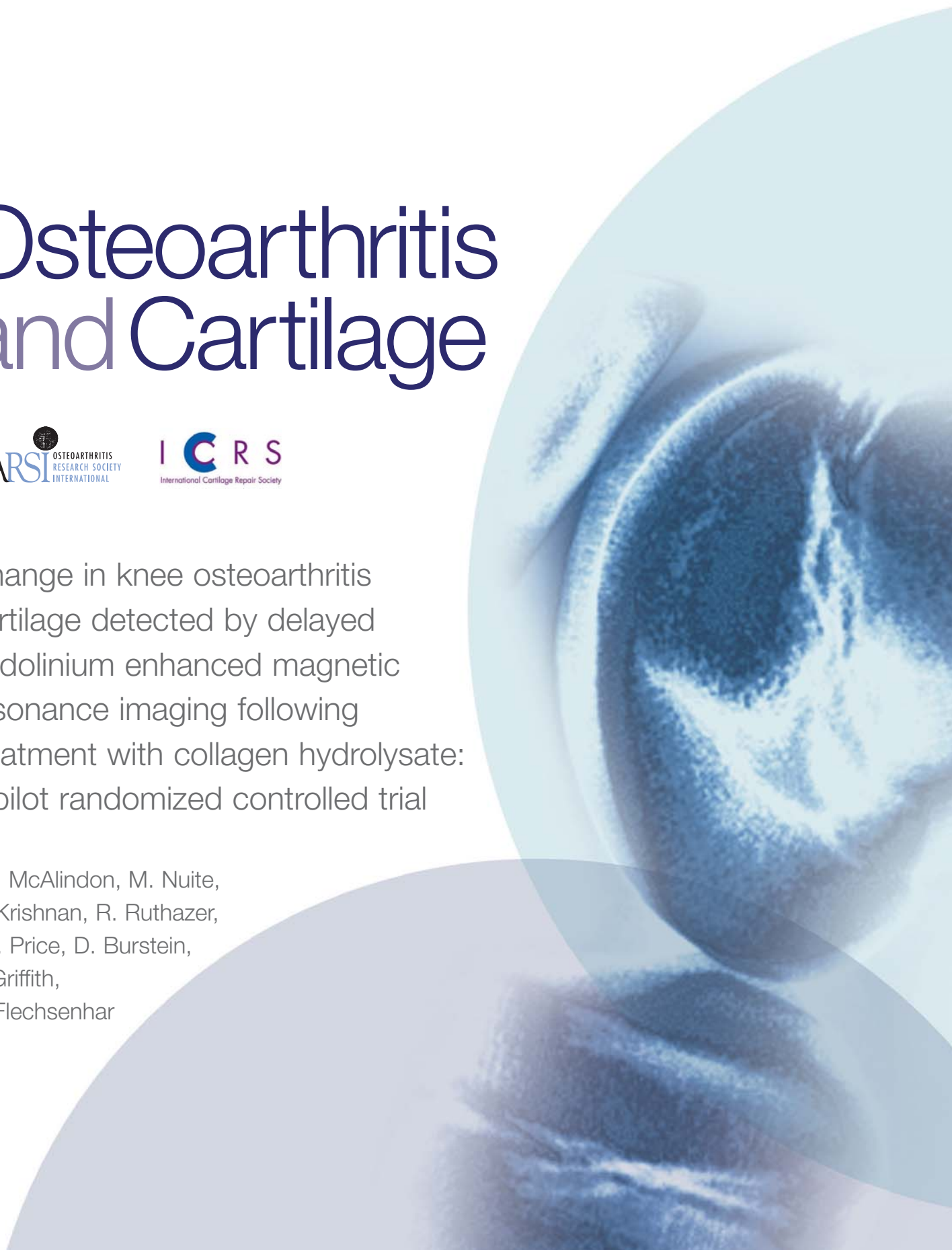# Osteoarthritis and Cartilage

Change in knee osteoarthritis cartilage detected by delayed gadolinium enhanced magnetic resonance imaging following treatment with collagen hydrolysate: a pilot randomized controlled trial

T.E. McAlindon, M. Nuite,
N. Krishnan, R. Ruthazer,
L.L. Price, D. Burstein,
J. Griffith,
K. Flechsenhar

Osteoarthritis and Cartilage

OARSI — Osteoarthritis Research Society International

Change in knee osteoarthritis cartilage detected by delayed gadolinium enhanced magnetic resonance imaging following treatment with collagen hydrolysate: a pilot randomized controlled trial

T.E. McAlindon †*, M. Nuite †, N. Krishnan ‡, R. Ruthazer §, L.L. Price §, D. Burstein ‖, J. Griffith ¶, K. Flechsenhar #

† Division of Rheumatology, Tufts Medical Center, Box 406, 800 Washington Street, Boston, MA 02111, United States
‡ Department of Radiology, Beth Israel Deaconess Medical Center, 300 Brookline Avenue, Boston, MA 02115, United States
§ Biostatistics Research Center, Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Box 063, 800 Washington Street, Boston, MA 02111, United States
‖ Department of Radiology, Beth Israel Deaconess Medical Center, 4 Blackfan Circle, Boston, MA 02115, United States
¶ Design and Data Resource Center, Tufts Clinical and Translational Science Institute, Tufts Medical Center, Box 063, 800 Washington Street, Boston, MA 02111, United States
# Department of Research and Business Discovery, GELITA AG, Gammelsbacherstrasse 2, D-69412 Eberbach, Germany

ARTICLE INFO

SUMMARY

Objective: To determine whether either of two magnetic resonance imaging approaches — delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC), or T2 mapping — can detect short-term changes in knee hyaline cartilage among individuals taking a formulation of collagen hydrolysate.

Design: Single center, prospective, randomized, placebo-controlled, double-blind, pilot trial of collagen hydrolysate for mild knee osteoarthritis (OA). Participants were allowed to continue the prior analgesic use. The primary outcome was change in dGEMRIC T1 relaxation time in the cartilage regions of interest at the 24-week timepoint. Secondary endpoints included the change in dGEMRIC T1 relaxation time between baseline and 48 weeks, the change in T2 relaxation time at 0, 24 and 48 weeks, the symptom and functional measures obtained at each of the visits, and overall analgesic use.

Results: Among a sample of 30 randomized subjects the dGEMRIC score increased in the medial and lateral tibial regions of interest (median increase of 29 and 41 ms respectively) in participants assigned to collagen hydrolysate but decreased (median decline 37 and 36 ms respectively) in the placebo arm with the changes between the two groups at 24 weeks reaching significance. No other significant changes between the two groups were seen in the other four regions, or in any of the T2 values or in the clinical outcomes.

Conclusions: These preliminary results suggest that the dGEMRIC technique may be able to detect change in proteoglycan content in knee cartilage among individuals taking collagen hydrolysate after 24 weeks.

© 2011 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

Given the scale of the public health impact of osteoarthritis (OA), it is remarkable that it benefits from few, if any, disease-modifying medical remedies. Although a number of factors may have obfuscated the testing of effective interventions for OA, the absence of a useful biomarker of articular health has been a primary obstacle to drug development. However, the recent application of sophisticated imaging technology to evaluate structural change in osteoarthritic joints offers an opportunity to measure pathological progression in critical structures such as articular cartilage. Two magnetic resonance imaging (MRI)-based approaches for which the preliminary data indicate such potential include delayed gadolinium enhanced MRI of cartilage (dGEMRIC)[1] and T2 mapping[2].

dGEMRIC exploits the tendency of the negatively-charged contrast agent Gd-DTPA^{2-} to accumulate in hyaline cartilage in concentrations that correlate inversely with its proteoglycan content[1]. *Ex-vivo* studies have shown a strong correlation of the dGEMRIC index with quantitative measures of proteoglycan content and with semi-quantitative histologic scores[3]. A lower dGEMRIC index has been associated with regions of hyaline

cartilage damage ascertained on knee arthroscopy[4] and among patients with hip dysplasia syndrome[5]. The technique appears to discriminate between knees subjected to different levels of physical activity, both cross-sectionally[6] and longitudinally[7]. The T2 relaxation time, on the other hand, reflects the water content of cartilage tissue and correlates with age and symptomatic degeneration, albeit with qualitative differences in the nature of T2 increases attributable to age vs degeneration[8]. The parameter also appears to be immediately sensitive to change following exercise and rest[9]. Since changes in proteoglycan and water distribution and content are features of early OA, these techniques have the potential to operate as proxy indicators of the state of cartilage health. Leveraging these properties could allow efficacy testing of putative structure-modifying interventions for OA at an early stage of disease development. However, this possibility has not yet been tested.

As a product promulgated for benefits on cartilage health, collagen hydrolysate (CH) is representative of many putative OA interventions whose development and clinical validation would be greatly facilitated by the availability of an objective measure of articular cartilage health. CH is a nutritional product derived from animal collagenous tissues comprising a range of polypeptides with molecular weights from 3 to 10 kDa. CH induces the novel synthesis of type 2 collagen and proteoglycans in the extracellular matrix in a dose-dependent manner[10], is readily absorbed across the gastrointestinal mucosa in murine models and distributes to hyaline cartilage, where it accumulates[11]. In the STR/ort mouse model of spontaneous OA, oral administration of CH reduced both the development and progression of cartilage damage[12,13]. A range of clinical reports and observations suggest that CH may benefit symptoms of OA, with one randomized clinical trial producing mixed results[14].

Since our knowledge of both the measurement properties of these imaging techniques, and the *in-vivo* efficacy of CH, is insufficient to design a definitive clinical trial, we performed a pilot study to determine if dGEMRIC and T2 mapping can discriminate changes in knee hyaline cartilage among participants taking a CH preparation (Fortigel®) compared to those taking placebo.

Methods

Overview

The study was designed as a single center, prospective, randomized, placebo-controlled, double-blind, pilot trial with a planned enrollment of 30 participants with mild knee OA, testing the ability of dGEMRIC and T2 mapping to detect change in cartilage attributable to CH. The primary endpoint occurred at 24 weeks, with the possibility of a further 24-week extension contingent on a blinded interim analysis by a data and safety monitoring board. The study was approved by the Institutional Review Boards of Tufts − New England Medical Center (protocol #7598) and Beth Israel Deaconess Medical Center (protocol #2006-P-000151). It was conducted at Tufts − New England Medical Center under an FDA investigational new drug registration (IND #74249), and registered on the ClinicalTrials.gov website (identifier NCT00536302).

Sample

We recruited individuals aged 49 years or older with mild to moderate severity knee OA, based on an affirmative response to a standard knee pain question "during the past 6 months, have you had any pain in the knee more than half the days of the month?", a Western Ontario and McMaster Universities (WOMAC) osteoarthritis index pain subscale score greater than one, and at least one definite osteophyte on postero-anterior, weight-bearing, semi-flexed or lateral knee radiographs. In order to constrain the sample to those with milder disease, we required that their radiographs demonstrate relative preservation of tibiofemoral joint space (>3 mm). By definition, these criteria are consistent with the American College of Rheumatology classification system for knee OA[15]. Other eligibility criteria included that participants be prepared, during the course of the study, to refrain from use of agents purported to have effects on articular cartilage (such as glucosamine and chondroitin) and maintain a stable analgesic regimen. We excluded applicants who reported taking agents purported to have chondroprotective effects (such as glucosamine and chondroitin) within 14 weeks, used opioids on a chronic basis, were expecting to have knee arthroscopy or arthroplasty, had any contraindications to undergoing MRI or receiving intravenous gadolinium, had active pathology that could confound interpretation of knee pain or any conditions that could interfere with adherence.

Study intervention

The CH formulation employed in this study (Fortigel®) was a liquid that comprised 10 g of CH, 0.4 g fructose, malt extract, citric acid, xanthan, potassium sorbate, acesulfame-K, sucralose and natural and artificial flavors in 10 ml water. The placebo, which was identical in appearance and flavor, contained the identical excipients but no CH.

Randomization and blinding

Participants were randomized on a 1:1 basis to receive either CH or placebo using a computer-generated blocked randomization list, stratified by body mass index around a cut-point of 32 kg/m². The randomization list was generated by Dr Imma Fischer, biomathematician at the Institute for Medical Information Processing, the University of Tuebingen in Germany. The randomization list was retained by the sponsor, Gelita AG. The active and placebo formulations were provided in identical ampoules by Gelita AG. They were shipped to the Tufts − New England Medical Center research pharmacy, whose staff stored them in preparation for dispensation. The research pharmacy dispensed the study medication to participants in an 8-week supply.

Concomitant analgesic use

During the trial we permitted participants to continue taking their regular analgesics or nonsteroidal anti-inflammatory drugs. We encouraged them to maintain a fixed regimen when taking these medications during the course of the trial and required that they account for all consumption of such medications prospectively, on a paper calendar supplied by the study team.

Study assessments

Study evaluations comprised a screening visit and a baseline assessment (week 0) followed by scheduled visits at weeks 8, 16, 24, 32, 40, and 48, with a window of 7 days around each study evaluation timepoint.

MRI protocol

dGEMRIC and T2 MRI scans were acquired at baseline, 24 and 48 weeks. Patients were injected with 0.2 mmoles/kg Gd-DTPA²⁻ (Magnevist, Berlex, NJ) and asked to walk for 10 min to aid in the transport of the contrast agent into the tissue. 90 min post-injection dGEMRIC imaging was performed using quad knee coil on a 1.5 T Twin Speed Excite scanner (GE Healthcare, Waukesha, WI).

Thirty-two 3.0 mm sagittal slices were acquired using inversion recovery prepared fast spoiled 3D gradient echo acquisition with a flip angle $=20°$, square field of view $=14$ cm, matrix $=512 \times 512$, bandwidth $=62.5$ kHz. Five inversion delays (TI $= 1650$, 650, 350, 150, 28 ms) were used with a variable repetition time between consecutive inversion pulses (Tr) ranging from 360 ms to 1920 ms and 7.3/3.1 ms (Ts/Te).

Multi-slice fast spin echo Fat Sat Dual Echo (Tr 3000 ms, Te 88.3 ms, Etl 6, Nex 1, Matrix 512×512) images were acquired that covered the same section of the knee as the dGEMRIC series.

dGEMRIC and T2 mapping computational analyses

We used a software analysis package (MRIMapper, ©Beth Israel Deaconess Medical Center & Massachusetts Institute of Technology, 2006) to generate cartilage maps reflecting the dGEMRIC T1 and the T2 relaxation times. Three regions of interest (ROIs) were analyzed in each of the medial and lateral sections of the knee; the central weight-bearing medial/lateral femoral cartilage (cMF/cLF), the posterior medial/lateral femoral cartilage (pMF/pLF) and the medial/lateral tibial plateau cartilage (MT/LT). The dGEMRIC maps generated in Matlab used a pixel-by-pixel three-parameter T1 fit. The T2 images were used as a guide for cartilage segmentation. The dGEMRIC data were corrected for dose bias due to the subjects' varying body mass indexes, as previously described[16]. The dGEMRIC value reported is the average of a given ROI. T2 data were also analyzed using the same software in the three ROIs per section. For follow-up scans, the choice of section for analysis was visually matched to prior scans.

Clinical assessments

Screening and baseline assessments included posterior–anterior semi-flexed radiography according to a standardized protocol[17], a physical examination to document clinical features of knee OA and rule out alternative causes of knee pain, and measurement of height and weight. At each visit we assessed pain during the prior 24 h using the WOMAC-Likert scale version 3.0 and a global pain visual analog scale. We measured functional capacity using both the timed 20 m walking and chair stand tests. We documented overall concomitant analgesic consumption at each visit aided by paper calendars provided to each participant.

Evaluation of adherence

Dosing instructions were reviewed at each study visit by study staff. We instructed participants to return at each visit the study ampoules that had been dispensed to them at the previous encounter. These were collected and counted by study staff. We evaluated adherence as the number of used ampoules returned divided by the number of days elapsed since the last visit.

Adverse event surveillance

We instructed participants to record in their study calendar any changes to their health or medications during the study. We asked participants at each study visit if they had experienced any adverse experiences since the previous encounter. Adverse events were subjectively assessed by the principal investigator prior to unblinding of treatment allocation. Reported adverse events were recorded and classified according to the affected body system, severity (including need for hospitalization), inferred relation to investigational product (blinded), action taken, status and outcome.

Analytic plan

Our *a priori* primary outcome was the change in dGEMRIC T1 relaxation time in the cartilage ROIs between baseline and the
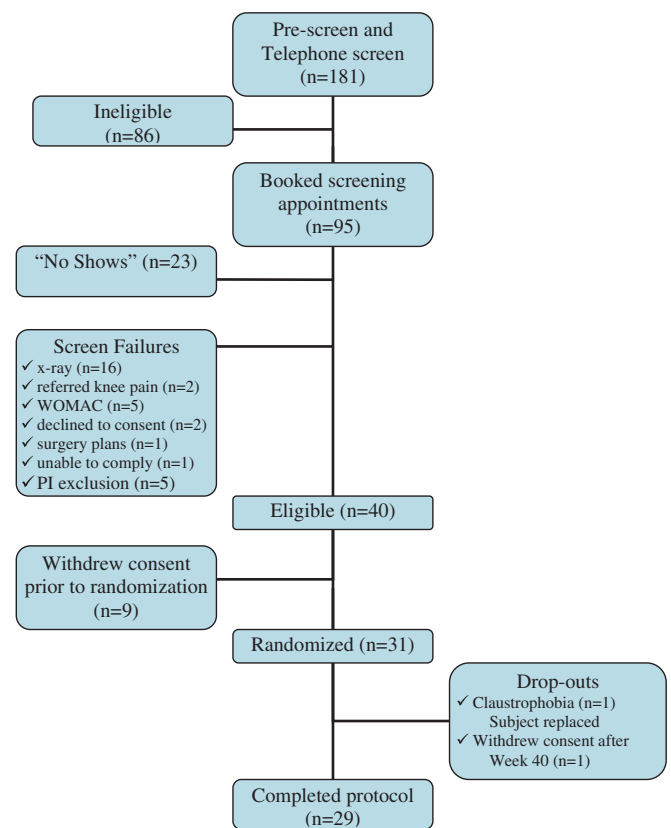

Fig. 1. Flow of recruitment.

24-week timepoint. Secondary endpoints included the change in the dGEMRIC T1 relaxation time between baseline and 48 weeks, the change in T2 relaxation time at 0, 24 and 48 weeks, the symptom and functional measures obtained at each of the visits, and overall analgesic use. Change was analyzed as both raw change from baseline as the primary measures and percent change from baseline as ancillary measures.

In order to evaluate trends in concomitant analgesic use in the groups, we transformed consumption of these pills into a single scale reflecting their relative efficacy in relation to acetaminophen.

Our general approach to evaluation of all endpoints was to test for differences between baseline and 24 weeks and baseline and 48 weeks by using a Students two-tailed *t*-test comparison of change between the two groups. However, in the event that the distribution of the outcomes did not conform to normality, we used non-parametric tests to compare groups.

Table I
Demographics and baseline characteristics of participants

	CH* ($n = 15$)	Placebo ($n = 15$)	P^*
Age, y, mean (SD)	58.9 (8.0)	60.3 (8.5)	0.65
Gender (female), n (%)	9 (60%)	9 (60%)	1.00
Race, n (%)			1.00
White	14 (93%)	13 (87%)	
Asian	1 (7%)	0	
Black	0	1 (7%)	
American Indian	0	1 (7%)	
BMI, kg/m², mean (SD)	30.1 (4.6)	31.2 (7.0)	0.60
KL score $= 2$ (vs 1), n (%)	14 (93%)	13 (87%)	0.54

* *P*-values are from tests comparing the CH and placebo groups using either the Student *t*-test (age, BMI), Fisher-exact test (race), or chi-square test (gender, KL).

Table II
Baseline values for the clinical outcome measures

	CH* ($n = 15$)	Placebo ($n = 15$)	P^*
WOMAC mean score (SD)			
Pain	4.6 (2.6)	5.8 (3.0)	0.24
Stiffness	2.9 (1.1)	2.8 (1.5)	0.89
Function	12.9 (8.7)	20.6 (10.9)	0.04
Total	20.3 (10.5)	29.2 (13.8)	0.06
20-m walk, mean seconds, (SD)	16.8 (2.4)	19.7 (4.4)	0.04
Chair stand mean seconds, (SD)	14.7 (3.5)	17.7 (5.9)	0.10
Analgesic consumption			
Median mg, <interquartile range>	0 (0–127587)	0 (0–6153)	0.70
Any, n (%)	7 (47%)	6 (40%)	0.71

* P-values from two-sample Student t-test (clinical measures), Wilcoxon rank-sum test (mg of analgesics) and chi-square test (any analgesics).

Statistical power

Prior studies provided insights into the extent of variability we would encounter in the dGEMRIC analyses. For example, a comparison of OA vs non-diseased cartilage found T1 values for non-diseased vs OA cartilage of 380 ms vs 290 ms, with standard deviation of 80 ms[4]. A sample size of 15 in each group, a two-tailed test and a significance level of 0.05, would provide 84% power to detect change of this magnitude. For a change of 40 ms (an effect size of 0.5) power fell to 38%.

Results

Sample

We screened 181 applicants between April and November 2007 to reach the target enrollment goal of 30 subjects (Fig. 1). Study participation ended 11/13/2008 per protocol. One randomized subject withdrew because of claustrophobia in the MRI scanner. This subject was replaced by randomizing an additional subject thus increasing the actual number of randomized subjects to 31 in

order to achieve the target number of 30 enrolled and analyzable subjects, or 15 subjects per group. Another participant withdrew consent after the Week 40 study visit. The active and placebo groups were reasonably matched on most demographic characteristics, although there was a nonsignificant trend toward greater body mass index in the placebo group (Table I). With respect to the balancing of disease severity, there was some evidence for slightly greater impairment in the group randomized to placebo (Table II). There were no significant differences between the active and control in the baseline values for the dGEMRIC index scores or T2 values (Tables III and IV).

Adherence

Overall, the dosing adherence for the study was 96.2% for all participants (range 49–100%). There was no difference in compliance between the groups (96.6 vs 95.8%).

dGEMRIC

Figure 2 displays examples of dGEMRIC images manifesting change. Since there was evidence of non-normality of distribution of the outcome measures, we conservatively employed a Wilcoxon rank-sum (non-parametric) test for the unadjusted analyses.

We found statistically significant differences in change scores between randomization groups in the medial tibia at 24 weeks ($P = 0.03$) and lateral tibia at 24 weeks ($P = 0.02$) (Table III). Although differences in the regions appeared to persist, the P-values associated with the group differences in change scores at 48 weeks were $P = 0.08$ for the medial tibia and $P = 0.07$ for the lateral tibia and thus did not meet statistical significance. All other group differences in change from baseline had P-values >0.10. However, there was a fair amount of variability between regions with apparent (nonsignificant) trends in opposing directions.

We further explored these data using percent change from baseline and using non-parametric and parametric methods (t-test

Table III
Median and mean baseline dGEMRIC index scores and 24 and 48 week change from baseline scores

	Median (25th–75th percentile) Mean ± standard deviation					
	CH			Placebo		
	Baseline ($n = 15$)	24 Weeks (change from baseline) ($n = 15$)	48 Weeks (change from baseline) ($n = 15$)	Baseline ($n = 15$)	24 Weeks (change from baseline) ($n = 14$)	48 Weeks (change from baseline) ($n = 13$)
Site						
Medial tibia	410.1	28.6*	47.6	427.6	−37.4	23.3
	(333.1 to 442.6)	(−29.5 to 74.4)	(15 to 81.9)	(375.4 to 495.2)	(−93.3 to 19.8)	(−27.2 to 44.7)
	396.5 ± 82.9	29.6 ± 70.5	41.4 ± 70.9	441.8 ± 75.6	−30.0 ± 62.6	2.4 ± 64.4
Central medial femur	457.5	−15.6	17.1	468.2	21.6	−4.7
	(420.2 to 496.2)	(−56.2 to 60.5)	(−71 to 33.3)	(384.2 to 512.6)	(−11.3 to 63.7)	(−56.1 to 22.3)
	451.4 ± 53.0	10.3 ± 72.3	−16.7 ± 75.2	447.3 ± 73.4	23.5 ± 47.0	−14.3 ± 52.1
Posterior medial femur	475.2	27.7	15.3	445.3	21.9	−5.7
	(415.6 to 517.1)	(−16.4 to 44.8)	(−27.8 to 64.7)	(404.4 to 534.6)	(−32.5 to 64.2)	(−32 to 20.2)
	465.1 ± 61.6	13.6 ± 57.6	17.5 ± 63.0	458.9 ± 74.6	6.8 ± 68.1	−2.2 ± 55.4
Lateral tibia	472.3	40.7*	28.3	508	−35.9	−1.3
	(362.5 to 550)	(−26.6 to 75.5)	(1.6 to 75.4)	(416.3 to 562.6)	(−60.3 to 18.5)	(−55.7 to 21.3)
	455.7 ± 90.4	25.5 ± 60.2	20.0 ± 65.0	483.8 ± 89.5	−28.5 ± 47.8	−17.3 ± 55.3
Central lateral femur	471.2	−18.2	−5.4	461.6	30.5	3.2
	(428.6 to 495.3)	(−30.5 to 103.4)	(−25.3 to 31.1)	(380.9 to 502.3)	(−8.3 to 56.7)	(−4.7 to 25.7)
	460.6 ± 66.9	7.2 ± 69.9	−2.5 ± 47.1	448.1 ± 72.7	31.3 ± 60.1	15.2 ± 43.7
Posterior lateral femur	446.6	38.4	30.8	440	27.2	19.0
	(364.4 to 477.5)	(−10.2 to 65.4)	(−54.6 to 55.7)	(394.2 to 477.4)	(−15.8 to 70.2)	(−25.1 to 93)
	443.9 ± 87.9	31.5 ± 68.1	6.2 ± 77.9	434.0 ± 52.3	24.3 ± 57.8	40.3 ± 88.3

* Statistically significant differences in change scores between randomization groups were found for medial tibia at 24 weeks ($P = 0.03$) and lateral tibia at 24 weeks ($P = 0.02$). No other statistically significant differences (at $P \leq 0.05$ level) were found. The P-values associated with the group differences in change scores between for medial tibia at 48 weeks ($P = 0.08$) and lateral tibia at 48 weeks ($P = 0.07$) were <0.10 and all other group differences in change from baseline had P-values >0.10. All reported P-values are from the Wilcoxon rank-sum test.

Table IV
Median baseline T2 index scores and 24- and 48-week change from baseline scores

	CH			Placebo		
	Baseline	24 Weeks (change from baseline)	48 Weeks (change from baseline)	Baseline	24 Weeks (change from baseline)	48 Weeks (change from baseline)
Sample size	14	14	11	15	15	11
Medial tibia	35.5	1.0	1.0	35.0	1.0	1.0
Central medial femur	41.0	−0.5	1.0	40.0	0.0	−1.0
Posterior medial femur	47.5	−0.5	−2.0	45.0	1.0	−2.0
Lateral tibia	33.0	2.0	1.0	35.0	0.0	−1.0
Central lateral femur	43.5	−1.5	−2.0	42.0	−1.0	0.0
Posterior lateral femur	46.5	1.0	−1.0[*]	45.0	0.0	0.0

[*] A statistically significant difference ($P < 0.05$) in change scores between randomization groups was found for the posterior lateral femur region at 48 weeks. No other statistically significant differences were detected. The Wilcoxon rank-sum test was used.

for unadjusted analyses). Their results were not substantially different to those of the prior analyses.

Secondary outcomes

When the T2 values over time were evaluated, it became apparent that there was very little change in the ROIs (Table IV). As with the T1 analysis, we chose to present results from the more conservative non-parametric analysis used to compare the change from baseline values between the two groups (although results from the *t*-test were very similar, data not shown). Statistical analysis with a Wilcoxon rank-sum test failed to show significant differences between the groups except at 48 weeks in the posterior lateral femur region where it decreased (median change = −1.0, mean change = −1.1) in the CH group and increased (median change = 0, mean change = +1.8) in the placebo group ($P = 0.05$).

The clinical indices broadly improved in both groups without any major differences between them, except perhaps a trend toward greater improvement in the stiffness subdomain of the WOMAC in the CH group (Table V). However, the groups were imbalanced at baseline for a number of these outcomes.

There was no significant difference in the amount of analgesic use (median of acetaminophen equivalents) at any of the study timepoints, nor in the total amount accumulated over the 48-week period.

Adverse events

There were 43 adverse events reports (13 participants) among the CH group and 45 (13 participants) among the placebo group. Only one event (appendicitis) was classified as serious and this occurred in the CH group and was considered unrelated to treatment. The relationship to treatment among the remaining 42 adverse events in the CH group was 'unrelated' for 39 and 'unlikely' for four. There were no serious adverse event reports among the placebo group. The relationship to treatment among the
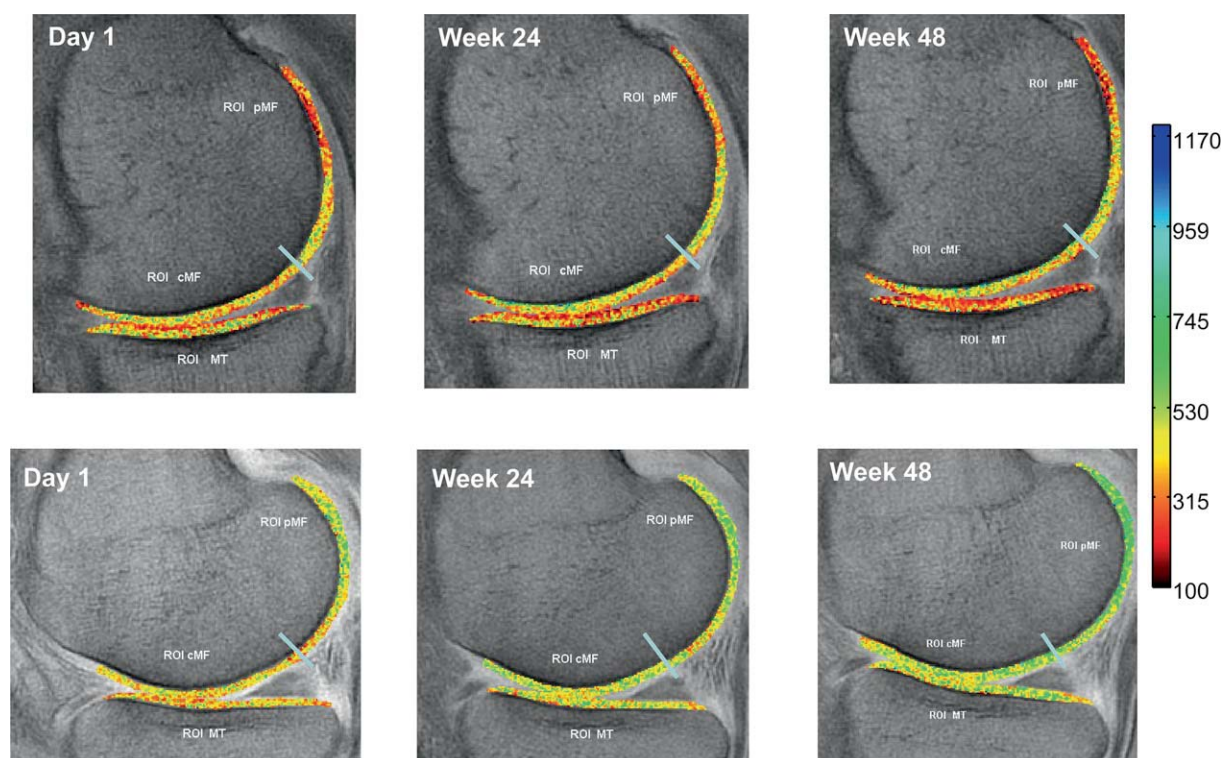

Fig. 2. Example of dGEMRIC images from the (top) placebo, and (bottom) CH group. The lines delineate the separation between the central femoral ROI and the posterior femoral ROI. Over time, more red areas in the tibial plateau in the placebo case illustrate the decrease in the dGEMRIC index, while the shift from red to yellow/green in the CH example illustrates an increase in the dGEMRIC index.

Table V
Change in clinical indices at 24 and 48 weeks

	CH		Placebo	
	24 Weeks	48 Weeks	24 Weeks	48 Weeks
Sample size	15	14	15	15
WOMAC				
Pain	−1.5 (2.8)	−2.3 (2.6)	−1.4 (3.5)	−1.9 (4.3)
Stiffness	−1.2 (0.9)	−1.2 (1.5)	−0.5 (1.1)	−0.6 (1.4)
Function	−3.4 (6.9)	−3.2 (4.6)	−4.7 (6.9)	−7.1 (9.5)
Total	−6.1 (8.9)	−6.7 (6.4)	−5.7 (9.8)	−9.6 (14.1)
20-m walk (s)	−1.3 (1.2)	−1.8 (0.9)	−2.1 (1.7)	−2.2 (2.4)
Chair stand (s)	−1.4 (2.1)	−2.1 (1.9)	−2.0 (2.1)	−2.8 (3.5)

All reported values are (mean ± SD). The change in 24-week WOMAC stiffness score between randomization groups was of borderline statistical significance ($P = 0.08$). No other between-group differences were detected. The Student t-test was used.

remaining 45 adverse events in the placebo group was 'unrelated' for 44 and 'unlikely' for one.

Discussion

We designed this pilot study to test of the potential of dGEMRIC to detect a change in cartilage status in the setting of a randomized clinical trial of CH, a developmental product targeted for OA. The dGEMRIC index has been validated as a technique to indicate proteoglycan concentration within hyaline cartilage, with seminal studies that included pathological correlation[1]. We chose to apply dGEMRIC to test CH because this compound is being developed specifically as an agent to promote cartilage health. CH is absorbed following oral administration and distributed to joints[11]. The compound stimulates cultured chondrocytes to synthesize type 2 collagen and proteoglycans[10]. In other *in-vivo* experiments, it retarded both the development and progression of OA lesions[12]. Our pre-specified primary outcome was change in the dGEMRIC index in the medial compartments at the 24-week timepoint.

Our results showed varying directions of change in the dGEMRIC index among the ROIs, with significant differences between groups only in the medial and lateral tibial cartilage regions. In these regions the dGEMRIC score increased among those assigned to CH but decreased in the placebo group. Similar trends, however, were not observed in the other cartilage ROIs. Indeed, there was considerable variability in the dGEMRIC outcome data between the different regions, with apparent, nonsignificant, trends in opposite directions. Since this was a small pilot study, it is possible that these changes might all have occurred within the range of random effects. Alternatively, these inconsistencies could reflect regionally directed benefits of CH, perhaps contingent on the distribution of extant pathology. Therefore, we view our results as an affirmation that the dGEMRIC technique, and the CH intervention, merit further testing in a larger study. However, because of the pilot nature of this study, and its small sample size, we do not regard these results to be definitive.

In contrast to the dGEMRIC results, the T2 cartilage measures showed little variability or change with time, and no differences in any region between groups.

Also negative were our secondary clinical outcome measures, including measures of pain and physical function. Both groups exhibited improvement in scores, likely reflecting regression to the mean. However, the participant groups were unbalanced at baseline with respect to clinical severity, and there was substantial variability in the clinical outcomes data, which may have limited our ability to detect differences. Also, we allowed the participants to continue their use of prior analgesics, which may have attenuated any clinical efficacy. Future studies should use an approach to

mitigate this possibility, such as the use of an analgesic washout period prior to each pain assessment.

It is pertinent that we constrained our sample to individuals with relatively mild knee OA. The underlying reasoning was twofold − firstly, the dGEMRIC technique requires sufficient remaining cartilage to provide reliable measurements − secondly, we infer from the laboratory data on CH that its potential to stimulate an effective chondrocyte response may be predicated on the presence of intact collagenous matrix. Consequently, the results of this study may be generalizable only to mild OA and not necessarily to those with disease of greater severity.

Additional limitations of this study relate to its small sample size and the absence of morphometric MRI sequences. These factors precluded an analysis of the relationship of macroscopic cartilage damage to the dGEMRIC and T2 measures, which are questions of interest that could be addressed in future appropriately-designed studies.

In conclusion, we have been able to deploy dGEMRIC in a pilot clinical trial of a developmental product for knee OA and have detected apparent changes in tibial cartilage proteoglycan concentration within a 6-month observation period.

Role of the funding source

Author contributions

All authors made substantial contributions to (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the manuscript as follows:

- Conception and design: Timothy E. McAlindon, Deborah Burstein, John Griffith, Klaus Flechsenhar.
- Analysis and interpretation of the data: Timothy E. McAlindon, Nitya Krishnan, Robin Ruthazer, Lori Lyn Price, Deborah Burstein, John Griffith.
- Drafting of the article: Timothy E. McAlindon, Melynn Nuite, Deborah Burstein, Klaus Flechsenhar.
- Critical revision of the article for important intellectual content: Timothy E. McAlindon, Melynn Nuite, Robin Ruthazer, Lori Lyn Price, Deborah Burstein, John Griffith, Klaus Flechsenhar.
- Final approval of the article: all authors.
- Provision of study materials or patients: Timothy E. McAlindon, Melynn Nuite, Klaus Flechsenhar.
- Statistical expertise: Robin Ruthazer, Lori Lyn Price, John Griffith.
- Obtaining of funding: Timothy E. McAlindon, Klaus Flechsenhar.
- Administrative, technical, or logistic support: Nitya Krishnan, Deborah Burstein, Klaus Flechsenhar.
- Collection and assembly of data: Melynn Nuite, Nitya Krishnan, Robin Ruthazer, Lori Lyn Price, Deborah Burstein.
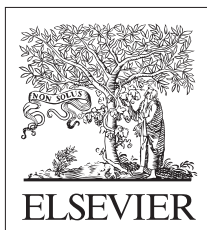
Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.joca.2011.01.001.

References

1. Gray ML, Burstein D, Kim YJ, Maroudas A, 2007 Elizabeth Winston Lanier Award Winner. Magnetic resonance imaging of cartilage glycosaminoglycan: basic principles, imaging technique, and clinical applications. J Orthop Res 2008;26:281–91.
2. David-Vaudey E, Ghosh S, Ries M, Majumdar S. T2 relaxation time measurements in osteoarthritis. Magn Reson Imaging 2004;22:673–82.
3. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 1999;41:857–65.
4. Tiderius CJ, Olsson LE, Leander P, Ekberg O, Dahlberg L. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) in early knee osteoarthritis. Magn Reson Med 2003;49:488–92.
5. Cunningham T, Jessel R, Zurakowski D, Millis MB, Kim YJ. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage to predict early failure of Bernese periacetabular osteotomy for hip dysplasia. J Bone Joint Surg Am 2006;88:1540–8.
6. Tiderius CJ, Svensson J, Leander P, Ola T, Dahlberg L. dGEMRIC (delayed gadolinium-enhanced MRI of cartilage) indicates adaptive capacity of human knee cartilage. Magn Reson Med 2004;51:286–90.
7. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis. Arthritis Rheum 2005;52:3507–14.
8. Mosher TJ, Dardzinski BJ. Cartilage MRI T2 relaxation time mapping: overview and applications. Semin Musculoskelet Radiol 2004;8:355–68.
9. Liess C, Lusse S, Karger N, Heller M, Gluer CC. Detection of changes in cartilage water content using MRI T2-mapping in vivo. Osteoarthritis Cartilage 2002;10:907–13.
10. Oesser S, Seifert J. Stimulation of type II collagen biosynthesis and secretion in bovine chondrocytes cultured with degraded collagen. Cell Tissue Res 2003;311:393–9.
11. Oesser S, Adam M, Babel W, Seifert J. Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). J Nutr 1999;129: 1891–5.
12. Oesser S, Raabe A, Schunck M. Orally administered collagen hydrolysate halts the progression of osteoarthritis in STR/ort mice. Osteoarthritis Cartilage 2007;15(Suppl C):94–5.
13. Oesser S, Proksch E, Schunck M. Prophylactic treatment with a special collagen hydrolysate decreases cartilage tissue degeneration in the knee joints. Osteoarthritis Cartilage 2008;16(Suppl 4):S45.
14. Moskowitz RW. Role of collagen hydrolysate in bone and joint disease. Semin Arthritis Rheum 2000;30:87–99.
15. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al.* Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29: 1039–49.
16. Tiderius C, Hori M, Williams A, Sharma L, Prasad PV, Finnell M, *et al.* dGEMRIC as a function of BMI. Osteoarthritis Cartilage 2006;14:1091–7.
17. Buckland-Wright C. Protocols for precise radio-anatomical positioning of the tibiofemoral and patellofemoral compartments of the knee. Osteoarthritis Cartilage 1995;3(Suppl A): 71–80.

951000440