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Preliminary results of a phase II randomized study to determine the efficacy and safety of genetically engineered allogeneic human chondrocytes expressing TGF-β1 in patients with grade 3 chronic degenerative joint disease of the knee

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SUMMARY

Objective: The aim of this study was to preliminarily evaluate the efficacy and outcomes of injectable genetically engineered chondrocytes virally transduced with TGF-β1 (GEC-TGF-β1) compared to placebo.
Design: A multi-center, double-blinded, placebo-controlled, randomized study of adults with knee osteoarthritis. A total of 102 patients were 2:1 randomized to GEC-TGF-β1 or placebo. Primary outcomes assessed were (1) function of the knee joint, scored using the International Knee Documentation Committee (IKDC); and (2) pain, measured by Visual Analog Scale (VAS). Secondary endpoints assessed were pain and analgesic use, quality of life (QOL), and adverse events (AEs) including need for total knee arthroplasty after treatment.
Results: IKDC showed significant improvement in the GEC-TGF-β1 group over the placebo at week 12 (least mean square difference (LSMD): 10.3; $P = 0.0342$), week 52 (LSMD: 13.6; $P = 0.0082$), and overall (LSMD: 8.6; $P = 0.0453$). VAS Analysis showed a significant improvement in GEC-TGF-β1 group compared to placebo at weeks 12 (LSMD: −13.8; $P = 0.0162$), 52 (LSMD: −13.1; $P = 0.0332$), and overall (LSMD: −10.1; $P = 0.0350$). Reduction in pain severity at week 12 and 52, frequency at 24 h and week 52, and the percentage of patients in the GEC-TGF-β1 group receiving analgesics at week 4 (27 vs 40%) and 12 (27 vs 37%) was observed.
Conclusions: GEC-TGF-β1 patients had more positive responses on the IKDC, VAS, and were less likely to require analgesics.
Trial Number: ClinicalTrials.gov (NCT01221441) – "Study of TG-C in Patients with Grade 3 Degenerative Joint Disease of the Knee".

Introduction

Articular cartilage damage leads to osteoarthritis, which ultimately results in decreased quality of life and marked disability, pain, and functional limitations[1]. As population size, obesity rate, and life expectancy all increase, the incidence of knee osteoarthritis will continue to rise[2,3]. Therefore, more patients will need joint arthroplasty. Despite prosthetic survivorship >15–20 years for total knee arthroplasty, younger patients may require additional surgery. It is therefore paramount that alternative treatments are developed to delay arthroplasty as long as possible[4,5].

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Cartilage has poor intrinsic and biomechanical repair capacity, thus, injuries lead to progressive, permanent damage[6–8]. Presently, standard non-operative treatment for knee osteoarthritis consists of several options, which may help alleviate disease symptoms, but do not restore damaged cartilage[9–11]. However, recently, an exploration of novel techniques of cell-mediated cytokine gene therapy for cartilage regeneration, including the use of various growth factors and bone morphogenetic proteins. Specifically, TGF-β proteins induce osteogenesis and chondrogenesis, and play a role in cell growth, differentiation, and extracellular matrix protein synthesis[12]. Moreover, studies suggest that TGF-β stimulates proteoglycan synthesis and chondrocyte proliferation, and may also possess anti-inflammatory and immunosuppressive properties[13].

One novel technique involves human chondrocytes transduced with a viral vector containing the gene for TGF-β1 transcription. This study preliminarily evaluated injectable genetically engineered allogeneic human chondrocytes expressing TGF-β1 (GEC-TGF-β1) compared to placebo in patients with grade 3 degenerative knee disease. Primary outcomes assessed efficacy with regards to knee functionality and symptoms of knee osteoarthritis pain, as well as safety of administration by observation of adverse events (AEs), findings and laboratory tests, including immune analyses. Secondary endpoints evaluated include incidence and analgesia dose and/or anti-inflammatory medications; cells expression outside the injection site; and efficacy of GEC-TGF-β1 by evaluating the need for secondary procedures.

Methods

Patient selection

This is the 1 year follow-up of a 2 year 2:1 ratio multi-center, double-blinded, placebo-controlled, randomized study of 102 adults with Kellgren–Lawrence grade III knee osteoarthritis. All institutions obtained institutional review board approval, and the study was registered in ClinicalTrials.gov (NCT01221441) as "Study of TG-C in Patients with Grade 3 Degenerative Joint Disease of the Knee". It was conducted in accordance with the International Conference on Harmonization Tripartite Guideline, Guideline for Good Clinical Practice, ethical principles with origin in the Declaration of Helsinki, as well as the USA Code of Federal Regulations.

Patients were included if all inclusion criteria were met. These were: age between 18 and 70 years; body mass index (BMI) between '18.5 and 45.5' kg/m^2; grade 3 radiographic knee osteoarthritis as determined by the criteria of Kellgren–Lawrence; pain symptoms for more than 4 consecutive months. Please see Appendix A for complete list of inclusion and exclusion criteria.

Patients

Patients (n = 102) were randomized to receive genetically engineered chondrocytes virally transduced with TGF-β1 (GEC-TGF-β1) (TissueGene-C; TissueGene Inc., Rockville, Maryland, USA (n = 67)), or placebo (2 ml normal saline (0.9%); n = 35) according to a randomization list managed by Comprehensive NeuroScience Inc., (CNS; Morrisville, North Carolina). Each site was provided with a randomization list managed by an unblinded pharmacist or lab technician. As patients were screened and prepared for enrollment, the unblinded pharmacist referred to the randomization list and completed the Investigational Product Request Form. TissueGene, Inc. shipped cells to the unblinded pharmacist at the site or placebo was prepared by the unblinded pharmacist. All injections were sequentially numbered to ensure standardization, and administered by the principal investigators, who were blinded to injection type. Follow-up evaluations were conducted by blinded clinicians, unaware of study group.

The study was initiated in May 2011 and concluded October 2013 once patient enrollment numbers were met. The GEC-TGF-β1cohort consisted of 67 patients (24 men and 43 women) who had a mean age of 57 years (range, 34–70), and BMI of 30 kg/m^2 (range, 19–43). The placebo cohort consisted of 35 patients (14 men and 21 women) who had a mean age of 56 years (range, 25–70) and BMI of 30 kg/m^2 (range, 20–43) (Table I). There were 17 (25.4%) patients in the treatment arm and nine patients (25.7%) in the control group who discontinued the study prematurely (See Consort Flow for patient allocation). The ratio of treatment to placebo in the withdrawn patient population is approximately 2:1 (same ratio for entire study). After excluding 26 individuals who did not complete the trial, 76 were randomized to GEC-TGF-β1 treatment group (n = 50) or the placebo group (n = 26) (Fig. 1).

Determination of sample size

The sample size was based on the assumption that the standard deviation for International Knee Documentation Committee (IKDC) Subjective Knee Evaluation is between 20 and 25 points and that the correlation between baseline and month 6 assessment is at least 0.5. A 2:1 ratio of TissueGene-C (TG-C) patients to control (67 TG-C and 33 control) is needed at least a 12.1–15.1 point difference from the control group (for SD = 20 and 25, respectively) with alpha = 0.05 and 80% power. This sample size allows detection of an 0.6 effect size for Visual Analog Scale (VAS), with alpha = 0.05 and 80% power.

Treatment

GEC-TGF-β1 is a 3:1 mixture of non-transduced allogeneic human chondrocytes and transduced allogeneic human chondrocytes expressing TGF-β1, irradiated during manufacturing to render replication incompetent. The GEC-TGF-β1 chondrocytes were derived from a single human donor, grown from cartilage tissue from an infant polydactyly finger. Testing for viruses absence and other adventitious donor agents and the cell line were conducted. Chondrocytes virally transduced with TGF-β1 represent a cell-mediated cytokine gene therapy approach designed for local intra-articular administration in patients who had osteoarthritis to stimulate cartilage regeneration via cytokine gene expression, TGF-β1, via a retroviral vector.

Patient's knee joints were aspirated to remove synovial fluid prior to GEC-TGF-β1 or placebo administration. Treatment or placebo (2 ml normal saline (0.9%)) was injected via 18 gauge needle using the inferolateral or inferomedial entry point with the knee in 90° flexion. Synovial fluid aspirated was analyzed to rule out infection. To avoid cell shearing, injection was performed over approximately 10 s. Both patient and physicians were blinded at the time of injection.

Table I
Demographics

Parameter	Statistic	Treatment (N = 67)	Placebo (N = 35)
Age (years)	Mean (range)	56.7 (34–71)	56.4 (25–70)
Sex	Female	43	21
	Male	24	14
Race	Caucasian/White	53	29
	Black	12	4
	Hispanic	2	2
Body mass index (kg/m^2)	Mean (range)	29.6 (19–43)	29.6 (20–43)

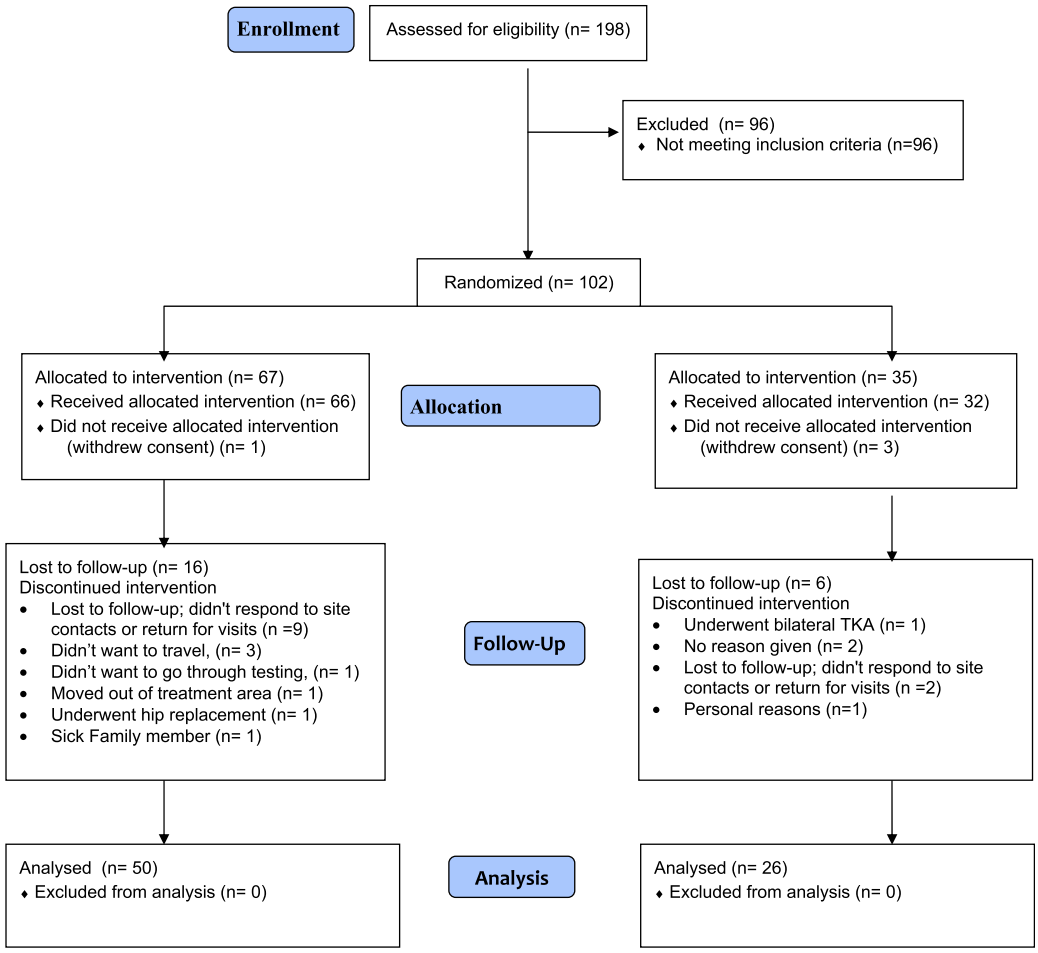

Fig. 1. Enrollment flowchart.

Study assessment

Patients underwent examination of endpoints at screening, baseline, 24 h post-dose, week 4, and months 3, 6, and 12, except Short-Form 36, which was assessed at baseline and months 6 and 12. Patients had a physical examination at screening, prior to dosing, 24 h after dosing, and at all study visits. A drug screen, hepatitis B virus (HBV)/hepatitis C virus (HCV) test, and human immunodeficiency virus (HIV) I/II test were performed at screening. Abnormalities observed during physical and laboratory examinations were recorded.

The primary efficacy endpoints assessed symptomatic improvement, specifically: (1) function of the knee using the IKDC subjective knee evaluation; and (2) pain measured by a 100 mm VAS. Secondary endpoints included assessment of symptoms, pain, and functionality using the Knee Injury and Osteoarthritis Outcome Score (KOOS), the Lysholm Knee Scale, and the Lower Extremity Functional Scale (LEFS), as well as incidence of AEs, use of analgesic medications, and need for subsequent surgery.

The IKDC Knee Examination Form was used at each visit[14,15]. To evaluate limitations associated with knee osteoarthritis, patients completed the KOOS[16,17] at all time points. The KOOS is subdivided into five subscales (symptoms, pain, function in daily living (activities of daily living – ADL), sports ability, and quality of life (QOL)), and each subscale is reported separately (range 0–100 points). Patients were evaluated according to the LEFS[18,19], Lysholm, and SF-36 questionnaires[20]. The LEFS is a patient-rated evaluation of ability to perform activities-of-daily-living. The Lysholm Score (0–100 points) queries related to locking, pain, and the effects of injury on knee-related activities. The SF-36 is a general, valid, and reliable multipurpose health survey that assess functional health and well-being, through mental and physical components evaluations.

Reduction in pain was measured by 100-mm VAS, pain questionnaires, KOOS subscores, and frequencies/doses of analgesics. The pain questionnaire describes severity, frequency, and the time and speed of pain onset.

To further assess any improvements in VAS or IKDC in withdrawn patients, they were analyzed for modified OMERACT-OARSI response[21]. A positive response is defined as either: (1) high pain improvement (VAS) or function (IKDC) $\geq$50% and absolute score changes $\geq$20, or; (2) moderate improvements in pain and function $\geq$20% and absolute score changes $\geq$10 for both VAS and IKDC.

Untoward medical occurrences were recorded even if not necessarily causally-related with treatment. AEs can be any unfavorable and unintended sign (including abnormal laboratory findings), symptoms, or disease temporally associated with use of an investigational product, whether or not related. Blood samples were analyzed for TGF-β1 expression by enzyme-linked immunosorbent assay (ELISA) and by polymerase chain reaction (PCR) for

vector DNA. Immune responses, specifically, T-cell responses, antibodies to HLA-A, B, and C, antibodies to TGF-b1, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and multiplex cytokine analyses were performed to observe for any allogeneic responses. Patients could undergo knee arthroplasty at any time.

Statistical analysis

Endpoint analyses were performed using repeated measures and mixed-model methodology. A linear mixed-model was used for IKDC or VAS with change from baseline as the dependent variable and post-treatment time point (weeks 4, 12, 24, and 52) and treatment, as well as interactions between time point and treatment as fixed factors and with subject included in the model as a random factor. A spatial power covariance structure for unequally spaced time intervals was used to model the repeated assessment over time. Denominator degrees of freedom were adjusted using Kenward–Roger's method. Treatment comparisons were made at each time point, with the primary comparison at week 52, and overall across all four time points. Each parameter was analyzed with linear mixed-model repeated measures. Comparisons at each time point were performed by contrasts within a single model.

Results

Primary endpoint

Knee evaluations and pain

The change from baseline IKDC showed a significant improvement in the least square (LS) mean of GEC-TGF-β1 group compared to placebo at week 12 (least mean square difference (LSMD [95% CI]: 10.3 [0.8–19.9]; $P = 0.0342$), week 52 (LSMD [95% CI]: 13.6 [3.6–23.6]; $P = 0.0082$), and overall (LSMD [95% CI]: 8.6 [0.2–17.0]; $P = 0.0453$). The LSMD at week 4 and 24 between the GEC-TGF-β1 and placebo were not statistically significant (Table II and Fig. 2).

Analysis of VAS showed a significant improvement in the GEC-TGF-β1 group compared to placebo at week 12 (LSMD [95% CI]: −13.8 [−25.0–2.6]; $P = 0.0162$), week 52 (LSMD [95% CI]: −13.1 [−25.1–−1.1]; $P = 0.0332$), and overall (LSMD [95% CI]: −10.1 [−19.4–−0.7]; $P = 0.0350$). While the LS mean differences in VAS between the GEC-TGF-β1 and placebo at week 4 and 24 were not statistically significant, there was a trend towards improvement (−5.8 [−16.7–5.20; $P = 0.2991$ and −7.7 [−19.0–3.7]; $P = 0.1832$], respectively) (Table III and Fig. 3).

Results from modified OMERACT-OARSI indicated for the withdrawn patients, the response rate was 58.3% for GEC-TGF-β1 group and 42.9% for placebo (lower than overall response rate). However, most patients withdrew on, or before 24 weeks; efficacy was observed to be higher in the remaining patients at later time points (week 24 and 52). Ten (10) withdrawn patients had improvements in pain and/or function that correlated to a positive response according to modified OMERACT-OARSI criteria, whereas, 16 patients did not. Patients who withdrew did not respond as well as the overall patient population.

The Lysholm, KOOS, and LEFS, did not demonstrate significant differences between the treatment and placebo at a majority of times ($P > 0.05$; Table III for values). However, evaluation of subjective knee functionality demonstrated that LSMD (95% CI) significantly improved from baseline for KOOS Pain between GEC-TGF-β1 and placebo (9.4 (0.4–18.4) at week 52 $P = 0.0416$). The LSMD (95% CI) between the GEC-TGF-β1 group and placebo for Lysholm at week 52 approached significance ($P = 0.0723$) (Appendix B).

AEs

Through 1 year in the GEC-TGF-β1 cohort, 58 of 67 patients (87%) experienced AEs. Most were mild (13%) or moderate (67%), with severe AEs occurring in 10%. In the placebo group, 27 of 35 (77%) patients reported AEs. The majority were mild (26%) or moderate (43%) in severity, with 9% being severe. In the study cohort, 45 patients (67%) were experiencing AEs related to study drug. A majority occurred within the first 4 weeks post-treatment administration, with 53 (79%) occurring in the GEC-TGF-β1cohort, while the placebo cohort had 18 (51%) events. Forty-one patients (61%) in the GEC-TGF-β1 cohort had an event after 4 weeks, while 21 (60%) of the placebo patients had events in the same period. Of 53 events in the GEC-TGF-β1cohort, 44 were considered possible, probably, or definitely related to the drug, while 9 of 41 events were considered to be related after 4 weeks. In the placebo cohort, 8 of 18 events were considered possible, probably, or definitely related to the placebo, while 1 of 21 were related to the placebo treatment. The most common AEs definitely related to treatment with GEC-TGF-β1 were joint inflammation (19 patients), arthralgia (14 patients), and effusion (14 patients). Three serious AEs were experienced, one GEC-TGF-β1 and two placebo patients. These serious adverse events (SAEs) were not considered related to study medication. No patients were withdrawn from the study due to an AE. No SAEs were reported for abnormal laboratory values. No significant trends were seen with respect to immunologic responses to GEC-TGF-β1. The AEs of increased C-reactive protein and increased numbers of eosinophils were seen in one GEC-TGF-β1 and no placebo patients. Increased interleukin or cytokine values were reported as AEs in six GEC-TGF-β1 and one placebo patient. Development or increase in TGF-β1 antibodies was not a significant clinical or efficacy concern in patients receiving GEC-TGF-β1. Antibody analysis indicates that there were no increases in antibodies to donor-specific HLA antigens.

Secondary endpoints

Pain questionnaire and analgesic medication

At week 12 and 52 visits, the mean pain difference significantly improved from baseline in treatment group compared to the placebo group −0.91 vs −0.42 and −8.6 vs −0.30 ($P = 0.0184$ and 0.0399). At the week 52 time point, the mean (SD) change from baseline for frequency of pain was −1.14 (1.175) and −0.35 (1.089) in the treatment group and placebo groups ($P = 0.0248$). There were no statistically significant differences between treatment and

Table II
Summary of IKDC outcomes

Visit	TissueGene-C	Placebo		95% CI of LS mean difference	*P*-value
	LS mean	LS mean	LS mean difference		
Week 4	13.8	11.2	2.6	−6.8–12.0	0.5846
Week 12	23.0	12.7	10.3	0.8–19.9	0.0342
Week 24	20.4	12.5	7.9	−1.8–17.6	0.1089
Week 52	23.3	9.7	13.6	3.6–23.6	0.0082
Overall	20.1	11.5	8.6	0.2–17.0	0.0453

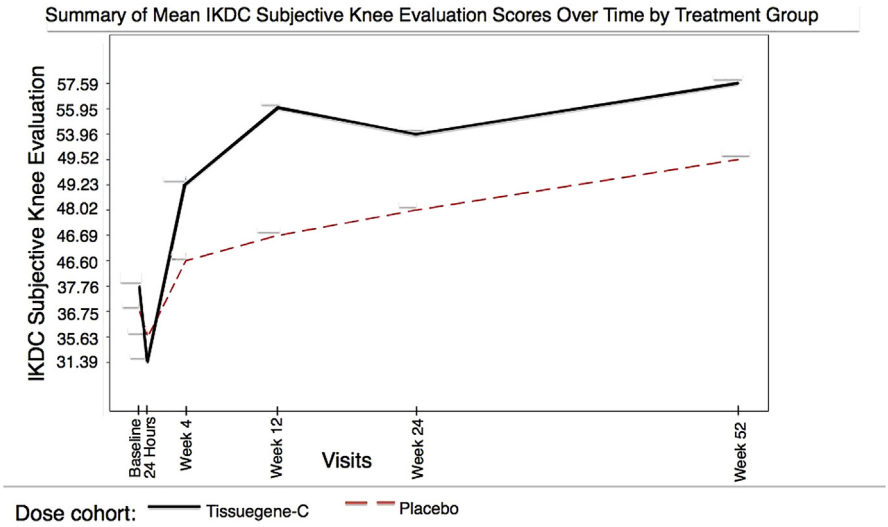

Fig. 2. Mean IKDC subjective knee evaluation scores over time by treatment group.

Table III
Summary of VAS outcomes

Visit	TissueGene-C	Placebo		95% CI of LS mean difference	*P*-value
	LS mean	LS mean	LS mean difference		
Week 4	−26.9	−21.1	−5.8	−16.7–5.2	0.2991
Week 12	−37.2	−23.4	−13.8	−25.0–−2.6	0.0164
Week 24	−35.5	−27.8	−7.7	−19.0–3.7	0.1832
Week 52	−39.9	−26.9	−13.1	−25.1–−1.1	0.0332
Overall	−34.9	−24.8	−10.1	−19.4–−0.7	0.0350

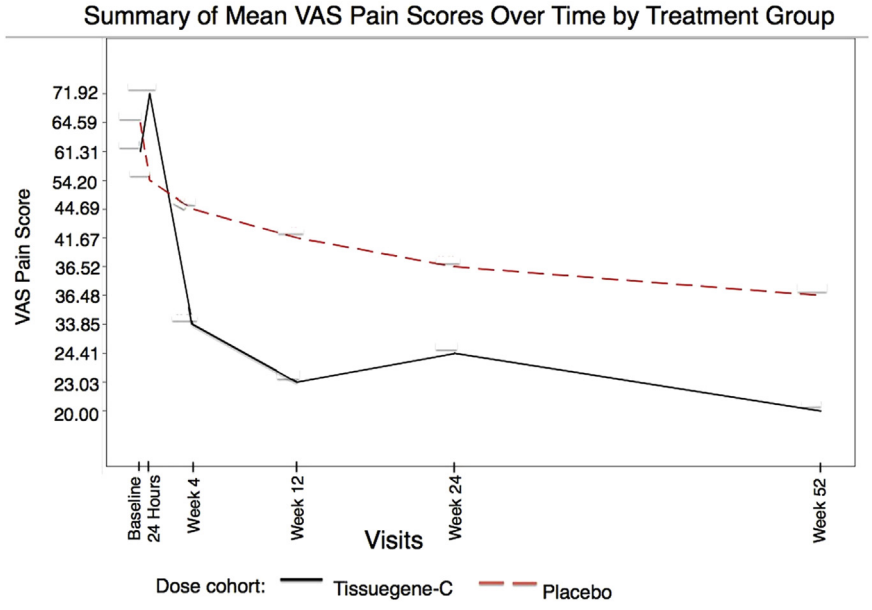

Fig. 3. Mean VAS pain scores over time by treatment group.

placebo groups for speed of onset of knee pain at any time point and severity and frequency of pain at remaining time points (Appendix B). We demonstrated that patients in the treatment group required less pain medication at week 4 and 12 compared to the placebo cohort (27 vs 40% and 27 vs 37 %), however, at remaining time points, analgesic use was similar (Table IV).

QOL assessment

The LS mean difference (95% CI) between treatment and placebo groups for overall SF-36 domain score was 0.5 (−4.2 to 5.2) at week 24, −0.4 (−5.4 to 4.6) at week 52, and 0.1 (−4.1 to 4.3) overall ($P = 0.837$, 0.879, and 0.981).

Expression of GEC-TGF-β1 outside of knee

One patient at 12 weeks in the GEC-TGF-β1 group and one patient at 24 weeks in the placebo group had an "indeterminate" value for the presence of replication competent retroviruses. All other assays were rated as "not detected" or were not performed.

Secondary procedures

One subject had arthroscopy on their treated knee. One placebo patient had bilateral total knee arthroplasty (TKA) after week 4 and was discontinued from the study, whereas, no patients in the GEC-TGF-β1 cohort underwent TKA.

Discussion

This study described the use of a novel injectable allogeneic human chondrocytes expressing TGF-β1 in patients with grade 3 chronic degenerative knee disease. We found that patients receiving GEC-TGF-β1 had a more positive response in IKDC evaluation, pain rated by VAS, and pain questionnaires, and were less likely to require analgesics compared to placebo. GEC-TGF-β1 appears to improve symptoms and pain related to knee osteoarthritis.

The potential positive effects of injectable retroviral transduced allogeneic human chondrocytes expressing TGF-β1 were first described by Noh *et al.*[22], who conducted a pre-clinical evaluation of cartilage in animal models involving induced knee articular damage. Articular defect examination demonstrated signs of regeneration at autopsy. At 8 weeks, biopsy findings suggested that TG-C resulted in proliferative foci of new chondrocytes in hyaline cartilage matrix in both rabbits and goats, and these were made up of young chondrocytes in hyaline matrix, which stained positive with toluidine blue and type II collagen. Human chondrocytes (TGF-β1) staining revealed involvement of small foci only at defect site, but not in other joint areas. The defect foci morphology and staining characteristics indicated hyaline cartilage consistent with articular cartilage was present. At 1 year, there was positive staining of the proliferating cartilage in five of eight animals, indicated production of type II collagen. The authors concluded that TG-C may potentially be an effective strategy to promote cartilage growth, type II collagen deposition, and hyaline cartilage formation for articular defects.

Following this initial pre-clinical study, Ha *et al.*[23] performed the first phase I trials in humans, evaluating safety and biologic activity of injectable TG-C in 12 knees. No severe AEs related to the TG-C treatment were noted, with the most common AEs being effusion, which resolved in all cases. This injectable TG-C, led to 10 of 12 patients showing improvements in Knee Society Clinical Ratings from baseline at 6 months, with trending improvements in range-of-motion and pain up to 1 year following dosing. Patients demonstrated marked improvements (>40%) in pain scores (VAS) for up to 3 months following dosing. These authors concluded that there were minimal localized AEs and that there may be a trend towards improvement in knee osteoarthritis symptoms. The findings of the present study are in concordance with this phase I trial, demonstrating that TG-C may be a beneficial treatment option, will the majority of AEs being effusion or inflammation which was self-limiting.

This study has shown that GEC-TGF-β1 was safe and generally well-tolerated. There were a greater proportion of patients in the GEC-TGF-β1 cohort that exhibited AEs, but most were classified as mild or moderate severity. The majority of GEC-TGF-β1 patients exhibited knee inflammation, which was related to the treatment. These events are local reactions that occurred shortly after administration and were not late-onset AEs due to drug or progression of disease. It is hypothesized that TGF-B1 in GEC-TGF-β1 may induce an inflammatory response. Animal studies have previously indicated that TGF-B1 produced by the transduced cells may induce an inflammatory response, however, these effects were transient and reversible. These results are consistent with the potential effects of TGF-B1. We also noted that the inflammation seen is not unexpected based on animal safety studies: acute toxicity studies indicated GEC-TGF-β1 may induce local inflammation as evidenced by mild-to-moderate synovial cell hyperplasia, synovial, vascularization, and pannus formation when injected intra-articularly in rabbits and edema, nodules, and chronic inflammation at the subcutaneous injection site and/or skin/subcutis when injected subcutaneously in mice. These findings were transient and were not unexpected due to the fact that th0e cells administered were xenogeneic and the TGF-β1 protein is known to induce synovial proliferation and inflammation. To avoid this, this novel technique irradiates the transduced cells prior to dosing to render their replication incompetent, which allows cells to express TGF-β1 for up to 2 weeks. This allows for action on normal human chondrocytes, while ensuring no prolonged or excessive TGF-β1 expression and that gene-modified cells do not persist *in vivo*.

Despite this study being a prospective, multi-center, double-blinded, placebo-controlled, randomized trial, there were several limitations. The cohort size was somewhat small, and a relatively large number of patients dropped out. Our evaluation only extended to 52 weeks, thus it is difficult to assess the longer-term efficacy of this treatment option. However, we plan to further follow these patients to evaluate long-term efficacy and safety. Furthermore, several endpoints were defined as primary outcomes, which may result in multiplicity issues. As a result, this may lead to an increased rate of false positive conclusions, such as claims for the effectiveness of a treatment. To address this, we have limited our

Table IV
Summary of patients taking analgesia medication

	TissueGene %	Placebo %
Patients taking Analgesia Medication at:		
Screening	19.4	25.7
Baseline	22.4	25.7
24 h	20.9	17.1
Week 4	26.9	40.0
Week 12	26.9	37.1
Week 24	29.9	28.6
Week 52	31.3	28.6

pre-defined primary endpoints to two variables on which power analyses were performed and declared the secondary variables as supportive, although we acknowledge that multiplicity may still be present. Also, our inclusion criteria included an extensive age range as well as patients who may have had undiagnosed concomitant hip osteoarthritis. We did not want to exclude any patients above 18 years of age with signs of osteoarthritis, and this may add bias, as the population is not homogenous. Furthermore, to the best of our knowledge the primary area of painful arthritis would have been the knee, however, there is a potential bias for unknown hip disease, but we believe that these findings would have had equal distributions in both cohorts. Our purpose was to assess the effect of this injection, compared to patients who would have undergone no treatment at all in a clinical setting. Thus, we believe that the use of normal saline was the appropriate injection to emulate 'no treatment', as providing no injection at all would compromise the blinding in this study. Saline has also been used in several randomized-controlled studies[22,24–26]. Furthermore, several studies, such as that by Noh *et al.* demonstrated no cartilage growth with chondrocytes alone, though this was in animal studies and as such, was the basis of our decision to compare GEC-TGF-β1 to placebo. However, we do appreciate that further comparisons to chondrocyte injections alone may be important, and thus future studies should evaluate this. Furthermore, detection of TGF-β in synovial fluid was not included in the study protocol or in the endpoints assessed, and incorporating this in future analyses may be beneficial.

We have seen various levels of cartilage improvement in the treatment group compared to the control, and preliminary analysis of radiographic data have demonstrated that the treatment injection had beneficial effects on hyaline cartilage regeneration. However, full radiographic analysis includes assessment of: (1) cartilage morphology; (2) cartilage lesions; (3) bone marrow lesions; (4) subchondral cysts; (5) osteophytes; (6) bone attrition; (7) effusions; and (8) synovitis as determined by 3T magnetic resonance imaging (MRI). Such extensive evaluations of all the patients are still under analysis, and radiographic assessments of cartilage growth will be incorporated in a future, follow-up study.

Currently, there are various options and surgical procedures to relieve the symptoms associated with knee osteoarthritis, however, these routes only manage symptoms, and in the case of surgery, may increase patient morbidity. One of the major limitations in knee osteoarthritis treatment is the lack of any intervention proven to directly impact the disease process and possibly reverse the current knee damage. Moreover, recent exploration into novel techniques of cell-mediated cytokine gene therapy and tissue engineering approaches for cartilage regeneration has been shown to have a promising future in knee osteoarthritis treatment[22,23,27]. Our study demonstrated that GEC-TGF-β1 may have positive effects on pain levels in patients who have knee osteoarthritis, as demonstrated by the VAS and IKDC scores, at 1 year follow-up when compared to the control cohort. Patients receiving GEC-TGF-β1 had more positive responses on the knee evaluation and pain, and they were less likely to require analgesics compared to placebo.

Specific contribution of each author is as follows

Jeffrey Cherian: data analysis, manuscript preparation.
Dale Bramlet: contributed patients, data collection.
Kwan Hee Lee: Chief medical officer of Trial.
Javad Parvizi: contributed patients, data collection, and manuscript revision.
David Romness: contributed patients, data collection.
Michael Mont: contributed patients, data analysis, manuscript preparation, and critical review of manuscript, supervision.

Disclosures

JJC: reports institutional grants from TissueGene, Inc., during the conduct of the study; is a paid consultant for DJO Global.

DB: reports institutional grants from TissueGene, Inc., during the conduct of the study.

KHL: is a paid employee of TissueGene, Inc.

DR: reports institutional grants from TissueGene, Inc., during the conduct of the study; grants and personal fees from TissueGene, outside the submitted work.

JP: reports grants from TissueGene, Inc., during the conduct of the study; grants and personal fees from TissueGene, outside the submitted work; and 3M: Research support, Alphaeon: Stock or stock Options, CD Diagnostics: Stock or stock Options, Cempra: Research support, CeramTec: Consultant; Research support, ConvaTec: Consultant, Corentec: Consultant; Stock or stock Options, Datatrace: Publishing royalties, financial or material support, DePuy, A Johnson & Johnson Company: Research support, Eastern Orthopaedic Association: Board or committee member, Elsevier: Publishing royalties, financial or material support, Hip Innovation Technology: Stock or stock Options, Jaypee Publishing: Publishing royalties, financial or material support, Johnson & Johnson: Consultant, Joint Purification Systems: Stock or stock Options, Journal of Arthroplasty: Editorial or governing board, Journal of Bone and Joint Surgery – American: Editorial or governing board Journal of Bone and Joint Surgery – British: Editorial or governing board, Medtronic: Consultant, Muller Foundation: Board or committee member, National Institutes of Health (NIAMS & NICHD): Research support, OREF: Research support, PRN: Stock or stock Options, SLACK Incorporated: Publishing royalties, financial or material support, Smith & Nephew: Consultant; Research support, StelKast: Research support, Stryker: Research support, TissueGene: Consultant, Wolters Kluwer Health – Lippincott Williams & Wilkins: Publishing royalties, financial or material support, Zimmer: Consultant; Research support.

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Conflicts of interest

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Appendix A. Inclusion and exclusion criteria

Inclusion	Exclusion
• Ages of 18–70 years • General good health ◦ Physical examination ◦ Normal hematology ◦ Serum chemistry ◦ Urinalysis screening a ◦ Negative history of significant organ system disorders • Body mass index (BMI) between '18.5 and 45.5' kg/m^2 • Blood pressure measurements ◦ Systolic blood pressure between 90 and 160 mmHg and ◦ Diastolic blood pressure between 50 and 90 mmHg) • Kellgren–Lawrence grade 3 chronic OA of the knee • Symptoms of pain for more than 4 consecutive months and a intensity of ≥40 and ≤90 on the 100-mm scale • Cleared for use protocol specified equipment (3T MRI) • Provided written informed consent after the nature of the study is fully explained and understood by the patient	• 71 years of age or older • Abnormal hematology, serum chemistry, or urinalysis screening laboratory values • Taken non-steroidal anti-inflammatory drugs (NSAIDs) within 14 days of baseline visit • Taken steroidal anti-inflammatory medications within 2 months of baseline visit • A recent (within 1 year) history of drug abuse and/or a positive urine drug test at the time of screening • Received injections to the treated knee within 2 months prior to study entry • Contraindications for 3T MRI • Pregnant or currently breast-feeding children • History of systemic, rheumatic or inflammatory disease of the knee or chondrocalcinosis, hemochromatosis, inflammatory arthritis, osteonecrosis of the femoral condyle, arthropathy of the knee associated with juxta-articular, Paget's disease of the femur or tibia, ochronosis, hemophilic arthropathy, infectious arthritis, Charcot's knee joint, villonodular synovitis, synovial chondromatosis, and/or history of inflammatory arthropathy • History of ongoing infectious disease pathology, including human immunodeficiency virus (HIV) and hepatitis B or C • Participated in a study of an experimental drug or medical device within 30 days of study entry

Appendix B. Outcomes of subjective functional outcomes and pain questionnaire

Summary of subjective functional results

Visit	TissueGene-C	Placebo		95% CI of LS mean difference	*P*-value
	LS mean	LS mean	LS mean difference		
KOOS subscale symptom					
Week 4	6.9	7.7	−0.7	−6.5–5.0	0.8
Week 12	13.5	8.4	5.2	−0.8–11.1	0.087
Week 24	10.5	13.2	−2.7	−8.6–3.3	0.374
Week 52	11.8	13.8	−2.0	−8.3–4.2	0.524
Overall	10.7	10.6	−0.1	−5.0–4.8	0.98
KOOS subscale pain					
Week 4	12.7	14.8	−2.1	−10.5–6.3	0.621
Week 12	21.8	14.9	6.9	−1.6–21.8	0.111
Week 24	20.7	19.1	1.5	−7.1–10.2	0.729
Week 52	25	15.7	9.4	0.4–18.4	0.042
Overall	20	16.1	3.9	−3.6–11.5	0.304
KOOS subscale ADL					
Week 4	12.2	13.6	−1.4	−9.3–6.5	0.723
Week 12	20	13.5	6.4	−1.6–9.5	0.116
Week 24	18.3	17.5	0.9	−7.3–9.0	0.833
Week 52	22.1	16.2	5.9	−2.6–14.5	0.172
Overall	18.1	15.2	3.0	−4.1–−10.0	0.405
KOOS subscale QOL					
Week 4	14.8	13.9	0.9	−8.9–10.8	0.849
Week 12	21.8	15.9	5.9	−4.1–15.8	0.846
Week 24	17.8	19.9	−2.1	−12.2–8.1	0.686
Week 52	24.3	21.1	3.2	−7.6–13.9	0.559
Overall	19.7	17.7	2.0	−6.7–−13.9	0.65
KOOS subscale sport					
Week 4	13	13.2	−0.3	−12.2–11.7	0.964
Week 12	21.2	12	9.2	−2.9–21.3	0.134
Week 24	18.2	21.1	−2.9	−15.2–9.4	0.644
Week 52	24.8	16.9	7.9	−5.2–21.0	0.238
Overall	19.3	15.8	3.5	−7.3–14.2	0.522
Lysholm scores					
Week 4	14.6	11.2	3.4	−4.9–11.7	0.422
Week 12	19.8	15.8	4.0	−4.4–12.5	0.349
Week 24	16.6	17.9	−1.3	−9.8–7.2	0.765
Week 52	21.3	13.2	8.1	−0.7–17.0	0.072
Overall	18.1	14.5	3.6	−4.0–11.1	0.352
Lower extremity functional score					
Week 4	9.8	10.9	−5.8	−16.7–5.2	0.774
Week 12	15.7	12.5	−13.8	−25.0–−2.6	0.381
Week 24	14.4	15.6	−7.7	−19.0–3.7	0.745
Week 52	16.7	13.5	−13.1	−25.1–−1.1	0.427
Overall	14.2	13.1	−10.1	−19.4–−0.7	0.755

Summary of pain questionnaire

Visit	TissueGene-C	Placebo		95% CI of LS mean difference	*P*-value
	Mean	Mean	Mean difference		
Severity of pain					
Week 4	−0.72	−0.41	−0.31	−0.84–0.22	0.2222
Week 12	−0.91	−0.42	−0.49	−0.90–−0.08	0.0184
Week 24	−0.6	−0.52	−0.18	−0.63–0.34	0.8260
Week 52	−8.6	−0.30	−0.56	−1.08–−0.04	0.0399
Frequency of pain					
Week 4	−0.40	−0.37	−0.03	−0.52–0.46	0.694
Week 12	−1.0	−0.71	−0.29	−0.87–0.29	0.286
Week 24	−0.74	−0.3	−0.34	−0.95–0.07	0.105
Week 52	−1.14	−0.35	−0.79	−1.43–−0.15	0.025
Time since knee pain of pain questionnaire					
Week 4	−0.17	−0.26	0.09	−0.61–0.79	0.963
Week 12	−0.34	0.04	−0.3	−1–0.24	0.589
Week 24	−0.09	0.04	−0.15	−0.78–0.52	0.34
Week 52	−0.03	0.11	−0.14	−0.98–0.84	0.532
Speed of onset pain questionnaire					
Week 4	0.05	−0.19	0.24	−0.21–0.69	0.255
Week 12	−0.12	−0.14	0.02	−0.48–0.52	0.856
Week 24	−0.02	−0.14	0.12	−0.38–0.62	0.838
Week 52	0.17	−0.16	0.33	−0.25–0.91	0.191

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.06.019.

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