SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

A. Device Generic Name: Sodium Hyaluronate

B. Device Trade Name: Nuflexxa

C. Applicant Name and Address: Savient Pharmaceuticals, Inc.
One Tower Center Boulevard
East Brunswick, NJ 08816

D. Date of Panel Recommendation: None

E. Premarket Approval (PMA) Number: P010029

F. Date of Notice of Approval to the Applicant: December 3, 2004

II. INDICATION FOR USE

Nuflexxa (1% sodium hyaluronate) is indicated for the treatment of pain in osteoarthritis of the knee in patients who have failed to respond adequately to conservative non-pharmacologic therapy and simple analgesics, e.g., acetaminophen.

III. CONTRAINDICATIONS

- Do not use Nuflexxa to treat patients who have a known hypersensitivity to hyaluronate preparations
- Do not use Nuflexxa to treat patients with knee joint infection, infections or skin disease in the area of the injection site.

IV. WARNINGS AND PRECAUTIONS

Refer to product labeling.

V. DEVICE DESCRIPTION

Nuflexxa consists of a one percent bacteria-derived sodium hyaluronate solution in phosphate buffered saline. Hyaluronic acid is composed of alternating D-glucuronate and N-acetylglucosamine residues, linked in alternating β (1→4) and (1→3) bonds. Figure 1 shows the structure of the repeating disaccharide unit.

Nuflexxa has a pH of 6.8 – 7.6, an osmolality of 258-381 mOsm/kg and a viscosity of 105,000 ± 30,000 cP at a shear rate of 0.1 sec^{-1} at 25°C. The average molecular weight of the sodium hyaluronate is approximately 3 million daltons (Md).

Each 1 mL of Nuflexxa contains:

Sodium hyaluronate	10 mg
Sodium chloride	8.5 mg
Disodium hydrogen phosphate dodecahydrate	0.56 mg
Sodium dihydrogen phosphate dihydrate	0.05 mg
Water for injections	q.s.

Each Nuflexxa unit consists of a sterile; 2.25 mL syringe containing 2.0 mL 1% bacteria derived sodium hyaluronate in phosphate buffered saline. Each package contains 3 blister packed syringes and product information (a package insert).

Throughout this document are reports of studies that were conducted to support the safety and effectiveness of Nuflexxa. The studies were conducted with a product that had various names; BioLon, OphtHA, BioHy, and 1% NaHA, all refer to the same product, and are identical to Nuflexxa in its current formulation.

VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

- Infection
- Arthralgia (knee pain)
- Arthrosis
- Joint (knee) disorder
- Joint (knee) swelling
- Joint (knee) effusion
- Joint (knee) stiffness
- Pain in limb
- Tendonitis
- Paraesthesia
- Phlebitis
- Pruritus
- Injection site erythema
- Injection site edema
- Injection site pain
- Injection site reaction
- Arthropathy
- Baker's cyst
- Bursitis
- Localized osteoarthritis

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- Aggravated osteoarthritis
- Immune Response

VII. ALTERNATE PRACTICES OR PROCEDURES

For patients who have failed to respond adequately to conservative non-pharmacological therapy and simple analgesics (e.g., acetaminophen), alternative practices and procedures include nonsteroidal anti-inflammatory drugs (NSAIDs); intra-articular injection of corticosteroid; avoidance of activities that cause joint pain; exercise; physical therapy; weight loss, and removal of excess fluid from the knee. For patients who have failed the above treatments, surgical interventions such as arthroscopic surgery and total knee replacement are also alternative treatments.

VIII. MARKETING HISTORY

Sodium hyaluronate, manufactured by Bio-Technology General (Israel) Ltd. (BTG) has been marketed as OphtHA and 1% NaHA solution. Since April 1993 the product has been marketed as BioLon for use in eye surgery. In June 1995, BioLon was approved as a medical device by MDC (Medical Device Certification), a notified body of the European Community, and a CE mark was issued. In July 1998, BioLon received PMA approval by the Center for Devices and Radiological Health for use in ophthalmology.

BioLon, OphtHA, and 1% NaHA have never been withdrawn from marketing for any reason related to safety or effectiveness of the device.

IX. SUMMARY OF PRECLINICAL TESTING

Studies were performed in accordance with U.S. or U.K. Good Laboratory Practices. Some of the studies refer to the product as OphtHA ---the name given to the product before the trade name was adopted. OphtHA, BioLon, and 1% NaHA solution all refer to the same product, and are identical to Nuflexxa in its current formulation. The purpose of the studies is to document the biocompatibility of Nuflexxa.

A. The following three tests are tests that are performed routinely during the production of Nuflexxa. They are included because they were used in to validate the method of production, evaluate hemolytic activity of the master cell bank, and can be considered to be preclinical tests.

1. Inflammation

As part of a validation study conducted on the method producing bulk NaHA, 14 bulk NaHA batches were tested using two methods; the mouse peritoneal inflammation assay and the owl monkey assay. The latter test involves injection of NaHA solution into the eye of the owl monkey, Actus trivirgatus. The number of leucocytes drawn to the injected eye is counted; the test results are an indicator of an inflammatory response. A limit of 200 cells per mL was established for NaHA. Under the conditions of this testing, the bulk NaHA was considered to be non-inflammatory.

2. *In Vitro* Hemocompatibility

The hemocompatibility of BioLon was determined by evaluating the interaction of BioLon with erythrocytes in human whole blood. The extent of hemolysis was determined by the measurement of the amount of hemoglobin leaking in to the incubation medium, measured by absorbance of the solutions at 430 nm. The positive control was distilled water, the negative control was a 0.9% saline solution, and the test solutions were solutions of 0.5% NaHA, 0.25% NaHA, and 0.125% NaHA. Under the conditions of this testing the results, BioLon at a concentration of 0.25% NaHA was considered to be hemocompatible.

3. Pyrogenicity

Three lots of syringes OphtHA were subjected to the standard rabbit pyrogen test. The standard test method was used, with one slight modification. Since intravenous injection of high volumes of 1 % NaHA was thought to be problematic due to high viscosity, it was necessary to modify the standard injection protocol by diluting the sample prior to injection to reduce viscosity. In preliminary experiments, it was determined that 1 mL of the solution could be diluted in 15 mL saline and injected into the rabbit ear vein without deleterious effect. The protocol recommended, therefore, that the test contractor mix the contents of each syringe (0.5 or 1.0 mL) with 15 mL sterile, pyrogen-free saline prior to injection. Under the conditions of this test, the lots of OphtHA tested were non-pyrogenic.

B. Biocompatibility Studies

Biocompatibility studies are summarized below. Although some of the studies were designed in order to verify the safety of the product for intraocular use, they were considered applicable for the use of the product under consideration.

1. In Vitro Chromosomal Aberration Study in Mammalian Cells (Solution)

A solution of Nuflexxa was prepared using McCoy's 5A Medium. A Chromosomal Aberration study was conducted to determine whether a solution of the test article would cause genotoxicity in Chinese Hamster Ovary (CHO) cells in the presence and absence of S9 metabolic activation. The methodology of Galloway et al. (1985) was followed. A monolayer of CHO cells was exposed to the test article solution (prepared the day of treatment) in triplicate cultures and in the presence and absence of S9 metabolic activation. Parallel testing was also conducted with a negative and positive control; culture medium was used as the negative control, Mitomycin C (MMC) served as the positive control in the absence of S9 and cyclophosphamide (CP) served as the positive control in the presence of S9.

Under the conditions of this assay, the test article solution was not considered genotoxic to Chinese Hamster Ovary cells in the presence or absence of S9 metabolic activation. The negative and positive controls performed as expected.

2. Cytotoxicity Test

An *in vitro* biocompatibility test, was conducted on Nuflexxa to determine the potential for a cytotoxicity response. A filter disc with a 0.1 mL aliquot of the test article, a filter disc control with 0.1 mL of 0.9% Sodium Chloride Irrigation USP, a negative control, and a positive control were each placed on triplicate agarose surfaces directly overlaying confluent monolayers of L-929 mouse fibroblast cells. After incubating at 37°C in 5% CO_2 for 24 hours, the cell culture was examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). The culture was then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the articles.

Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity. The test article met the requirements of the USP since the grade was less than a grade 2 (mild reactivity). The filter disc control, the negative control and the positive control performed as anticipated.

3. In Vitro Hemolysis Study

Nuflexxa - 1% hyaluronic acid in saline was added to saline which was then added to diluted rabbit blood to evaluated whether direct contact with the test article would cause in vitro red blood cell hemolysis. Blood was obtained from three rabbits, pooled, diluted and added to duplicate test tubes containing Nuflexxa. The negative control (high density polyethylene) and the positive control Purified Water/Sterile Water for Injection) were similarly prepared. Each tube was inverted to uniformly mix the contents with the blood. The tubes were then maintained in a stationary position for 4 hours at 37°C. Following incubation, the suspensions were mixed, transferred to conical tubes, and centrifuged. The resulting supernatant was added to Drabkin's Reagent. The percent transmission of the extract was spectrophotometrically measured at a wavelength of 540 nm.

Under the conditions of this study, the mean hemolytic index for the Nuflexxa in saline solution was 0% and Nuflexxa was determined to be non-hemolytic. The negative and positive controls performed as anticipated.

4. Acute Intraperitoneal Toxicity in Mice

Ten CD-1 mice (five male and five female) were given a single intraperitoneal injection of 40 mL/kg BioLon (the maximum volume that could be administered). The animals in the control group received 40-mL/kg saline. Animals were observed daily for 14 days. Body weights were recorded on days 1,2,3, 5,8, 11 and 15. The animals were then sacrificed and necropsies performed. During the entire study no animals died, nor did they show any other sign of reaction to treatment. Body weight gains were normal. No abnormalities were observed at necropsy. As a result, intraperitoneal dose of 40 mL/kg BioLon was determined to be non-toxic to mice.

5. Acute Intravenous Toxicity

a. Acute Intravenous Toxicity in Mice

BioLon was serially diluted and injected intravenously at 20 mL/kg into mice. In the main study, groups of mice (five males and five females per dose) were injected with a volume dosage of 20 mL/kg, using dilutions corresponding to 0%, 0.0312%, 0.0418%, 0.05% and 0.0625% NaHA. Animals were inspected four times on the day of dosing and twice thereafter until termination on day 15. Body weights were recorded on day 1, 2, 3, 5, 8,11 and 15. Necropsy was performed on dying animals on the day of death and on the surviving mice on day 15. Another study was performed in a similar manner using Healon. Healon is a similar ophthalmic product containing sodium hyaluronate. Two groups of mice were injected with 20 mL/kg at dilutions of Healon corresponding to 0.062% and 0.046% sodium hyaluronate.

Mortality occurred in both sexes at concentrations of sodium hyaluronate exceeding 0.0418%. Mortality occurred immediately after dosing and was preceded by clonic convulsions and gasping. Surviving animals showed no pathological changes, and body weight gain was normal. The acute intravenous median lethal dose (LD_{50}) for BioLon was found to be a 20 mL/kg dose if 0.045% sodium hyaluronate, i.e., equivalent to 9 mg NaHA/kg body weight. In the Healon groups, mortality was observed with similar concentrations of sodium hyaluronate; Healon thus exhibits an LD_{50} not different from that of BioLon. The proximity of death to time of injection and the convulsive nature of death led to the proposal that mortality was the result of the increase in blood viscosity caused by the high molecular weight sodium hyaluronate.

The LD_{50} value of 9 mg NaHA/kg body weight observed in this study is thirty times higher than the dose of BioHy used in intra-articular injections for treatment of osteoarthritis of the knee and not for intravenous injection. As a result it was considered that this study did not provide data that would indicate that BioHy is toxic in the concentration intended to be used for the intended treatment of osteoarthritis of the knee.

b. Acute Intravenous Toxicity in Rats

Dilutions of bulk NaHA were prepared in pyrogen-free saline to final NaHA concentrations of 0.15%, 0.18%, 0.20%m 0.23%, 0.25%, 0.30%, and were injected into groups of OFA rats, five males and five females per group. Animals were periodically observed (at 30 minutes, 2 hours, 4 hours and 6 hours after injection, and twice daily for 14 days) for clinical signs and were weighed before and after the study. Gross pathology was observed on all animals at the end of the study.

At concentrations of NaHA higher than 0.15% (i.e., at 0.18% and higher), animals of both sexes died with 10 minutes of dosing; mortality was preceded by clonic convulsions and gasping. Surviving animals displayed similar symptoms, but recovered fully and exhibited normal weight gains.

The LD50 for injection at 5 mL/kg was found to be 0.19% for males and 0.21% for females, i.e., 9.5 mg NaHA/kg body weight and 10.5 mg NaHA/kg body weight, respectively. The pre-mortem signs observed in this study led to the proposal that death was due to the viscosity increases in the blood following injection; as was noted in the mouse intravenous toxicity study reported above and as a result it was considered that this study did not provide data that would indicate that BioHy is toxic in the concentration intended to be used for the intended treatment of osteoarthritis of the knee.

c. Additional Information on Intravenous Administration

In the studies carried out in mice and rats as noted above, it was believed that the mortality observed was caused by the increase in blood viscosity due to the rapid injection of high molecular weight NaHA. To further evaluate this belief, a limited study (not fully GLP compliant; there was no written protocol) was conducted to determine the effects of NaHA injection on 200-260 g male Wistar rats. Groups of five animals were injected with NaHA solutions at a range of concentrations equivalent to 12.5 —40 mg/kg. When high volumes (20mL/kg) were injected slowly into the venous blood flow, no lethality was observed. Lethality occurred only in cases where injection was rapid. Administration of NaHA caused no lethality even at concentrations exceeding the putative LD_{50} values observed in the intravenous studies. It is, therefore, proposed that it is likely that the mortality in the mouse and rat acute intravenous studies was a function of injection rate resulting in high viscosity, and not a direct effect of the NaHA molecule itself.

Further evidence supporting this belief obtained from the rabbit pyrogen test conducted with BioLon. The rabbit pyrogen test (as described above) was conducted by diluting the NaHA solution administered; this was done in order to reduce any potential effects of elevated viscosity. The lack of any reaction in the rabbit pyrogen test supports the hypothesis that elevated viscosity caused mortality in the mouse or rat studies. Prevention of elevated viscosity either by dilution of the test solution or by slowing the injection rate eliminated the viscosity effect of NaHA injection.

6. Acute Intraocular Toxicity

a. Acute Intraocular Toxicity in Rabbit Eyes

Two groups (each containing three males and three females) of New Zealand rabbits were anesthetized and a 0.2 mL portion of the aqueous humor was removed from the right eye of each animal by paracentesis, using a 30-gauge needle attached to a 1 mL tuberculin syringe. The removed humor was replaced with 0.2 mL BioLon (experimental group) or saline (control); this injection protocol thus mimicked the intended surgical use of BioLon. Intraocular pressure (IOP) and corneal thickness determinations were made before injection at 0.5 h, 2 h, 6 h, 24 h, 7 days and 14 days; at each time point microscopic evaluations of lens transparency, flare reaction and iris hyperemia were also performed. Endothelial cell density was measured prior to paracentesis and on day 14, eye tissue was examined histologically at the end of the study.

IOP levels in the experimental group rose rapidly in the first 6 hours following injection, and after 24 hours returned to the levels of the control group. IOP in both control and experimental groups returned to preoperative levels after 7 days. A transient rise in IOP is a well-known phenomenon in viscoelastic surgery utilizing NaHA. Corneal thickness and endothelial cell density were normal and identical in both groups, as were the biomicroscopic and histological evaluations. Based on the results of the study, BioLon was judged safe for use in ophthalmic surgery.

b. Acute Intraocular Toxicity in Cat Eyes

Three groups of Iva:Fec (Tif) cats were used in the study. Three males and three females were included in the BioLon group, and three males and one female each were in the Healon and saline control groups. Animals were anesthetized and a 0.2 mL portion of the aqueous humor removed from each right eye by paracentesis, using a 30-gauge needle attached to a 1 mL tuberculin syringe. To mimic the intended surgical use of BioLon, the removed humor was replaced with 0.2 mL BioLon (experimental group), Healon or saline (controls). Intraocular pressure (IOP) and corneal thickness determinations were made before injection and at 0.5 h, 2 h, 6 h, 24h, 7 days and 14 days; and microscopic evaluations of lens transparency, flare reaction, and iris hyperemia were also performed at each time point. Endothelial cell density was measured prior to paracentesis and on day 14, and eye tissue was examined histologically at the end of the study.

LOP levels of the saline group remained low and gradually returned to preoperative values. LOP in the BioLon and Healon groups rose during the first 48 hours following injection, returning to preoperative levels after 7 days. This transient rise in IOP is a well-known phenomenon in viscoelastic surgery utilizing NaHA. Corneal thickness and endothelial cell density were normal and identical in all three groups, as were the biomicroscopic and histological evaluations. Based on the results of the study, BioLon was judged safe for use in ophthalmic surgery.

7. Eye Irritation in Rabbits

Three New Zealand white rabbits each received 0.1 mL of a 0.2% NaHA solution, placed into the lower everted lid of one eye of each animal. The eyes were examined after 1 hour and 1,2, 3, 4 and 7 days after instillation of the test material. In this study, effects on the eye were tested at more dilute concentrations of NaHA, because the viscous nature of 1 % NaHA precludes its use in this kind of experiment.

The sample did not elicit a positive response in any of the three treated animals according to OECD test criteria.

8. Mouse Bone Marrow Micronucleus Study

Nuflexxa was dissolved in 0.9% sodium chloride USP solution and evaluated for genotoxicity using the Mouse Bone Marrow Micronucleus model. For 2 consecutive days (days 1 and 2), ten mice (five per sex) were injected intraperitoneally with the test article solution at a dose of 12.50 mL/kg. Similarly, ten mice were dosed with saline as the negative control condition. On day 2, ten additional mice were dosed with the positive control, 50 mg/kg cyclophosphamide (12.50 mL/kg). All animals were observed immediately following injection and daily for general health. On day 3, the animals were euthanized. The bone marrow was collected from the femurs and smears were prepared. The polychromatic erythrocytes were evaluated microscopically for the presence of micronuclei. The percentage of polychromatic erythrocytes among total erythrocytes was determined.

The negative and positive controls performed as expected. There was no evidence of cellular toxicity in the negative control groups. There was evidence of cellular toxicity in the positive control groups. Under the conditions of this study, the Nuflexxa test solution was not considered to be genotoxic to the mouse.

9. Subacute toxicity (14 days) in Rats and Rabbits

Bulk NaHA was dissolved and diluted in pyrogen-free saline to a 0.18% (1.8 mg/mL) NaHA concentration. The solution was used to intraperitoneally inject a group of OFA rats (five males and five females) with a 10 mg NaHA/kg body weight dose daily for 14 days. A similar control group of animals was injected daily with 1 mL saline. Animals were inspected daily for clinical symptoms and sacrificed at the end of the study. Various organs were examined for gross pathology; certain organs were weighed and ratios of organ/body weight calculated. A variety of blood cell analyses and clinical chemical tests were also performed. No clinical or other effects were noted during daily examinations of the animals over the entire course of the study. Body and organ weights in the treated group were normal and identical to those of the control group, as were the blood cell analyses, blood chemistry and histopathology data. It was concluded that the NaHA caused no pathological change or other deleterious effect under the conditions of the study.

Bulk NaHA was dissolved and diluted in pyrogen-free saline to a concentration of 0.1% (1.0 mg/mL) NaHA. The solution was used to intravenously inject a group of New Zealand white rabbits (three males and three females) with a 10 mg NaHA/kg body weight dose daily for 14 days. A similar group of animals was injected daily with 10 mL saline/kg body weight as a control. Animals were inspected daily for clinical symptoms and sacrificed at the end of the study. Various organs were examined for gross pathology; certain organs were weighed and ratios of organ/body weight calculated. A variety of blood cell analyses and clinical chemistry tests were also performed. No clinical or other effects were noted during daily examinations of the animals over the entire course of the study. Body and organ weights in the treated group were normal and identical to those in the control group, as were the blood cell analyses, blood chemistry and histopathology data. It was concluded that the NaHA caused no pathological change or other deleterious effect under the conditions of the study.

10. Sodium Hyaluronate Bacterial Mutation Assay

The Ames test involves the exposure of selected mutant strains of Salmonella typhimurium to the test compound (NaHA) and the quantification of revertants due to the exposure; mutagenic materials cause a large number of reverse mutations. One gram of bulk NaHA was dissolved in 100 mL sterile distilled water and the Ames test was performed at dose levels of 25, 75, 250, 750, 2500 and 5000 mcg NaHA in order to assess the mutagenic effect of the material. Under the test conditions, no evidence of mutagenic activity was observed at any dose level of NaHA.

11. NaHA Absorption, Distribution, Metabolism and Excretion (ADME)

The ADME (absorption, distribution, metabolism and excretion) of BioLon was investigated using carbon labeled NaHA that had been prepared according to fermentation and purification procedures similar to those used for the manufacture of bulk NaHA. The following studies were performed; distribution and metabolism studies after intravenous administration in rats and rabbits; and excretion study in rats and absorption studies after intraocular administration into rabbit eyes.

12. Intravenous Studies

a. Intravenous Study in Rabbits

In this study two male New Zealand albino rabbits were used. The right femoral vein of each was exposed and fitted with an intravenous cannula. A ^{14}C labelled NaHA solution ($7.2x10^6$ counts per minute (cpm) in 2 mL) was injected into the ear vein, and blood samples were drawn through the femoral cannula into syringes containing disodium EDTA at 1, 2, 4, 6, 8, 10, 15, 20, 30 and 60 minutes after injection. At the end of the 60 minute period, the animals were sacrificed and their livers removed for analysis.

b. Intravenous Study in Rats

Thirty nine male Wistar rats (200-220 g body weight) were divided into 13 groups of 3 rats each. Labeled NaHA (983,000 cpm in 0.5 mL) was injected into the tail vein. Blood samples were taken either by cardiac puncture (1 mL) after 2, 5, 7 or 15 minutes, or from the aorta (5-6 mL) after 10, 20, 30, 60 minutes and 4, 8, 24, 48, 72 hours. The rats were sacrificed and various organs were removed for analysis.

The results of the intravenous studies in rabbits and rats indicate that intravenously-administered ^{14}C-NaHA was rapidly eliminated from the circulation of rats and rabbits. The half-life in the rabbit was about 5 minutes. A half-life value of 3.7 minutes was reported for the rat. No specific localization of undegraded NaHA was found in a variety of rat organs examined, except for the liver, which was the major locus of metabolism.

The results of analysis of rabbit plasma indicate that up to 10 minutes post injection most of the radioactivity was associated with high molecular weight NaHA. The NaHA was progressively degraded following that time point resulting in a shortening of the polymer chain at 30 minutes and down to monomeric units at 60 minutes. The pattern of radioactive metabolites in the rat plasma was similar to that in the rabbit during the first 60 minutes. Analysis of blood radioactivity at time periods after one hour revealed the appearance of labeled metabolites of increasing molecular weights.

To evaluate the chemical nature of the high molecular weight metabolites, the high molecular weight fractions of the 5 minute and 4 hour samples were subjected to hyaluronidase digestion and re-chromatographed. The results revealed that the 5 minute fraction sample was susceptible to enzymatic digestion, whereas the 4 hour sample was hyaluronidase resistant.

Evaluation of the distribution of radioactivity in the various organs of the rats at various time points after administration of the labelled NaHA revealed that most of the administered radioactivity accumulated in the liver, reaching a maximum (72% of the injected dose) at 20 minutes. The levels in the liver gradually declined thereafter, which was reported to be indicative of active metabolism of the accumulated material in this organ. Some radioactivity was found in the spleen, heart and kidneys, reaching a maximum about 1 hour post-injection (2.6%, 1.4% and 1.3%, respectively).

The results of chromatographic analyses of rat liver extracts obtained at 10 minutes, 1, 4, and 24 hours indicate that at 10 minutes post-administration, all the radioactivity in the liver was associated with high molecular weight species. The material which accumulated in the liver was progressively degraded. At 1 hour most of the radioactivity in the extracts was associated with low molecular weight degradation products at 1 h.

13. Excretion Study in Rats

A gas-tight metabolic chamber was constructed by equipping a large polypropylene-polycarbonate desiccator with two gas connectors on the top and an outlet for urine at the bottom. A wire net floor was used for collection of feces. Food and water were mixed together and put in an immobilized container inside the chamber. Air was passed through the chamber at a flow rate of 30 L/hr by suction through two serially-connected traps containing ethanolamine for trapping of CO_2.

A rat was placed put inside the metabolic chamber following intravenous administration of ^{14}C-(9.83×10^5 counts per minute (cpm) in 0.5 mL). The ethanolamine vessels were sampled at 2, 4, and 8 hours post-injection; at 8, 24 and 48 hours the ethanolamine was replaced with fresh material. Urine was collected at 8, 24, 48 and 72 hours.

The results of the excretion study indicate that most of the ^{14}C-radioactivity in labelled NaHA was excreted via respiration, while some of the label was excreted through the urinary tract. Approximately 60% of the injected radioactivity was already removed from the body by 8 hours (total of respiratory and urine radioactivity); 69.6-86.1% was excreted at 24 h and 81.6-103.3% was recovered by 72 hours.

14. Intraocular Studies in Rabbits

Two studies were performed with the intraocular route. In the first study, the pharmacokinetics of disappearance from the anterior chamber and the fate of residual NaHA were examined, while the second study involved the appearance of metabolites in the blood.

In the pharmacokinetics study, eight male New Zealand rabbits were used. The animals were anaesthetized and 0.2 mL of the aqueous humour was removed from one eye using a 27 gauge needle. The syringe was then detached, leaving the needle in situ, and 0.2 mL of ^{14}C-NaHA solution (10 mg/mL sodium hyaluronate; 400,000 cpm per 0.2 mL) was introduced as replacement. This operation was repeated in the second eye. The animals were sacrificed at 0, 1, 5, 15, 24, 34, 48 and 72 hours, and the contents of the anterior chamber of each eye were then removed using a 27 gauge needle followed by two washes of 0.3 mL saline. The aqueous humour and washes of each eye were combined and taken for analysis.

In the second study, ^{14}C-NaHA of higher specific activity (0.2 mL of 10 mg/mL NaHA, 4 x 10^6 cpm) was injected into each eye of two rabbits, as described above, and blood samples were drawn from the ear veins at 1, 3, 5, 8, 11, 15.5, 24, 32, 50 and 74 hours post-injection.

The results of this study reported that the disappearance of the 1% NaHA solution from the anterior chamber of rabbit eyes follows first order kinetics (single compartment model) during the first 24 h, with a half life of 10.5 hr. By 48 hr, the amount of residual ^{14}C-NaHA in the eye was 0.4% of the initial dose; no radioactivity could be detected by 72 h. Chromatographic analysis of the aqueous humour samples taken at 1 h and 24 h showed that no degradation of NaHA occurred in the anterior chamber.

The results of these studies indicate that intravenously-injected ^{14}C-NaHA was rapidly eliminated from the circulation by the liver, where the accumulated NaHA was further digested to yield sugar oligomers; which were further utilized via general carbohydrate metabolic pathways, which was reported to be apparent from the appearance with time of low molecular weight entities and further incorporation into new hyaluronidase-resistant high molecular weight moieties which was indicated by the chromatographic profiles of the liver and plasma extracts. The sponsor makes comparison to the similarities of these results to the metabolic fate and disposition eukaryotic hyaluronate.

15. Clearance Rate following Intra-articular injections in Rabbits

To determine the clearance rate of Nuflexxa a study was conducted in which intra-articular injections of Nuflexxa were injected in rabbits; 0.5 mL of Nuflexxa were injected unilaterally into the rabbit knee. After various time points (1 hr, 24 hr, 2, 4, 7, 14, 30, and 60 days), the sodium hyaluronate (Na-HA) content, the active component of Nuflexxa, was determined in repeated rinsing solutions from the knee-joint (treated and untreated knees in comparison).

The test method used to determine the sodium hyaluronate content in the rinsing solutions was a modification of the standard assay for the determination of sodium hyaluronate as described in the European Pharmacopoeia 2000 (monograph of sodium hyaluronate). The principle of the test method is a reaction of sodium hyaluronate with carbazole followed by the measurement of the absorbance of the received red solution at 530 nm. After calibration of the UN/Vis spectrophotometer with known amounts of sodium hyaluronate the sodium hyaluronate amount in the rinsing solutions can be calculated.

The results are as follows: the highest levels of Na-HA were detected 24 hr after injecting the material. After 4 days, the amount: of Na-HA in the treated knees was determined to be comparable to the amounts determined in the untreated knees. This situation remained essentially unchanged until the end of the study (60 days). The total Na-HA amounts of the treated knees were compared statistically to the results of the untreated knees within each animal group. It was

shown that the differences were extremely significance ($P < 0.001$) for the results (treated vs. untreated knee) of the first three time points (1 hr, 24 hr, and 2 days). For the other time points ($\geq$ 4 days) no significant differences between the Na-HA content of the treated knees and the Na-HA content of the untreated knees were found ($P>0.05$). However, there was a tendency to higher absolute Na-HA amounts in the treated knees as compared to the untreated knees.

16. Test for Local Effects 7 Days and 4 Weeks After Injection

A test to evaluate the potential for Nuflexxa to produce local effects after injection was conducted in 6 New Zealand White rabbits (3 for each evaluation period). Four 0.1 mL solutions of Nuflexxa and four 0.1 mL solutions of the control (NaCl 0.9 %) were injected into the paravertebral muscle on opposite sides of the spine of each rabbit. This yielded a total of eight test areas for the evaluation of the biological response.

Follow-up times were 7 days and 4 weeks respectively. Post injection each animal was observed at least once daily during the test period. At the termination of the experimental period, the animals were euthanized. At this time period the biological response of Nuflexxa was evaluated by macroscopic and histopathological assessment in comparison to the control material.

There were no responses reported for Nuflexxa or control samples.

17. Delayed Sensitization in Guinea Pigs (Modified Magnusson-Kligman)

Three groups of guinea pigs were used. The treatment regime consisted of: a) primary induction on day 1 by intradermal injection of BioLon with Freund's complete adjuvant; b) secondary induction on day 8 by topical application of BioLon onto the dermal injection sites; c) challenge on day 22 by topical application; and d) rechallenge on day 29 by intradermal injection. At primary induction, three dermal sites were used. One site received Freund's adjuvant only, the second site received BioLon only, and the third was injected with both materials. Of the three groups of animals, one served as the test group and was exposed to induction, challenge and rechallenge. The second group was exposed to the challenge (topical) only, and thus served as control for the topical treatment, while the third group was exposed to the rechallenge (intradermal) only and served as control for the intradermal treatment. The dermal reactions were scored at predetermined times after the inductions and challenges and recorded. Following challenge, no dermal responses were observed in test animals 24 or 48 hours after bandage removal. Following rechallenge, no dermal response was observed 24 hours after injection. It was concluded that BioLon did not cause a delayed contact hypersensitivity reaction in the guinea pig.

18. Immunogenicity of BioLon™ in Rabbits

To evaluate the immunogenicity of BioLon, two rabbits were injected intradermally three times, three weeks apart, with BioLon emulsified with Freund's complete adjuvant. Sera were collected one week after the last injection. For comparison, another group of rabbits was immunized under a similar regimen with bovine serum albumin (BSA), and the resultant sera served as a positive control. The two sera were analyzed for the presence of antibodies to the corresponding antigens using three different methods: a) the Ouchterlony method; b) the interface ring test; and c) passive hemagglutination of sheep RBC. Using a large range of serial dilutions of BioLon and of the sera of BioLon-treated rabbits, no cross-reaction was observed in any of the three methods used, while a strong reaction was obtained between BSA and the BSA antisera. Under the conditions of this study, BioLon was not considered to be immunogenic.

19. Safety Evaluation of Repeated Intra-Articular Injections in the Rabbit

Nuflexxa was evaluated for biocompatibility, local and systemic effects following repeated intra-articular injections in the knee joint of the rabbit. Pretreatment blood specimens were collected for hematology and clinical chemistry analysis and serum harvested and stored for possible future analysis. Three rabbits were injected with the test article, bilaterally into the knee joint at the lateral-anterior aspect of each knee. The control article, 0.9% sodium chloride for injection, was similarly injected into both knee joints of the three additional rabbits. The animals were similarly dosed at 1 and 2 weeks following the initial injections. One test animal was found dead following the week 1 injections. The animal was replaced and subjected to the same repeated injection procedure previously described. The animals were observed daily for general health. Detailed examinations for clinical signs of disease or abnormality were conducted daily for the first two weeks and weekly thereafter. Body weights were recorded prior to injection, weekly, and at termination. At 6 weeks following the initial injections, the animals were terminated and blood specimens collected for hematology and clinical chemistry analysis. The serum from the specimens was harvested and stored for possible future analysis. A necropsy was conducted and selected organs were excised, weighed, and processed histologically. Synovial fluid was obtained from each knee joint and examined macroscopically and microscopically. The knee joint and surrounding tissue around each injection site was also excised from each rabbit, and examined macroscopically and microscopically. Microscopic evaluation of selected organs was also conducted. Body weights, organ, weights, hematology values, and clinical chemistry values were analyzed subjectively. The animal that was found dead was not included in the final evaluation of the test article, but the data collected was included with this report.

All animals were reported to be clinically normal following the intra-articular injections. While one death occurred in a test animal, this death was considered by the sponsor to be anesthetic related rather than an effect of the test article. There was no swelling, signs of discomfort, or reactions noted for any of the knee joints (test or control). The repeated injections were reported to be well tolerated by the rabbits without any evidence of ill effects. At necropsy, the synovial fluid appeared normal and the joints demonstrated no abnormalities. Examination of the viscera and other tissues revealed no abnormalities and organ weights were similar between and within test and control groups. Clinical pathology revealed no abnormalities and results were similar between groups and intervals. No treatment differences were identified in the cytological evaluation of the synovial fluid from the test and control groups. Microscopic examination of the synovial fluid, knee joint and associated tissues revealed no biologically significant differences identified between the test and control tissue samples.

3 weekly intra-articular injections of Nuflexxa (in the rabbit) were well tolerated and there was no evidence that the test article induced biologically significant alterations in the knee joint. There was no evidence of systemic alterations.

20. Evaluation of Antibody Response to Repeated Injections of Nuflexxa in the Rabbit

Nuflexxa was evaluated for the potential to elicit a humoral immune response when injected into rabbits. The study was conducted in 3 phases. The first two phases of the study: antibody production and ELISA establishment were conducted to produce a method for detection of antibodies to the test article. The final phase used this ELISA to analyze serum samples from rabbits receiving repeated intra-articular injections of the test article to determine if antibodies had been produced against the test article.

Phase I - Antibody Production

One rabbit demonstrated a moderate antibody response (positive at a 1:320 dilution), one had a mild positive response (positive at a 1:80 to 1:160 dilution), and the third rabbit injected had no antibody response. Comparison of pre-immune sera and sera obtained 3 weeks after the third booster injection confirmed an authentic anti-Nuflexxa immune response.

Phase II- ELISA establishment

The ELISA was constructed according to the following scheme:
Coating with antigen (5 μg) -> wash $\rightarrow$+ block $\rightarrow$ wash $\rightarrow$ primary antibody (α-Nuflexxa) $\rightarrow$ wash $\rightarrow$ enzyme-linked anti-rabbit I(.,G (secondary antibody) $\rightarrow$> wash $\rightarrow$ o-phenylenediamine (OPD) + H_2O_2 $\rightarrow$ A490

The rabbit with the moderate response was used for creation of the ELISA. The ELISA was optimized with respect to the concentrations of the reagents used.

Phase III - detection of immune responses in repeat injected animals
None of the animals injected with Nuflexxa exhibited an immune response. The sponsor stated that "while a weak antibody response was seen in the immunization protocol, no antibody response to the test article was seen when given by the clinical intra-articular route." And therefore, the sponsor concludes that "the test article appears to present no immunogenic response when used under clinical conditions".

The sponsor has observed an immune response in 2 of 3 animals immunized with Nuflexxa. Previously it was believed that the product would not elicit an immune response. Serum from the animal that exhibited the more significant immune response (was used to construct an ELISA. No immune response was observed in three animals which had received repeat intra-articular injections of the product. The information is too limited to draw the conclusion that presentation of Nuflexxa via in the intra-articular route in humans will not elicit an immune response. The sponsor should collect serum samples for evaluation of a possible anti-Nuflexxa immune response in individuals receiving intra-articular injections of Nuflexxa

X. SUMMARY OF CLINICAL STUDIES

The safety and effectiveness of Nuflexxa as a treatment for pain in osteoarthritis (OA) of the knee was investigated in a multi-center clinical trial conducted in Germany and a single center clinical trial conducted in Israel.

The single-center study was a, single-masked, placebo control, prospective, two parallel treatment arm clinical trial enrolling a total of 49 subjects (25, Nuflexxa; 24, placebo) who were randomized into two treatment groups in a ratio of 1:1 Nuflexxa or placebo. Due to the limited number of patients that were enrolled in this investigation and differences in the protocol, only the adverse events reported during this study were evaluated for determining the safety of this product.

A. Study Design

This multi center clinical investigation was a prospective randomized, double masked, active control (commercially available hyaluronan) study conducted at 10 centers in Germany. A total of 321 patients with stage 2 – 3 osteoarthritis of the knee according to the Kellgren and Lawrence grading system, meeting the Altman Criteria for Classification of Idiopathic Osteoarthritis of the knee, and scoring an average score of 41 – 80 mm on the 100 mm WOMAC VAS pain index were randomized into groups of equal size to receive either Nuflexxa (160 patients) or the active control (161 patients). The study was designed to show that Nuflexxa is no less effective than the active control (Δ=8.00).

1. Inclusion Criteria

   (1) Males and females between the age of 50 to 80 years.

   (2) Clinical evidence of chronic idiopathic osteoarthritis of the study knee as classified according to Altman criteria.

   (3) Radiologically verified osteoarthritis of the study knee (stages 2 or 3 according to Kellgren and Lawrence grading system for osteoarthritis of the knee).

   (4) Moderate to severe knee joint pain (using the 100 mm WOMAC VAS index) as evaluated by the examining physician. The average score of the 5 pain parameters will range between 41-80 mm on the VAS allowing only one parameter to be below 20 mm or above 80 mm.

   (5) No previous history of surgical treatment to the knee or arthroscopy intervention (except for removal of loose parts, meniscus repair, ligament repair) or injections into the study knee in the 3 months prior to initiation of the study.

   (6) Willingness to discontinue NSAID and analgesic 14 days prior to the first injection and throughout the study.

2. Exclusion Criteria

   (1) Patients with osteoarthritis originating from knee trauma, rheumatoid arthritis, joint infection and other inflammatory and metabolic arthritis.

   (2) Inability to perform the 50 foot walk test.

(3) Prior hyaluronic acid injections into the study knee (within the last 6 months).

(4) No steroid medication taken three months prior to enrollment.

(5) Chronic active fibromyalgia.

(6) Osteonecrosis of either knee.

(7) Patients with clinically diagnosed or were in the past diagnosed to have osteoarthritis of the hip joint.

B. Patient Population and Demographics

The demographics of trial participants were comparable across treatment groups with regard to age, gender, Kellgren & Lawrence grading system, stiffness, crepitus, bony enlargement, and no palpable warmth. (refer to Table 1).

Table 1. Patient Baseline Characteristics

Parameter	Number of Patients (%)	
	Nuflexxa	Active Control
*Kellgren & Lawrence Grading System		
Definite osteophytes (Stage 2)	88(55.0%)	84(52.2%)
Moderate multiple osteophytes (Stage 3)	72(45.0%)	77(47.8%)
Study knee		
Left	73(45.6%)	80(49.7%)
Right	87(54.4%)	81 (50.3%)
Age (n = number of patients)	62.7 ± 7.5 (160)	63.7 ± 7.3 (161)
Female (n)	62.9 ± 7.9 (99)	64.3 ± 7.3 (108)
Males (n)	62.5 ± 6.8 (61)	62.5 ±7.3 (53)
Osteoarthritis duration		
Study knee (months prior to enrollment)	57.1 ± 45.9	60.7 ± 53.5
Radiological diagnosis		
Study knee (months prior to enrollment)	3.9 ± 3.8	4.4 ± 6.4
**Altman Criteria		
Knee pain	160(100%)	161 (100%)
Stiffness < 30 minutes	151 (94.4%)	151 (93.8%)
Crepitus	154(96.3%)	159(98.8%)
Bony tenderness	134(83.8%)	145(90.1%)
Bony enlargement	72(45.0%)	76(47.2%)
No palpable warmth	153 (95.6%)	149(92.5%)

*Kellgren and Lawrence (Ref. 2)-. Based on radiological findings, osteoarthritis stages were defined as follows: 0 =normal, 1 =doubtful narrowing of joint space and possible osteophytic lipping, 2 =definite osteophytes and possible narrowing of joint space, 3 =moderate multiple osteophytes and definite narrowing of joint space, some sclerosis and possible deformity of bone contour, 4 =large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour.

**Altman, et al., (Ref. 1)- Clinical criteria for classification of idiopathic osteoarthritis (OA) of the knee were defined as follows: Knee pain + at least 3 of the following 5 parameters: Age >50 years, Stiffness <30 minutes, Crepitus, Bony tenderness, Bony enlargement, No palpable warmth.

C. Treatment and Evaluation Schedule

Patients who were eligible to participate in the study were stratified on the basis of the average pain severity (as evaluated in the pre-screening assessment) and were randomized within the center into equal treatment groups. Each treatment arm had an approximately equal number of patients with an average score of 41-60 mm and 61-80 mm. Nuflexxa or the active control was administered by intra-articular injection once a week (one week apart) at Week 0, 1 and 2, for a total of three injections using aseptic technique. Effusion was aspirated if present. Follow-up evaluator assessments were conducted at Weeks 3, 6, and 12; patient self-assessments were performed at Weeks 1-3, 6 and 12. Study duration was 12 weeks.

1. Treatment

   At weeks 0, 1, and 2 the patients were given an intra-articular injection into the study knee of 2 mL of Nuflexxa or the active control. The treatment was carried out in two stages:

   a. The needle of investigator's choice (17-21g) was used to aspirate synovial fluid from the knee joint under sterile conditions.

   b. The Nuflexxa or active control was injected into the study (signal) knee joint under sterile conditions. The investigators determined the optimal joint positioning and site for needle insertion for each knee according to the anatomic and pathologic condition. Patients were required to rest at home for 24 hours after the treatment.

   In order to maintain double masked conditions and eliminate the risk of bias, handling and injection was performed by a different doctor from the one performing the assessment (examining doctor).

2. Concomitant Medication and Rescue Medication

   A two week withdrawal period was required from all analgesic and anti-inflammatory medications prior to initiation of intra-articular HA injections. During the 2 week washout period all patients could receive up to 4 grams per day of acetaminophen for pain relief. Acetaminophen was used at this dose throughout the trial as rescue medication. Some patients with osteoarthritis enrolled in this study were expected to have exacerbations of symptoms (flares) which may require additional treatment other than rescue medication. In the event that the investigator prescribes the use of NSAID or analgesic the treatment was recorded and documented and the patient followed-up. Only 100 mg per day of acetylsalicylic acid as thrombosis prophylaxis was allowed.

Primary Performance Endpoint:

The primary performance endpoint measurement is change from baseline in the average of patient's self-evaluation of 5 pain parameters at week 12 (or last visit for early dropouts) using the WOMAC index on a 0-100 mm horizontal visual analog scale (VAS). The 5 pain parameters are:

1) Walking on a flat surface
2) Going up and down stairs
3) Rest during night
4) Sitting or lying
5) Standing upright

D. Safety Results

Adverse events were monitored and recorded throughout the study. Local signs and symptoms were recorded on diary cards for 3 consecutive days following each injection. Pre-and post -Treatment laboratory values were monitored and vital signs 30 minutes post-injection were also recorded.

1. Multicenter Clinical Investigation

Three hundred twenty-one patients were randomized into groups of equal size to receive either Nuflexxa (n = 160) product or the active control (n = 161). A total of 119 patients reported 196 adverse events; this number represents 54 (33.8%) of the Nuflexxa group and 65 (44.4%) of the active control group. There were no deaths reported during the study. Incidences of each event were similar for both groups, except for knee joint effusion which was reported by 14 patients (17 incidents) in the active control group and one patient in the Nuflexxa treatment group. Fifty-two adverse events were considered device-related. Table 2 shows the incidence of adverse events reported by >1% of patients in each to treatment group (the numerator is the number of incidents; the denominator is the number of patients in the respective treatment arm).

Table 2. Incidence of Adverse Events Occurring in Greater Than 1% of All Subjects

Body System	Adverse Event	Patients, n (%)	
		Nuflexxa n = 160 patients	Active Control N = 161 patients
Gastrointestinal disorders	Nausea	3 (1.88)	0
General disorders and administration site	Fatigue	2 (1.25)	0
Infections and infestations	Bronchitis	1 (0.63)	2 (1.24)
	Infection	2 (1.25)	0
Investigations	Blood pressure increased	6 (3.75)	1 (0.62)
Musculoskeletal, connective tissue and bone	Arthralgia (knee pain)	14 (8.75)	17 (10.6)
	Arthrosis	2 (1.25)	0
	Back pain	8 (5.00)	11 (6.83)
	Joint disorder	2 (1.25)	2 (1.24)
	Joint (knee) effusion	1 (0.63)	14 (8.07)
	Joint (knee) swelling	3 (1.88)	3 (1.86)
	Pain in limb	2 (1.25)	0
	Tendonitis	3 (1.88)	2 (1.24)
Nervous system disorders	Headache	1 (0.63)	3 (1.86)
	Paraesthesia	2 (1.25)	1 (0.62)
Respiratory, thoracic and mediastinal	Rhinitis	5 (3.13)	7 (4.35)
Skin and subcutaneous tissue disorders	Erythema	0	2 (1.24)
	Pruritus	0	3 (1.86)
Vascular disorders	Phlebitis	0	2 (1.24)
Total		57	70

A total of 160 patients received 478 injections of Nuflexxa. There were 27 patients reporting adverse events considered to be related to Nuflexxa injections: arthralgia – 11 (6.9%); back pain – 1 (0.63%); blood pressure increase – 3 (1.88%); joint effusion – 1 (0.63%); joint swelling – 3 (1.88%); nausea – 1 (0.63%); paresthesia – 2 (1.25%); feeling of sickness after injection– 3 (1.88%); skin irritation – 1 (0.63%); tenderness in study knee– 1(0.63%). Four adverse events were reported for the Nuflexxa-group for which the relationship to treatment was considered to be unknown: fatigue – 3 (1.88%); nausea – 1 (0.63%). Table 3 reports the number of patients reporting adverse events considered to be treatment related by treatment group.

Table 3. Relationship of Adverse Effects to Treatment Groups That Were Considered to be Treatment Related

Adverse Event	Nuflexxa (Number of patients reporting the event) n = 160 patients	Active Control (Number of patients reporting the event) n = 161 patients
Arthralgia (knee pain)	11	9
Back Pain	1	0
Baker's Cyst	0	1
Blood Pressure Increase	3	0
Erythema	0	1
Inflammation Localized	0	1
Joint Effusion (knee)	1	9
Joint Swelling (knee)	3	2
Nausea	1	0
Edema Lower Limb	0	1
Paresthesia	2	0
Pruritis	0	1
Sickness	3	0
Skin Irritation	1	0
Tenderness	1	0
TOTAL	**27**	**25**

2. Single Center Clinical Investigation

In a single-center placebo control clinical trial a total of 49 subjects were randomized to either placebo or Nuflexxa. Due to the limited number of patients that were enrolled in this investigation and differences in protocol only the adverse events reported during this study were evaluated for determining the safety of this product.

Adverse events were reported by 17 (68 %) and 15 (63 %) of the patients in the Nuflexxa and placebo groups respectively. The most common adverse events were knee pain (29) and upper respiratory tract infection (6). Table 4 lists the incidence of adverse events that were reported during this study where the numerator is the number of incidents and the denominator is the number of patients in the respective treatment group.

Of the 65 reported adverse events, 20 were regarded as either "possibly, probably or definitely related to treatment." Table 5 shows the relationship of the incidence of adverse events considered to be treatment related to the treatment group.

Table 4. Incidence of Adverse Events by Treatment Group

Term	Nuflexxa n (%) n=25 patients	Placebo n (%) n=24 patients	Total n=49 patients
Knee Pain	18 (72)	11 (46)	29
Upper Respiratory Tract Infection	4 (16)	2 (8)	6
Back Pain	2 (8)	1 (4)	3
Asthenia	1 (4)	2 (8)	3
Rash	1 (4)	1 (4)	2
Herpes Simplex	1 (4)	0	1
Herpes Zoster	1 (4)	0	1
Peptic Ulcer	1 (4)	0	1
Rhinitis	1 (4)	0	1
Skeletal Pain	1 (4)	0	1
Swollen Eyelids	1 (4)	0	1
Total Knee Replacement	1 (4)	0	1
Knee Swelling	1 (4)	0	1
Hypokinesia of Knee	1 (4)	0	1
Surgery	0	2 (8)	2
Knee Trauma	0	1(4)	1
Elective Non-Surgical Procedures	0	1 (4)	1
Gingivitis	0	1 (4)	1
Chest Pain	0	1 (4)	1
Headache	0	1 (4)	1
Pruritus	0	1 (4)	1
Sudden Sensorial Verbal Hearing Loss	0	1 (4)	1
Bitter Taste	0	1 (4)	1
Vertigo	0	1 (4)	1
Appendicitis	0	1 (4)	1
Hip Pain	0	1 (4)	1
TOTAL	34	31	65

Table 5. Relationship of Adverse Effects to Treatment Groups That Were Considered to be Treatment Related

Adverse Event	Nuflexxa n = 25 patients	Placebo n = 24 patients
Hip pain	0	1
Hypokinesia of knee	1	0
Knee pain	10	5
Knee swelling	1	0
Rash	0	1
Bitter taste	0	1
TOTAL	12	8

E. Efficacy Results

For this trial, the main performance analysis for determining non-inferiority was determined using the improvement in the average of the 5 patient's self-evaluation pain parameters measured by the VAS WOMAC index at week 12 from baseline. This analysis was performed for both the intent-to-treat population, i.e. every subject who has received the injection, and the evaluable population, i.e. those subjects who have average pain scores of 41-80 allowing only one parameter to be below 20 or above 80 at both the pre-screening visit and visit 1. For those patients who dropped out of the study before week 12, the last evaluation was used. For those patients who requested NSAID or analgesic during the study, the last evaluation before start of NSAID/analgesic was used for the analysis. The results when using a $\Delta 0.08$ indicates that the effects of Nuflexxa on pain relief was not inferior to that of a commercially available hyaluronan.

Males and females showed a similar reduction in pain throughout the study in both the Nuflexxa and active control groups. The main performance end point was tested by repeated measurement model with the following possible covariates: baseline pain index, gender, age, center, and severity of pain at baseline; the treatment effect was not affected by the covariate analysis.

Table 6. Changes from baseline to last visit in average of five WOMAC pain scores (primary end point)

	Nuflexxa		Active Control (Commercially Available Hyaluronan)		Standard deviation	P value (non inferiority)
	N	Change from baseline (mm)	N	Change from baseline (mm)		
ITT-patient	160	29.9	161	28.4	21	0.0032
Evaluable-patient	103	33.5	105	32.18	20	0.0083

XI. CONCLUSIONS DRAWN FROM THE STUDY

The data obtained from the multicenter, double masked, active control study provide evidence of the safety and effectiveness of Nuflexxa for the treatment of pain in osteoarthritis of the knee in patients who have failed to adequately respond to conservative non-pharmacological therapy and simple analgesics (e.g., acetaminophen). The study results indicate that the effects of Nuflexxa on pain relief was not inferior to that of a commercially available hyaluronan.

Baseline parameters of pain, stiffness and physical function were balanced for the two treatment groups and equal within the acceptable limit range.

The safety profile of both treatment groups with respect to laboratory measurements and reported adverse events was similar. The incidences and types of device related adverse events were similar in both groups.

XII. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515 (c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Orthopaedic and Rehabilitation Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicated information previously reviewed by this panel.

XIII. CDRH DECISION

CDRH approved a single course of three weekly injections of Nuflexxa for the treatment of pain in osteoarthritis of the knee in patients who have failed to adequately respond to conservative non-pharmacological therapy and simple analgesics (e.g., acetaminophen).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See product labeling

Hazard to Health from Use of the Device: See Indications, Contraindications, Precautions, and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.

XV. REFERENCES

1. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum 1986;29:1039-1049.

2. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthritis. Ann Rheum Dis 1957;16:494-501.