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Safety and efficacy of retreatment with a bioengineered hyaluronate for painful osteoarthritis of the knee: results of the open-label Extension Study of the FLEXX Trial[1]

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SUMMARY

Objective: To evaluate the safety of repeated intra-articular (IA) injections of Euflexxa® (1% sodium hyaluronate; IA–BioHA) for painful knee osteoarthritis (OA).
Design: Participants who completed the randomized, double-blind, 26-week FLEXX Trial comparing IA-BioHA to IA saline (IA-SA) for knee OA[1] received three weekly IA-BioHA injections in a 26-week Extension Study. Adverse events (AEs) were recorded and the effect of treatment on knee pain was measured immediately following a 50-foot walk test using a 100 mm visual analog scale (VAS). Responder rate, Medical Outcomes Study Short Form 36 scores, Patient's Global Assessment, and intake of rescue medication were also evaluated.
Results: The Extension Study included 433 subjects, 219 who received IA-BioHA and 214 who received IA-SA during the FLEXX Trial. Safety results from the Extension Study indicated that 43.4% (188/433) of subjects had AEs, of which 4.8% (21/433) were deemed treatment-related AEs. Two AEs in the Extension Study led to discontinuation, and no joint effusion was reported. Patients who continued with IA-BioHA in the Extension Study maintained their improvement from baseline, with an average reduction in pain in the VAS score of −3.5 mm. Patients initially treated with IA-SA in the FLEXX Trial also had a reduction in VAS score of −9.0 mm. Secondary efficacy variables also improved during the Extension Study.
Conclusions: Repeat injections of IA-BioHA were effective, safe, well tolerated, and not associated with an increase in AEs, such as synovial effusions. Additional symptom improvements were noted for subjects who received either IA-BioHA or IA-SA in the FLEXX Trial.
Clinical Trial Registration Number: NCT00379236

Introduction

Intra-articular (IA) injection of hyaluronate (HA) has been shown to be safe and effective for relieving pain in patients with osteoarthritis (OA) of the knee[1–6] and is recommended for patients who cannot be effectively managed with non-pharmacologic interventions or simple analgesics[7].

Euflexxa (BioHA) is a bioengineered 1% sodium HA that is produced by biological fermentation and does not require cross-linking[8]. It has a molecular weight range of 2.4–3.6 million daltons that is achieved by controlled fermentation, recovery, and purification processes. The safety and efficacy of a single course of BioHA was evaluated in a 26-week, randomized, double-blind, multicenter, saline-controlled FLEXX Trial[1]. The study included 588 subjects with painful knee OA who received three weekly IA injections of either BioHA or buffered saline (IA-SA). Results from the FLEXX Trial showed that IA-BioHA decreased mean pain

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100 mm visual analog scale (VAS) scores immediately after a 50-foot walk test by −25.7 mm vs −18.5 mm for the IA-SA group. Both treatments were well tolerated with about 1% of subjects in each group reporting injection-site reactions.

Although many studies support the safety and efficacy of single course IA-HA injections, fewer trials have evaluated the risks and benefits of repeated series of injections. Some evidence assessing the safety and tolerability of repeat IA-HA injections exists from an open-label 12-month study of 108 subjects with knee OA, in which a second course of treatment 4–8 months after the initial intervention reduced symptoms beyond that observed after the first course of treatment[9]. Another long-term study showed repeat injections of either avian or non-avian-derived HA also improved resting pain between the first and tenth series of injections[10]. However, 4.8% of subjects reported adverse events (AEs) (most often pain, effusion, and erythema) for avian-derived HA vs 1.7% for non-avian-derived HA between the second and tenth series of injections[10].One recent study suggests that re-injection with HA may slow progression of structural damage in subjects with milder knee OA[11]. Thus, it is important to understand and assess both the risks and potential benefits of repeated IA-HA injections.

To further address the safety of a repeated series of IA-BioHA injections, we conducted a multicenter, 26-week, open-label Extension Study of the FLEXX Trial. Subjects completing the FLEXX Trial were offered the option to receive an additional three-injection series (one injection per week for 3 weeks) of IA-BioHA and were followed for an additional 23 weeks after the injections during the Extension Study. This report provides new information about the safety and efficacy of re-injection of IA-BioHA in subjects who received IA-BioHA in the FLEXX Trial as well as the effects of a single series of IA-BioHA injections in the 26-week Extension Study for subjects who received IA-SA in the FLEXX Trial.

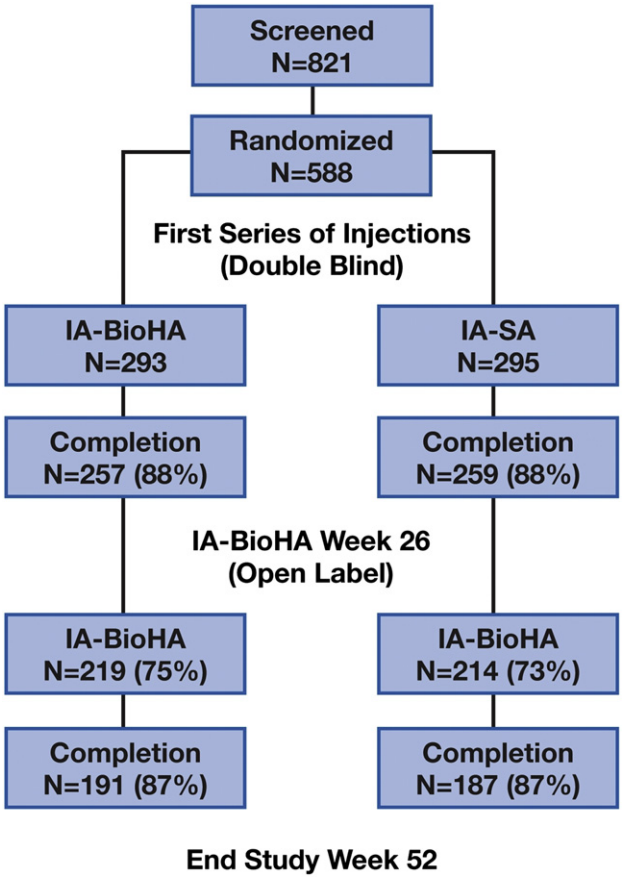

Fig. 1. Flow of participants.

Subjects and methods

Summary of the FLEXX Trial

The FLEXX Trial[1] was a randomized (1:1), double-blind, multicenter, saline-controlled study that enrolled subjects with OA of the knee according to American College of Rheumatology criteria: moderate-to-severe joint pain (a score of 41–90 on a 100 mm VAS) immediately following a 50-foot walk, and a bilateral standing anterior-posterior radiograph demonstrating Kellgren and Lawrence grade 2 or 3 OA of the target knee. Five hundred eighty-eight (588) subjects were randomized to either IA-BioHA ($n = 293$) or IA-SA ($n = 295$). Two hundred fifty-seven subjects (88%) who received IA-BioHA and 259 subjects who received IA-SA (88%), a total of 516 subjects, completed the FLEXX Trial (Fig. 1).

The primary efficacy endpoint of the FLEXX Trial was the difference between IA-BioHA and IA-SA in subjects' pain scores, measured on a 100-mm VAS after a 50-foot walk test and compared to baseline at 26 weeks following treatment. Safety was assessed by monitoring and reporting vital signs, physical examination of the target knee following injection, AEs, and concomitant medications. The sample size for the FLEXX Trial was based on the requirement for 90% power to detect an 8.0 mm difference between average IA-BioHA and IA-SA scores at the two-sided 5% significance level and an estimated 30% dropout rate by week 26[1].

Study design for the FLEXX Trial Extension Study

The Extension Study was a multicenter open-label 26-week trial. All 516 subjects who completed the FLEXX Trial were eligible for the Extension Study and remained without knowledge of whether they received IA-BioHA or IA-SA in the initial series of injections (Fig. 1).

The FLEXX Trial Extension Study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and in compliance with the approved protocol, Good Clinical Practice, and applicable regulatory requirements. Consent was obtained for participation in the Extension Study from each subject in accordance with local Institutional Review Board oversight.

Treatment

IA-BioHA is a 20 mg per 2 mL viscoelastic sterile solution of highly purified, non-cross-linked, high molecular weight (2.4–3.6 million daltons) hyaluronan extracted from bacterial cells in phosphate-buffered saline. All subjects enrolled in the Extension Study received one 2 mL injection of IA-BioHA per week for 3 weeks in the same knee targeted during the FLEXX Trial. Skilled 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. IA-BioHA was injected by either a supra- or infra-patellar approach without fluoroscopic or ultrasound guidance. If aspiration of the joint was necessary, the joint capsule could be infiltrated with lidocaine using a 20- to 23-gauge needle. However, if an effusion was present, IA-BioHA was injected using a 16- to 18-gauge needle. Injections were administered at baseline (week 26) and weeks 27 and 28. Subjects were interviewed by telephone at week 34 or 35 and office visits were carried out at weeks 41 and 52 (all time points refer to the time from initiation of the FLEXX Trial).

Outcome measurements

Efficacy

Efficacy measures included knee pain when walking a 50-foot distance recorded on a 100 mm VAS at Extension Study baseline (week 26) and weeks 27, 28, 41, and 52; the Outcome Measures in Rheumatology (OMERACT)–Osteoarthritis Research Society International (OARSI) responder rate at Extension Study baseline and weeks 27, 28, 41, and 52[12]; the Western Ontario and McMaster Universities osteoarthritis index (WOMAC) subscales of Pain, Stiffness, and Disability at Extension Study baseline and week 52; Patient's Global Assessment ("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 trial") recorded on a 100 mm VAS at Extension Study baseline and week 52; the Short Form Survey Instrument SF-36v2 Health Survey (scored per instructions in the Second Edition of the User's Manual for the SF-36v2 Health Survey) at Extension Study baseline and week 52[13]; and the weekly number of tablets of acetaminophen rescue medication used between visits.

Safety

All AEs were recorded at each visit/interview. Safety variables also included laboratory parameters, vital signs, and physical examination of the knee.

Statistical methods

Efficacy variables were analyzed using an intent-to-treat (ITT) approach. The population for efficacy analysis included subjects who had an evaluation at baseline of the Extension Study (week 26), received at least one injection of IA-BioHA, and had at least one post-baseline evaluation during the Extension Study. Safety analyses included all 433 subjects enrolled in the Extension Study.

Differences in baseline characteristics between the 219 subjects treated with IA-BioHA and the 214 subjects treated with IA-SA during the FLEXX Trial were assessed with Chi-square tests for categorical variables and Student's 2-sample *t*-tests for continuous variables, with or without Satterthwaite's correction for unequal variances, as appropriate.

Data summaries for evaluation of safety in the Extension Study included the overall number and percentage of subjects experiencing at least one treatment-emergent AE (TEAE) and listing of AEs that occurred with a prevalence of >1% in the Extension Study. Percentages of patients experiencing TEAEs during the double-blind trial are also included for comparison. Ninety-five percent confidence intervals (CIs) for all TEAE percentages were calculated using the (second-order correct) Wilson interval.

For evaluation of efficacy, average changes from baseline in pain VAS scores were estimated with least squares means (LSM) ± standard error of the mean (SEM), derived with a repeated-measures, mixed-effects analysis of covariance (ANCOVA) model, with no data imputation for each time point. The structure of the correlation pattern of patients repeated VAS scores of changes from baseline is user specified. That which passed goodness-of-fit criteria resulted in using an exchangeable correlation structure (compound symmetry) for the repeated measurements. The model included the following factors: baseline pain score on the 50-foot walk test, trial center, core treatment group, trial week, and treatment group-by-trial week interaction. The trial center was classified as a random factor and trial week as a repeated measure. The interactions between study center and treatment group and between the covariate and treatment group were also to be included in the model if they were significant, but this was not the case for either interaction.

The other efficacy outcomes of OARSI–OMERACT responder rates, the three WOMAC domains of Pain, Stiffness, and Disability, Patient's Global Assessment, the average number of study-specific acetaminophen (rescue medication) tablets consumed per week, and SF-36 Health Survey average scores for Physical Functioning (PF) and Bodily Pain (BP) were summarized with descriptive statistics as changes from baseline during the Extension Study (between week 26 and week 52) and percentage changes from the FLEXX Trial baseline (week 0). Prior to analysis, all SF-36 items were transformed into norm-based values using data from the 1988 United States general population.

Results

Subjects

Three hundred seventy-eight (378) subjects (87%) completed the Extension Study, including 187 subjects treated with IA-SA and 191 treated with IA-BioHA in the FLEXX Trial (Fig. 1). Eleven subjects who did not receive all three injections were included in the group of discontinuations [study-related AEs (two), unrelated AEs (three), withdrew consent (two), lost to follow-up (four)]. Eight subjects failed to meet criteria for inclusion in the ITT efficacy analysis and the population evaluated for efficacy thus included 425 subjects. Two of the 425 subjects in the efficacy analysis received only two injections and nine subjects received only one injection during the Extension Study.

There were no differences in the demographics of the subjects treated with IA-SA or IA-BioHA during the FLEXX Trial who agreed to continue in the 26-week Extension Study (Tables I and II).

Safety

Overall, 43.4% of subjects reported at least one TEAE during the Extension Study compared to 55.4% who reported at least one TEAE during the double-blind FLEXX Trial (Table III). The percentages of subjects experiencing at least one AE in the Extension Study were similar for those who previously received IA-BioHA (43.8%) or IA-SA (43.0%) during the FLEXX Trial. The AEs that occurred most often in the Extension Study were arthralgia (9.9%), injury (4.4%), nasopharyngitis (3.5%), upper respiratory infections (3%), and joint swelling in the soft tissue at the injection site (2.3%) (Table III). There were no differences in the AE profiles for subjects receiving IA-SA as their initial injection during the FLEXX Trial or IA-BioHA re-injection during the Extension Study (Table III).

Among subjects with at least one AE in the Extension Study, 21 (4.8%) had an event considered related to the IA-BioHA. The most frequent treatment-related AEs were arthralgia (2.8%), joint swelling (1.2%), peripheral edema (0.7%), and injection site pain

Table I
Characteristics of the Extension Study population

	IA-BioHA/IA-BioHA $N = 219$	IA-SA/IA-BioHA $N = 214$	All $N = 433$
Men *n* (%)	82 (37.4)	78 (36.4)	160
Women *n* (%)	137 (62.6)	136 (63.6)	273
Age Y Mean (SD)	62.7 (11.0)	60.7 (10.2)	61.7 (10.7)
Race (%)			
Caucasian	170 (77.6)	163 (76.2)	333 (76.9)
African American	20 (9.1)	23 (10.7)	43 (9.9)
Asian	5 (2.3)	3 (1.4)	8 (1.8)
Hispanic	23 (10.5)	22 (10.3)	45 (10.4)
Other	1 (0.5)	3 (1.4)	4 (0.9)
Weight kg Mean (SD)	91.2 (21.8)	92.4 (22.1)	91.8 (22.0)
Height cm Mean (SD)	167.2 (11.3)	167.7 (10.9)	167.5 (11.1)
BMI kg/m^2 Mean (SD)	32.7 (7.6)	33.0 (7.6)	32.8 (7.6)

SD = standard deviation; BMI = body mass index.

Table II
Discontinuation in the Extension Study

Reason for discontinuation	Total *N* (%)
AE	13 (3.0)*
Use of exclusionary medication	3 (0.7)
Protocol violation	7 (1.6)
Withdrawal of consent	13 (3.0)
Lost to follow-up	12 (2.8)
Other	7 (1.6)

* There were two discontinuations due to TEAEs, both for arthralgia.

(0.5%). There was no detectable pre-treatment or post-treatment synovitis or synovial effusions in either the FLEXX Trial or the Extension Study. Twenty events classified as serious TEAEs were reported in 12 subjects (2.8%) during the Extension Study; none were considered related to study treatment. There were no deaths. Two subjects re-injected with IA-BioHA had treatment-related AEs of joint pain that led to discontinuation from the Extension Study.

Efficacy

Pain VAS

At the end of the FLEXX Trial, there was a −26.5 mm reduction in pain VAS scores for subjects treated with IA-BioHA and a −21.5 mm pain reduction in those who received IA-SA (Fig. 2). Subjects treated with BioHA in the FLEXX Trial who continued into the Extension Study had a further −3.5 mm decrease in the pain scale scores between weeks 26 and 52. Those treated with IA-SA in the FLEXX Trial had a further −9.0 mm decrease in pain scale scores between weeks 26 and 52 during the Extension Study (Fig. 2). For both groups, improvement occurred by week 27 and was sustained to week 52.

OMERACT–OARSI responder rate

Among the 425 subjects analyzed for efficacy during the Extension Study, the OMERACT–OARSI responder rate during the FLEXX Trial (from week 0 to week 26) was 67% for the subjects who received IA-BioHA and 59% for those treated with IA-SA. The responder rate for all subjects was 75.3% at the completion of the Extension Study (week 52) (Table IV).

WOMAC pain, stiffness, and disability

Between the end of the FLEXX Trial and the end of the Extension Study, the mean WOMAC Pain score decreased an additional −6.1 mm, WOMAC Stiffness decreased an additional −5.9 mm, and WOMAC Disability decreased an additional −5.6 mm. Overall WOMAC Pain, Stiffness, and Disability scores decreased by 47.2%, 42.5%, and 44.1%, respectively, from the FLEXX Trial baseline to the end of the Extension Study (Table IV).

Table III
TEAEs in the Extension Study experienced by >1% of patients, with corresponding percentages from the double-blind FLEXX Trial: number of subjects, percentages and 95% CIs

			FLEXX Trial			Extension Study		
			IA-BioHA *N* = 293 *n* (%)	IA-SA *N* = 295 *n* (%)	All treatments *N* = 588 *n* (%)	IA-BioHA/ IA-BioHA *N* = 219 *n* (%)	IA-SA/ IA-BioHA *N* = 214 *n* (%)	All treatments *N* = 433 *n* (%)
Subjects experience at least one TEAE (any prevalence)			157 (53.6) [47.9,59.2]	169 (57.3) [51.6,62.8]	326 (55.4) [51.4,59.4]	96 (43.8) [37.4,50.5]	92 (43.0) [36.5,49.7]	188 (43.4) [38.8,48.1]
Organ-system AEs (prevalence >1% in Extension Study)	Gastrointestinal disorders	Nausea	5 (1.7) [0.7,3.9]	7 (2.4) [1.2,4.8]	12 (2.0) [1.2,3.5]	4 (1.8) [0.7,4.6]	3 (1.4) [0.5,4.0]	7 (1.6) [0.8,3.3]
		Diarrhea	12 (4.1) [2.4,7.0]	2 (0.7) [0.2,2.4]	14 (2.4) [1.4,4.0]	3 (1.4) [0.5,4.0]	1 (0.5) [0.1,2.6]	4 (0.9) [0.4,2.4]
	General disorders and administrative site	Pain	4 (1.4) [0.5,3.5]	1 (0.3) [0.1,1.9]	5 (0.9) [0.4,2.0]	4 (1.8) [0.7,4.6]	1 (0.5) [0.1,2.6]	5 (1.2) [0.5,2.7]
		Injection site pain	2 (0.7) [0.2,2.5]	0 [0.0,1.3]	2 (0.3) [0.1,1.2]	0 [0.0,1.7]	3 (1.4) [0.5,4.0]	3 (0.7) [0.2,2.0]
	Immune system disorders	Hypersensitivity	1 (0.3) [0.1,1.9]	1 (0.3) [0.1,1.9]	2 (0.3) [0.1,1.2]	3 (1.4) [0.5,4.0]	1 (0.5) [0.1,2.6]	4 (0.9) [0.4,2.4]
	Infections	Nasopharyngitis	4 (1.4) [0.5,3.5]	13 (4.4) [2.6,7.4]	17 (2.9) [1.8,4.6]	10 (4.6) [2.5,8.2]	5 (2.3) [1.0,5.4]	15 (3.5) [2.1,5.6]
		Upper respiratory infection	12 (4.1) [2.4,7.0]	11 (3.7) [2.1,6.6]	23 (3.9) [2.6,5.8]	6 (2.7) [1.3,5.9]	7 (3.3) [1.6,6.6]	13 (3.0) [1.8,5.1]
		Sinusitis	6 (2.1) [0.9,4.4]	10 (3.4) [1.9,6.1]	16 (2.7) [1.7,4.4]	5 (2.3) [1.0,5.2]	3 (1.4) [0.5,4.0]	8 (1.9) [0.9,3.6]
		Urinary tract infection	6 (2.1) [0.9,4.4]	6 (2.0) [0.9,4.4]	12 (2.0) [1.2,3.5]	3 (1.4) [0.5,4.0]	1 (0.5) [0.1,2.6]	4 (0.9) [0.4,2.4]
	Injury and procedural complications	Injury	8 (2.7) [1.4,5.3]	9 (3.1) [1.6,5.7]	17 (2.9) [1.8,4.6]	9 (4.1) [2.2,7.6]	10 (4.7) [2.6,8.4]	19 (4.4) [2.8,6.8]
	Musculoskeletal and connective tissue disorders	Arthralgia	27 (9.2) [6.4,13.1]	35 (11.9) [8.7,16.1]	62 (10.5) [8.3,13.3]	19 (8.7) [5.6,13.2]	24 (11.2) [7.7,16.1]	43 (9.9) [7.5,13.1]
		Joint swelling	4 (1.4) [0.5,3.5]	3 (1.0) [0.4,3.0]	7 (1.2) [0.6,2.4]	6 (2.7) [1.3,5.9]	4 (1.9) [0.7,4.7]	10 (2.3) [1.3,4.2]
		Back pain	12 (4.1) [2.4,7.0]	11 (3.7) [2.1,6.6]	23 (3.9) [2.6,5.8]	6 (2.7) [1.3,5.9]	1 (0.5) [0.1,2.6]	7 (1.6) [0.8,3.3]
		Pain in extremity	3 (1.0) [0.4,3.0]	10 (3.4) [1.9,6.1]	13 (2.2) [1.3,3.8]	3 (1.4) [0.5,4.0]	4 (1.9) [0.7,4.7]	7 (1.6) [0.8,3.3]
	Nervous system disorders	Headache	6 (2.1) [0.9,4.4]	11 (3.7) [2.1,6.6]	17 (2.9) [1.8,4.6]	3 (1.4) [0.5,4.0]	2 (0.9) [0.3,3.3]	5 (1.2) [0.5,2.7]
	Respiratory disorders	Cough	7 (2.4) [1.2,4.9]	3 (1.0) [0.4,3.0]	10 (1.7) [0.9,3.1]	3 (1.4) [0.5,4.0]	1 (0.5) [0.1,2.6]	4 (0.9) [0.4,2.4]
	Vascular disorders	Hypertension	13 (4.4) [2.6,7.4]	5 (1.7) [0.7,3.9]	18 (3.1) [1.9,4.8]	1 (0.5) [0.1,2.5]	4 (1.9) [0.7,4.7]	5 (1.2) [0.5,2.7]

N = number of subjects in the safety population; *n* = number of subjects having experienced the TEAE within the organ system class; (%) = percentage of subjects = (*n*/*N*) 100. AEs categorized by the treatment arm for the double-blind trial.

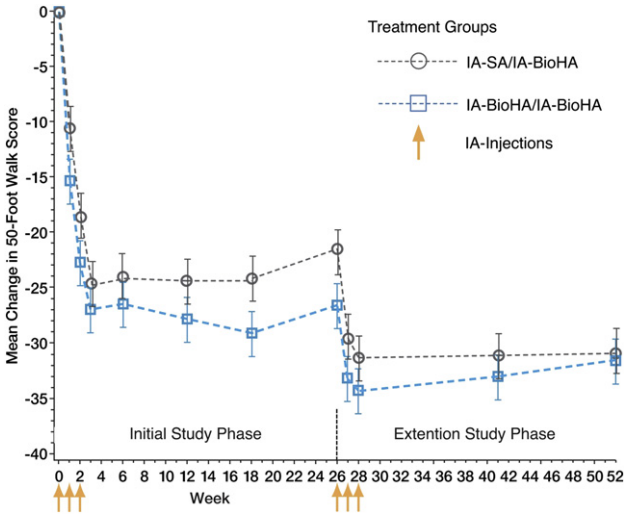

Fig. 2. Mean change in VAS pain scores following 50-foot walk test from week 0 to week 52.
During the FLEXX Trial, the randomized groups received one injection of either IA-BioHA or IA-SA at weeks 0, 1 and 2, with re-injection of IA-BioHA at weeks 26, 27, and 28 only in subjects who elected to participate in the Extension Study. Data points represent LSM ± SEM, estimated with a repeated-measures, mixed-model ANCOVA model, with no data imputation. The model included the following factors: baseline pain score on the 50-foot walk test as a covariate, trial center, core treatment group, trial week, and treatment group-by-trial week interaction. Trial center was classified as a random factor, and trial week as a repeated measure.

Patient's Global Assessment

Patient's Global Assessment improved an additional −8.1 mm, an overall 51.6% decrease from initiation of the FLEXX Trial to the end of the Extension Study.

Use of rescue medication

Mean acetaminophen use was reduced from 14.6 to 12.8 tablets per week over the course of the FLEXX Trial. At the end of the Extension Study, acetaminophen use was further reduced to 9.5 tablets per week, representing an overall 34.9% reduction from the beginning of the FLEXX Trial (Table IV).

SF-36

At FLEXX Trial baseline, the mean SF-36 PF and BP scale scores were 32.7 and 36.9, respectively. At the end of the FLEXX Trial, these scores improved to 36.7 and 40.4. At the end of the Extension Study, the scores for the PF and BP scales were 37.7 and 42.7. The mean changes from the end of the FLEXX Trial to the end of the Extension Study were 0.9 and 2.2 for SF-36 PF and BP, respectively. From FLEXX Trial baseline to the end of the Extension Study, there were 15.2% and 15.7% improvements for SF-36 PF and BP, respectively.

Table IV
Summary of results for secondary efficacy endpoints

OMERACT–OARSI Responders (%)	75.3
Measure	% Improvement from Baseline of Double-blind Study
WOMAC Pain	47.2
WOMAC Stiffness	42.0
WOMAC Disability	44.1
Patient's Global Assessment	51.6
Acetaminophen use	34.9 (reduction)
SF-36 PF	15.2
SF-36 BP	15.7

Discussion

Although efficacy of IA-HA injections has been established previously, safety of repeated HA injections, including BioHA, is understudied. The FLEXX Trial Extension Study showed that a repeated series of three weekly injections of IA-BioHA given 23 weeks after an initial three-injection treatment course was effective, safe, and well tolerated in subjects with chronic painful OA of the knee. Pain reduction observed from an initial injection of IA-BioHA during the FLEXX Trial was sustained for an additional 23 weeks during the Extension Study after IA-BioHA re-injection. No subject reported a joint effusion during either the initial FLEXX Trial or following re-injection of IA-BioHA during the Extension Study.

The positive results obtained with re-injection of IA-BioHA in the present study are consistent with those from prior trials that have addressed this issue. An observational study of 108 subjects with knee OA showed decreased pain in four subjects who received a repeat series of five injections of HA[9]. Another study of 306 subjects with knee OA who received four series of five HA injections every 6 months indicated that HA was significantly superior to saline with respect to achievement of OARSI responder criteria over 3.5 years of follow-up[14]. A third study, which included 897 subjects with knee OA who received two series of a 500,000–730,000 dalton non-avian-derived HA, showed that improvements in walking and resting pain following the second series of injections were at least equivalent to those after the first series[15]. Safety results and AEs associated with HA re-injection were not reported in these studies.

Some data regarding safety of repeated HA injections have been reported previously. Results of one small-scale study of 39 subjects with knee OA who received a series of three injections with either saline or HA every 3 months over 1 year indicated that HA re-injection was not significantly superior to *placebo* for improving pain or function. In this trial, 40% of the 20 HA-treated subjects reported pain during or immediately after the injection for at least one of the nine injections for a total of 17 events[16]. A trial of 75 subjects with knee OA treated with a three-injection series of a straight chain avian-derived HA every 6 months for 30 months showed that pain decreased following the first injection and continued to decline throughout the study. Neither local nor systemic AEs were observed with re-injection; however, five subjects (6.6%) complained of localized pain after IA injection[17].

Several published studies report differences in AE risk associated with hylan and different HA preparations. A systematic review and meta-analysis of thirteen randomized, controlled clinical trials with a pooled total of 2085 patients revealed an increased risk associated with hylan for any local AE (relative risk [RR] 1.91; 95% CI 1.04, 3.49; $I = 28\%$) and for flares (RR 2.04; 95% CI 1.18, 3.53; $I = 0\%$), however there was no difference observed in efficacy for hylan and HAs[18]. One multicenter, subject-blinded, randomized control trial included 660 subjects with symptomatic knee OA who were randomized to three IA injections of either a high molecular weight (average molecular weight 6 million daltons) avian-derived, cross-linked hylan or one of two HA preparations; a non-cross-linked medium molecular weight (1.0–2.9 million daltons) avian-derived HA or a non-cross-linked low molecular weight (about 1.5 million daltons) HA obtained through bacterial fermentation. Subjects completing the initial phase of the trial were offered an additional three-injection series during months 7–12 of the study. Although pain relief was similar in all treatment groups, re-injection of the high molecular weight, cross-linked, avian-derived hylan was associated with more local AEs (9.1%), effusions (7.3%), and flares (6.4%) than the HA preparations (2.7%, 2.7%, and 0%, respectively)[2]. Repeated injections of avian and non-avian-derived HA were also compared in a non-randomized study that included 4412 subjects treated over the period from 1997 to 2007. The HA preparation used

was based on subject preference and subsequent series of injections were at the subjects' discretion. Study results indicated that both the avian and non-avian preparations improved resting pain over as many as 10 series of injections. AEs were reported for 4.8% of subjects receiving avian-derived HA vs 1.7% for those treated with the non-avian preparation[10].

Although the FLEXX Trial Extension Study provided new information about the safety and efficacy of BioHA re-injection, the trial had limitations that should be acknowledged. The Extension Study was an open-label trial and lacked a control group. This aspect of the study design may have influenced subjects' perceptions and reporting of efficacy, but it was necessary to maintain subject participation, as it is very difficult to enroll subjects in a long-term study if they are not guaranteed active therapy. The open-label design of the Extension Study was unlikely to have influenced AE reporting, especially since the subjects remained blinded to previous therapy, and subjects from both the IA-SA and IA-BioHA treated groups reported similar numbers of AEs. A second limitation of the Extension Study was that it included only a single course of retreatment. Thus, it is not known whether further series of re-injections would result in different efficacy or safety findings. Finally, while the sample size for this trial was large relative to several of the other long-term evaluations of repeated injections of IA-HA, conclusions regarding safety must be tempered. With sample sizes of 200, 300, and 400, for example, there is 95% chance of observing at least one event with rates of 1.5%, 1% and 0.75%, respectively. Detection of very rare AEs would require study of many thousands of subjects[19].

In conclusion, results from this FLEXX Trial Extension Study showed that re-injection with BioHA was safe and effective. Comparison of Extension Study results with those from prior trials suggests further that the efficacy of IA-BioHA re-injection was at least equivalent to that reported for re-injection of other IA-HA agents, and that the AE profile for re-injection of IA-BioHA was at least equal, if not superior, to that reported for other IA-HA preparations. Results from subjects who received their initial treatment with IA-BioHA in the FLEXX Trail Extension Study also supported the efficacy and safety of this preparation.

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Author contribution statement

RDA, JER, DAB, and HTH all contributed to the conception and design of the study, acquisition of data, and analysis and interpretation of data as well as reviewing the manuscript and revising it critically for important intellectual content. Statistical expertise was provided by DAB. RDA approved the final version of the manuscript submitted for publication and is responsible for the integrity of the work as a whole.

Conflict of interest

Roy D. Altman, MD is a paid consultant to Ferring Pharmaceuticals Inc., Rotta Pharmaceuticals, Inc., Novartis Pharmaceuticals Corporation, Endo Pharmaceuticals, AstraZeneca Pharmaceuticals LP, and Abbott Theralogix, LLC as well as receives clinical trial funding from Ferring Pharmaceuticals and Novartis Pharmaceuticals Corporation.
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Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.joca.2011.07.001.

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