A Prospective, Randomized, Open-Label, Multicenter, Phase III Noninferiority Trial to Compare the Clinical Efficacy of Matrix-Associated Autologous Chondrocyte Implantation With Spheroid Technology Versus Arthroscopic Microfracture for Cartilage Defects of the Knee

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Background: Autologous chondrocyte implantation (ACI) and microfracture are established treatments for large, full-thickness cartilage defects, but there is still a need to expand the clinical and health economic knowledge of these procedures.

Purpose: To confirm the noninferiority of ACI compared with microfracture.

Study Design: Randomized controlled trial; Level of evidence, 2.

Methods: Patients were randomized to be treated with matrix-associated ACI using spheroid technology (n = 52) or microfracture (n = 50). Both procedures followed standard methods. Patients were assessed by the Knee injury and Osteoarthritis Outcome Score (KOOS), MOCART (magnetic resonance observation of cartilage repair tissue) scoring system, Bern score, modified Lysholm score, International Cartilage Repair Society (ICRS) rating (histological and immunochemical scoring after rebiopsy 24 months after implantation), and International Knee Documentation Committee (IKDC) examination form. The main assessments were conducted 24 months after study treatment.

Results: In the primary intention-to-treat analysis, the overall KOOS score for both ACI and microfracture yielded a statistically significant improvement relative to baseline. According to the between-group analysis, ACI passed the test of noninferiority compared with microfracture; thus, the primary goal of the study was achieved. The KOOS subscores yielded the same qualitative results as the overall KOOS score (ie, for each of these, noninferiority was demonstrated), and in 1 case (Activities of Daily Living subscore), the threshold for superiority was passed. The subgroup analyses did not yield any clear evidence of an association between treatment effect and any of the categories investigated (age, diagnosis, defect localization, sex). A histological analysis of biopsies from 16 patients (ACI: n = 9; microfracture: n = 7) suggested a better quality of repair in the patients treated with ACI.

Conclusion: The efficacy of both ACI and microfracture was demonstrated with respect to both functional outcomes and morphological repair. The primary analysis confirmed the statistical hypothesis of the noninferiority of ACI, even for relatively small cartilage defects (1-4 cm^2) treated in this study, the indication for which microfracture is generally accepted as the standard of care. ACI showed significant superiority in the KOOS subscores of Activities of Daily Living at 24 months and Knee-related Quality of Life at 12 months.

Registration: NCT01222559 (ClinicalTrials.gov identifier).

Keywords: autologous chondrocyte implantation; cartilage lesion; knee surgery; MOCART; KOOS; randomized clinical trial

Autologous chondrocyte implantation (ACI) was introduced by Brittberg et al[6] in 1994 and has since occupied an increasingly important position in the treatment of

cartilage defects. Currently, ACI is a standard procedure for the treatment of moderately large to large cartilage defects of the knee[18] and is recommended by national and international medical associations. Such recommendations are based on ever-stronger clinical and scientific evidence. Long-term results of the treatment are now available, including patient data obtained up to 20 years. Moreover, many prospective randomized studies have been reported in which this therapeutic approach has been compared systematically with other methods—above all, the techniques of bone marrow stimulation.[3,13,14,24,25] Hitherto, studies have revealed unambiguously that the structural results of ACI are superior to those of therapies based on bone marrow stimulation.[10,22,25,26] However, not all these studies yielded a clear demonstration of the clinical superiority of ACI, and this appears to have been dependent, at least in part, on the exact microfracture method used. The superiority of advanced (ie, second- or third-generation) ACI compared with bone marrow stimulation is further supported by studies in which a direct comparison was made between the various ACI techniques and by meta-analyses that revealed the superiority of the more recent ACI modalities.[17,20,22] These studies confirmed the superiority of ACI, particularly for larger defect sizes; in contrast, the hypothesis of superiority for smaller defect sizes remains unconfirmed, so that expert associations recommend ACI only as a second-line treatment for this indication.[18]

Against this background, it seems important also to examine more recent developments of ACI and to adduce scientific evidence for its effectiveness. The matrix-associated ACI procedure used in this study is based on spheroid technology (Spherox; CO.DON).[27] The general principle is based on the acquisition of the patient's own healthy chondrocytes derived from a nonweightbearing part of the knee. These chondrocytes are cultured in vitro in monolayer and 3-dimensional culture, where they form spheroids (=active substance), which are then transplanted into the cartilage defect. The cultivation process does not employ any exogenous stimuli or growth factors. The finished product is applied as spheroids consisting of chondrocytes and their own extracellular matrix in a physiological NaCl solution. The transplanted spheroids adhere to the debrided surface of the defect, synthesize hyaline-like matrix components de novo, and thereby are integrated into the surrounding tissue, filling the clefts of the defect. The product is able to adhere to the various cartilage defect areas, where it fills the defects with an extracellular matrix protein without the need for an exogenous matrix material (as in other matrix-associated ACI procedures) or exogenous fixation with a periosteal flap (as in first-generation ACI).[1,2,27]

Several nonrandomized studies, as well as a recently completed prospective, randomized, multicenter, phase II study, have confirmed the effective and safe treatment by matrix-associated ACI with spheroid technology, especially for defect sizes up to 10 cm^2.[4,19]

In the present trial, our aim was not only to assess the clinical results of the treatment in direct comparison with arthroscopic microfracture by using a prospective randomized study design but also to judge the quality of the regenerated cartilage by means of standardized magnetic resonance imaging (MRI).

Since 2007, ACI has been classified by European regulators as an advanced therapy medicinal product and placed formally under medical law. Moreover, as ACI, unlike microfracture, involves 2 surgical procedures, the present study focused in particular on the aspect of patient safety in connection with the use of the product.

METHODS

Study Design and Surgical Treatment

This multicenter, phase III clinical trial was designed prospectively to compare the efficacy and safety of 2 treatments: (1) ACI using Spherox (formerly called chondrosphere) and (2) microfracture. The trial was randomized with a fixed block size (6) stratified prospectively by age into 2 classes (18-34 and 35-50 years). Patients were allocated randomly to the ACI or microfracture group on a 1:1 basis. Randomization was performed by telephone by the research staff immediately during surgery because defect size (criterion for inclusion in this study: 1-4 cm^2) could not be determined earlier.

The different procedures required by the 2 treatments prevented blinding. However, MRI scans were assessed by an independent reader, and histological assessments

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were conducted by an independent pathologist, both of whom were blinded to the treatment performed.

Sample size calculation considered the overall Knee injury and Osteoarthritis Outcome Score (KOOS) result (change from baseline) and was focused on the test of non-inferiority to compare ACI with microfracture (1-sided α = .025; power = 80%; lower confidence limit = –8.5; expected mean difference = 0; SD = 15). The margin was derived from a minimal important difference of 8 to 10 points reported for the KOOS. It was possible to reduce the sample size by using repeated-measures analysis of covariance (ANCOVA) to estimate the overall effect (10% reduction). Hence, a minimum sample size of 90 (ie, 45 microfracture and 45 ACI) was calculated; this included an assumed drop-out rate of 7.5% for the microfracture group and 15.5% for the ACI group (greater for ACI than for microfracture because of the risk of insufficient cell culture). This led to a final sample size of 102 (50 microfracture and 52 ACI).

The study was conducted in full compliance with standard protocols, the principles laid down in the Declaration of Helsinki, Good Clinical Practice guidelines, and all relevant laws and regulations. The protocol and informed consent form for this study were approved in writing by the appropriate ethics committees in Germany and Poland, where the study was conducted, before any patient was recruited.

After approval of the protocol and informed consent form by the appropriate ethics committees and registration of the study, patients who consented to take part in the trial were included, between December 2010 and December 2014, at 8 German and 3 Polish orthopaedic centers. Follow-up was planned for up to 5 years, to be completed in August 2020. The results presented here are derived from the 2-year follow-up analysis, which was completed in August 2017.

In all patients, the indication for study participation was determined during routine arthroscopic surgery of the affected knee joint. Only symptomatic, unipolar, full-thickness focal cartilage defects of International Cartilage Repair Society (ICRS) grade 3 or 4, with a defect size between 1 and 4 cm^2 and with intact adjacent cartilage, were included. Final eligibility was assessed by arthroscopic surgery of the affected knee. Inclusion and exclusion criteria are summarized in Table 1. Patients were stratified at recruitment by age group (see above); there was no other stratification of recruitment.

Surgical Techniques and Rehabilitation

Patients were randomized (1:1) to treatment by ACI or microfracture. Treatment by ACI required 2 surgical interventions: biopsy to obtain cartilage cells that were used to grow chondrocytes in vitro and subsequent implantation of the chondrocytes; details have been given in an earlier publication.[19] Both biopsy and transplantation could be performed either mini-open or arthroscopically. Microfracture, a single intervention, was performed according to Steadman et al.[28] The number of spheroids implanted was to be within the manufacturer's normal recommended

TABLE 1
Main Inclusion and Exclusion Criteria[a]

Inclusion Criteria
– Age of 18-50 years inclusive (male or female patient)
– Isolated, symptomatic full-thickness cartilage defects (ICRS grade 3 or 4)
– Chondral defect size of 1-4 cm^2 after debridement to healthy cartilage and maximum depth of 6 mm
– Nearly intact chondral structure surrounding the defect as well as the corresponding joint area
– Willingness to accept restrictions on analgesics (only paracetamol and/or topical nonsteroidal anti-inflammatory drugs allowed during trial and discontinuation of pain medication required 1 week before each visit) and to follow the strict rehabilitation protocol and follow-up program

Exclusion Criteria
– Defects in both knees at the same time
– Radiological signs of osteoarthritis
– Any signs of knee instability
– Valgus or varus malalignment (>5° over the mechanical axis)
– Clinically relevant second cartilage lesion on the same knee
– More than 50% resection of the meniscus in the affected knee or an incomplete meniscal rim
– Rheumatoid arthritis, parainfectious or infectious arthritis, or a condition after these diseases
– Pregnancy and planned pregnancy (because MRI was thus impossible)
– Obesity (body mass index >30 kg/m^2)
– Previous treatment with ACI in the affected knee
– Microfracture performed less than 1 year before screening in the affected knee
– Meniscal implant in the affected knee
– Meniscal suture (in the affected knee) 3 months before baseline
– Mosaicplasty (osteoarticular implant system) in the affected knee
– Hyaluronic acid intra-articular injections in the affected knee within 3 months before baseline
– Specific osteoarthritis drugs (such as chondroitin sulfate, diacerein, N-glucosamine, piascledine, or capsaicin) in the 2 weeks before baseline
– Corticosteroid treatment by an intra-articular route within the month before baseline or systemic (all routes) corticosteroids within 2 weeks before baseline
– Chronic use of anticoagulants
– Current diagnosis of osteomyelitis, human immunodeficiency virus (1 or 2), and/or hepatitis C infection

[a]Complete inclusion and exclusion criteria are provided in the public database at ClinicalTrials.gov (identifier: NCT01222559). ACI, autologous chondrocyte implantation; ICRS, International Cartilage Repair Society; MRI, magnetic resonance imaging.

range of 10 to 70 per cm^2, which was confirmed in the phase II clinical trial.[4,19]

After surgery, all patients followed a standardized rehabilitation protocol appropriate for their respective surgical treatment. Partial weightbearing was recommended for 6 weeks with 10 to 20 kg, starting on the first day after surgery. Regaining full weightbearing was recommended within weeks 7 to 8 and at the latest after 12 weeks.

Continuous passive motion was started from the day after surgery for 6 weeks, increasing from 0°-0°-60° in the first week to 0°-0°-90° by week 6. Within the first 6 weeks, physical therapy was aimed primarily at the reduction of swelling, isometric quadriceps activity, and mobilization. From week 7 onward, an increase to full range of motion was encouraged, proprioceptive and muscular training was increased, and cycling or aquajogging was permitted. Physical therapy was adjusted to the individual joint status and complaints, while return to high-impact sports was recommended after 12 months at the earliest.

Assessment Criteria

Patients were assessed at baseline and then 6 weeks and 3, 6, 12, 18, and 24 months after treatment. The principal assessment was performed by using the KOOS, which has been validated for focal cartilage lesions.[5,12,23] Other assessments included the MOCART (magnetic resonance observation of cartilage repair tissue) scoring system,[9] the modified Lysholm score, the International Knee Documentation Committee (IKDC) examination form, the histological Bern score, and the ICRS (histological and immunochemical) rating.[8,23] Safety variables were adverse events, vital signs, electrocardiography findings, physical examination findings, concomitant pain medications, and laboratory values.

Statistical Analysis

The primary variable was the overall KOOS score at 24 ± 2 months after treatment. The primary analysis was performed according to a prospectively defined hierarchical scheme. First, the ACI group was tested for relevant clinical improvement from baseline. If a significant difference ($P < .05$) was found, then the 2 treatment groups were compared by repeated-measures ANCOVA: if the lower 95% confidence limit for the difference between the changes in the overall KOOS score was above –8.5, ACI was to be regarded as significantly noninferior to microfracture, and if this lower confidence limit was positive, then ACI was to be regarded as superior to microfracture. The study was powered for noninferiority but not for superiority. All testing other than the above was at the descriptive level or analyzed exploratively at the full level of significance ($\alpha = 5\%$).

The analyses described here were performed with the intention-to-treat (ITT) population, defined as comprising all patients who (1) were successfully randomized, (2) underwent either ACI on the day of implantation or microfracture on the day of arthroscopic surgery, and (3) completed the KOOS questionnaire at baseline. A supporting per-protocol (PP) analysis was performed. The overall KOOS and MOCART scores were investigated for prospectively defined subgroups for the ITT and PP populations (age as stratified variable [as defined above], diagnosis, defect localization, sex). All parameters were tabulated by treatment group, in which categorically scaled variables are presented as absolute and relative frequencies and continuously scaled variables are reported as mean

± SD. In case of relevant clinical improvement, the least squares mean (difference in the KOOS score from baseline to 24 months) was used to estimate the adjusted 1-sided 97.5% CI. The mean overall KOOS score as well as the change from baseline were illustrated using error bars by treatment.

The clinical improvement from baseline was also analyzed for secondary variables using a 1-sample t test: the Bern score, modified Lysholm score, and IKDC score (current health assessment form, subjective knee evaluation form). Furthermore, noninferiority and superiority analyses were performed for the KOOS subscales (Pain, Other Symptoms, Function in Activities of Daily Living, Function in Sports and Recreation, and Knee-related Quality of Life).

The Kruskal-Wallis test was applied for the final grade or IKDC knee examination form. The change in the grade from baseline was analyzed by the Wilcoxon signed-rank test.

The change from baseline of the overall KOOS and MOCART scores was further investigated for prospectively defined subgroups: age as a stratification variable (18-34 and 35-50 years), diagnosis (traumatic cartilage lesion, osteochondritis dissecans, osteoarthritis, avascular necrosis, and other), defect localization (femur, tibia, and patella), and sex (male and female). For the overall KOOS and MOCART scores, a Spearman correlation analysis was performed for each visit and dosage group.

The frequencies of adverse events (or serious adverse events) were tabulated by severity, relationship to the study drug, preferred term/system organ class, and outcome for each treatment group as well as overall. Statistical analyses were performed by StatConsult.

RESULTS

Patient Population

The total population comprised 102 patients (61 male, 41 female) aged 37 ± 9 years. Figure 1 shows an illustration of the grouping and flow of patients within the trial. Demographic and baseline data are summarized in Table 2. The treatment groups were well balanced with respect to demographics and disease background. A minor imbalance with respect to smoking habit was considered irrelevant. Primary defect locations were all in the femur (70% medial and 30% lateral in both groups), with 1 patient also showing a patellar defect (and thus violating an inclusion criterion); defects of the patella alone or the tibia were not represented. ICRS grades were adequately balanced between the treatment groups, with more than one-half of defects in both groups being of grade 4.

Defect sizes before debridement ranged from 0.5 to 4.0 cm^2 and were likewise well balanced between groups. Compliance with the study treatment and subsequent rehabilitation measures (which were completed by all the study patients) was good. Major protocol violations were mainly missed visits including loss to follow-up (8 and 5
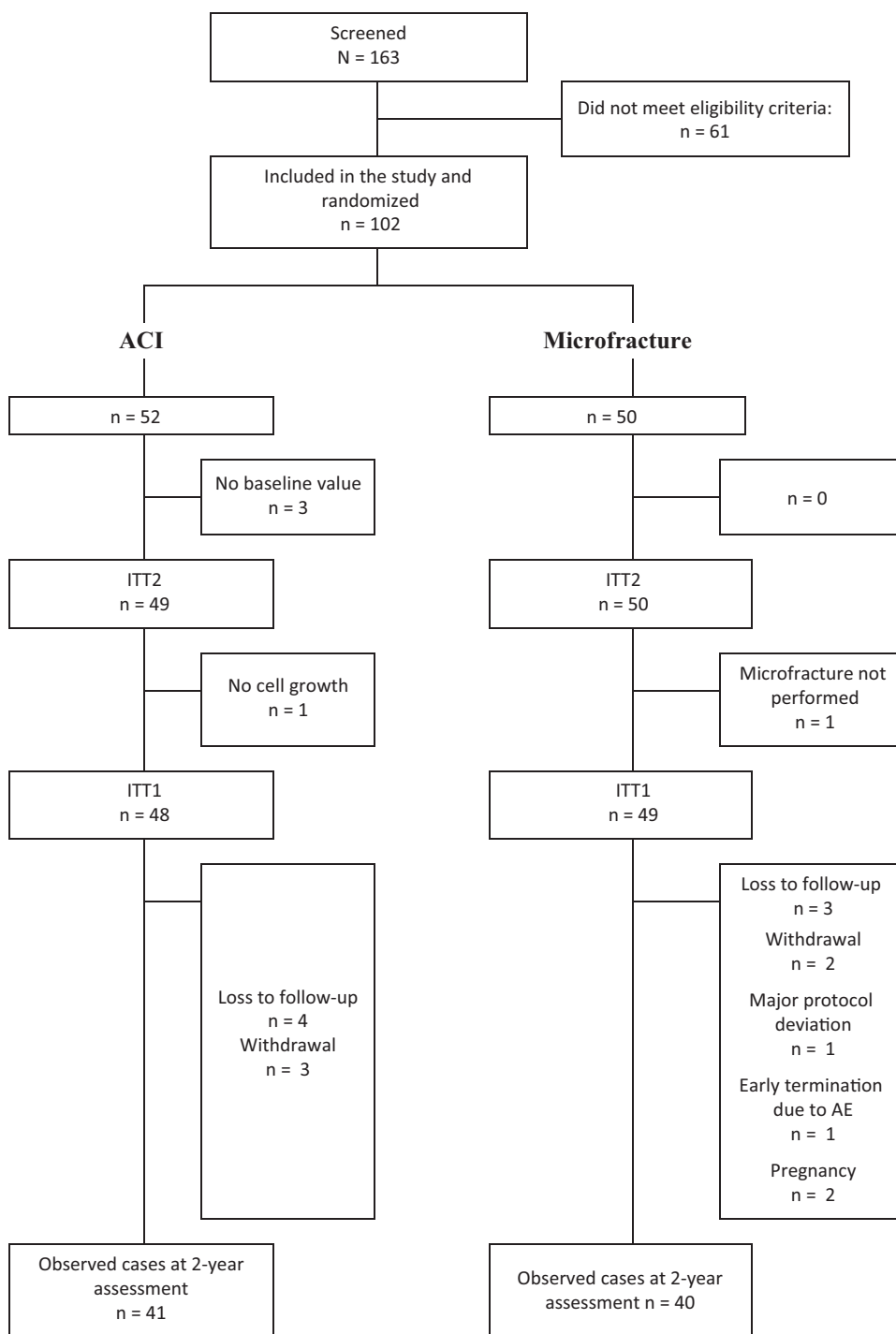
Figure 1. Patient disposition. ACI, autologous chondrocyte implantation; AE, adverse event; ITT, intention-to-treat.

patients in the ACI and microfracture groups, respectively) or prohibited analgesics (4 and 8 patients, respectively); for 2 patients in the ACI group, the minimum spheroid dose was not attained, as the defect was found to be larger than expected, and 1 patient in the ACI group was found after debridement to have a defect larger than allowed (5 cm^2).

The treatment groups were well balanced with respect to medical history; the only conspicuous difference was in immune system disorders, which were more frequent in the ACI group: 17 patients in the ACI group reported 33 prior or concomitant immune system–related events, while 16 patients in the microfracture group reported 17 such events (most frequent in both groups: drug hypersensitivity,

TABLE 2
Patient Demographic and Baseline Data[a]

	ACI (n = 52)	Microfracture (n = 50)	All Patients (N = 102)
Sex, n			
Female	19	22	41
Male	33	28	61
Age, mean ± SD, y	36 ± 10	37 ± 9	37 ± 9
Body mass index,[b] kg/m^2	25.7 ± 3.3 (18.8-31.2)	25.8 ± 3.0 (18.2-30.0)	25.8 ± 3.1 (18.2-31.2)
Smoker, n			
Yes	14	20	34
No	38	30	68
Defect size, cm^2			
Before debridement	2.2 ± 0.7 (0.5-3.5)	2.0 ± 0.8 (0.8-4.0)	2.1 ± 0.8 (0.5-4.0)
After debridement	2.7 ± 0.8 (1.4-5.0)	2.4 ± 0.8 (1.0-4.0)	2.6 ± 0.8 (1.0-5.0)
Defect location, n			
Femur	52	49	101
Femur and patella	—	1	1
Tibia or patella	—	—	—
ICRS grade,[b] n			
3	17	20	37
4	31	29	60
Presence of further defects with ICRS grade <3, n			
Femur	—	—	—
Tibia	2	3	5
Patella	10	10	20

[a]Data are shown as mean ± SD (range) unless otherwise indicated. ACI, autologous chondrocyte implantation; ICRS, International Cartilage Repair Society.
[b]Only assessed for treated patients (intention-to-treat population).

seasonal allergy). This difference was not considered likely to have affected the study results.

Clinical Efficacy Results: KOOS

The overall KOOS score and its subscores are measured on a scale from 0 (worst) to 100 (best). Results for the overall KOOS after 24 months compared with baseline are shown in Table 3; the interim 12-month results are also shown. An improvement in both treatment groups was seen, although notably greater for ACI than for microfracture; the improvement in both groups relative to baseline was statistically significant ($P < .0001$). The lower confidence limit was determined by repeated-measures ANCOVA to be –0.4, clearly greater than the prespecified noninferiority margin of –8.5 (see Methods) and thus implying that the treatment by ACI is to be regarded as significantly noninferior to the treatment by microfracture. The corresponding PP analysis (Table 3) revealed a lower confidence limit of –0.01, very close to implying the significant superiority of ACI, for which, however, the study was not powered. The time courses of the KOOS (absolute values and changes from baseline) for the 2 treatment groups are plotted in Figure 2. The 12-month results, obtained in an interim analysis, are similar (Table 3); in fact, for the ITT population, the significance threshold for the superiority of ACI was narrowly crossed. Subgroup analyses (by age, diagnosis, defect localization, sex) were performed but did not yield any clear evidence of the dependence of the treatment effect on any of the categories mentioned.

At the study visit 24 months after treatment, there was continued improvement in both treatment groups compared with the 12-month assessment, but the difference at the 24-month assessment was smaller, as the results in the microfracture group showed a more pronounced improvement during the second year after treatment. However, the results for microfracture at the 24-month visit remained inferior to those for ACI at any given visit.

KOOS subscores for the ITT population are shown in Table 4. For each subscale, a result similar to that for the overall KOOS was obtained: the explorative statistical test at the descriptive level implied the significant noninferiority of ACI. For the Function in Activities of Daily Living subscale, the threshold for superiority was crossed, as was also the case for the Knee-related Quality of Life subscale in the interim 12-month analysis.

Clinical Efficacy Results: MOCART

For the MOCART score, 0 represents the worst possible diagnosis and 100 a normal joint. Results for all MOCART assessments up to 24 months after treatment are shown in Table 5. The validity of the MOCART analysis is limited, owing to the reduced numbers of patients (Table 5) and the fact that scores were not assessable at baseline. In view of the patients' clinical condition and the assessment of the cartilage defect (ICRS grade 3 or 4), the baseline score must be regarded as having been very poor. As Table 5 shows, there was a wide range of MOCART scores, and no clear conclusion could be drawn. The MOCART items showing

TABLE 3
KOOS Scores 12 and 24 Months After Treatment[a]

	ACI (n = 48)		Microfracture (n = 49)	
	Score	Change From Baseline	Score	Change From Baseline
ITT population				
Baseline	56.6 ± 15.4	—	51.7 ± 16.5	—
12 mo	78.7 ± 18.6	22.2 ± 18.3	68.1 ± 18.6	16.4 ± 15.1
Treatment difference	7.8 ($P < .0001$) with lower confidence limit equal to +1.4[b]			
24 mo	81.5 ± 17.3	24.9 ± 17.4	73.2 ± 18.8	21.5 ± 15.7
Treatment difference	6.1 ($P < .0001$) with lower confidence limit equal to –0.4[c]			
PP population				
Baseline[d]	56.2 ± 14.9	—	55.2 ± 14.9	—
12 mo	78.2 ± 18.3	21.9 ± 17.6	71.3 ± 17.2	16.1 ± 15.9
Treatment difference	6.3 ($P < .0001$) with lower confidence limit equal to –0.7[c]			
Baseline[d]	56.5 ± 14.4	—	53.4 ± 14.7	—
24 mo	84.7 ± 14.9	28.2 ± 16.1	75.9 ± 18.8	22.4 ± 14.3
Treatment difference	7.0 ($P < .0001$) with lower confidence limit equal to –0.01[c]			

[a]Data are shown as mean ± SD unless otherwise indicated. ACI, autologous chondrocyte implantation; ITT, intention-to-treat; KOOS, Knee injury and Osteoarthritis Outcome Score; PP, per-protocol.

[b]Suggesting significant superiority of ACI compared with microfracture (lower limit > 0).

[c]Suggesting significant noninferiority of ACI compared with microfracture (0 > lower limit > –8.5).

[d]Based on the respective PP population (n = 39 vs 34 for ACI at 12 and 24 months, respectively, and n = 41 vs 32 for MF at 12 and 24 months, respectively).

the greatest improvements were defect repair, surface, signal intensity, subchondral bone, and synovitis. However, a systematic difference between the 2 treatment groups could not be established.

No correlation between the clinical outcome, measured by the KOOS, and the structural outcome, measured by the MOCART scoring system, was observed.

Clinical Efficacy Results: Other Criteria

Arthroscopic assessments were performed at the 24-month examination for a subset of patients (ACI group, n = 9; microfracture group, n = 7) who consented to this additional invasive procedure, which included a biopsy of regenerated tissue (R biopsy). In the ACI group, 2 patients showed normal (ICRS grade 1) and 8 patients showed nearly normal (ICRS grade 2) cartilage, while in the microfracture group, 1 patient showed cartilage that was normal, 4 nearly normal, and 2 abnormal (ICRS grade 3).

The R biopsies showed the normal presence of predominantly viable cells (9/9 patients from the ACI group and 6/7 patients from the microfracture group) and normal cartilage mineralization (all patients). Subchondral bone was also predominantly normal (6/6 and 5/7 patients, respectively). Thus, both treatments resulted in cartilage repair tissue of good to mixed quality; results tended to be slightly better in the ACI group than in the microfracture group (with the exception of surface), but the small number of patients investigated renders this observation uncertain.

Further analyses (visual histological assessment, Bern score, and histological staining) were conducted on these samples but likewise without clear differences emerging between the 2 treatment groups. The IKDC examination form revealed a score that improved in both treatment

groups between baseline and the 24-month visit; the improvement was somewhat greater in the ACI group than in the microfracture group. Similar results were obtained for the IKDC subjective knee evaluation form (changes from baseline of 24.2 ± 16.9 for ACI and 20.3 ± 16.8 for microfracture) and modified Lysholm score (improvements on a 24-point scale of 4.9 ± 4.3 and 4.4 ± 3.5 points, respectively).

In summary, all efficacy assessments showed a clear response in both treatment groups. At 12 months, a better response was seen in the ACI group than in the microfracture group for the KOOS and for some other study variables. After 24 months, there was a continued improvement in the KOOS in both treatment groups, but the difference between groups was smaller, as the results in the microfracture group showed more pronounced improvement during the second year after treatment. Nevertheless, the change from baseline in the KOOS for ACI remained greater than that for microfracture at any given visit. Statistical significance testing, both at the formal level of the primary analysis and at the descriptive level of the numerous secondary and post hoc analyses, supported the noninferiority of ACI compared with microfracture. Significant superiority was approached for the KOOS (in the PP analysis, closely), but as noted, this study was not powered for superiority.

Safety Results

All patients in the ACI group received the standard spheroid dose, except for 2 who received less (see above). The mean number of spheroids administered was 64 ± 40, with a range of 12 to 175. No adverse events were fatal, and none in the ACI group led to withdrawal from the study or permanent sequelae; in the microfracture group, 1 event (joint adhesion, considered possibly treatment related)
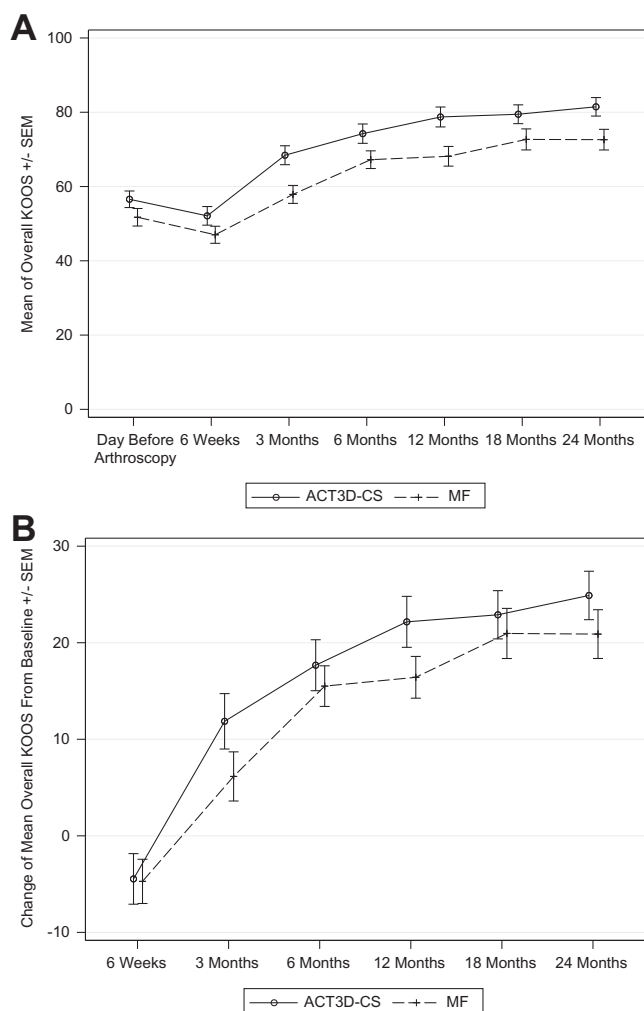
Figure 2. Improvement in KOOS results for the 2 treatment groups (A) overall and (B) compared with baseline. ACT3D-CS, autologous chondrocyte implantation-chondrosphere; KOOS, Knee injury and Osteoarthritis Outcome Score; MF, microfracture.

TABLE 4
KOOS Subscores 24 Months After Treatment[a]

	Difference	Lower Confidence Limit
Pain	4.6	−2.0
Other Symptoms	5.3	−0.3
Function in Activities of Daily Living	6.8	1.5
Function in Sports and Recreation	8.3	−1.9
Knee-related Quality of Life	8.0	−1.1

[a]Subscores for intention-to-treat population. KOOS, Knee injury and Osteoarthritis Outcome Score.

TABLE 5
MOCART Scores Up to 24 Months After Treatment[a]

	Mean ± SD (Range)
Visit 2 (3 mo)	
ACI (n = 31)	66 ± 16 (30-95)
Microfracture (n = 31)	62 ± 11 (45-95)
Visit 4 (12 mo)	
ACI (n = 33)	79 ± 14 (50-100)
Microfracture (n = 36)	76 ± 13 (45-100)
Visit 5 (18 mo)	
ACI (n = 42)	77 ± 14 (35-100)
Microfracture (n = 41)	76 ± 14 (55-100)
Visit 6 (24 mo)	
ACI (n = 46)	76 ± 16 (25-100)
Microfracture (n = 43)	79 ± 13 (50-100)

[a]Differences from total population sizes are because of missing results. ACI, autologous chondrocyte implantation; MOCART, magnetic resonance observation of cartilage repair tissue.

resulted in permanent sequelae, and 2 patients were withdrawn from the study because of adverse events.

The overall incidence of adverse events, of patients with any adverse events and of patients with treatment-related adverse events, did not differ substantially between the treatment groups. In both groups, the system organ class most frequently affected was, as expected, musculoskeletal and connective tissue disorders, affecting 32 patients in the ACI group and 27 in the microfracture group; specifically, joint effusion affected 18 and 15 patients, arthralgia 16 and 18 patients, joint swelling 9 and 8 patients, and back pain 3 and 2 patients, respectively. Such a pattern is to be expected in view of the procedures carried out. Other adverse events were notably less frequent and appeared to reflect the long (24-month) observation period. Adverse events considered related to the study treatment or procedures were almost entirely in the above categories, and most others were not recorded more than once each.

No patient experienced more than 1 serious adverse event. In the ACI group, there were 4 such events: abdominal neoplasm, cystitis, Hodgkin disease, and malaise (all treatment unrelated); in the microfracture group, there were 6 events: cartilage injury, cellulitis, meniscal lesion, deep vein thrombosis, joint adhesion, and arthralgia, of which the last 3 were considered to be at least possibly treatment related. The 2 severe adverse events in the ACI group were conjunctivitis and Hodgkin disease (both considered treatment unrelated). The 13 severe events in the microfracture group affected 7 patients; of these events, skin dystrophy, nausea, bone marrow edema, arthralgia, and joint swelling were considered at least possibly treatment related.

While none of the reported serious adverse events were assessed as treatment related in the ACI group, in the microfracture group, 3 at least possibly treatment-related serious adverse events were reported, affecting 2 patients, all occurring within 6 weeks after treatment: one patient suffered from deep vein thrombosis (moderate intensity, probably related), while the other patient suffered from a cartilage injury and persistent pain after treatment (moderate intensity, possibly related), which must be interpreted as treatment failure.

Specifically, no cartilage formation outside the region of the implant was found; similarly, MRI revealed no case of hypertrophy of the regenerated tissue, such as was seen in first-generation ACI procedures. Vital signs, electrocardiography findings, and recorded concomitant pain medications were monitored but did not yield any sign of a safety concern associated with either treatment.

DISCUSSION

We describe here the 2-year results of a prospectively designed randomized study to compare the efficacy and safety of 2 treatment modalities for cartilage defects of the knee: (1) matrix-associated ACI with spheroid technology and (2) microfracture. Our most important finding was the observation of a trend toward the advantages of spheroid-based ACI in direct comparison with arthroscopic microfracture. The principal results of this study can be summarized as follows:

- Both ACI and microfracture led to lasting improvement in the clinical function of the joint treated over the entire 2-year observation period.
- There were no differences between the 2 treatments with respect to complication rates or the incidence of adverse events.
- The primary analysis confirmed the statistical hypothesis of the noninferiority of ACI, even for the relatively small cartilage defects treated in this study. This was supported by the secondary analyses.
- At the descriptive level, ACI showed superiority in the KOOS subscores of Function in Activities of Daily Living after 24 months and Knee-related Quality of Life after 12 months.

Today, it is generally recognized that microfracture is not well suited to treating relatively large cartilage defects[16,18] and that ACI appears to offer advantages (especially for larger defects) and for this reason is generally recommended for such indications by expert associations. A study of this kind nonetheless remains the best method of obtaining well-grounded information about the efficacy of ACI, especially because there are currently no other standard therapies for large cartilage defects except ACI, and in their absence, a randomized comparative study in this defect size range would be ethically indefensible. Against this background, this study included only patients with cartilage defects up to 4 cm^2 in size and, with existing knowledge of the efficacy of microfracture in this defect size range,[18] was conducted as a noninferiority study. Analogous trials to test other ACI procedures have been described by other authors.[7,24,25]

These findings are in broad agreement with the results of earlier studies in which recent ACI products and microfracture were compared directly for the same indication. While a study comparing these procedures using first-generation ACI did not reveal any significant difference between the treatments, even in the long term,[13,14] studies in which recent products were used did show systematic advantages of ACI in subgroups or on subscales, even though they did not reveal the clear overall superiority of ACI in treating small defects.[3,7,24,25]

Thus, Saris et al[25] and Vanlauwe et al[29] found superiority of the product ChondroCelect (TiGenix) after 2 years, and also superiority in functional outcomes after 5 years, in the subgroup of patients whose symptoms had only been short term at the time of surgery. In the same study, the structural superiority of ACI (ie, better histological quality of the regenerated cartilage) after 12 months was also demonstrated.[26] In another study by Saris et al[24] using matrix-associated ACI that employed a collagen type 1/3 membrane (MACI; Genzyme), ACI likewise showed an advantage in functional outcomes as measured by 2 KOOS subscales (Pain and Function in Activities of Daily Living) after 24 months, thus confirming the results of an earlier study by Basad and coworkers[3] in which a clear superiority of ACI over microfracture after 24 months was found. Interestingly, a closer examination of these studies shows a direct relationship between the superiority of ACI on one hand and the size of the defects treated on the other hand. In the present study, the mean defect size was 2.1 cm^2, and in the study of Saris et al,[25] using characterized chondrocytes, it was 2.6 cm^2. Both studies revealed scattered significances supporting the superiority of ACI. However, in studies on the efficacy of MACI (mean defect size, 4.8 cm^2),[24] and especially the study by Basad et al[3] (which alone recruited only patients within the indication range of microfracture), ACI proved clearly superior in terms of functional outcomes, which irrespective of the question addressed is difficult to demonstrate, owing to the large number of factors influencing the result.

The present study concerned not only the question of efficacy but also that of patient safety in the 2 procedures applied. ACI today continues to require 2 invasive procedures, each of which is less harmful for the subchondral bone than microfracture but together are more so. Therefore, attention must be paid to the incidence of adverse events and undesirable complications, and this must naturally also enter into a global assessment of the 2 procedures and, for individual patients, the choice of therapy. In this study, no difference was seen between the 2 treatment groups with respect to adverse events and complications, confirming the observations already published that ACI is largely free of complications and provides a high degree of patient safety.[4] Although with the limited number of cases observed the possibility of hypertrophy cannot be excluded, and it was described for this product in an earlier case report,[21] its complete absence in the 52-patient cohort of this study does imply that its general incidence rate is notably lower than that so far reported in the literature.[20] Also, the other typical complications of ACI (disturbed fusion, delamination, and graft failure) were not recorded in the present clinical trial, while 1 case of treatment failure and 1 serious case of deep vein thrombosis were reported as related to microfracture.

Despite the prospective, controlled randomized design of this study with its high evidence level, some limitations are to be noted. At the time when the study was set up (in 2010), the evidence for clinical superiority was not as clear as it is today (partly, of course, through the results of

precisely this study). In 2010, as still is the case today, the recommended treatment for cartilage therapy in smaller defects was microfracture[16]; therefore, at the time, powering for superiority was considered overambitious and would also have raised an ethical issue concerning the number of patients to be recruited. Therefore, the formal aim of the study was chosen to be noninferiority, albeit with a hierarchical statistical design allowing additional testing for superiority if noninferiority was achieved (as it was). However, for the reasons stated, this was chosen as a secondary objective and was not included in the powering (sample size calculation), so that an assessment of superiority was only possible at the descriptive level. Nonetheless, it is conspicuous that (descriptively) significant superiority was, despite the low powering, only narrowly missed, and most of the comparisons, irrespective of statistical testing, showed a better result for ACI than for microfracture. This provides an important signal for future studies.

From a clinical perspective, in view of the greater operative effort and financial costs of ACI compared with microfracture, a demonstration of superiority would naturally have been preferred to justify the use of ACI in a clinical setting. However, the economic question includes other aspects (such as follow-up costs for physical therapy, medication, reoperation rate, etc) that are well outside the scope of a randomized controlled trial, which necessarily focuses primarily on the medical and scientific issues. To assess the broader picture, a detailed economic comparison between ACI and microfracture is required. The fact that superiority was found, or closely approached, in spite of inadequate powering for this, underlines the significance of the results found and is of particular interest in view of the fact (see above) that large cartilage defects were not included in this study.

Another limitation of the study was the impossibility of blinding the patients because of the different numbers of procedures involved; thus, the possibility of bias due to a placebo effect cannot be completely ruled out. However, this appears unlikely to explain the superiority of ACI, as the radiological assessment, which confirmed the efficacy of ACI, was conducted in a fully blinded manner. However, even here, there is a limitation because, as in all cartilage regeneration studies, the MOCART scoring system before treatment is technically impossible because the score is designed to assess the quality of repair tissue, which does not yet exist before treatment. Thus, a strict "before-after" comparison is impossible. The lack of correlation between the MOCART score and patient-reported outcome scores is consistent with numerous literature reports.[11,15,19,30] Thus, the evidence whether a radiological score is reliable in predicting clinical outcomes (patient's situation including pain and functionality) after cartilage repair is still lacking.[9] Moreover, there may have been minor differences between study centers with respect to the operative technique or (despite standardization) rehabilitation therapy.

Even when the above factors are taken into account, this study leads to the following important conclusions: (1) The use of matrix-associated ACI with spheroid technology for the treatment of full-thickness cartilage defects of the knee seems safe and efficient with respect to an unambiguous improvement in symptoms, compared with the preoperative situation, on all objective and subjective scores. (2) Even though the defect sizes in this study lay within the range for which bone marrow stimulation is regarded as an appropriate treatment, the relevant subscores with clinical benefit after 24 months showed the superiority of ACI compared with microfracture at the descriptive level. These results are thus in line with earlier studies in which the efficacy and safety of other ACI products were investigated, and they underline the clinical relevance and suitability of this procedure for the treatment of cartilage defects of the knee.

ACKNOWLEDGMENT

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