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A Multicenter, Single-Blind, Phase IIa Clinical Trial to Evaluate the Efficacy and Safety of a Cell-Mediated Gene Therapy in Degenerative Knee Arthritis Patients

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Abstract

Osteoarthritis leads to articular cartilage wear, and newer therapies are aimed at slowing this degeneration. Growth factors and cytokines influence cartilage formation, and researchers are studying their use on cartilage regeneration in osteoarthritis. One method uses genetically engineered cells to deliver growth factors to damaged cartilage. This technique utilizes transforming growth factor-β proteins in modified chondrocytes to stimulate cartilage growth via an intra-articular injection. We evaluated the efficacy and outcomes of this injection on patients who had International Cartilage Repair Society grade 4 knee osteoarthritis. We evaluated 27 patients (6 men, 21 women) who had late-stage knee osteoarthritis. Patients were randomized to receive genetically engineered chondrocytes doses of 6×10^6 cells (group 1) or 1.8×10^7 cells (group 2) at a 1:1 ratio. Primary endpoints were subjective and functional evaluations, assessed by the International Knee Documentation Committee (IKDC) score. Secondary endpoints were pain severity and physical function, using the Western Ontario and McMaster osteoarthritis (WOMAC) index and the 100 mm visual analog scale (VAS). Patients were followed at 2, 4, 12, and 24 weeks postinjection. Both groups had significant improvements in outcomes. Scores improved at 12 and 24 weeks from baseline in IKDC ($+10$ and $+14$ points in group 1; $+11$ and $+13$ points in group 2), WOMAC (-12 and -13 points in group 1; -10 and -12 points in group 2), and VAS (-19 and -24 points in group 1; -20 and -20 in group 2) scores. Additionally, there were no serious adverse events, and no significant difference in adverse event incidence between the groups. Both groups expressed a mean improvement in pain, function, and physical ability following treatment injection. This modality appears to be a promising treatment for cartilage degeneration. However, further larger, multicenter, randomized studies are needed to truly evaluate the efficacy of this novel approach.

Introduction

Osteoarthritis of the knee is a debilitating condition that leads to pain, loss of function, and reduced mobility, which ultimately may require a total knee arthroplasty to restore functional ability.[1] The treatment of younger patients proves to be exceptionally challenging, as they are more likely to outlive the limited lifetime of their prostheses, which leads orthopedic surgeons or other practitioners to search for longer-lasting treatment options.[2] Fortunately, osteoarthritis is a slowly developing disease, which provides a larger window of opportunity to potentially alter its course before arthroplasty is needed.[3] Novel techniques, such as cartilage regeneration, are becoming increasingly compelling as less invasive measures to control, and potentially reverse, this disease, especially as the incidence of osteoarthritis is postulated to rise significantly in the coming decades.[2,4]

Articular cartilage destruction is well known to be central to the osteoarthritic process. However, despite over one million procedures involving articular cartilage damage performed in the United States each year,[5,6] restoration of the joint articular surface continues to pose difficulties. Studies have targeted several modulators and biochemical factors that influence the growth of cartilage. These factors include bone morphogenic proteins, which are effective stimulators of bone formation, and transforming growth factor-β

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(TGF-β) proteins, which stimulate cell growth and extracellular matrix formation.[7] Specifically, TGF-β1 is thought to stimulate proteoglycan synthesis, growth of articular chondrocytes, and tissue regeneration.[4,8] Additionally, it has been postulated to have immunosuppressive and anti-inflammatory properties.[9] Other growth factors, such as epidermal growth factor, insulin-like growth factor I, and basic fibroblast growth factor, stimulate chondrogenesis, but they have not been shown to have an effect on cartilage defects.[3,10,11] Additionally, administration of these factors has been problematic as the concentration, rate of release, and mode of delivery have been difficult to determine.[6] Researchers have attempted to engineer delivery of these through liposomes, dissolved in media, and in genetically modified human chondrocyte cells, with promising results shown in animals.[10–12] However, little has been done to evaluate the effect of these growth factors on humans.

The use of genetically modified chondrocytes is a novel technique that aims to successfully stimulate cartilage regeneration by incorporating cell-mediated gene therapy. One method involves the use of allogeneic human chondrocytes transduced with a retroviral vector engineered to express TGF-β1 mixed with normal allogeneic human chondrocytes. The nontransduced chondrocytes are included as supplementary cells to fill the cartilage defect and also as additional targets for the TGF-β1 expressed from the transduced cells. This method presents an opportunity to deliver cartilage regeneration in a minimally invasive manner.[4,13]

Following the success of a phase I trial,[4] the purpose of this clinical trial was to demonstrate the efficacy and safety of a 3:1 mixture of nontransduced allogeneic human chondrocytes and allogeneic human chondrocytes transduced to express TGF-β1 (GEC-TGF- β1), in patients with osteoarthritis of the knee. Specifically, we compared the effect of varied doses of GEC-TGF-β1 on (1) objective functional improvements; (2) subjective functional improvements; (3) levels of pain; (4) improvement in activity levels; (5) changes in stiffness and range of motion; and (6) the incidence of adverse events (AEs) in our study patients.

Methods

See Supplementary Methods, available online at www.liebertpub.com/humc

Results

International Knee Documentation Committee evaluation

In both groups, knee symptoms, function evaluation, and ability to carry out activities significantly improved at 12 and 24 weeks' follow-up (Fig. 1). In group 1, the mean International Knee Documentation Committee (IKDC) score (converted to 100-point-based scores) was 45 points at baseline, 55 points at week 12, and 59 points at week 24, and the mean change from baseline at weeks 12 and 24 was significant ($+10$ and $+14$ points, respectively; $p<0.001$) (Table 1).

In group 2, the mean score was 49 points before commencing treatment, 60 points at week 12, and 62 points at week 24. The mean improvements in scores from baseline at weeks 12 and 24 were significant ($+11$ and $+13$ points, respectively; $p<0.0006$). However, there was no significant difference in improvement from baseline at 12 and 24 weeks between groups 1 and 2 ($p>0.05$) (Table 1).

Table 1. International Knee Documentation Committee Subjective Evaluation

IKDC	*Group 1 (n = 13), mean*	*Group 2 (n = 14), mean*	p-*Value between the groups*
Baseline	45	49	0.2803*
Week 12	55	60	
Week 24	59	62	
Change 1	10	11	0.7935*
p-Value (in group)	**0.0006****	**0.0006****	
Change 2	14	13	0.8572*
p-Value (in group)	**0.0009****	**0.0003****	

Change 1, week 12–baseline; change 2, week 24–baseline; IKDC, International Knee Documentation Committee.
Bold numerals indicate statistically significant values.
*Unpaired *t*-test; **paired *t*-test.

Western Ontario and McMaster osteoarthritis questionnaire evaluation

Following injection of the GEC-TGF-β1, both groups experienced an improvement in severity of pain and physical function. In group 1, the mean total Western Ontario and McMaster osteoarthritis (WOMAC) scores changed significantly from 32 points before treatment, to 20 points at week 12 and 18 points at week 24 (-12 and -13 points, respectively; $p<0.0002$) (Table 2). In group 2, the mean total WOMAC scores changed from 30 points before treatment, to 20 points at week 12 and 18 points at week 24. The mean change in scores at weeks 12 and 24 was -10 and -12 points, respectively ($p<0.0001$). However, the change in results from baseline at 12 and 24 weeks was not significantly different between both groups ($p>0.05$) (Table 2). The subscores of severity of pain and physical function improved significantly in groups 1 and 2 ($p<0.05$). Stiffness improved significantly in group 2 (-1 point at weeks 12 and 24, respectively; $p<0.0032$), but no significant change was found in group 1 at 12 and 24 weeks ($p>0.05$).

100 mm visual analog scale score evaluation

We can conclude from our results that both groups experienced a significant improvement in pain, expressed with visual analog scale (VAS) scores, but there was no significant difference in results between the two groups ($p>0.05$). In group 1, the mean VAS scores were 52 points before treatment, 33 points at week 12, and 27 points at week 24 post-treatment. The mean change from baseline at weeks 12 and 24 was -19 and -24 points, respectively ($p<0.0004$) (Table 3). In group 2, the mean VAS scores were 48 points before treatment, 28 points at week 12, and 28 points at week 24, with a mean change of -20 points at weeks 12 and 24, respectively ($p=0.0008$ and 0.0005, respectively) (Table 3).

Adverse events

All the major AEs were associated with the injection site and were classified as either mild or moderate, and all cases resolved without treatment or medication. The highest incidence

Table 2. Western Ontario and McMaster Osteoarthritis Questionnaire Responses Evaluation

WOMAC	*Group 1 (n = 13), mean*	*Group 2 (n = 14), mean*	p-*Value between the groups*
Total score			
Baseline	32	30	0.6783*
Week 12	20	20	
Week 24	18	18	
Change 1	−12	−10	0.5403*
p-Value in the group	**<0.0001****	**<0.0001****	
Change 2	−13	−12	0.6918*
p-Value in the group	**0.0002****	**<0.0001****	
Severity of pain			
Baseline	6	6	0.9773*
Week 12	4	4	
Week 24	3	4	
Change 1	−3	−3	0.8379*
p-Value in the group	**<0.0001****	**<0.0001****	
Change 2	−3	−3	0.5818*
p-Value in the group	**0.0002****	**0.0002****	
Stiffness			
Baseline	2	2	0.8859*
Week 12	2	1	
Week 24	1	1	
Change 1	−1	−1	0.5653*
p-Value in the group	0.0692**	**0.0011****	
Change 2	−1	−1	0.6234*
p-Value in the group	0.0646**	**0.0032****	
Physical function			
Baseline	23	22	0.5830*
Week 12	15	15	
Week 24	14	13	
Change 1	−8	−7	0.4408*
p-Value in the group	**<0.0001****	**0.0016****	
Change 2	−10	−9	0.6690*
p-Value in the group	**0.0004****	**<0.0001****	

Change 1, week 12-baseline; change 2, week 24–baseline; WOMAC, Western Ontario and McMaster osteoarthritis.
Bold numerals indicate statistically significant values.
*Unpaired *t*-test; **paired *t*-test.

of AEs in both groups was administration-site conditions, which comprised injection-site joint swelling, joint pain, and peripheral edema, and musculoskeletal and connective tissue disorders, which included joint stiffness, arthralgias, and musculoskeletal discomfort. The high-dose treatment group had a higher incidence of AEs, which may be attributed to a dose-dependent increase in TGF-β. However, these differences were not significant for either AEs or adverse drug reactions (ADRs) and are unlikely to be clinically relevant. Additionally, there were no serious AEs noted, and no subject was withdrawn from the study secondary to an AE (Tables 4 and 5).

Table 3. 100 mm Visual Analog Scale Evaluation

100 mm VAS	*Group 1 (n = 13), mean*	*Group 2 (n = 14), mean*	p-*Value between the Groups*
Baseline	52	48	0.5225*
Week 12	33	28	
Week 24	27	28	
Change 1	−19	−20	0.8637*
p-Value in the group	0.0002**	0.0008**	
Change 2	−24	−20	0.4891*
p-Value in the group	0.0004**	0.0005**	

Change 1, week 12–baseline; change 2, week 24–baseline; VAS, visual analog scale.
*Unpaired *t*-test; **paired *t*-test.

TGF-β1 and vector DNA PCR results

In both groups, the change in TGF-β1 (ELISA) values between baseline and week 24 was within the clinically normal range, and all patients had negative vector DNA PCR results both before and after treatment.

Laboratory results

There were no significant changes in biochemistry or urinalysis in either dose group, and we considered changes in hematology results not being clinically significant enough to affect the safety of the patients. In group 1, there were no significant changes in hematology results from baseline at week 24. In group 2, the mean international normalized ratio significantly decreased at week 24 (0.94; range, 0.83–1) when compared with baseline (0.97; range, 0.91–1.09), but there was no significant change in the prothrombin time or partial thromboplastin time ($p > 0.05$).

Discussion

Previously thought to be impossible, cartilage regeneration may play a plausible role in the treatment of osteoarthritis. Our study has shown that the administration of GEC-TGF-β1 yielded improved symptoms, activity levels, and knee function in patients with degenerative knee arthritis. Additionally, these patients experienced significant improvement in stiffness, motor function, and pain, as demonstrated by the IKDC, WOMAC, and VAS scores. No subjects experienced significant or serious AEs, and major AEs were associated with the injection site and resolved within a

Table 4. Major Adverse Events

Major adverse events	*Group 1 (n = 14)* n	*Group 1* %	*Group 2 (n = 14)* n	*Group 2* %	p-*Value*
No	6	43	4	29	0.4302[a]
Yes	8	57	10	71	
Injection-site adverse events	8	57	10	71	

[a]Pearson's chi-square test.

Table 5. Incidence/Number of Cases of Adverse Events by Organ

	Group 1 (N = *13*)			*Group 2* (N = *14*)		
Body system/preferred term	n	*%*	*No. of cases*	n	*%*	*No. of cases*
General disorders and administration-site conditions	9	64	11	10	71	16
Injection-site joint swelling	7	50	7	10	71	11
Injection-site joint pain	3	21	3	3	21	3
Peripheral edema	1	7	1	1	7	1
Feeling cold	0	0	0	1	7	1
Musculoskeletal and connective tissue disorders	1	7	2	3	21	3
Joint stiffness	1	7	2	1	7	1
Arthralgia	0	0	0	1	7	1
Musculoskeletal discomfort	0	0	0	1	7	1
Total	9	64	13	10	71	19

few days without any action needed. Given these results, the use of allogeneic human chondrocytes expressing TGF-β is promising for the treatment of osteoarthritis.

There were several limitations of this study. Our assessment of posttreatment outcomes extended to only 24 weeks following injection, and the lack of long-term follow-up did not allow us to predict whether outcomes will continue to change or remain static. A longer follow-up period may have also allowed us to see changes in the articular cartilage surface on MRI. However, this study is intended to provide only preliminary data on the effects of this treatment injection, and these patients are presently being followed up, which will continue for five years to allow for prolonged evaluation. Our study did not include a control cohort, which prevented us from comparing results to patients with no intervention. Consequently, it is difficult to rule out the possibility of a placebo effect. However, both cohorts did demonstrate significant functional improvements, which we believe is worthy of reporting. Furthermore, a study was recently concluded that compared the highest treatment dose from this study to a placebo injection, and the treatment cohort had superior results in several of the outcome measures.[15] Therefore, we believe that the placebo effect is less likely to be the sole cause of the observed improvements. In addition, the patient population was relatively small, and future evaluation of this technique may benefit from larger study cohorts. Nevertheless, we believe that the outcomes of this study are valuable given the paucity of reports on this new therapy.

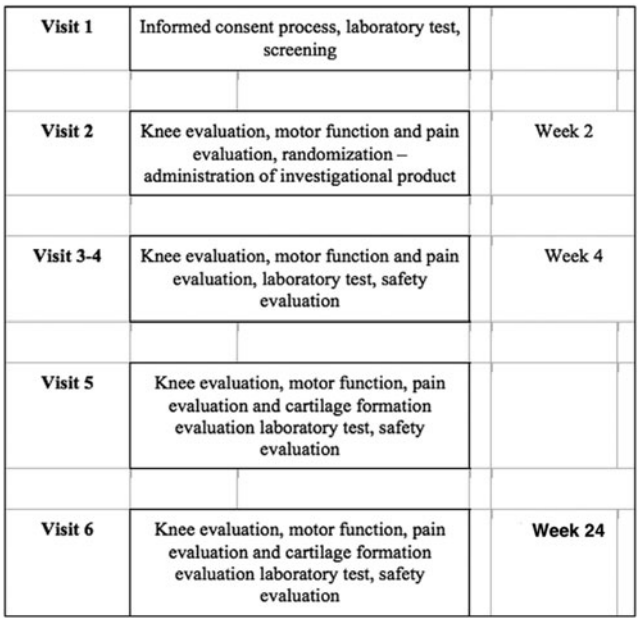

FIG. 1. Study design.

Multiple factors and cytokines have been implicated in cartilage differentiation and regeneration. Of these, TGF-β has been considered to be a multifunctional cytokine with numerous effects on cell development, as demonstrated by animal studies.[6,11] These include the induction of osteogenesis and chondrogenesis, stimulation of chondrocyte proteoglycan synthesis, extracellular matrix protein synthesis, and stimulation of endochondral ossification, which eventually leads to bone formation.[11,16,17] Furthermore, the TGF-β1 treatment may exhibit anti-inflammatory effects by inducing M2 macrophage recruitment and IL-10 in the joint fluid, and thus inhibit elimination by the immune system. Conversely, some evidence has implied that TGF-β may have an inhibitory effect, downregulating chondrocyte matrix protein production.[18] The effect appears to be dose dependent, with studies demonstrating that direct delivery of excessive TGF-β into the joint space may result in osteophyte production and secondary degenerative changes.[6,19] In addition, overexpression of TGF-β has been linked to synovial fibrosis.[20] To avoid these possible complications, this new technique renders the transduced cells incompetent by irradiating them, therefore allowing TGF-β1 expression for up to 2 weeks only. This avoids the risk of excessive expression while still allowing for its action on normal human chondrocytes.

The use of transduced human chondrocytes expressing TGF-β1 has been only recently introduced. A study by Song et al.[12] investigated the efficacy of cartilage regeneration when using a mixture of human chondrocytes with and without TGF-β1-expressing cells (mixed cells), compared with either TGF-β1-expressing chondrocytes (hChonJb#7) or nontransduced human chondrocytes (hChonJ) alone. With the mixed cells and hChonJb#7 cells, cartilage-like tissue developed 1 week following injection and continued to grow for 7–8 weeks. No growth was seen with the chondrocytes alone. Following this, the authors tested the same combinations on artificially created defects in the

femoral condyle of rabbit knee joints. Using histological grading, it was found that the combination of hChonJ and hChonJb#7 cells yielded the highest scores, followed by hChonJb#7 cells alone, and lastly, hChonJ cells alone (14 vs. 9 vs. 8 points, respectively). Conclusively, they deduced that mixed cells were capable of inducing articular cartilage growth more efficiently than either one alone.[12] Additionally, Lee et al. evaluated the cell-mediated delivery of TGF-β1 in rabbits using NIH 3T3 fibroblasts.[11] They achieved regeneration of hyaline cartilage at defect sites between 3 and 6 weeks following administration, as well as integration with the normal adjacent cartilage. They also noted that the fibroblasts continued to express the TGF-β1 up to 4 weeks after injection. From these studies, the use of cells to transmit TGF-β1 appears to be a potential treatment method for cartilage damage.

Noh et al. first examined the use of GEC-TGF-β1 (TissueGene-C) on rabbits and goats. In both animals, use of GEC-TGF-β1 yielded proliferation of new chondrocytes in the hyaline cartilage matrix of the defects studied.[21] Specifically, injected cells were shown to attach to defect sites, to colonize damaged cartilage, and to regenerate cartilage tissue. However, immunohistochemical staining at 12 months showed no difference between treated and untreated groups, alluding to the possibility that positive effects on joint cartilage only last between 6 and 12 months. The authors believed that the TGF-β1 acts in a paracrine fashion, also stimulating adjacent human chondrocytes that have not been genetically altered. They concluded that the GEC-TGF-β1 promoted chondrocyte proliferation, deposition of type II collagen, and hyaline cartilage formation within an eight-week time frame.

Ha et al. conducted a phase I study assessing the safety, biologic activity, and dose response of intra-articularly administered TissueGene-C in 12 human adult patients.[4] Patients received one of three doses: 3×10^6 cells, 1×10^7 cells, or 3×10^7 cells. Four patients were initially treated with the lowest dose level, and following review of safety data one month after administration, the dosing levels were then increased to the following amount. On completion of the study, no dose-limiting toxicity was observed, and dosing was proceeded to the highest level. AEs were limited to the injection sites and included itching, warm sensations, and joint swelling, which all resolved within a few days without further treatment. No abnormalities were noted in laboratory parameters; ELISA analysis for TGF-β1 showed levels within normal range, and PCR analysis revealed no vector DNA in any of the patients' blood tests. Additional parameters evaluated were the Knee Society Clinical Rating System, which showed clinical symptom improvement in the mid- and high-dose groups after 12 months, the WOMAC index, which showed no clear trend after 12 months, and VAS, which revealed a greater than 40% improvement in pain scores for patients treated with the high dose. This was the first clinical trial to describe human treatment with TGF-β1-expressing allogeneic chondrocytes and has provided evidence that it was well tolerated at various dose levels.

A study by Abe et al. evaluated the use of liposomes as a mode of delivery for TGF-β1 in 53 rabbits.[10] The treatment group received liposomes containing TGF-β1, and the controls received PBS solution, liposomal PBS solution, or PBS solution containing TGF-β1. Histologically, the cartilage defects that received liposomal TGF-β1 were filled with thick fibrocartilaginous tissue, while the rabbits that received PBS showed poor filling of the defects. Using the Wakitani histological scoring method, the liposomal TGF-β1 group had significantly better scores than all of the control groups ($p<0.05$). Additionally, liposomal delivery of the TGF-β1 resulted in production of type II collagen, as highlighted by immunohistochemical staining. It was concluded that only the TGF-β encapsulated in liposomes had the ability to promote cartilage restoration, but further studies were needed to assess its potential for replication in humans.

There are several techniques that have been hypothesized to influence cartilage regeneration, and the current literature suggests that it is a multifactorial process that is still in the early stages. However, we believe that TGF-β1-expressing chondrocytes are a current, viable treatment option using cell-mediated gene therapy. In conclusion, this study has demonstrated that this intra-articular injection is safe, is easy to use, and has a significant impact on improving function, pain, and physical ability in patients with osteoarthritis of the knee. Although still in its preliminary stages of testing, we hope to see the use of GEC-TGF-β1 explored in further *in vivo*, prospective studies.

Author Disclosure Statement

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