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A double-blind randomized controlled trial comparing alternate forms of high molecular weight hyaluronan for the treatment of osteoarthritis of the knee[1]

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Summary

Objective: To compare the safety and effectiveness of a high molecular weight hyaluronan produced by biological fermentation (Bio-HA) with those of avian-derived hyaluronan that uses cross-linking to achieve high molecular weight (CL-HA).

Design: This was a prospective, multicenter, randomized, double-blind trial evaluating patients with confirmed osteoarthritis of the knee. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC Index) pain subscale was the primary effectiveness measure (visual analog scale). Both products were administered via three weekly injections, with follow-up evaluations at weeks 3, 6 and 12. Acetaminophen was permitted as rescue medication and quantitated by pill counts.

Results: Analyses were performed on the intent-to-treat population, defined as all patients receiving at least one injection. Of the 321 patients randomized to treatment, 314 patients (98%) completed the final study assessment. Improvement in the average WOMAC Index pain score was 29.8 mm (−61.6%) for Bio-HA and 28.8 mm (−54.9%) for CL-HA, meeting the prospective criteria for non-inferiority. For the secondary outcome measures, statistically significant differences favored Bio-HA for the number of patients requiring acetaminophen ($P = 0.013$) and patient global satisfaction evaluations ($P = 0.03$). Local reactions differed between the products in that 15 effusions were reported in 13 CL-HA patients (8.1%) after injection, compared to one effusion (0.6%) after Bio-HA injection ($P = 0.0015$).

Conclusion: The effectiveness of Bio-HA was not inferior to that of CL-HA. The significantly higher incidence of post-injection effusion in the CL-HA group provides a safety advantage for Bio-HA. These data suggest that Bio-HA has an improved benefit-risk profile compared with CL-HA.

Key words: Osteoarthritis, Viscosupplementation, Hyaluronan.

Introduction

Intra-articular hyaluronan (IA-HA) injections are now licensed worldwide for the treatment of osteoarthritis (OA). In the United States, IA-HA is indicated for pain relief in patients with OA of the knee who fail to respond to conservative non-pharmacologic therapy or simple analgesics (e.g., acetaminophen), and is included in the guidelines of the American College of Rheumatology and the American Academy of Orthopedic Surgery. The goal of therapy is to reduce pain and improve physical function by temporarily supplementing the viscosity and elasticity of synovial fluid, which are reduced in OA[1]. A course of treatment consists of a series of three to five weekly intra-articular injections with a viscoelastic solution of hyaluronan or its derivatives. Efficacy trials comparing IA-HA injections with saline injections demonstrate a statistically significant difference over a 3–6-month period, depending on the trial design[2–5]. In clinical practice, patients can experience symptomatic benefits for a year or longer[6–8].

Commonly referred to as viscosupplementation, the therapeutic benefits of IA-HA injections are believed to be dependent on the viscoelastic properties of the hyaluronan injected[9]. It is widely believed that higher molecular weight hyaluronan preparations will provide improved clinical benefits[10,11]. Questions regarding the importance of molecular weight for IA-HA products are of particular clinical relevance, because products can differ substantially in this parameter. Four IA-HA products are currently available in the United States: Hyalgan® (Fidia SpA, Padua, Italy), Supartz® (Seikagaku Corporation, Tokyo, Japan), Orthovisc® (Anika Therapeutics, Woburn, MA) and Synvisc® (Genzyme Corporation, Cambridge, MA). Supartz, Hyalgan and Orthovisc contain unmodified hyaluronan derived from chicken combs, with molecular weight ranges specified on their respective labels as 0.62–1.2 million Daltons for Supartz, 0.5–0.72 million Daltons for Hyalgan, and 1–2.9 million Daltons for Orthovisc[12–13]. Synvisc is composed of two cross-linked derivatives of hyaluronan (CL-HA): solid hylan gel particles and soluble hylan molecules described as having a molecular weight of 6 million Daltons[14]. Several recent publications have noted acute local reactions after hylan CL-HA injection[15,16], particularly in patients receiving repeat treatment[17]. Inflammatory reactions around hylan gel particles have also been histologically observed in synovial

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biopsies[18,19]. These reports make it especially important to consider safety differences among IA-HA products, and alternate ways of producing high molecular weight hyaluronan for viscosupplementation.

All four of the IA-HA products currently available in the United States are produced from chicken combs and therefore require the removal of inflammatory and immunogenic impurities endogenous to the avian tissue source[20]. With the aim of producing hyaluronan from a non-avian source, methods have been developed to produce high molecular weight hyaluronan using biological fermentation (Bio-HA)[21,22]. EUFLEXXA™ bioengineered 1% sodium hyaluronate (Ferring Pharmaceuticals, Inc., Suffern, NY) is a high molecular weight IA-HA product produced by biological fermentation. It has been approved in the European Union since November 2000 and Israel since June 2001 and has a molecular weight range of 2.4–3.6 million Daltons. The high molecular weight of Bio-HA is achieved by careful control of the fermentation, recovery and purification processes and does not require the use of any cross-linking processes.

A small single-blind trial comparing Bio-HA with placebo in 49 patients was conducted to estimate the efficacy of the product. The results were favorable but the study was underpowered to declare statistically significant differences[23]. Randomized clinical trials (RCTs) comparing IA-HA products with placebo injections have not been uniformly positive, and recent meta-analyses have likewise reached divergent conclusions[3,24,25]. Because the "placebo" intra-articular intervention in these RCTs can be considered an active treatment in patients presenting with a synovial effusion[26], RCTs of IA-HA present methodological challenges that remain incompletely resolved. Despite any ongoing controversy, Food and Drug Administration (FDA) has recently accepted RCTs designed to test for non-inferiority as part of the marketing application for IA-HA products in the United States.

In addition to the above consideration regarding non-inferiority, it was also deemed unethical to conduct a placebo-controlled trial of Bio-HA in a setting where IA-HA products are used in routine clinical practice. Our primary objective was therefore limited to comparing the safety and effectiveness of Bio-HA with those of CL-HA. This particular IA-HA preparation was chosen for comparison because several recent meta-analyses noted that the effect size for IA-HA is greatest for the higher molecular weight preparations, and CL-HA is the highest molecular weight hyaluronan preparation currently available[3,24,25].

Methods

TRIAL DESIGN

This was a multicenter, prospective, randomized, controlled, double-blind (blinded patient/blinded evaluator) study conducted in adult patients with symptomatic OA of the knee. Patients were centrally randomized to receive either EUFLEXXA™ (Bio-HA, Bio-engineered HA, Ferring Pharmaceuticals, Inc., Suffern, NY) or Synvisc® (CL-HA, Hylan G-F 20, Genzyme Corporation, Cambridge, MA). For the blinding procedure, unmarked boxes containing three blister-packaged syringes of either Bio-HA or CL-HA were delivered to the investigational sites. A computer-generated randomization number was centrally assigned to each box, and the randomization code was centrally maintained by the sponsor and concealed from the study sites. Randomization was blocked within the sites in groups of four. The physician who performed evaluations was separate from the physician who performed injections in order to maintain double-blinding (blinded patient, blinded evaluator). All study-related case report forms recorded only the randomization number.

Both products were administered as a course of three 2 ml injections administered weekly. Before administration of each injection, any synovial fluid that was present in the knee was aspirated. Patients were advised to rest for 24 h following each injection, consistent with the label instructions for most IA-HA products. Assessments were performed at screening, at baseline (prior to the first injection), and at 1, 2, 3, 6, and 12 weeks after the initial injection. Only acetaminophen was permitted for rescue analgesia, up to 4 g daily, with usage quantitated by pill counts. Acetaminophen (as 500 mg tablets) was provided to study patients according to the following schedule: 28 tablets were provided at treatment initiation, week 1 and week 2; 84 tablets were provided at week 3; and 168 tablets were provided at week 6. Non-steroidal anti-inflammatory drugs (NSAIDs) and other non-acetaminophen pain medications were prohibited during the study, and patients taking such agents were considered dropouts from the point of medication usage. The study was carried out in accordance with the International Conference on Hormonization (ICH) Guidelines for Good Clinical Practice (May 1, 1996, amended September 1997) and the Declaration of Helsinki concerning medical research in humans (1966).

PATIENTS

Patients were enrolled at 10 sites across Germany. The study protocol and informed consent form were approved by the relevant ethics committees. The study was open to patients of either sex, age 50–80 years, with confirmed OA in one or both knees. OA diagnosis date and radiological diagnosis date for the study knee and other knee were recorded on the study case report form at baseline. In patients with bilateral OA, the more symptomatic knee was assigned as the study knee at the screening visit based on the investigator's clinical judgment. Patients were included regardless of whether the tibio-femoral or patello-femoral compartment was predominantly affected. Criteria for inclusion were as follows: clinical evidence of chronic idiopathic OA of the study knee according to the criteria of Altman; radiologically verified OA of the study knee of grade 2 or 3 according to a modification of the grading system of Kellgren and Lawrence (grade 2 defined as definite osteophytes with unimpaired joint space and grade 3 defined as definite osteophytes with moderate joint space narrowing[27]); symptoms in the study knee for at least 1 year; willingness to discontinue all OA treatments other than acetaminophen; and moderate-to-severe knee pain as reflected by a visual analog scale (VAS) pain score of 41–80 (on a scale of 0 mm [no pain] to 100 mm [worst pain]) for the average of the five pain questions of the Western Ontario and McMaster University Osteoarthritis Index (WOMAC Index)[28], with only one pain parameter permitted to be below 20 mm or above 80 mm on the VAS. The five questions in the WOMAC Index pain scale are regarding pain during (1) walking on a flat surface, (2) going up and down stairs, (3) rest at night, (4) sitting or lying, and (5) standing upright.

Patients were excluded from the study if they had secondary OA originating from a known injury to the knee, rheumatoid arthritis, history of joint infection, dermatologic disorders or skin infection in proximity to the study knee, osteonecrosis, chronic active fibromyalgia, any inflammatory

or metabolic arthritides and known hypersensitivity to acetaminophen or hyaluronan. Patients were also excluded if they had hyaluronan injections to the study knee within 6 months of the screening visit, corticosteroid injections and surgery or arthroscopy to the study knee within 3 months of screening. Non-ambulatory patients were excluded, as judged by their inability to perform a 50-foot walk test. Patients with symptomatic OA of the hip or any other health condition that would have interfered with the study assessments were excluded, including patients with uncontrolled hematological, cardiovascular, neoplastic, pulmonary, neurological, renal, hepatic or systemic disease. Clinical laboratory values used as exclusion criteria were fasting blood glucose concentration above 160 mg/dl, alkaline phosphatase above 250 U/l, alanine aminotransferase above 30 U/l, or aspartate aminotransferase above 30 U/l. Patients were precluded from participating in any other study during the study period and during the 4 weeks prior to study enrollment.

OUTCOME MEASURES

The primary effectiveness outcome measure was the change in patients' average score on the five questions in the WOMAC Index pain scale, as measured on a 0–100 mm VAS for WOMAC subscale and full index analyses. Secondary effectiveness measures included the full WOMAC Index, the patients' global assessment of treatment and consumption of acetaminophen for pain relief as quantitated by pill counts. For the patient global evaluations, patients were asked to respond to the question "Are you satisfied with the results of the injections?" and to grade their response on the four-point ordinal scale: (1) dissatisfied, (2) slightly satisfied, (3) satisfied or (4) very satisfied. These provided the core outcomes measures of pain, function, and global assessment suggested for OA clinical trials[29,30].

Safety was assessed by collecting adverse event data at each study visit, or whenever reported by patients. Adverse events were defined as any emergent sign or symptom, whether related or unrelated to the study treatment. A serious adverse event was defined as one that (1) was fatal, (2) was life threatening, (3) resulted in persistent or significant disability/incapacity, (4) required or prolonged inpatient hospitalization or (5) was a congenital anomaly or birth defect. Adverse events were coded using MedDRA (*Medical Dictionary for Regulatory Activities*) terminology, with coding performed before unblinding the study. Patients' blood chemistry and hematological parameters were assessed at the beginning and end of the study, and vital signs were monitored for 30 min after each injection.

STATISTICAL METHODS

The analyses presented were performed on the intent-to-treat (ITT) population, defined as all patients randomized to treatment and receiving at least one injection. All data presented are on an ITT/last observation carried forward basis. Patients using prohibited pain medications during the trial were considered dropouts from the point of medication usage.

Patient demographic characteristics and disease status at baseline were examined for treatment group comparability using a one-way analysis of variance (ANOVA) for the continuous variables and a Cochran–Mantel–Haenszel test, stratified by center, for the categorical variables.

The primary effectiveness outcome measure of the study was the improvement in the WOMAC Index pain score from baseline (week 0) to the last patient visit (week 12). The study was powered to test for non-inferiority of Bio-HA compared with CL-HA treatment, the latter having been licensed by the FDA in the United States and therefore considered as the active comparator. To compare the effectiveness of the two treatments, a one-way ANOVA (Generalized Lineared Models (GLM) one-way ANOVA) was used to test a null hypothesis, H_0, defined as:

$$H_0: \mu_R - \mu_T > 8.00$$

where μ_R was the mean outcome measure for the active comparator and μ_T was the mean for the fermentation-derived treatment. The value of 8 mm, which is less than the minimal clinically important difference of 9–12 mm[31], was selected as the criterion for non-inferiority. Thus, if a one-sided 95% upper confidence boundary for the treatment difference were less than the limit of the allowable range (<4.16 mm), Bio-HA could be declared non-inferior to CL-HA. The same approach was applied to the other WOMAC indices.

The three WOMAC subscale scores, i.e., pain, stiffness, and physical function were calculated as the averages of the component item scores within each subscale. The total WOMAC score was calculated as the average of the 24 component item scores (five in the pain subscale, two in the stiffness subscale, and 17 in the physical function subscale). A one-sample paired *t* test was used to compare the within-group pain reductions from baseline to week 12. A Cochran–Mantel–Haenszel test was applied to the analysis of the percentage of patients who were symptom free (VAS score <20) as determined by the average score of the five WOMAC pain parameters. Patients with a VAS score of <20 were defined as "symptom free" because a 0–20 score on the VAS WOMAC pain subscale would correspond to a "none" rating on the Likert WOMAC pain subscale[32], and because this definition was previously applied in trials of IA-HA products[5,33]. The consumption of rescue medication by the ITT population and patients with unilateral knee OA was analyzed using the Cochrane–Mantel–Haenszel test. Patient global overall assessments evaluated at the end of the study were analyzed using Wilcoxon's two-sample test. Adverse events data for the two groups were compared using a two-tailed Fisher's exact test, including the number of patients for whom joint effusions were reported.

Sample size calculation

Assuming a standard deviation of 24 mm for the primary outcome measure, the required sample size for the study was estimated to be at least 250 patients for a power of 0.8 and a significance level of 0.05 (one sided). Anticipating protocol violators and early discontinuations for 25%, it was projected that 320 patients should be enrolled.

Results

PATIENTS

Patient disposition for the study is presented in Fig. 1. Of 404 patients screened at study entry, a total of 321 patients met the criteria for study entry and were randomized, 160 to the Bio-HA group and 161 to the CL-HA group. Of the 83 patients not meeting the trial's inclusion or exclusion criteria, 17 were excluded because they did not meet the inclusion criteria for WOMAC Index pain scores at baseline. Of

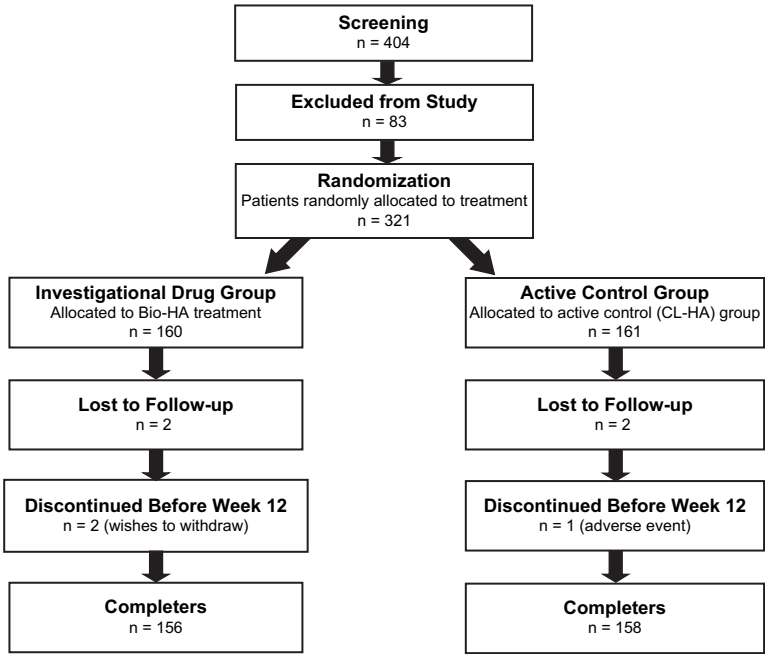

Fig. 1. Flow chart of patient disposition.

the 321 patients randomized and receiving at least one injection, 314 (98%) completed the final study visit, with one patient in the CL-HA group discontinued because of an adverse event (effusion in Baker's cyst in the injected knee, possibly treatment related). Two patients in each group were lost to follow-up and two patients in the Bio-HA group decided to withdraw from the study for unknown reasons. The analyses presented here were performed on the ITT population, defined as all patients randomized and receiving at least one injection.

Baseline characteristics for the ITT population are provided in Table I. The patients were predominately female (2:1 ratio), with a mean age of 63.2 years and a mean duration of OA in the study knee of 58.9 months. Kellgren and Lawrence radiologic grades were approximately equally distributed between grades II and III. All the patients met at least four of the six Altman criteria for the diagnosis of knee OA. During the physical examination prior to treatment, patients were evaluated with respect to presence of effusion in the study knee (none/small/large). Eleven Bio-HA patients and 15 CL-HA patients were judged to have a small effusion at baseline. No significant differences in baseline characteristics were noted between the two study groups.

Baseline scores for the WOMAC Index pain, stiffness, and physical function scales are shown in Table II. The scores were similar for the two treatment groups and indicative of moderate-to-severe impairment at baseline.

PRIMARY OUTCOME MEASURE

Table III provides the absolute change and percent improvement from baseline for the two study groups for each question in the WOMAC pain subscale and for the average of the five WOMAC pain questions (the primary outcome measure). Both groups experienced statistically significant and clinically important improvements from baseline ($P < 0.0001$). At the study end point, the mean improvement in the primary effectiveness measure for the Bio-HA group was 29.9 mm (62% improvement from baseline), as compared to 28.4 mm (55% improvement from baseline) for the CL-HA group. The one-sided 95% upper confidence boundary for the treatment difference was 2.4 mm, which

Table I
Patient demographic baseline characteristics

Parameter	Number of patients (%)		
	Bio-HA ($n = 160$)	CL-HA ($n = 161$)	Total ($n = 321$)
Gender			
Female	99 (61.9%)	108 (67.1%)	207(64.5%)
Male	61 (38.1%)	53 (32.9%)	4(35.5%)
Age (years; mean ± Standard Deviation (SD))	62.7 ± 7.5	63.7 ± 7.3	63.2 ± 7.4
Body Mass Index (mean [kg/m^2] ± SD)	28.5 ± 4.7	27.8 ± 4.4	28.1 ± 4.6
Study knee			
Left	73 (45.6%)	80 (49.7%)	153 (47.7%)
Right	87 (54.4%)	81 (50.3%)	168 (52.3%)
Kellgren and Lawrence grading system			
(Grade 2)	88 (55.0%)	84 (52.2%)	172 (53.6%)
(Grade 3)	72 (45.0%)	77 (47.8%)	149 (46.4%)
Clinical symptomatology			
Knee pain	160 (100%)	161 (100%)	321 (100%)
Stiffness <30 minutes	151 (94.4%)	151 (93.8%)	302 (94.0%)
Crepitus	154 (96.3%)	159 (98.8%)	313 (97.5%)
Bony tenderness	134 (83.8%)	145 (90.1%)	279 (86.9%)
Bony enlargement	72 (45.0%)	76 (47.2%)	148 (46.1%)
No palpable warmth	153 (95.6%)	149 (92.5%)	302 (94.0%)
Duration of OA in study knee			
Months prior to enrollment (mean ± SD)	57.1 ± 45.9	60.7 ± 53.5	58.9 ± 49.8

There were no statistically significant between-group differences for any of the above parameters.

Table II
Mean baseline score for full WOMAC Index (100 mm VAS)

WOMAC Index scores	Mean baseline VAS score (±SE)	
	Bio-HA ($n = 160$)	CL-HA ($n = 161$)
Average of 5 WOMAC Index pain scores	49.2 ± 1.1	51.9 ± 1.1
Average of 2 WOMAC Index stiffness scores	43.2 ± 1.5	47.8 ± 1.5
Average of 17 WOMAC Index physical function scores	47.0 ± 1.2	50.8 ± 1.2
Average scores for full WOMAC Index (pain, stiffness and physical function)	47.2 ± 1.1	50.8 ± 1.1

There were no statistically significant between-group differences in any baseline parameter.

was within the allowable range (<4.16 mm), meeting the prospective criteria for non-inferiority.

Figure 2 displays the mean change in VAS score for the primary effectiveness measure during the full study period, illustrating that clinically important reductions in pain continued after completion of the 15-day injection period for patients in both treatment groups. By the 12-week study end point, the mean score for the WOMAC pain average had dropped to approximately 20 mm in both treatment groups.

The percentage of patients whose VAS score for the average of the five WOMAC pain questions was <20 (defined previously as "symptom-free" patients) was compared for the two treatment groups in a *post hoc* analysis. At the study end point, 63% of patients in the Bio-HA group were symptom free compared to 52% in the CL-HA group, a statistically significant difference ($P = 0.038$; 95% Confidence Interval (C.I.) = 0.3%, 21.7%) (Table IV). This difference between the groups with respect to symptom-free patients for the WOMAC pain subscale was confirmed by evaluating the percentage of patients with an average WOMAC function subscale score less than <20 mm at week 12: 64.3% (101/157) for the Bio-HA group vs 47.5% (75/158) for the CL-HA group ($P = 0.003$).

SECONDARY OUTCOME MEASURES

WOMAC Index

The improvement in the full WOMAC Index score (pain, stiffness and function subscales) over the study period is illustrated in Fig. 3. Table V provides the average

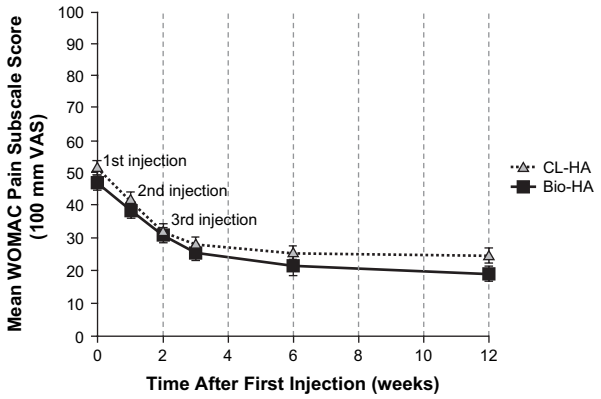

Fig. 2. Change from baseline in mean WOMAC Index pain subscale VAS scores throughout the study period (average of the five WOMAC pain scale questions). Error bars represent standard error of the mean. VAS = 100 mm VAS.

improvement for the three subscales of the WOMAC Index as a percent improvement from baseline and the percent improvement in the aggregate WOMAC score. The Bio-HA group experienced a mean improvement of 27.3 mm in the full WOMAC Index (59% improvement from baseline) compared to 25.9 mm (52%) for the CL-HA group. There were no significant differences between the two groups with respect to the WOMAC Index or its subscales.

Patient global assessments

The results of the patient global assessments performed at the study end point (12 weeks) are illustrated in Table VI. Approximately 94% of patients reported some degree of satisfaction with treatment and approximately 80% reported being either satisfied or very satisfied in both study groups. Analysis across all categories of improvement revealed a statistically significant difference favoring the Bio-HA group ($P = 0.03$). The between-group difference was apparent when comparing the percentage of patients who reported being 'very satisfied' (50% in the Bio-HA group compared to 37% in the CL-HA group), and corroborates the higher percentage of "symptom-free" patients found in the Bio-HA group with respect to the WOMAC Index pain scale.

Use of rescue medication

The use of rescue medication (acetaminophen) in the study population is detailed in Table VII for all evaluation

Table III
Reduction from baseline in individual questions and aggregate scores for the WOMAC pain subscale (100 mm VAS) at week 12

WOMAC Index pain scores	Mean* (%) reduction from baseline (±Standard Error (SE))	
	Bio-HA ($n = 160$)	CL-HA ($n = 161$)
Walking on a flat surface	31.2 ± 2.0 (59.7 ± 4.4)	28.7 ± 2.0 (49.5 ± 4.4)
Going up and down stairs	36.1 ± 2.2 (58.0 ± 3.7)	32.2 ± 2.2 (46.2 ± 3.7)
Rest during night	25.6 ± 1.9 (51.0 ± 5.8)	27.1 ± 1.9 (48.4 ± 5.8)
Sitting or lying	26.4 ± 1.9 (55.1 ± 5.0)	25.7 ± 1.9 (44.3 ± 5.0)
Standing upright	30.1 ± 2.1 (59.3 ± 4.7)	28.4 ± 2.1 (44.9 ± 4.7)
Average of five WOMAC Index pain scores	29.9 ± 1.7 (61.5 ± 3.0)	28.4 ± 1.7 (54.2 ± 3.0)

Both treatment groups demonstrated statistically significant and clinically meaningful improvements from baseline ($P < 0.0001$). At the study end point, the mean improvement in the primary effectiveness measure (average of five WOMAC Index pain scores) for Bio-HA was 29.9 mm (62% improvement from baseline) compared to 28.4 mm (54% improvement from baseline) for CL-HA.

Table IV
*Number of patients who were symptom free (VAS score below 20 mm) in WOMAC pain questions (individual categories and average scores) at week 12**

WOMAC Index pain scores	Number (%) of patients that were symptom free (VAS score between 0 and 20 mm)	
	Bio-HA ($n = 160$)	CL-HA ($n = 161$)
Walking on a flat surface	101 (63.1%)	88 (54.7%)
Going up and down stairs	80 (50.0%)	61 (37.9%)
Rest during night	117 (73.1%)	100 (62.1%)
Sitting or lying	109 (68.1%)	98 (60.9%)
Standing upright	104 (65.0%)	90 (55.9%)
Average of five WOMAC Index pain scores	101 (63.1%)*	84 (52.2%)*

*At the study end point, 63.1% of Bio-HA patients were symptom free (VAS score for the average of the five WOMAC pain questions was <20 mm) compared to 52.2% in the CL-HA group ($P = 0.038$; 95% C.I. = 0.3%, 21.7%).

time points. This table also describes acetaminophen use in the patient subgroup with unilateral knee OA. For the full study population, 61% (97/159) of Bio-HA and 73% (118/161) of CL-HA patients required rescue medication at some point during the study ($P = 0.013$). On average, Bio-HA patients used 4.2 acetaminophen tablets per week during the trial, compared to 5.8 tablets per week for CL-HA patients. Over the full study period, the CL-HA patients used significantly more acetaminophen tablets than the Bio-HA patients (Bio-HA, mean of 51.0 tablets; CL-HA, mean of 67.7 tablets; $P < 0.001$; two-tailed, two-sample t test) (Table VII). In the subgroup analysis performed on patients with unilateral OA, 49% of Bio-HA patients (33/67) used rescue medication at any time in the 12-week study period, compared to 82% of CL-HA patients (59/72), a statistically significant difference ($P = 0.001$).

SAFETY OUTCOMES

Adverse events were coded using MedDRA terminology before the study was unblinded. A total of 196 treatment-emergent adverse events were reported in 119 patients; 33.8% of Bio-HA patients (54/160) and 40.4% of CL-HA patients (65/161) experienced an adverse event. The severity of these adverse events was coded by the investigator as mild or moderate in 77/91 cases (84.6%) for the Bio-HA group and 95/105 cases (90.5%) for the CL-HA group. There were no deaths in either treatment group. Five serious adverse events were reported during the trial, three in the Bio-HA group and two in the CL-HA group; none of these events were considered related to the study treatment, and none resulted in withdrawal of the patient from the study. No significant systemic reactions were noted for either group (one investigator considered three incidences of high blood pressure in the Bio-HA group to be remotely related to the treatment, with no explanation). There were no significant within or between-group changes in mean values for clinical laboratory evaluations or vital signs over the course of the study.

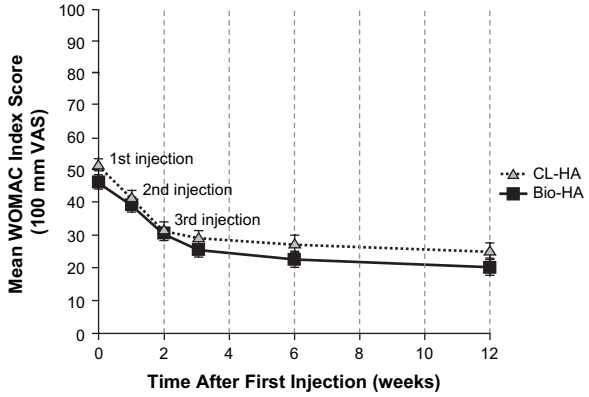

Fig. 3. Change from baseline in mean WOMAC Index VAS scores (pain, stiffness, and function scales). Error bars represent standard error of the mean. VAS = 100 mm VAS.

Adverse events occurring in more than 5% of patients are listed in Table VIII. The most commonly reported events were arthralgia, back pain and joint effusion. The incidence of joint effusion was significantly higher in the CL-HA group (8.1% vs 0.6%; $P = 0.0015$), with one episode reported in one patient for the Bio-HA group compared with 15 episodes in 13 patients for the CL-HA group. One patient in the CL-HA group was reported to have an effusion and then developed a Baker's cyst (possibly attributed to study therapy); this patient withdrew from the study.

Discussion

Several IA-HA products are currently available in the United States for the treatment of knee OA, and more than 20 additional products are available in other parts of the world. These preparations can differ significantly in their molecular weight, purity, and concentration[12–14,34]. It is therefore essential to identify any clinically important differences among IA-HA preparations and establish standards that ensure safety and effectiveness.

The trial reported here compares two high molecular weight hyaluronan preparations used for treating patients with OA of the knee. Because the IA-HA products tested are derived from different sources (microbial fermentation vs extraction from avian tissue), and because the avian-source

Table V
Reduction from baseline in score for full WOMAC Index (100 mm VAS) at week 12

WOMAC Index scores	Mean* (%) reduction from baseline (±SE)	
	Bio-HA ($n = 160$)	CL-HA ($n = 161$)
Average of five WOMAC Index pain scores	29.9 ± 1.7 (61.5 ± 3.0)	28.4 ± 1.7 (54.2 ± 3.0)
Average of two WOMAC Index stiffness scores	24.8 ± 1.9 (52.8 ± 10.6)	25.4 ± 1.9 (30.3 ± 10.5)
Average of 17 WOMAC Index physical function scores	26.9 ± 1.6 (57.6 ± 3.1)	25.2 ± 1.6 (49.4 ± 3.1)
Average scores or full WOMAC Index (pain, stiffness and physical function)	27.3 ± 1.6 (58.8 ± 3.0)	25.9 ± 1.6 (51.1 ± 3.0)

At the study end point, the Bio-HA patients experienced a mean improvement from baseline of 59% in the full WOMAC Index compared to 52% for the CL-HA group. There were no statistically significant between-group differences.

Table VI
*Subjective patient assessment of treatment**

	Overall patient assessment of treatment		
	Bio-HA	CL-HA	Total
No. of patients assessed	157 (100%)	158 (100%)	315 (100%)
Dissatisfied	12 (7.6%)	11 (7.0%)	23 (7.3%)
Slightly satisfied	18 (11.5%)	28 (17.7%)	46 (14.6%)
Satisfied	48 (30.6%)	61 (38.6%)	109 (34.6%)
Very satisfied	79 (50.3%)*	58 (36.7%)*	137 (43.5%)

*At the end of the study, there was a statistically significant between-group difference in favor of Bio-HA ($P = 0.03$) patients who were very satisfied with treatment results.

Table VIII
*Adverse events occurring in more than 5% of patients (any causality)**

Adverse event	Bio-HA ($n = 160$)		CL-HA ($n = 161$)	
	No. of patients (%)	No. of events	No. of patients (%)	No. of events
Total	54 (33.8)	91	65 (40.4)	105
Arthralgia	14 (8.8)	18	17 (10.6)	19
Back pain	8 (5.0)	8	11 (6.8)	11
Joint effusion	1 (0.6)*	1	13 (8.1)*	15

*There was a statistically significant between-group difference between the number of Bio-HA and CL-HA patients with joint effusions ($P = 0.0015$). Adverse event coding was performed while results were blinded.

hyaluronan uses cross-linking processes to increase molecular weight by chemical derivatization, it is essential to evaluate any clinically important differences between the two IA-HA products. This trial was designed and powered to test for non-inferiority and met the predefined criteria for demonstrating that the effectiveness of Bio-HA was not inferior to that of CL-HA for the primary variable (change in average VAS score on the five questions in the WOMAC Index pain scale). With respect to the trial's secondary outcome measures, statistically significant differences favoring the Bio-HA group were found for the patient global assessments and the percentage of patients requiring acetaminophen as rescue medication. The latter is notable because it indicates that the similar effectiveness observed for the primary outcome measure and differences in effectiveness for some secondary outcome measures were observed despite the greater usage of acetaminophen in the CL-HA group. Statistically significant and clinically important differences in safety outcomes were also found in the trial, though it was not specifically powered to detect such differences. The number of local reactions accompanied by effusion was significantly higher in the CL-HA group compared to the Bio-HA group (15 effusions in 13 patients for CL-HA, compared to one effusion). These data corroborate recent reports of acute local inflammatory reactions to CL-HA injection[15–19,35], but are the first clear demonstration of this important sequela in a RCT setting. Because effusions may necessitate arthrocentesis, the reduced number of effusions in the Bio-HA group suggests that patients treated with Bio-HA are at reduced risk for local reactions requiring physician intervention and follow-up. Treatment of adverse reactions can add significantly to the total cost of IA-HA treatment, suggesting that Bio-HA may also have health economic advantages over CL-HA.

Several recent meta-analyses have evaluated the effect size when IA-HA is compared with saline injections[3,24,25]. Though the magnitude of the effect size remains a subject of debate, all of these meta-analyses reported a statistically significant difference favoring IA-HA over saline injections. The absence of an adequately powered, placebo-controlled RCT for Bio-HA remains a limitation on the interpretation of our data; however, the results of these recent meta-analyses support the decision to perform the clinical study reported here as a non-inferiority trial.

Managing patients with OA of the knee presents a growing challenge to clinicians and health policy decision makers. Pharmacotherapy with NSAIDs remains a mainstay of therapy, despite the iatrogenic morbidities of long-term NSAID administration[36,37]. Safety concerns associated with the use of systemic medications are exacerbated by comorbidities in the affected population and potentially dangerous drug interactions. The trial data presented here provide evidence that Bio-HA can reduce pain and improve function in patients with knee OA without the iatrogenic local reactions associated with cross-linked IA-HA products.

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Table VII
*Number (%) of patients using rescue medication (acetaminophen)**

Time	Single knee		All subjects	
	Bio-HA	CL-HA	Bio-HA	CL-HA
Week 1	19/67 (28.4%)	36/71 (50.7%)	50/158 (31.6%)	71/156 (45.5%)
Week 2	16/66 (24.2%)	35/71 (49.3%)	47/157 (29.9%)	78/160 (48.8%)
Week 3	22/67 (32.8%)	33/71 (46.5%)	55/158 (34.8%)	75/160 (46.9%)
Week 6	24/67 (35.8%)	41/69 (59.4%)	65/158 (41.1%)	89/156 (57.1%)
Week 12	23/65 (35.4%)	42/67 (62.7%)	64/154 (41.6%)	91/155 (58.7%)
During study	33/67 (49.3%)	59/72 (81.9%)	97/159* (61.0%)	118/161* (73.3%)

*There was a statistically significant between-group difference in favor of Bio-HA ($P = 0.013$) in the number of patients who required rescue medications during the study. Total patient numbers varied throughout the course of the study due to loss of pill bottles by individual patients.

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