A Double-Blind, Randomized, Saline-Controlled Study of the Efficacy and Safety of EUFLEXXA® for Treatment of Painful Osteoarthritis of the Knee, With an Open-Label Safety Extension (The FLEXX Trial)

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Objective: To report the FLEXX trial, the first well-controlled study assessing the safety and efficacy of Euflexxa (1% sodium hyaluronate; IA-BioHA) therapy for knee osteoarthritis (OA) at 26 weeks.

Methods: This was a randomized, double-blind, multicenter, saline-controlled study. Subjects with chronic knee OA were randomized to 3 weekly intra-articular (IA) injections of either buffered saline (IA-SA) or IA-BioHA (20 mg/2 ml). The primary efficacy outcome was subject recorded difference in least-squares means between IA-BioHA and IA-SA in subjects' change from baseline to week 26 following a 50-foot walk test, measured via 100-mm visual analog scale (VAS). Secondary outcome measures included Osteoarthritis Research Society International responder index, Western Ontario McMaster University Osteoarthritis Index VA 3.1 subscales, patient global assessment, rescue medication, and health-related quality of life (HRQoL) by the SF-36. Safety was assessed by monitoring and reporting vital signs, physical examination of the target knee following injection, adverse events, and concomitant medications.

Results: Five hundred eighty-eight subjects were randomized to either IA-BioHA ($n = 293$) or IA-SA ($n = 295$), with an 88% 26 week completion rate. No statistical differences were noted between the treatment groups at baseline. In the IA-BioHA group, mean VAS scores decreased by 25.7 mm, compared with 18.5 mm in the IA-SA group. This corresponded to a median reduction of 53% from baseline for IA-BioHA and a 38% reduction for IA-SA. The difference in least-squares means was −6.6 mm ($P = 0.002$). Secondary outcome measures were consistent with significant improvement in Osteoarthritis Research Society International responder index, HRQoL, and function. Both IA-SA and IA-BioHA injections were well tolerated, with a low incidence of adverse events that were equally distributed between groups. Injection-site reactions were reported by 1 (<1%) subject in the IA-SA group and 2 (1%) in the IA-BioHA group.

Conclusions: IA-BioHA therapy resulted in significant OA knee pain relief at 26 weeks compared with IA-SA. Subjects treated with IA-BioHA also experienced significant improvements in joint function, treatment satisfaction, and HRQoL.

 Semin Arthritis Rheum 39:1-9

Keywords: *hyaluronic acid, hyaluronate, osteoarthritis, therapy, knee, intra-articular therapy*

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This study was supported by Ferring Pharmaceuticals Inc., Parsippany, New Jersey.
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0049-0172/09/$-see front matter
doi:10.1016/j.semarthrit.2009.04.001

Osteoarthritis (OA) is the most prevalent chronic joint disorder worldwide (1) and manifests primarily as pain and loss of joint function. This condition affects more than 20 million Americans (2) and is second only to heart disease as a cause of work disability (3,4). Advanced age and obesity are among the major demographic risk factors for knee OA. Because of United States secular trends toward increased longevity and obesity, it is likely that the incidence and impact of knee OA will increase dramatically over the next decade (5-8). Biomechanical trauma is another major risk factor for OA of the knee, and despite the aging of the population, a significant proportion of Americans participate in sports and physical activities beyond the age of 65 years (6). The combination of pharmacologic and nonpharmacologic treatments is used frequently in clinical practice and is universally recommended in all of the 12 existing guidelines for the management of hip and/or knee OA (9). Although the mechanism of action of intra-articular (IA) sodium hyaluronate (IA-HA) is not completely understood, IA-HA occupies multiple CD44 linking sites, thereby preventing receptor cross-linking and intracellular activation of messengers such as nuclear factor kappaB (10). In addition, evidence suggests that high-molecular-weight (MW) HA interferes with nuclear factor kappaB activation through binding to Toll receptors (11). This activity retards activation of multiple inflammatory mediators, with demonstrated reduction in pain transmission, synovial inflammation, and chondrocyte release of degradative enzymes such as matrix metalloproteinases (12-15). Although IA-HA therapy provides biomechanical improvement in joint function, their relatively short residence time in the joint indicates that viscosupplementation is a minor function (15). Systematic reviews and meta-analyses such as the Cochrane Review have confirmed the efficacy of IA-HA for relieving OA-related pain and improving joint function (8,16). Although multimodal therapy of OA is generally recommended, studies have not adequately examined combination therapy of hyaluronate with other approaches, such as IA depocorticosteroids.

EUFLEXXA® [bioengineered 1% sodium hyaluronate (IA-BioHA); Ferring Pharmaceuticals, Inc., Parsippany, NJ] is a high MW HA product derived from nonpyogenic streptococcus zooepidemicus and produced by a highly purified biologic fermentation process. The high MW of IA-BioHA (range: 2.4-3.6 million d) is achieved by careful control of the fermentation, recovery, and purification processes and does not require the use of any cross-linking processes. The efficacy of IA-BioHA for the treatment of OA of the knee was previously demonstrated in a 12-week double-blind, randomized study using the active comparator Synvisc® (Hylan G-F 20, Genzyme Corp., Cambridge, MA), which found similar efficacy between the agents for the primary endpoint measure, but a significantly higher incidence of postinjection effusion in the Hylan G-F 20 group (17). The current study (the FLEXX trial) is a relatively large, controlled study assessing the safety and efficacy of IA-BioHA compared with intra-articular saline (IA-SA) at 26 weeks. An additional 26-week extension of this study will be reported separately.

SUBJECTS AND METHODS

The study was conducted in the United States from October 2006 to May 2008 in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki concerning medical research in humans. Subjects were enrolled at 36 sites. Criteria for inclusion were as follows: OA of the knee by American College of Rheumatology criteria (18); moderate to severe pain score of 41 to 90 mm recorded on 100-mm visual analog scale (VAS) immediately following a 50-foot walk; bilateral standing anterior-posterior radiograph demonstrating Kellgren and Lawrence grade 2 or 3 OA of the target knee (19); ability and willingness to use only acetaminophen as the analgesic (rescue) study medication; unassisted walking 50 feet on a flat surface and going up and down stairs; and willingness and ability to complete efficacy and safety questionnaires. Subjects having radiographic confirmation of OA in the nontarget (contralateral) knee were eligible as long as the target knee was the more symptomatic knee and met the criteria listed above. Pain in the nontarget knee must have been limited to <40 mm following the 50-foot walk test at screening. All procedures were approved by the institutional review board at each study site. Each subject provided informed consent before enrollment. Subjects who met all inclusion criteria were randomized using a computer-generated statistical analysis system to either IA-BioHA or IA-SA.

Subjects were excluded if they reported any major injury to the target knee within the prior 12 months; any surgery to the target knee within the prior 12 months or surgery to the contralateral knee or other weight-bearing inflammatory arthropathies; gout or pseudogout within the previous 6 months; radiographic acute fracture, severe loss of bone density, avascular necrosis, and/or severe bone or joint deformity in the target knee; osteonecrosis of either knee; fibromyalgia, pes anserine bursitis, lumbar radiculopathy, and/or neurogenic or vascular claudication; significant anterior knee pain due to diagnosed isolated patella-femoral syndrome or chondromalacia in the target knee; target knee joint infection or skin disorder/infection within the previous 6 months; symptomatic OA of the hips, spine, or ankle; known hypersensitivity to acetaminophen, IA-BioHA, or phosphate-buffered saline solution; women of childbearing potential who are pregnant, nursing, or planning to become pregnant, and those who do not agree to remain on an acceptable method of birth control throughout the study; history of immune disorders; vascular insufficiency of lower limbs or peripheral neuropathy; current treatment or treatment of cancer within the previous 2 years (excluding basal cell or squa-

mous cell carcinoma of the skin); active liver or renal disease; any clinically significant abnormal laboratory value; any intercurrent chronic disease or condition that might interfere with the completion of the study; and participation in any experimental device study within the prior 6 months or any experimental drug study within the prior month.

Acetylsalicylic acid (aspirin) at a maximum of 325 mg/d was allowed for cardiovascular protection. Nonprescription nutraceuticals (eg, glucosamine, chondroitin), topical analgesics, and nasal or inhaled corticosteroids were allowed if the dosage had been stable for at least 1 month and the identical regimen was to be continued throughout the study period. Nonpharmacologic treatments (physical therapy, acupuncture, osteopathic, and chiropractic manipulations) were allowed if treatment had been stable for at least 1 month and there was no plan to change frequency throughout the course of the study.

Safety measures were collected at every visit (screening, baseline/week 0, weeks 1, 2, 3, 6, 12, 18, and 26). Observed values for the 50-foot walk test, Western Ontario McMaster University Osteoarthritis (WOMAC) subscales, and patient global assessment were recorded at each visit to ensure consistency of data, while comparative statistical analysis was conducted according to a predefined statistical analysis plan for weeks 12 and 26. SF-36 data were recorded at baseline and at weeks 12 and 26.

Before performing the 50-foot walk test at the screening visit, subjects had to have discontinued the use of the following pharmacologic agents for the periods stated: nonsteroidal anti-inflammatory drugs (1 week), opioid narcotics (4 weeks), local corticosteroid knee injections (3 months), systemic corticosteroids (1 month), and IA-HA (6 months). Maximum allowable acetaminophen dose (only used for knee pain) was 4 g/d (4000 mg). Subjects must have been willing and able to discontinue acetaminophen, ice packs, or topical heat at least 24 hours before all study-specific visits.

A complete medical history, physical examination, complete blood count, and metabolic profile were performed at screening. Vital signs were measured at all study visits. The injection site on the target knee was examined immediately before and 5 minutes after injection for untoward effects of the injection. The blinded investigator performed all target knee assessments for a particular subject. All efforts were made to have the same investigator perform each evaluation for a particular subject.

Subjects received 3 weekly injections of either IA-BioHA (20 mg/2 ml of 1% sodium hyaluronate) or IA-SA (2 mL of a phosphate buffered saline) into the target knee. Treatment began at the baseline visit (first injection; week 0), with a second injection at week 1 and the third injection at week 2. The study devices were supplied in boxes containing 3 doses of either IA-BioHA or IA-SA. At the start of the study, blinded investigators and unblinded injectors were determined at each study site. Blinded knee evaluators and subjects remained blinded to the treatment group assignment. Unblinded injectors were instructed to cleanse the skin around the injection site with betadine or alcohol before administering lidocaine 1% (without epinephrine) into the skin and subcutaneous tissue. If aspiration was necessary, the joint capsule was also infiltrated with lidocaine. A 16- to 18-gauge needle was utilized if an effusion was present; otherwise, 20- to 23-gauge needles were used for IA injections that were performed using either a suprapatellar or infrapatellar approach.

Acetaminophen (500-mg tablets) was provided to subjects in bottles of 56 tablets (1-week supply) and/or 168 tablets (3-week supply). Each bottle dispensed to a subject was documented, and tablets were counted at each visit.

Outcome Measures

Primary Efficacy Outcome

The primary efficacy outcome variable was the difference in least-squares means between IA-BioHA and IA-SA in each subject's change from baseline to week 26 on 100-mm VAS following the 50-foot walk test. For the intent-to-treat (ITT) population, which includes all subjects who were randomized and received at least 1 injection of IA-BioHA or IA-SA and had at least 1 post baseline evaluation, all data available at the various time points were included in a repeated-measures model; the model included study center as a random effect and all other factors as fixed effects, including baseline pain score on the 50-foot walk test, treatment group, study week, and treatment group by study week interaction.

Secondary Efficacy Outcomes

The Outcome Measures in Rheumatology—Osteoarthritis Research Society International (OARSI) responder index was assessed at weeks 12 and 26 (20). In addition, changes in WOMAC Index subscales of pain, stiffness, and physical function scores were assessed at baseline and weeks 12 and 26, as was patient global assessment. The number of tablets of rescue medications used between visits and change in the Short Form-36 v2 (SF-36) Acute Form Health Survey mean scores were assessed at baseline and weeks 12 and 26 (21). The norm-based SF-36 scores were obtained from the Second Edition of User's Manual for the SF-36 v2 Health Survey.

Safety

Safety was assessed in both arms of the trial by physical examination of the target knee following each IA injection. Adverse events (AEs), injection-site reactions, vital signs, and concomitant medications were monitored and recorded. All randomized subjects were included in the safety population. AEs were coded using MedDRA.

Statistical Methods

Randomization

The randomization numbers were generated using a statistical analysis system allocating a 1:1 treatment allocation ratio.

Primary Outcome Hypothesis, Sample Size Justification, and Statistical Method

The primary objective of this trial was to demonstrate that IA-BioHA is superior to IA-SA with respect to the mean change in pain scores on the 50-foot walk test, measured on a 100-mm horizontal VAS (from 0 mm = no pain to 100 mm = extreme pain), from baseline (week 0, first injection) to the final study visit (week 26) for the ITT population. Past and current literature reports the use of a VAS measurement of pain following a 50-foot walk test in evaluating the short- and long-term analgesic efficacy of IA-HA (22,23). The primary null hypothesis (H_0) was that the effect of the 2 treatment groups at 26 weeks would be equal, and the corresponding alternative hypothesis (H_A) was that the effect of the 2 treatment groups at 26 weeks is unequal. The hypothesis to be tested for the primary efficacy variable was

$$H_0 : \mu_P = \mu_E \text{ vs. } H_A : \mu_P \neq \mu_E$$

where μ_E is the least-squares mean change from baseline to week 26 for IA-BioHA and μ_P is the least-squares mean change from baseline to week 26 for IA-SA.

The sample size was based on previous study results that yielded an estimate of the standard deviation of 25 mm for pain scores on the 50-foot walk test. Requiring 90% power to detect an 8.0-mm difference between average IA-BioHA and IA-SA scores at the 2-sided 5% significance level and a 30% dropout rate by week 26 resulted in a required sample size of 295 per arm to complete 206 subjects.

Secondary Outcomes

Treatment groups were compared using OARSI responder rates at weeks 12 and 26 using logistic regression analyses. The model included the factors of treatment group and study center. Treatment differences were expressed as odds ratios (OR) with 95% confidence intervals (CIs) (likelihood ratio based). The OR, 95% CI, and P value associated with the χ^2 test were reported along with the OARSI responder rates by treatment group at weeks 12 and 26.

The OARSI responder outcome was coded as "*Yes*" if there was high improvement in pain or function ≥50% and absolute change ≥20 mm or improvement in at least 2 of the 3 following categories: pain ≥20% and absolute change ≥10 mm; function ≥20% and absolute change ≥10 mm; and/or patient's global assessment ≥20% and absolute change ≥10 mm (20). Otherwise, the OARSI responder outcome was coded as "*No.*" Treatment groups were compared using logistic regression analyses adjusting for treatment group and study center.

The analysis of WOMAC subscale scores of pain, joint stiffness, and physical function; patient global assessment; and number of tablets of rescue medication followed similar methods as that of the primary efficacy variable. The covariate in each model was the respective baseline value. In addition to the comparison between the treatment groups at week 26, comparison between the treatment groups at week 12 was performed. The 95% CI and P value from the F-test for each comparison from the model are presented. For patient global assessment, the following question was asked: "*On the scale below, we want you to tell us your opinion on how significant the pain is in your knee today. Please only comment on the knee that is receiving injections for the study.*" Results were recorded using a 100-mm VAS.

Safety

The safety analysis was performed using data from all subjects. The safety analysis was performed by contrasting the percentages of subjects experiencing a specific type of AE, and results are displayed in tabular form.

RESULTS

Subject disposition is reflected in Figure 1. To randomize 588 subjects (IA-BioHA n = 293, IA-SA = 295), 821 were screened. Overall, there was an 88% completion rate in each treatment group. There were no differences in demographics between those completing and those discontinuing the study. There were 34 discontinuations in

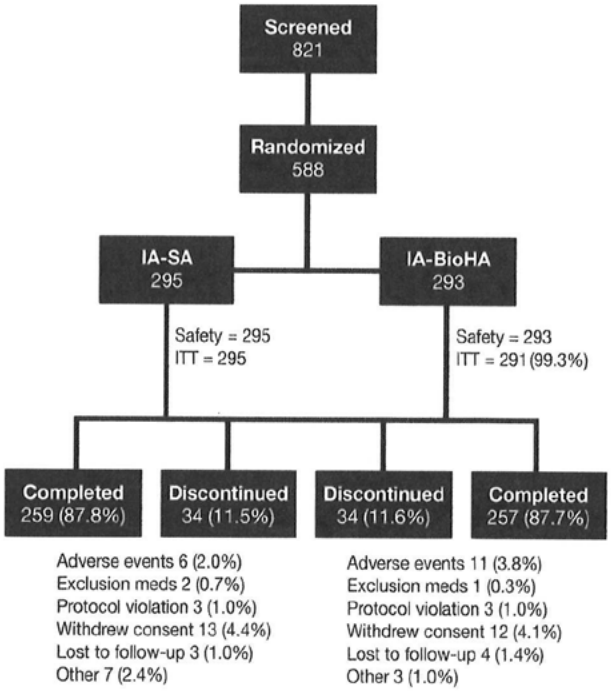

Figure 1 Flow of participants. IA-SA, intra-articular buffered saline; IA-BioHA, intra-articular 1% sodium hyaluronate; ITT, intent to treat.

Table 1 Baseline Demographics (ITT)

	IA-SA (N = 295)	IA-BioHA (N = 291)
Men, *n* (%)	109 (37)	107 (37)
Women, *n* (%)	186 (63)	184 (63)
Age, y, mean (SD)	60.8 (10.0)	62.5 (11.0)
BMI, kg/m^2 (mean)	33.0 (7.0)	32.4 (7.0)
Kellgren Lawrence grade, *n* (%)		
2	115 (39)	119 (41)
3	180 (61)	172 (59)
Prior year target knee treatment, *n* (%)	203 (69)	199 (68)
Physical therapy	23 (8)	16 (6)
Arthrocentesis	8 (3)	16 (6)
Steroid injection	52 (18)	55 (19)
Other injection	8 (3)	10 (3)
NSAID	176 (60)	172 (59.0)
Mean baseline pain (100 mm VAS 50 foot walk), mean (SD)	54.7 (22.0)	55.6 (22.0)

ITT, intent to treat; IA-SA, intra-articular buffered saline; IA-BioHA, intra-articular 1% sodium hyaluronate; SD, standard deviation; BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug; VAS, visual analog scale.

each group. AE discontinuations included 6 in the IA-SA group and 11 in the IA-BioHA group. Protocol violations caused discontinuation in 6 (3 in each group). Two subjects in the IA-BioHA group were excluded from the ITT population because of lack of follow-up data. Thus, the ITT population comprised 291 IA-BioHA–treated subjects and 295 IA-SA–treated subjects.

There were no statistical differences in demographics noted between the treatment groups at baseline (Table 1). The mean subject age and body mass index were 61.6 and 32.7, respectively. At baseline, 39% of IA-SA and 41% of IA-BioHA subjects had Kellgren-Lawrence radiographic grade 2, and 61 and 59%, respectively, had radiographic grade 3 OA of the knee. The most prevalent prior knee OA treatments within the previous year were nonsteroidal anti-inflammatory drugs and depot corticosteroid injections.

Primary Outcome Measure

The IA-BioHA group had a mean ± SD 50-foot walk pain score of 30.0 ± 26.1 mm, with a change from baseline of −25.7 ± 28.9 mm. In comparison, the score for the IA-SA group was 36.1 ± 28.6 mm, with a change from baseline of −18.5 ± 32.5 mm. This corresponded to a 53% median percent reduction from baseline for the IA-BioHA group compared with a 38% median reduction for the IA-SA group. The result of the ITT analysis was a difference (−36.4 mm IA-BioHA versus −29.7 mm IA-SA) in least-squares means of −6.6 mm (95% CI, −10.8 to −2.5 mm; P = 0.002). In the IA-BioHA group, 6 did not have a 50-foot walk recorded at 26 weeks.

By the end of the IA series of injections, both treatment groups demonstrated a reduction in pain scores (Fig. 2). Subsequently, the IA-BioHA group continuously separated from the IA-SA group, the latter demonstrating a lessening of effect over time.

At 26 weeks, 145 (58%) IA-BioHA subjects reported a ≥20-mm improvement in pain based on the VAS scoring of the 50-foot walk test, compared with 120 (46%) in the IA-SA group (P = 0.006, OR 1.7, 95% CI, 1.2-2.4). At 26 weeks, 47% of the IA-BioHA subjects were "pain free" (100-mm VAS <20 mm based on 50-foot walk) compared with 39% in the IA-SA group (P = 0.066, OR 1.4, 95% CI, 1.0-2.0).

IA-BioHA–treated subjects with baseline Kellgren-Lawrence radiographic grade 2 scores had significant improvement (−37 mm VAS change from baseline) in 50-foot walk pain scores compared with IA-SA (−22 mm) at 26 weeks. The difference (IA-BioHA versus IA-SA) of −9.7 mm (95% CI, −16.0 to -3.4 mm) was significant (P = 0.003). By contrast, radiographic grade 3 subjects demonstrated a lesser response (IA-BioHA −22.3 VAS change from baseline, IA-SA −16.3 mm). The difference in least-squares mean of −4.6 mm was not significant (P = 0.100, 95% CI, −10.0 to 0.9 mm).

Secondary Outcome Measures

The percentage of OARSI responders (Table 2) for the IA-BioHA group was significantly greater than that of the IA-SA group at week 26 (67% versus 59%; P = 0.047, OR 1.4, 95% CI, 1.0-2.1). OARSI response rates remained statistically significantly different even after adjustment for baseline pain and bilateral OA.

The decrease in WOMAC pain subscale at week 26 for the IS-BioHA group was a mean (SD) 19.2 (26.8) mm, compared with 16.3 (26.8) mm for the IA-SA group. The

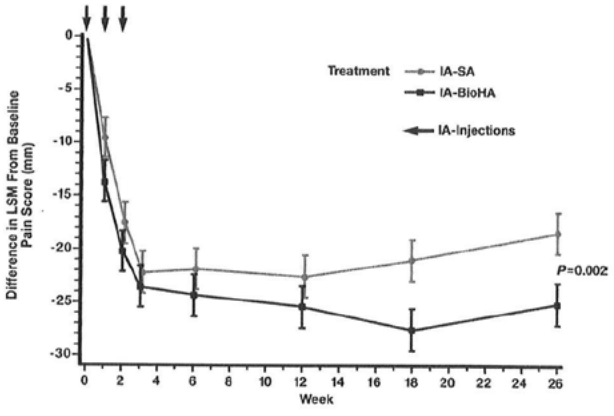

Figure 2 P value between study groups at 26 weeks. Differences of least-squares means (LSM) from baseline at assessment time points for pain scores on the 50-foot walk test on the intent-to-treat population. Arrows indicate intra-articular injection. Vertical lines indicate the 95% confidence intervals (CI). ITT, intent to treat; IA-SA, intra-articular buffered saline; IA-BioHA, intra-articular 1% sodium hyaluronate.

Table 2 OARSI Responder Rates Using 50-foot Walk Test (ITT Population)

Visit	Response/Statistic	IA-SA (N = 295)	IA-BioHA (N = 291)	All Treatments (N = 586)	Overall Comparison
Week 12	No. of subjects with data	274	263	537	
	Yes, *n* (%)	167 (61)	173 (66)	340 (63)	
	No, *n* (%)	107 (39)	90 (34)	197 (37)	
	Odds ratio (95% CI)				1.3 (0.9 to 1.8)
	P value				0.202
Week 26	No. of subjects with data	264	254	518	
	Yes, *n* (%)	155 (59)	169 (67)	324 (63)	
	No, *n* (%)	109 (41)	85 (34)	194 (38)	
	Odds ratio (95% CI)				1.4 (1.0 to 2.1)
	P value				0.047

OARSI, Osteoarthritis Research Society International; ITT, intent to treat; IA-SA, intra-articular buffered saline; IA-BioHA, intra-articular 1% sodium hyaluronate; *N*, number of subjects in a given treatment group for the population analyzed; *n*, number of subjects; (%), percentage of subjects based on *N*; CI, confidence interval.

Note: The *P* value for the odds ratio corresponds to the Wald chi-square test for IA-BioHA versus IA-SA with respect to OARSI responder rates from a logistic regression adjusting for treatment group and study center.

A subject was considered a responder if there was high improvement in pain or function ≥50% and absolute change ≥20 mm or improvement in at least 2 of the 3 following categories: pain ≥20% and absolute change ≥10 mm, function ≥20% and absolute change ≥10 mm, and/or patient global assessment ≥20% and absolute change ≥10 mm.

difference in least-squares means of −3.3 mm (95% CI, −7.0 to 0.5 mm) was not significant ($P = 0.085$). There was no significant between-group difference at week 12; however, the WOMAC pain subscale is composed of 5 individual questions (A1-A5), which were analyzed individually post-hoc. Analysis of question A1 (pain on walking on a flat surface) revealed significant reduction for IA-BioHA compared with IA-SA ($P = 0.036$), and significance levels were between $P = 0.07$ and 0.08 for questions A2 (pain going up or down stairs), A4 (pain while sitting or lying down), and A5 (pain while standing) but not for A3 (pain at night while in bed, $P = 0.321$).

Reduction in WOMAC joint stiffness subscale scores from baseline to 26 weeks were 19.6 (31.27) mm for the IA-BioHA and 15.4 (29.33) mm for the IA-SA group. The difference in least-squares means of −3.8 mm was not significant ($P = 0.075$, 95% CI, −8.0 to 0.4 mm).

At week 26, the mean (SD) reduction in WOMAC physical function subscale scores from baseline was larger in the IA-BioHA group than the IA-SA group: 19.5 (24.7) mm versus 14.6 (25.8) mm. The difference in least-squares means of −4.3 mm (95% CI, −7.9 to −0.7 mm) was statistically significant ($P = 0.019$). There was no significant difference at week 12.

The decrease in patient global assessment from baseline was greater in the IA-BioHA group than in the IA-SA group at 26 weeks: −22.0 (30.4) mm versus −17.8 (28.8) mm. The difference in least-squares means of −4.5 mm (95% CI, −8.6 to −0.3 mm) was statistically significant ($P = 0.035$). There was no significant difference at week 12.

At week 26, the mean (SD) change from baseline in the number of acetaminophen tablets used per week by the IA-BioHA group was 12.7 (14.56) tablets per week, and the mean (SD) change for the IA-SA group was 12.9 (15.23) tablets per week. The group difference in the least-squares mean change from baseline of −0.3 tablets per week (95% CI = −2.8 to 2.2 tablets per week) was not significant (*P* value = 0.822) nor was the group difference at week 12 ($P = 0.65$) or week 18 ($P = 0.89$).

Subjects in the FLEXX trial reported significant physical limitations at baseline as compared with both population norms in most of the SF-36 domains. The trial subjects also had lower scores in physical function (PF), role physical (RP), bodily pain (BP), and physical components summary (PCS) than those of existing OA population norms ($P < 0.05$). Both treated groups experienced significant improvement (increase) over their baseline values in several domains of the SF-36, predominantly belonging to physical functioning. The mean improvement (±SD) scores [range, 0-100; higher scores indicate better health-related quality of life (HRQoL)] between baseline and week 26 for the 4 domains related to physical functioning, and their PCS in the IA-BioHA group were significant ($P < 0.05$): PF 4.77 ± 9.65; RP 4.13 ± 10.49; BP 3.85 ± 9.93; general health (GH) 1.38 ± 6.22; and PCS 4.55 ± 8.50. The mean improvement scores between baseline and week 26 for the 4 domains related to physical functioning, and PCS in the IA-SA group were also significant ($P < 0.05$): PF 3.18 ± 9.05; RP 2.81 ± 10.52; BP 2.94 ± 9.56; GH 0.19 ± 6.75; PCS 2.71 ± 8.58. Repeated measures tested with a mixed-effects model indicated that the IA-BioHA group experienced statistically significant improvements over the IA-SA group at week 26 in the PCS score (1.609; 95% CI, 0.245-2.973, $P = 0.021$).

Safety Outcomes

In general, both IA-SA and IA-BioHA injections were well tolerated, with a low incidence of AEs throughout the 26-week study period. Table 3 provides the summary of treatment-emergent adverse events (TEAEs). One death

Table 3 Overall Summary of Treatment-Emergent Adverse Events (Safety Population)

	IA-SA (N = 295)		IA-BioHA (N = 293)		All Treatments (N = 588)	
	n (%)	Events	n (%)	Events	n (%)	Events
Any TEAE	169 (57.0)	379	157 (54.0)	363	326 (55.0)	742
Death	1 (0)	1	0	0	1 (0)	1
Serious TEAE	9 (3)	11	9 (3)	11	18 (3)	22
TEAE leading to withdrawal	5 (2)	5	3 (1)	4	8 (1)	9
Severe TEAE	19 (6)	21	11 (4)	15	30 (5)	36
Related TEAE	32 (11)	48	29 (10)	49	61 (10)	97

N, number of subjects in a given treatment group for the population analyzed; *n*, number of subjects; (%), percentage of subjects based on *N*; TEAE, treatment-emergent adverse event.

Note: An adverse event was counted as a TEAE if it was either not present at baseline (prior to the first injection) or present at baseline but increased in severity during the treatment period.

occurred in the IA-SA group as a result of a motor vehicle accident, which was considered unrelated to treatment. Eighteen (3%) subjects experienced 22 serious TEAEs, with similar proportions in the IA-BioHA ($n = 9$, 3%) and IA-SA ($n = 9$, 3%) groups. The most common serious TEAEs were pneumonia and transient ischemic attack [each affecting 2 (0.3%) subjects]. However, these events were not notably more frequent than any other serious TEAEs (each with an incidence of 0.2%). None of the serious TEAE were considered related to study treatment.

There was no difference between the treatment groups with respect to the incidence of nonserious TEAEs, and arthralgia was the only TEAE reported by at least 5% of subjects (IA-SA 12%, IA-BioHA 9%). TEAEs led to 5 (2%) IA-SA subjects withdrawing from the study prematurely compared with 3 (1%) in the IA-BioHA group. Four subjects discontinued from the study due to nonserious TEAEs (3 in IA-SA group due to worsening of knee pain, body aches, and withdrawal of consent and 1 in IA-BioHA group due to unrelieved knee pain). Of note, there were no joint effusions reported with IA-BioHA (Table 4).

DISCUSSION

Treatment with IA-BioHA was superior to IA-SA in reducing knee pain due to OA at 26 weeks. Secondary measurements were generally supportive of the primary outcome measurement. IA-BioHA was well tolerated, with no joint effusions.

It is of interest that 6 months following the series of 3 IA-BioHA injections, almost half (47%) the subjects were pain free (100-mm VAS of <20 mm based on 50-foot walk). This compares favorably with 39% pain free with IA-SA. The OARSI responder criteria require a 50% reduction of pain and/or increased function with at least a 20-mm change on a 100-mm scale. Achieving this degree of pain reduction appears to be clinically relevant (20) since reductions in chronic pain intensity of at least 10 to 20% have been reported to reflect minimally important changes (24). Reductions of at least 30% appear to reflect at least moderate clinically important differences, and it is recommended that the percentages of patients responding with this degree of pain relief be considered a success criterion in clinical trials of chronic pain treatments (24). In this study, improvement in pain exceeded these criteria. The results of this dichotomous variable (OARSI responder criteria) and the significant improvements in subjects' HRQoL and function support the effectiveness of IA-BioHA treatment for knee OA.

Not surprisingly, prior randomized clinical trials comparing other HA products with IA injections have not

Table 4 Musculoskeletal and Connective Tissue Adverse Events

	IA-SA (N = 295) n (%)	IA-BioHA (N = 291) n (%)
Musculoskeletal and connective tissue disorders	72 (24.0)	62 (21)
Arthralgia	35 (12)	27 (9)
Back pain	11 (4)	12 (4)
Pain in extremity	10 (3)	3 (1)
Musculoskeletal pain	4 (1)	6 (2)
Osteoarthritis	7 (2)	2 (1)
Joint swelling	4 (1)	4 (1)
Joint stiffness	4 (1)	3 (1)
Muscle spasm	3 (1)	3 (1)
Neck pain	4 (1)	1 (0)
Myalgia	2(1)	2 (1)
Bursitis	2 (1)	1 (0)
Tendonitis	2 (1)	1 (0)
Arthropathy	0	2 (1)
Joint effusion	2 (1)	0
Musculoskeletal stiffness	0	2 (0)
Arthritis	0	1 (0)
Bone pain	0	0
Bone swelling	0	0

IA-SA, intra-articular buffered saline; IA-BioHA, intra-articular 1% sodium hyaluronate.

been uniformly positive (2,25,26). This study was powered to detect a statistically significant difference between groups at 26 weeks, and despite the dramatic effect of IA-SA, IA-BioHA was superior at this time point. The separation of IA-BioHA from IA-SA began shortly after the third IA injection and became more evident over time. As a result, the robust IA-SA response likely contributed to the lack of statistical superiority of IA-BioHA over IA-SA at the $P < 0.05$ level at 12 weeks in some secondary endpoint measures as well as the overall WOMAC pain subscale.

In a meta-analysis of 198 clinical trials, the placebo effect size (the standardized mean difference between baseline and endpoint) in OA trials was determined to be 0.51 (95% CI 0.46-0.55) (27). Interestingly, the effect size of IA-SA is significantly higher (0.73; 95% CI 0.56-0.91). This effect can be attributed to several factors including placebo effect, the fact that joint aspiration alone improves OA knee pain, and regression to the mean (patients with severe symptoms are chosen for injection and in a variable condition tend to move toward the mean level of severity independently of the procedure) (28,29). In this study, 62 (21%) IA-BioHA and 51 (17%) IA-SA subjects reported aspiration of joint fluid during 1 or more of the injection series. Additional influences on the placebo effect include fluctuations in symptoms over time, lack of a natural history comparison, patient desire to please, and the use of rescue medication (30). Also, there is probably a therapeutic benefit to IA-SA that may be sustained for 6 months or more (28,29,31). Hence, when referring to IA injection, aspiration, and installation of buffered saline, the term "placebo" might be considered a misnomer. IA-SA might better be considered an active comparator rather than placebo.

Several previous studies have analyzed severity of OA to try to identify patients most likely to respond to therapy (32-34). It is suspected that those with severe radiographic change (Kellgren-Lawrence 4) are less responsive to HA therapy (32-34). In this study of Kellgren-Lawrence 2 and 3 subjects, those with less severe radiographic change (Kellgren-Lawrence 2), were more responsive to therapy. A limitation of this study was the exclusion of subjects with Kellgren-Lawrence 4 radiographic change. If supported by additional research, projecting responsiveness to therapy is helpful to the clinician in setting patient expectations.

Following database lock, it was noted that 20 subjects were errantly enrolled into the study with 50-foot walk pain scores less than 41 mm. Since randomization of these subjects (14 IA-BioHA, 6 IA-SA) represented major protocol violations and potentially lessened the overall treatment effect, post-hoc analysis was conducted to determine the impact on the primary outcome measure. Excluding these 20 subjects had little effect on the results or statistical significance levels; therefore, they were not included in this report.

Treated subjects experienced significant improvement in functionality as measured with the PCS score of the SF-36. The PCS scores and its individual domains have been found to be responsive across different severity levels of arthritis and to discriminate between arthritis treatment responders and nonresponders (35).

Of the 5 products available in the United States, the MW, molecular structure, and sourcing of the HA differ substantially. IA-BioHA has an MW range between 2.4 and 3.6 MDa, the highest MW of any of the non-cross-linked products available. Of the products available in the United States, 3 (Synvisc®, Hyalgan®, Suprartz®) are produced from avian sources (chicken or rooster combs) and therefore potentially contain the inflammatory and immunogenic impurities endogenous to avian tissue (36). IA-BioHA is produced by biologic fermentation, free of any avian proteins. A previously reported head-to-head study versus Synvisc® (hylan G-F 20) established IA-BioHA's efficacy at 12 weeks and also demonstrated statistically and clinically significant differences favoring IA-BioHA based on patient satisfaction, percentage of subjects "pain-free" at study completion (VAS pain score of $\leq$20), and percentage of subjects requiring acetaminophen rescue medication. Statistically significant and clinically important differences in safety outcomes were also found in that trial. The number of local reactions accompanied by effusion was significantly higher in the hylan G-F 20 group (8%) than in the IA-BioHA group (1%) (17). The lack of joint effusions noted in the current study confirms the prior safety profile of IA-BioHA.

Results of the FLEXX trial demonstrate significant OA knee pain relief with IA-BioHA therapy, which is sustained for 6 months. The utility of IA-BioHA therapy for knee OA is further supported by significant improvements in subject function, subject satisfaction with treatment, and HRQoL. The results of this study also support the favorable safety profile of IA-BioHA.

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