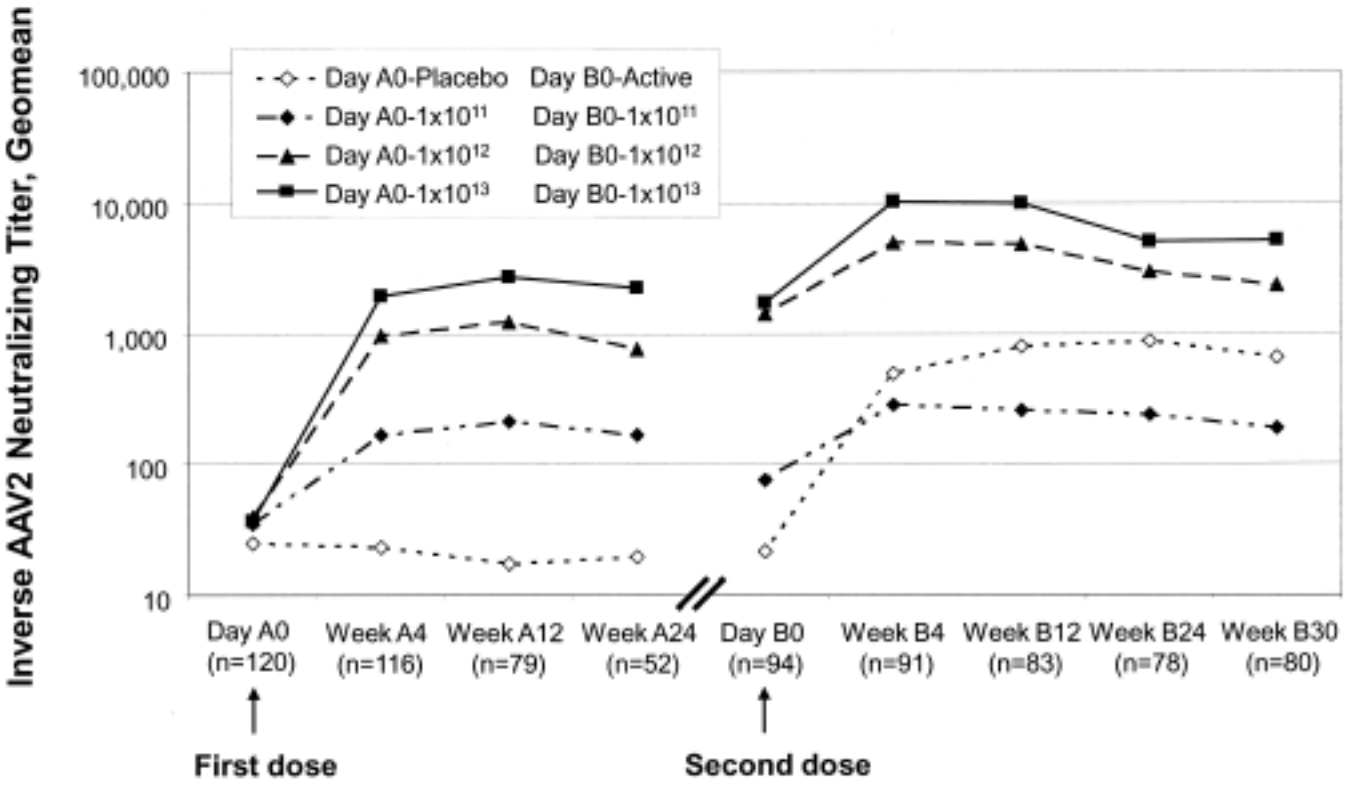

Figure 1. Change in inverse geometric mean anti-AAV2 capsid-neutralizing titer after injection of blinded study drug (placebo or rAAV2-TNFR:Fc) at Day A0 followed open-label rAAV2-TNFR:Fc at Day B0. Open-label rAAV2-TNFR:Fc was administered 12 to 30 weeks after injection of blinded study drug, depending on when the target joint met criteria for reinjection.

mean 1/591) among rAAV2-TNFR:Fc recipients, reflecting administration of blinded study drug. After administration of open-label rAAV2-TNFR:Fc, anti-AAV2 capsid-neutralizing titers rose again, particularly for subjects who received placebo first injection. No adverse effects were associated with the development of anti-AAV2 capsid-neutralizing titers.

Subjects exposed to rAAV2-TNFR:Fc exhibited minimal cellular response to AAV2 capsid. Preexisting IFN-γ CD8+ T cells reactive to AAV2 capsid peptides were detected in 2/56 (3.6%) subjects, ranging from 79 to 127 mean spot-forming units/1×10^6 cells. IFN-γ-secreting CD8+ T cells were induced in 0/14 (0%), 0/16 (0%), 3/13 (23%), and 2/13 (15%) subjects after administration of placebo and rAAV2-TNFR:Fc at doses of 1×10^{11}, 1×10^{12}, and 1×10^{13} DRP/ml joint volume, respectively. Responses were to 1 to 3 peptide pools, and were low in magnitude, ranging from 40 to 80 mean SFU/1×10^6 cells above the cutoff after administration of study drug. Neither of the 2 subjects with preexisting IFN-γ CD8+ T cells exhibited a response to AAV2 capsid peptides after receipt of placebo (n = 1) or rAAV2-TNFR:Fc at a dose of 1×10^{13} DRP/ml (n = 1). There was no apparent association with adverse events, preexisting or development of anti-AAV2-neutralizing titers, or lack of response, but the number of subjects evaluated was small.

Clinical outcomes. Local response after the first injection of study drug as determined by physical examination is summarized in Figures 2 and 3. In the dose-escalation cohorts (Figure 2), a 2-point or greater decrease in target joint swelling was observed in 13% to 27% of subjects who received rAAV2-TNFR:Fc, compared to none of placebo recipients. This trend was not observed in the phase 2 cohorts (Figure 3), where a 2-point or greater decrease in target joint swelling was observed in 12% to 19% of rAAV2-TNFR:Fc recipients and 19% of placebo recipients. A 2-point or greater decrease in target joint tenderness was observed in similar proportions of active and placebo subjects in both the dose escalation cohorts and the phase 2 expansion (Figures 2 and 3).

In the phase 2 cohorts, local response as determined by patient-reported outcomes is summarized in Figure 4. A 30% or greater improvement in target joint global VAS was observed in 21/50 (42%) rAAV2-TNFR:Fc subjects and 3/16 (19%) placebo subjects 12 weeks after first injection ($p = 0.14$). A 30% or greater improvement in target joint function VAS was observed in 16/50 (32%) rAAV2-TNFR:Fc subjects and 3/16 (19%) placebo subjects 12 weeks after first injection ($p = 0.36$). A 2-point decrease in target joint pain was observed in 6/50 (12%) rAAV2-TNFR:Fc subjects and 1/16 (6%) placebo subjects 12 weeks after first injection ($p = 0.67$). Response rates were similar among subjects taking TNF antagonists compared to those who were not.

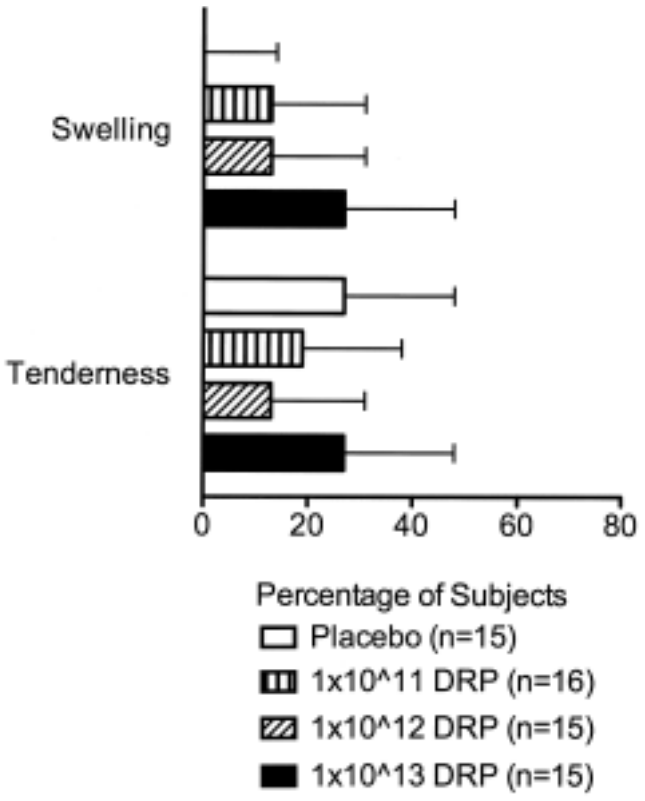

Figure 2. Percentage of subjects with 2-point or greater decrease in target joint swelling or 2-point or greater decrease in target joint tenderness 12 weeks after first injection by dose group in the dose escalation cohorts. Error bars represent upper limits of 95% confidence interval. DRP: DNase-resistant particles.

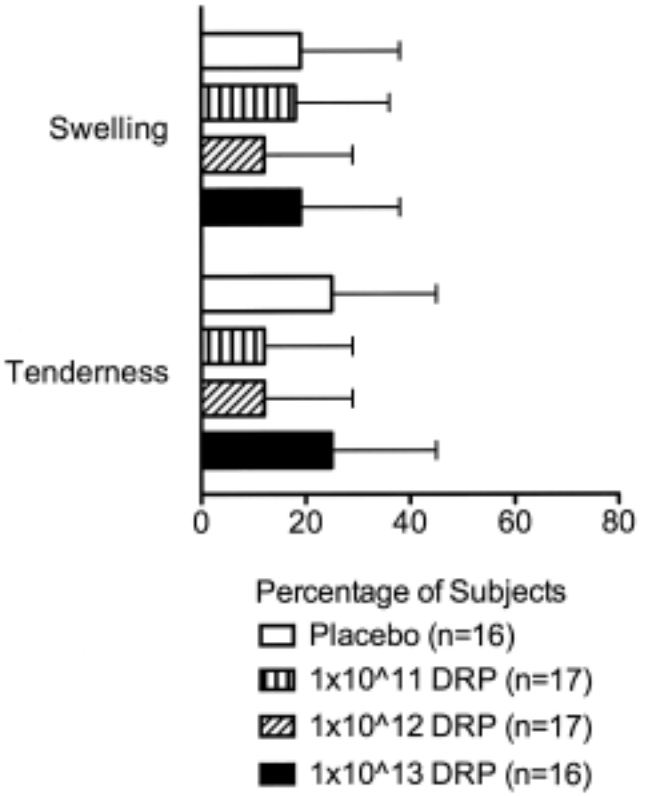

Figure 3. Percentage of subjects with 2-point or greater decrease in target joint swelling or 2-point or greater decrease in target joint tenderness 12 weeks after first injection by dose group in the phase 2 expansion cohorts. Error bars represent upper limits of 95% confidence interval. DRP: DNase-resistant particles.

Modest improvements in systemic measures of inflammatory arthritis were noted in subjects in all treatment groups in both the dose escalation cohorts and phase 2 cohorts (Table 5) and were not related to the dose of rAAV2-TNFR:Fc. Responses were observed among subjects currently taking TNF antagonists as well as those who were not.

The responses to a second injection of rAAV2-TNFR:Fc among subjects with grade 2 or greater swelling of the target joint just prior to the second injection are summarized in Table 6. Responses to the 2 physical examination measures and the 3 patient-reported outcomes were reported in all dose groups and in subjects with elevated anti-AAV2 capsid-neutralizing titers from their first injection of rAAV2-TNFR:Fc.

DISCUSSION

In this study, IA administration of rAAV2-TNFR:Fc appeared to be generally well tolerated. Administration site reactions, consisting of transient increases in injected joint tenderness and swelling, sometimes accompanied by warmth, redness, or itching, occurred in dose-dependent frequency in up to 20% of subjects, but were self-limited. No other dose-dependent adverse events were noted.

One subject experienced culture-negative septic arthritis of the injected knee 15 weeks after administration of rAAV2-TNFR:Fc at a dose of 1×10^{12} DRP/ml. The investigator and medical monitor considered the event probably related to rAAV2-TNFR:Fc, since expression of TNFR:Fc protein in the joint could lead to increased risk of infection. Since patients with RA are at increased risk for infection as a result of the disease and its treatment[7], further study is required to determine if IA administration of rAAV2-TNFR:Fc is associated with a further increase in the risk of septic arthritis.

A second subject died from disseminated histoplasmosis and retroperitoneal hematoma while on study. Although the etiology and relationship to study drug were initially uncertain, the events were ultimately determined to be unrelated to the study agent rAAV2-TNFR:Fc[33,34]. The clinical course experienced by this subject was consistent with progressive disseminated histoplasmosis[35-37], an infection for which she was at risk given her residence in an area endemic for *Histoplasma* and systemic immunosuppression with adalimumab, MTX, and prednisone[38,39]. The large retroperitoneal hematoma, an unusual manifestation of disseminated histoplasmosis, may have been the result of an undetected mycotic aneurysm or bleeding from coagulopathy, which

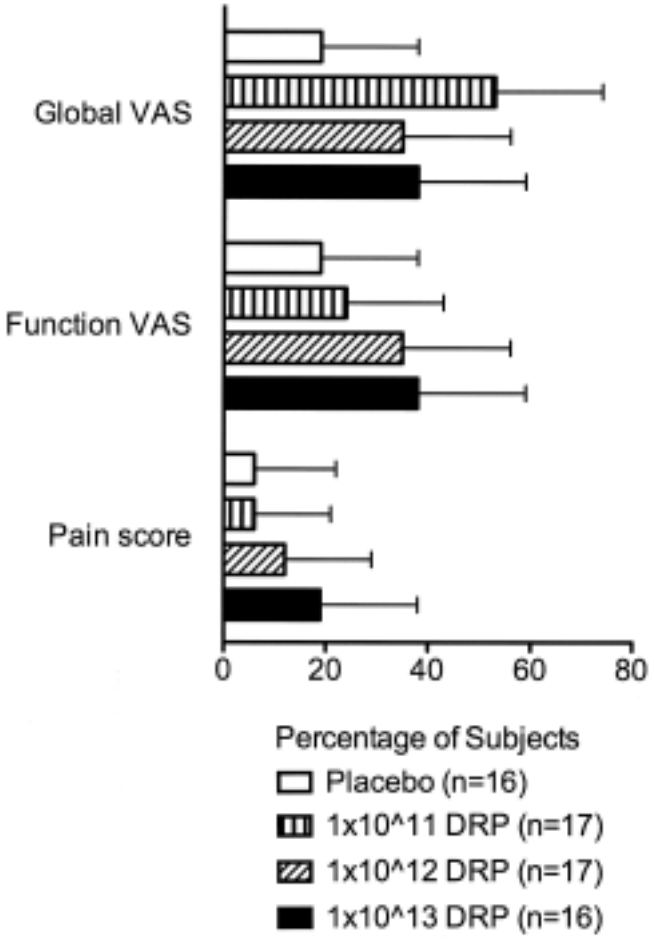

Figure 4. Percentage of subjects with 30% or greater improvement in target joint global visual analog scale (VAS), 30% or greater improvement in target joint function VAS, or 2-point or greater decrease in target joint pain 12 weeks after first injection by dose group in the phase 2 expansion cohorts. Error bars represent upper limits of 95% confidence interval. DRP: DNase-resistant particles.

has been described in cases of disseminated histoplamosis[36,40-42]. There was no evidence that her level of systemic immunosuppression was increased by IA administration of rAAV2-TNFR:Fc. In this subject and in others, systemic biodistribution of rAAV2-TNFR:Fc DNA after IA delivery was extremely low and transient. Further, TNFR:Fc protein was not detected in the serum of subjects not taking systemic TNF antagonists and anti-TNF activity was not increased over expected values in subjects who were taking systemic TNF antagonists, including the subject who died, after IA delivery of rAAV2-TNFR:Fc.

Local gene expression, as determined by a radioimmunoassay for TNFR:Fc protein in synovial fluid and an RT-PCR assay for mRNA specific to rAAV2-TNFR:Fc in synovial tissue, was not detected in the very limited number of samples that became available during the study. DNA-PCR verified the presence of rAAV2-TNFR:Fc genomic DNA in both synovial fluid and synovial tissue after administration of the highest dose of rAAV2-TNFR:Fc. At timepoints less than 2 weeks, expression may not have been detected because the single-stranded DNA in the vector had not yet been converted to double-stranded DNA, the first step in expression of AAV vectors, which is usually rate-limiting[16]. Alternatively, it is possible that peak expression was missed, since a well controlled tissue harvest was not conducted, or that the types of cells transduced are not capable of robust transgene expression[43]. Of note, TNFR:Fc mRNA was detected by quantitative PCR assay in joints for up to 1 year after IA administration of rAAV2-TNFR:Fc in animal studies conducted in support of this study (data not shown).

Humoral responses to AAV2 capsid were noted in all subjects who received rAAV2-TNFR:Fc, as expected. Only a small percentage of subjects developed low level of IFN-γ CD8+ T cells reactive to AAV2 capsid peptides. No adverse effects, including elevated liver enzymes, were associated with the development of T cell responses, unlike the report of 1 subject in a study of delivery of a higher dose of an AAV2 vector directly into the hepatic artery[44,45].

Validated measures to assess clinical response of a single joint to local therapies are in development[46,47]. In this study, patient-reported outcome measures of clinical response yielded greater differentiation between study agent and placebo than physical examination. Improvement in injected-joint patient-reported outcomes (target joint global VAS, target joint function VAS, target joint pain) was observed in the second phase of this study, but the improvement in target joint swelling observed in the first phase of the study was not reproduced in the second. Improvement in injected-joint patient-reported outcomes was also observed after a second injection of rAAV2-TNFR:Fc, despite the predictable elevation of anti-AAV2 capsid-neutralizing titers from the first injection of rAAV2-TNFR:Fc.

Gene transfer of therapeutic proteins has been developed as a potential therapeutic approach to treatment of inflammatory arthritis since the 1990s[48,49]. Local administration of a gene transfer agent offers the potential for targeted intervention to problematic joints while minimizing systemic side effects. The data from this phase 1/2 study demonstrates that gene transfer is generally safe and feasible and that further evaluation of gene transfer agents for the treatment of inflammatory arthritis is a viable option. In parallel with developing validated single-joint outcome measures, future studies should focus on analysis and improvement of transgene expression.

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Table 5. Clinical outcomes after first injection.

	Dose Escalation				Phase 2 Expansion			
	Placebo, N = 15	1×10^{11}, N = 16	1×10^{12}, N = 15	1×10^{13}, N = 15	Placebo, N = 16	1×10^{11}, N = 17	1×10^{12}, N = 17	1×10^{13}, N = 16
AAV2-neutralizing titers prior to 1st injection, geometric mean	1/15	1/41	1/20	1/58	1/40	1/29	1/74	1/23
Change in target joint assessments 12 weeks after 1st injection								
2-point or greater decrease in target joint swelling, n (%)	0	2 (13)	2 (13)	4 (27)	3 (19)	3 (18)	2 (12)	3 (19)
2-point or greater decrease in target joint tenderness, n (%)	4 (27)	3 (19)	2 (13)	4 (27)	4 (25)	2 (12)	2 (12)	4 (25)
Change in target joint patient-reported outcomes 12 weeks after 1st injection								
30% or greater improvement in target joint global VAS, n (%)	NT	NT	NT	NT	3 (19)	9 (53)	6 (35)	6 (38)
30% or greater improvement in target joint function VAS, n (%)	NT	NT	NT	NT	3 (19)	4 (24)	6 (35)	6 (38)
2-point or greater decrease in target joint pain, n (%)	NT	NT	NT	NT	1 (6)	1 (6)	2 (12)	3 (19)
Change in systemic efficacy measures 12 weeks after 1st injection								
Swollen joints (28 count), mean ± SD	1.4 ± 5.2	0.6 ± 3.5	–1.6 ± 4.6	–0.8 ± 1.3	–2.2 ± 3.3	–3.1 ± 3.8	–2.7 ± 5.3	–3.0 ± 4.5
Tender joints (28 count), mean ± SD	–2.3 ± 5.8	0.0 ± 4.0	–0.5 ± 6.8	–0.2 ± 2.7	–1.5 ± 7.1	–2.5 ± 5.3	–2.9 ± 5.0	–2.6 ± 6.0
Patient global assessment (cm), mean + SD	–0.5 ± 3.6	–1.1 ± 1.8	–0.7 ± 1.9	–2.9 ± 1.1	–0.3 ± 1.6	–1.3 ± 1.5	–2.5 ± 2.0	–0.9 ± 3.4
Patient assessment of pain (cm), mean ± SD	–0.0 ± 3.8	0.5 ± 1.7	–0.6 ± 2.7	–2.7 ± 1.3	0.1 ± 1.9	–0.8 ± 1.4	–2.4 ± 2.3	–1.2 ± 2.6
Health assessment questionnaire, mean ± SD	–0.1 ± 0.4	0.0 ± 0.2	–0.3 ± 0.3	–0.2 ± 0.4	–0.0 ± 0.2	–0.2 ± 0.3	–0.2 ± 0.4	–0.1 ± 0.3
Physician global assessment (cm), mean ± SD	–1.4 ± 1.4	–1.0 ± 1.4	–0.9 ± 2.1	–2.4 ± 0.8	–0.7 ± 2.1	–0.8 ± 1.2	–2.6 ± 1.9	–0.5 ± 1.4
Erythrocyte sedimentation rate (mm/h), mean ± SD	–14 ± 17	2 ± 7	–2 ± 5	–8 ± 13	–7 ± 9	9 ± 19	–1 ± 8	–3 ± 38
Disease Activity Score, mean ± SD	–0.7 ± 0.9	–0.2 ± 1.2	–0.4 ± 1.0	–0.7 ± 0.8	–0.5 ± 0.8	–0.4 ± 0.9	–0.7 ± 1.2	–0.4 ± 1.1

* Target joint qualified for 2nd injection if swelling the same or worse than prior to 1st injection. NT: not tested.

Table 6. Clinical outcomes after second injection among subjects with moderate or greater target joint swelling at the time of second injection who received 2 doses of rAAV2-TNFR:Fc.

	Dose Escalation			Phase 2 Expansion		
	1×10^{11}, N = 8	1×10^{12}, N = 8	1×10^{13}, N = 6	1×10^{11}, N = 11	1×10^{12}, N = 11	1×10^{13}, N = 11
AAV2-neutralizing titers prior to 2nd injection, geometric mean	1/45	1/861	1/8192	1/56	1/2181	1/903
Change in target joint assessment 12 weeks after 2nd injection						
2-point or greater decrease in target joint swelling, n (%)	0	0	0	0	1 (9)	2 (18)
2-point or greater decrease in target joint tenderness, n (%)	1 (13)	0	1 (17)	4 (36)	3 (27)	3 (27)
Change in target joint patient-reported outcomes 12 weeks after 2nd injection						
30% or greater improvement in target joint global VAS, n (%)	NT	NT	NT	2 (18)	6 (55)	6 (55)
30% or greater improvement in target joint function VAS, n (%)	NT	NT	NT	2 (18)	6 (55)	5 (45)
2-point or greater decrease in target joint pain, n (%)	NT	NT	NT	0	2 (18)	2 (18)

NT: not tested.

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