DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Roberto Fiorentini, M.D.
President
FIDIA Pharmaceutical Corporation
2000 K Street, N.W., Suite 700
Washington, D.C. 20006

MAY 28 1997

Re: P950027
Hyalgan®
Filed: July 28, 1995
Amended: September 1, October 5, 6 and 16, and November 6, 1995, June 28, August 7 and 28, September 5 and 23, October 8, 9, 11, 17 and 23, and December 4, 1996 and February 13, April 22, and May 8, 9 and 16, 1997

Dear Dr. Fiorentini:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for Hyalgan®. This device is indicated for the treatment of pain in osteoarthritis (OA) of the knee in patients who have failed to respond adequately to conservative nonpharmacologic therapy, and to simple analgesics, e.g., acetaminophen. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL

REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Stephen Hinckley, M.S., at (301) 594-1296.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

## CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA-approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but **not the replacement** of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

**The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:**

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

P950027

# SUMMARY OF SAFETY AND EFFECTIVENESS

## I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | Sodium Hyaluronate |
| Device Trade Name: | Hyalgan® |
| Applicant's Name and Address: | FIDIA Pharmaceutical Corporation<br>2000 K Street, NW, Suite 700<br>Washington, DC 20006 |
| Premarket Approval (PMA) Application Number: | P950027 |
| Date of Panel Recommendation: | November 21, 1996 |
| Date of Notice of Approval to the Applicant: | May 28, 1997 |

## II. INDICATIONS FOR USE

Hyalgan® is indicated for the treatment of pain in osteoarthritis (OA) of the knee in patients who have failed to respond adequately to conservative nonpharmacologic therapy, and to simple analgesics, e.g., acetaminophen.

## III. DEVICE DESCRIPTION

Hyalgan® is a sterile, non-pyrogenic, viscous solution consisting of a high molecular weight (500,000–730,000 daltons) fraction of purified sodium hyaluronate in phosphate buffered physiological sodium chloride , having a pH of 6.8–7.5. The sodium hyaluronate is extracted from rooster combs. Hyaluronic acid is a natural complex sugar of the glycosaminoglycan family and is a long-chain polymer containing repeating disaccharide units of Na-glucuronate-N-acetylglucosamine. It is supplied in colorless, borosilicate Type I glass vials, with rubber stoppers and flip-off aluminum seals, containing 2 mL of Hyalgan®. It is also available in 2 mL pre-filled, sealed B-D syringes made of colorless borosilicate Type I glass.

| Two milliliters of Hyalgan® contain: | |
|---|---|
| Sodium hyaluronate | 20.0 mg |
| Sodium chloride | 17.0 mg |
| Dibasic sodium phosphate ×12H2O | 1.2 mg |
| Monobasic sodium phosphate ×2H2O | 0.1 mg |
| Water for injection | q.s.* to 2.0 mL |

*q.s. = up to

## IV. CONTRAINDICATIONS

Do not administer to patients with known hypersensitivity to hyaluronate preparations.

Intra-articular injections are contraindicated in cases of past and present infections or skin diseases in the area of the injection site.



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V. **WARNINGS:** See attached labeling.

VI. **PRECAUTIONS:** See attached labeling.

## VII. ALTERNATIVE PRACTICES AND PROCEDURES.

For patients who have failed to respond adequately to conservative nonpharmacological therapy and simple analgesics, e.g., acetaminophen, alternative therapies to Hyalgan® include nonsteroidal anti-inflammatory drugs (NSAIDS); intraarticular injections of corticosteroids or injections of modified hyaluronan. For patients who have failed the above treatments, surgical interventions such as arthroscopic surgery and total knee replacement surgery are also alternative treatments.

## VIII. POTENTIAL ADVERSE EFFECTS

Hyalgan® was investigated in a single U.S. clinical investigation in which there were three treatment arms (164 subjects treated with Hyalgan®; 168 with placebo; and 163 with naproxen). Common adverse events reported for the Hyalgan®-treated subjects were: gastrointestinal complaints, injection site pain, headache, local joint pain and knee swelling/effusion, rash, pruritus, and ecchymosis (refer to Table 1). Hyalgan®-treated patients had 48/164 (29%) incidents of gastrointestinal complaints which were not statistically different from the placebo-treated group. A statistically significant difference in the occurrence of pain at the injection site was noted in the Hyalgan®-treated patients: 38/164 (23%) in comparison to 22/168 (13%) in the placebo-treated patients ($p=0.022$) There were 6/164 (3.7%) premature discontinuations in Hyalgan®-treated subjects due to injection site pain in comparison to 1/168 (<1%) in the placebo-treated subjects. These differences were not statistically significant. Headache, local joint pain and knee swelling/effusion, rash, and pruritus occurred in approximately equal frequency in both the Hyalgan® and placebo-treated groups.

In 2/164 (1.2%) Hyalgan®-treated subjects positive bacterial cultures were obtained from synovial fluid of effusion aspirated from the treated knee. Neither subject exhibited evidence of infection clinically or subsequently required treatment with antibiotics. Refer to Section XI. H. for a detailed discussion of the adverse events noted in the U.S. clinical investigation.

Hyalgan® has been in clinical use in Europe since 1987. Analysis of the adverse events that have been reported with the use of Hyalgan® in Europe reveals that most of the events are related to local symptoms such as pain, swelling/effusion, and warmth or redness at the injection site. In the two events reported as anaphylactoid reactions, Hyalgan® treatment was discontinued and both had favorable outcomes. Three cases of allergic reactions were reported in which the patients were discontinued from Hyalgan® treatment and the incidents resolved. Seven cases of fever were reported in which three of the cases were reported to be associated with local reactions; pyogenic arthritis was reported to be ruled out in these three cases. All fever patients were discontinued from Hyalgan® treatment and all incidents resolved. One incident of shock (which was described as a "hypotensive crisis") was reported. The incident resolved and Hyalgan® treatment was continued.

Forty non-U.S. clinical trials with Hyalgan® have been conducted. Adverse events reported in these studies are similar to those reported in the U.S. clinical investigation and those noted with clinical use in Europe. Refer to Section XIII for a detailed discussion of the adverse events for a detailed discussion of the adverse events noted in the forty non-U.S. clinical trials.



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## IX. MARKETING HISTORY

This product is marketed in Europe, Asia, and South America under the trademark Hyalgan® or Hyalart®. Hyalgan® is also called Polyreumin (Brazil) and Replasyn (Canada). Hyalgan® was introduced in Italy in 1987 and since that time, Hyalgan® or Hyalart® have been registered for use in twenty countries. The device has not been withdrawn from any country for any reason related to the safety and effectiveness of the device.

## X. SUMMARY OF PRECLINICAL STUDIES

### A. SAFETY STUDIES

Introduction

Various studies were conducted to assess the safety of Hyalectin® (sodium hyaluronate), Hyalovet® (a veterinary preparation with a composition identical to Hyalgan®), and Hyalgan®. *In vitro* and *in vivo* studies were performed to evaluate the potential for Hyalgan® to generate toxic or genetic effects.

**Hemolysis:**

**Blood compatibility/hemolytic potential study.**

The miscibility of Hyalectin® was evaluated using 10 and 20 mg/mL concentrations of Hyalectin® in physiologic saline at five different dilutions to human or canine serum or plasma (1:1 - 1:5). Each concentration was evaluated grossly for precipitation, coagulation, and separation of Hyalectin®. The hemolytic potential of Hyalectin® was evaluated using 10 and 20 mg/mL concentrations of Hyalectin® in physiologic saline. Each concentration was evaluated for hemolysis both grossly and using quantitative spectrophotometric methods at the same five dilutions described above using human and canine whole blood. In addition, various negative control solutions were also evaluated to account for the different components of blood used.

At 10 and 20 mg/mL, Hyalectin® combined with human or canine serum or plasma demonstrated no apparent miscibility problems as indicated by the absence of a separation of components and lack of precipitation or coagulation. In addition, Hyalgan® did not appear to possess any apparent potential for erythrocyte hemolysis.

**Genotoxicity:**

**Ames metabolic activation test to assess the potential mutagenic effect of Hyalectin®.**

The mutagenic potential of Hyalectin® (0, 50, 150, 500, 1,500, and 5,000 µg/plate) was tested using five bacterial strains of *S. typhimurium* (TA 1535, TA 1537, TA 1538, TA 98, and TA 100). Tests were performed in the presence and absence of exogenous metabolic activation in the form of Aroclor 1254-induced rat liver S-9.

No evidence of mutagenic potential for Hyalectin® was observed in the *S. typhimurium* bacterial mutation assay.



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**An assessment of the mutagenic potential of Hyalectin® in mammalian cells *in vitro* using the Chinese hamster ovary/HPRT locus assay.**

The ability of Hyalectin® (0, 100, 125, 150, 175, and 200 µg/mL) to induce forward mutation at the functionally hemizygous hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells was investigated in the presence and absence of exogenous metabolic activation in the form of Aroclor 1254-induced rat liver S-9.

Hyalectin® failed to demonstrate mutagenic potential in the CHO/HPRT gene mutation assay.

**Hyalectin® metaphase chromosome analysis of human lymphocytes cultured *in vitro*.**

Hyalectin® (5, 20, 100, and 200 µg/mL in the absence of the S-9 mix and 20, 100, and 200 µg/mL in its presence) was tested for its ability to induce chromosomal aberrations in human lymphocytes cultured *in vitro*. Cultured human lymphocytes, stimulated to divide by addition of phytohemagglutinin, were exposed to the test compound in both the presence and absence of an exogenous metabolic activation system in the form of Aroclor 1254-induced rat liver S-9.

Cultures treated with Hyalectin® showed no significant increases in the proportions of cells with aberrant chromosomes compared with solvent controls. There was a single exception of a very slight increase at the intermediate dose level (100 µg/mL) in the presence of S-9 mix when gap damage was included in the calculation. However, the actual value (2.5%) was low and not considered to be evidence of clastogenic activity. Therefore, no evidence of clastogenic activity induced by Hyalectin® was detected in human lymphocytes *in vitro*.

**Autoradiographic assessment of unscheduled DNA repair synthesis in cultured mammalian cells after exposure to Hyalectin®.**

The potential mutagenicity of Hyalectin® (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, and 2000 µg/mL) was assessed in cultured human epithelioid (HeLa) cells by unscheduled DNA synthesis or DNA repair assays in both the presence and absence of metabolic activation (S-9), in duplicate autoradiographic tests using tritiated thymidine.

No evidence of mutagenic potential for Hyalectin® was detected in a mammalian cell model of unscheduled DNA synthesis *in vitro*.

**Micronucleus test on Hyalectin®.**

The effect of Hyalectin® (400 mg/kg) on the incidence of micronucleated polychromatic erythrocytes (PCE) in Charles River mice was investigated. Bone marrow smears were obtained from the test compound and negative control groups at 24, 48 and 72 hours after dosing and examined for the presence of micronuclei. Bone marrow smears from the positive control were obtained only 24 hours after dosing and examined for the presence of micronuclei.

There were no clinical signs or mortalities observed at any time during the 72-hour observation period. Hyalectin® did not cause any statistically significant increases in the number of micronucleated PCE's or micronucleated normochromatic erythrocytes at any of

the three sacrifice times. Hyalectin® failed to cause any significant decreases in the ratio of polychromatic to normochromatic erythrocytes ($p>0.05$). It was concluded that Hyalectin® failed to demonstrate mutagenic potential or bone marrow cell toxicity *in vivo* when administered by subcutaneous injection.

**Implantation:**

**Assessment of the effects of an intra-articular injection of Hyalectin® into the stifle joint of the rabbit.**

Single i.a. injections of 5 mg Hyalectin® were administered into the right hind limb stifle joint of 4 rabbits/sex. The animals were sacrificed 3 and 10 days post injection.

All animals survived until the scheduled date of sacrifice. No ambulatory problems were observed in any of the animals after the injection, and no signs of toxicity were noted. Gross and microscopic examination of joint tissue did not reveal any changes that could be attributed to the presence of Hyalectin®.

**Hyalectin® toxicity to beagle dogs by intra-articular injection once a week for 13 weeks.**

Weekly i.a. injections of saline, or 5, and 15 mg of Hyalectin® were administered for up to 13 weeks to 4 male and female dogs per treatment dose (4 dogs/sex/dose); 12 dogs were treated for 4 weeks and sacrificed; 12 dogs were treated for 13 weeks and sacrificed.

Clinical signs were restricted to occasional vomiting, transient swelling, and occasional post injection limping in all treatment groups. No adverse affects on body weight gain, food intake, ophthalmoscopy, hematological, or urinary parameters were noted. Serum biochemistry values showed a decrease in globulin and plasma protein in the highest dose group. In addition, increased sodium and chloride levels were found in some Hyalectin® treated animals at week four, however, no intergroup differences were noted for in these values at week thirteen. Postmortem findings showed signs of subcutaneous hemorrhage at injection sites and minor weight deviations in single organ weights that were reported not related to treatment. No significant histopathological findings were noted. It was concluded that Hyalectin® did not cause any significant local or systemic toxicological effects.

**The effect of multiple dosages and dosage intervals of hyaluronic acid in horses.**

Four groups of 4 horses/sex/group were dosed daily with saline, 20, 60, or 100 mg

Hyalovet® for 4 days followed by twice weekly injections for 4 weeks; injections alternated between right and left radial carpal joints.

All animals survived to the scheduled date of sacrifice. No evidence of local or systemic toxicity that could be attributed to Hyalovet® was reported. No treatment-related effects on physical examination results, body weight, synovial fluid analysis, carpal flexion were observed. Mild synovitis characterized by slight elevations in lymphocyte count and protein levels; and reduced maximal carpal flexion was noted in all groups. This was attributed to the frequency of injection rather than the test substance.



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**Sensitization:**

***In vitro* assay for anaphylactic potential on Hyalgan® hyaluronic acid sodium salts.**

The ability of Hyalgan® to convert complement C3, C4, and C5 to active anaphylatoxins was measured *in vitro* using radioimmunoassays. Human serum was incubated for 30 minutes at 37°C with 1.0 mg/mL of inulin (positive control), saline (negative control), or a 1:1 solution of Hyalgan®. Following incubation, aliquots of the serum samples were transferred to vials containing precipitating agents for C3, C4, and C5. Precipitated serum samples were centrifuged, and supernatants were tested for the presence of activated complement using a radioimmunoassay. Increases in complement concentrations were determined relative to concentrations in the negative control.

Radioimmunoassays indicated that essentially 100% of the C4 was converted to the active anaphylatoxin (C4a). A two-fold increase in C3a and only a slight increase in C5a concentrations were noted as compared to the negative control. These data indicate that Hyalgan® activates the classical complement pathway *in vitro*. No significant evidence of activation of the alternative complement cascade was noted.

**Dermal sensitization study of Hyalgan® in guinea pigs- maximization test.**

A sensitization study of Hyalgan® was conducted in albino guinea pigs according to the methods described by Magnusson and Kligman. Thirty animals were divided into either a test group, a positive control group, or a negative control group (five animals/sex/group). Test animals were sensitized in duplicate with 0.05 mL Freund's Complete Adjuvant (FCA), 0.05 mL test Hyalgan® in sterile water, and Hyalgan® in sterile water with FCA. The positive control group was sensitized in duplicate with 0.05 mL FCA, 0.05 mL sulfathiazole in sterile water, and 0.05 mL sulfathiazole in sterile water and FCA. The negative control group was untreated. On Day 7, undiluted Hyalgan® was applied dermally to the injection site of the test animals. The positive control was similarly treated with sulfathiazole. The negative control group was untreated. On Day 21, all animals received challenges on the right flank. The test and negative control groups were challenged with Hyalgan®. The positive control group was challenged with sulfathiazole. Test sites were evaluated for evidence of erythema and edema 48 and 72 hours post-challenge.

None of the test or negative control animals exhibited evidence of a dermal reaction in response to challenge with Hyalgan®. All animals in the positive control group exhibited a slight to moderate dermal reaction in response to sulfathiazole challenge. These data indicate that Hyalgan® is not a skin sensitizer in guinea pigs.

**Test of the antigenicity and tolerance of Hyalovet 20 when injected intra-articularly in the horse.**

The potential antigenicity of Hyalovet® (20 mg/2 mL of Hyalectin®) was assessed in horses. Six healthy horses were immunized intra-articularly with Hyalovet® 3 times at 12 to 16 day intervals. Contralateral joints were injected with Ringer's solution and served as negative controls. Prior to the first, and after the last immunization, animals were challenged by intradermal injection at six sites with 0.05 mL of a challenge material (Ringer's solution [negative control], histamine solution [positive control], or hyaluronic acid). In addition to immunologic challenge, the following tests were performed on all test animals following

Hyalovet® injection: hematology; synovial fluid analysis; radiography; general clinical examination; joint inspection for heat and swelling; observation of gait.

No evidence of sensitization was observed following challenge with Hyalovet®. Hematological analysis, synovial fluid analysis, and radiographic findings did not indicate any adverse or lasting local or systemic effects associated with administration of the test material into normal joints. Transient swelling was observed in joints treated with the test material and negative control. Joint swelling was presumed to be the result of injection trauma and lack of exercise. These data indicate that Hyalovet® or its principal component, Hyalectin® do not possess antigenic properties in horses.

**Pharmaco-kinetics:**

**The pharmacokinetics of $^{3}$H-Hyalectin® after intra-articular administration to dogs.**

Single i.a. injections of 1 mg/kg were administered to 3 male and 3 female dogs; distribution and excretion was studied up to 8 days following injection.

$^{3}$H-Hyalectin® was rapidly cleared from the intra-articular injection site in dogs. The half-life of $^{3}$H-Hyalectin® in synovial fluid was approximately 17 hours. In two dogs sacrificed at either 120 hours or 192 hours after treatment, the rates of excretion of radioactivity in urine declined with a terminal half-life of approximately 100 hours after injection. $^{3}$H-Hyalectin® was distributed into the liver and kidneys, and also into the lymph nodes and bone marrow, from which it appeared to be removed relatively slowly. $^{3}$H-Hyalectin® was excreted mainly in the urine; the amounts of radioactivity excreted in feces were low.

**Acute Systemic Toxicity:**

**Report on the toxicity of hyaluronic acid (Hyalectin®).**

Sprague-Dawley rats and Swiss mice (5/sex/dose) were administered a single dose of up to 50 mg/kg (i.v.) or 100 mg/kg (s.c.) of Hyalectin® and observed for 14 days.

None of the 60 rats or 60 mice died after treatment by either administration route and no clinical signs of toxicity were observed. $LD_{50}$ values were in excess of 50 mg/kg by the i.v. route and in excess of 100 mg/kg by the s.c. route for both species. Thus, Hyalectin® administered intravenously or subcutaneously, exhibited no acute toxicity in Sprague-Dawley rats or Swiss mice at the highest doses tested, 50 mg/kg i.v. and 100 mg/kg s.c.

**Subacute Systemic Toxicity:**

**Report on the toxicity of hyaluronic acid (Hyalectin®).**

Sprague-Dawley rats (5/sex/dose) were administered saline or 5, 10, or 20 mg/kg/day of Hyalectin® by subcutaneous injection for 30 days. Mortality, behavior, and body weight gain were monitored and hematology, clinical chemistry, urinalysis, gross necropsy were performed. Animals received total doses of 150, 300, or 600 mg/kg Hyalectin®. The cumulative exposure tested in this study is more than 100–400 times the anticipated clinical exposure dose of 1.43 mg/kg for one cycle of treatment.

None of the 40 rats tested died, and results obtained for behavior, body weight, hematology, clinical chemistry, urinalysis, and macroscopic evaluation were within normal ranges. No adverse reactions were recorded.

**Hyalectin® toxicity to rats by repeated subcutaneous injection for 4 weeks.**

Forty rats were divided into four groups and treated for 4 weeks. Three of the groups, each consisting of five male and five female rats, received daily subcutaneous injection into the right or left flank at doses of saline or 4.5, 1.5, and 0.5 mg/kg/day of Hyalectin® for 4 weeks. Endpoints evaluated included mortality, body weight gain, food utilization, and post-mortem macroscopic findings. Total exposure doses of 14, 42, and 126 mg/kg were administered to the rats which are approximately 10–100 times the anticipated total clinical exposure dose of 1.43 mg/kg per treatment cycle.

There were no clinical findings indicative of any reaction to treatment, and no deaths occurred during the study. The food consumption and body weight gains of females receiving 1.5 or 4.5 mg/kg/day were greater than those in the control group. The efficiency of food utilization of these animals was, however, similar to that of the control group. There were no intergroup differences in organ weights at the postmortem examination, and macroscopic findings were normal. Histopathological examination of the subcutaneous injection sites revealed no treatment-related changes. Hyalectin® treatment with 0.5, 1.5, or 4.5 mg/kg/day s.c. for four weeks was well tolerated in rats, and no treatment-related adverse reactions were recorded for any endpoint examined.

**Toxicity of hyaluronic acid (Hyalectin®) in Beagle Dogs.**

Beagle dogs (1/sex/dose) were administered saline or 1.5, 3, and 6 mg/kg/day of Hyalectin® by intramuscular injection for 30 days. Mortality, behavior, and body weight gain were monitored and hematology, clinical chemistry, urinalysis, and gross and histological examinations were performed.

No deaths occurred, and no signs of adverse effects in any of the parameters measured were observed for any of the treated dogs. Additionally, no changes were identified in any of the postmortem macroscopic examinations of the principal thoracic and abdominal organs. Microscopic examination results revealed no Hyalectin®-related changes in tissues from the liver, kidneys, heart, adrenals, or spleen. No adverse reactions were observed.

**Subchronic Systemic Toxicity:**

**Toxicity of hyaluronic acid (Hyalectin®) in rats.**

Sprague-Dawley rats (5/sex/dose) were administered saline or 4, 8, or 16 mg/kg/day of Hyalectin® by subcutaneous injection for 180 days. Mortality, behavior, and body weight gain were monitored and hematology, clinical chemistry, urinalysis, and gross and microscopic examinations were performed. The rats received total exposure doses of 720, 1440, or 2880 mg/kg, and dogs received total exposure doses of 45, 90, or 180 mg/kg during the study.

No deaths occurred, and no signs of adverse effects in any of the parameters measured were observed for any of the treated rats. Additionally, no changes were identified in any of the

post-mortem macroscopic examinations of the principal thoracic and abdominal organs. Microscopic examination results revealed no Hyalectin®-related changes in tissues from the liver, kidneys, heart, adrenals, or spleen.

**Hyalectin® toxicity to rats by daily subcutaneous injection for 26 weeks.**

Three groups, each consisting of 20 male and 20 female rats, received Hyalectin® by daily subcutaneous injection at doses of 0, 0.5, 1.5, or 4.5 mg/kg/day for 26 weeks. The following observations were made during the study: mortality, clinical signs, body weight gain, food consumption, efficiency of food utilization, water consumption, ophthalmoscopy, laboratory investigations (hematology, serum chemistry, urinalysis), and passive cutaneous anaphylaxis (PCA) test. On completion of 26 weeks of treatment, all surviving rats were sacrificed for postmortem examination including organ weight analysis, and gross and microscopic histopathology. Total exposure doses of 91, 273, and 819 mg/kg were tested which are approximately 64–573 times the anticipated total clinical exposure of 1.43 mg/kg per treatment cycle.

No treatment-related clinical signs were observed. There were five deaths during the study, none of which were related to treatment. The food consumption, body weight gain, and efficiency of food utilization were similar for treated rats and controls. Water consumption investigations in week 4 revealed a slight, but not statistically significant, decrease in the group treated with 4.5 mg/kg/day when compared to controls. Investigations in Weeks 11 and 24 were extended to include all groups, but no evidence of a treatment-related effect was apparent. There were no treatment-related effects on hematological parameters. Decreased phosphorus levels were noted in all groups of treated male rats compared to controls in all investigations. In addition, reduced calcium levels and increased chloride levels were noted in all treated groups for males and in females receiving 1.5 or 4.5 mg/kg at week 26. The magnitude of all these changes was small, and no clear dose-response relationship was apparent for males at any dose. These changes are considered to be of equivocal toxicological significance. There was no treatment-related effect on urinalysis parameters. There was no positive blueing at any injection site for any of the samples taken in the PCA test; therefore, Hyalectin® did not activate antibody formation at the levels employed. A slight reduction in kidney weights was noted for all groups of treated males. However, no dosage dependency or microscopic changes were apparent, and this finding was considered to be of no toxicological significance. No treatment-related changes attributable to the daily subcutaneous injection of Hyalectin® were detected in the internal organs or injection sites. The changes recorded at the injection sites were those due to the repeated trauma of subcutaneous injections rather than the compound injected. These changes, recorded in rats from the control and treated groups, consisted of subcutaneous collagen deposition, sometimes with minimal subcutaneous hemorrhage, subcutaneous necrosis, or focal muscle degeneration. Daily treatment of rats with 0.5, 1.5, or 4.5 mg/kg Hyalectin® by subcutaneous administration for 26 weeks did not result in any systemic toxicity.

**Reproductive/ Developmental Toxicity:**

**Report on the toxicity of hyaluronic acid (Hyalectin®) in the rat.**

Sprague Dawley Rats (10 pregnant females/dose) were administered saline or 4, 8, and 16 mg/kg/day of Hyalectin® by s.c. injection on Days 6 through 15 of gestation. Fetuses were removed and examined after sacrifice on Day 20. Body weight gain, implantations, and

reabsorptions were monitored in the mothers and number of live fetuses, number of dead fetuses, body weight gain, and visceral and skeletal malformations were assessed in the fetuses.

No differences were observed between the treated or control groups for any of the parameters measured for the mothers. Also, the number, growth, and viability of the fetuses from the treated mothers did not differ from the corresponding controls. No fetal malformations occurred in either species. Statistical analysis of the results, comparing the various frequencies observed in the Hyalectin® treated groups failed to reveal any significant differences in either species. No fetal toxicity or teratogenic activity was observed.

**Effect of Hyalectin® on fertility and general reproductive performance of the rat.**

Four groups of 15 males and four groups of 30 females were treated with saline or 0.5, 1.5, or 4.5 mg/kg/day of Hyalectin® by subcutaneous injections. Males were treated for 9 weeks prior to mating, through the mating, and to day 21 postpartum. Females were dosed for 2 weeks prior to mating. They were either sacrificed on day 20 of pregnancy (14 females/group) or allowed to give birth and rear their young to weaning (16 females/group) prior to sacrifice. The following observations were made during the study: $F_0$ generation: Clinical signs, water and food consumption, body weight change, pregnancy rate, mating performance, duration of pregnancy, terminal autopsy to determine number of corpora lutea, number and distribution of live young, number and distribution of embryonic/fetal deaths, individual fetal weight, and gross fetal abnormalities; Litter data: Number of live young, sex, body weight, external abnormalities, surface righting reflex, startle reflex, air righting reflex, and pupil reflex; $F_1$ generation: (12 male, 12 female per treatment group); clinical signs of toxicity, developmental/behavioral examination (accelerating rotarod, actimat test, passive avoidance test), reproductive capacity, and litter data. On or shortly after day 21 postpartum, all $F_2$ pups and $F_1$ parents were sacrificed.

Among $F_0$ generation animals, there were no effects of treatment at any dosage on behavior, food intake, body weight change, mating performance, or duration of pregnancy. Among females, there was a higher incidence of minor injection site anomalies. In each group, including controls, these were diffuse hemorrhage/yellow discolorations. With the exception of three females in the 4.5 mg/kg group, these anomalies were detected in all females sacrificed at day 20 of pregnancy. Since these anomalies occurred in the control group as well as the treatment groups they were not considered to be attributed to Hyalectin® treatment. Litter values at day 20 of pregnancy and also from birth to weaning were unaffected by treatment of parent animals with Hyalectin®. Among the selected $F_1$ generation, general disposition, food intake, body weight change, performance of specific behavioral tests, mating performance, duration of pregnancy, and litter values from birth to weaning were similar for all groups. These data indicate that Hyalectin® does not affect fertility or general reproductive performance in rats.

**Effect of Hyalectin® on pregnancy of the rat.**

Four groups of 25 adult pregnant female rats were treated subcutaneously with saline or 0.5, 1.5, or 4.5 mg/kg/day of Hyalectin® from Day 6 through day 15 of pregnancy. Food and water consumption and body weight were observed in the parent animals during this period. On Day 20, the dams were sacrificed, dissected, and examined for congenital abnormalities

and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined immediately to determine number of corpora lutea, number and distribution of live young, number and distribution of embryonic/fetal deaths, individual fetal weight from which the litter weights were calculated, and fetal abnormalities. Live young were examined externally and weighed. Fetal pathology was also examined.

Female adults. There were 17 to 23 dams per group with live young at termination. There were no treatment-related signs of toxicity, and no deaths at any dosage level. At 4.5 mg/kg/day, food consumption and body weight gain after the start of treatment were marginally lower than among controls, but differences were too slight to be attributed to treatment. Findings at autopsy were occasional and not obviously related to treatment with Hyalectin®. This included hemorrhage at injection sites.

Litter data. There were no cases of total litter loss. Intergroup differences in litter size, sex ratio, implantation loss, litter weight, and average fetal weight were slight and not attributable to treatment with Hyalectin®. Fetal pathological examination showed occasional anomalies in all groups, the type and distribution of which showed no association with treatment. At 4.5 mg/kg/day, the marginally higher incidence of fetuses with an extra (14th) rib was within background control rates. Three fetuses (one in 4.5 mg/kg group, one in 0.5 mg/kg group, and one control) had more complex changes that, in isolation, were not attributable to treatment.

**Effect of Hyalectin® on peri- and post-natal development of the rat.**

In this assessment of the effect of Hyalectin® on peri- and post-natal development of the rat, doses of 0 (Control), 0.5, 1.5, or 4.5 mg/kg/day were administered to four groups, each consisting of 25 females, by subcutaneous injection from day 15 of pregnancy through weaning. The following observations were made during the study: Parent animals: Clinical signs, food and water consumption, body weights, pregnancy rate, and gestation period; Litter data: Total litter loss, pup mortality, litter and mean pup weights, sex ratio, external abnormalities, surface righting reflex, startle reflex, air righting reflex, and pupil reflex. On or shortly after day 21 postpartum, dams and litters were sacrificed and examined externally and internally for abnormalities.

A single adult female had a palpable lump at the injection site during lactation. Apart from this finding, there were no signs of reaction to the treatment at any dosage level in dams, and no deaths occurred. Food consumption and body weight gain were not affected by treatment with Hyalectin®. All births occurred on day 21 or 22 of pregnancy. Autopsy findings in the dams were occasional and not obviously related to the treatment. These included incidences of subcutaneous hemorrhage and edema at the injection site. A single female had total litter loss (1.5 mg/kg/day group). In the 4.5 mg/kg/day group, litter size at birth through weaning was slightly lower than the corresponding control value. From birth to weaning, pup mortality was low and similar in all groups. Litter and mean pup weights and sex ratios were similar in all groups. Occasional findings detected among pups found dead prior to termination were considered to be unrelated to treatment of parent females. At termination, occasional necropsy findings included nine incidents of increased renal pelvic dilatation (7 in the 4.5 mg/kg/day group, 1 in the 1.5 mg/kg/day group and 1 in the control group) and five cases of unilateral small eye (4 in the 4.5 mg/kg/day group, 1 in the 0.5 mg/kg/day group).

Thus, treatment of rats with total doses of Hyalectin® did not adversely affect peri- or post-natal development of the exposed pups. The occasional findings observed were considered not to be related to treatment with Hyalectin®.

**Report on the toxicity of hyaluronic acid (Hyalectin®) in rabbits.**

New Zealand white rabbits (6 pregnant females/dose) were administered 0, 4, 8, and 16 mg/kg/day by s.c. injection on Days 6 through 18 of gestation. Fetuses were removed and examined after sacrifice. Body weight gain, implantations, and reabsorptions were monitored in the mothers and number of live fetuses, number of dead fetuses, body weight gain, and visceral and skeletal malformations were assessed in the fetuses.

No differences were observed between the treated or control groups for any of the parameters measured for the mothers. Also, the number, growth, and viability of the fetuses from the treated mothers did not differ from the corresponding controls. No fetal malformations occurred in either species. Statistical analysis of the results, comparing the various frequencies observed in the Hyalectin® treated groups failed to reveal any significant differences in either species. Thus, the s.c. treatment of rabbits with Hyalectin® did not interfere with the normal course of pregnancy. No fetal toxicity or teratogenic activity was observed.

**Effect of Hyalectin® on pregnancy of the rabbit.**

The effect of Hyalectin® was tested on the course of pregnancy and *in utero* development of the rabbit. Doses of 0, 0.5, 1.5, or 4.5 mg/kg/day were administered by subcutaneous injections once a day from day 6 through day 18 of pregnancy to 78 rabbits. The following observations were made on parent animals during the study: clinical signs of toxicity, mortality, body weight, and food consumption. On Day 29, dams were sacrificed, litter values (number of corpora lutea, number and distribution of live young, number and distribution of embryonic/fetal deaths, individual fetal weights, and fetal abnormalities) were determined, and fetuses were subjected to visceral and skeletal examination.
There were 13 deaths (1, 7, 4, and 1 in Groups 1-4 respectively): 12 of the animals were sacrificed on humane grounds as a result of subcutaneous reaction (subcutaneous adhesion, thickening and infection) associated with the anterior (scapular) injection sites. This reaction did not occur at the posterior injection sites or after relocation of the anterior sites to the mid-dorso-lateral region. The pattern of pathology suggested the incidence of an infection caused by injection technique. On Day 29, dams were sacrificed, litter values were determined, and fetuses were subjected to visceral and skeletal examination. There were 14 or 15 dams per group with live young at the end of treatment. Among these dams, there were no clinical signs of toxicity, autopsy findings, or intergroup differences in food consumption or body weight gain attributable to treatment with Hyalectin®. Intergroup differences in implantation rate, live litter size, sex ratio, litter weight and mean fetal weight were slight, showed no treatment- or dose-related trends, and were not statistically significant. Despite the fact that the study was confounded by poor health of the animals and poor injection technique, intergroup differences in the incidence of fetuses with malformations, anomalies, and skeletal variants were too slight to be attributed to treatment with Hyalectin®. These results indicate that Hyalectin® does not adversely affect pregnancy outcome and pup development in the rabbit.

B. EFFECTIVENESS STUDIES

Introduction

Several studies were conducted to examine the effectiveness of Hyalectin® (the principal component of Hyalgan®) and Hyalovet® (a veterinary preparation with a composition identical to Hyalgan®) in traumatic or degenerative joint disease in horses and dogs.

**Implantation Models of Arthropathies:**

**Intra-articular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs[1,2].**

An established anterior cruciate ligament deficiency-induced cartilage degeneration (Pond-Nuki) experimental model was used to evaluate Hyalectin®'s effects on cartilage destructionTwenty-six dogs were divided into four groups and were either untreated or given weekly i.a. injections of 7 mg Hyalectin®; they were sacrificed at 7, 13, or 17 weeks following surgery.

Seven weeks after surgery, the cartilage damage, graded according to Mankin's scale, was significantly reduced in knee joints treated with hyaluronic acid from the second week post-surgery compared to untreated joints. When intra-articular therapy was initiated after the seventh week, osteoarthritis progression was still reduced compared to controls. Tissue evaluation demonstrated a beneficial effect of sodium hyaluronate treatment on cartilage response and osteoarthritis progression measured post-mortem according to Mankin's scale. An inhibitory effect on the development of a fibroblast-like cell layer observed in untreated joints was noted in Hyalovet® treated joints.

Radiographic examination showed that in untreated joints, the disease became progressively worse. All of the animals treated with hyaluronic acid showed a progressive decrease in lameness and the painful reaction to flexion-extension decreased with time, disappearing at almost 8 weeks. Untreated joints showed a marked increase in both total soluble glycosoaminoglycan (GAG measured as uronic acid) and in the associative fraction. In both treated groups, there was a reduced amount of soluble GAG. Cessation of treatment after 7 weeks caused gradual regression, with an increasing amount of $CaCl_2$ soluble material in the associative fraction, while inception at 7 weeks gave biochemical evidence of reversal, with increasing GAG present in the guanidine-soluble (dissociative) fraction on the insoluble residue.

**Effect of intra-articular injection of hyaluronic acid on experimentally induced osteoarthritis in horses.**

Osteoarthritis was induced in 12 healthy horses by surgically fracturing the third carpal bone. Following surgery, animals were exercised daily for 12 weeks. Subsequent osteoarthritis ranged from moderate to almost imperceptible. Animals were divided into treatment and control groups (6 animals/group) and dosed intra-articularly with 2 mL (10 mg/mL) Hyalovet® (Hyalectin®) or 2 mL physiological saline, respectively. Animals were clinically evaluated prior to treatment and periodically for 6 weeks after treatment.

No signs of toxicity were reported. No detectable differences in results between the treated and control group were noted in lameness, joint circumference, joint flexion, synovial fluid analysis, and radiographic evaluations. Bone imaging revealed a consistent decrease in uptake of radionuclide in treated joints during the duration of the study. An increase in radionuclide uptake was observed in control joints.

**Clinical evaluation of intra-articular injection of hyaluronic acid in horses for the treatment of non-infectious synovitis associated with osteoarthritis.**

Sixty-two horses with clinical evidence of lameness in one specific carpal (n=37) or fetlock (n=25) joint were treated intra-articularly with 2 mL (20 mg) hyaluronic acid (Hyalovet®). Nineteen carpal and 14 fetlock joints required a second treatment 14 days following the first. Clinical examinations were conducted 3, 7, 14, and 28 days post-treatment, as required.

No signs of toxicity were reported. Two horses experienced transient swelling in treated joints immediately following injection. Clinically, response to treatment was rated as excellent or good in 90% of the cases. Lameness scores improved in all of the horses. Joint heat, observed in 34 joints prior to injection, decreased in 31 joints after treatment. Pain on palpation, observed in 20 joints, showed a decrease 14 days after treatment. The pain on flexion, observed in 57 joints, improved in 91 % of the treated joints. No measurable changes in the angle of flexion measured prior to treatment and at each evaluation were reported. No changes in joint circumference were detected in either treated or contralateral control carpel or fetlock joints. Results of synovial fluid analysis revealed a significant increase in viscosity ($p < 0.05$) 14 days following treatment and a decrease in synovial fluid proteins.

**Clinical evaluation of sodium hyaluronate in horses.**

Horses (18/group) with clinical evidence of lameness in one specific carpal joint and either low synovial fluid viscosity (<10 cS) or high synovial fluid protein content (>5 g/dL) were divided into two groups. One group was injected twice i.a. with 2 mL (20 mg) Hyalovet® at an interval of 1 week. The second group was similarly treated with Hyvisc® (an approved reference control hyaluronic acid preparation). Animals were clinically evaluated prior to treatment and three times a week for 2 weeks following treatment.

No adverse effects were noted in either group. Clinically, treatment with either preparation resulted in marked reduction of lameness, joint pain, joint heat, and joint swelling. Synovial viscosity decreased slightly in response to both treatments, and there was no change in synovial protein levels or lymphocyte counts.

**Use of hyaluronic acid in the treatment of equine arthropathy[3].**

Seventy-six horses (106 joints) with arthropathy (e.g., arthritis, arthrosis, tenosynovitis, and arthrosynovitis) were treated with a single injection of 2 to 4 mL of Hyalovet® (10 mg/mL Hyalectin®). Fifteen of the horses received injections following intra-articular surgery. Animals were monitored for up to 4 months.

No general systemic reactions were noted, although cases of pericapsular edema, hypothermia, and localized pain were observed. These reactions were transitory and did not impair the therapeutic effect. In the surgically-treated cases, hyaluronic acid allowed the

reintegration, if not complete, of the synovial fluid lost during surgery and the recovery of a correct intra-synovial lubrication. The results were judged as very good (total disappearance of pain, recovery of lameness) in 87.5% of the 40 arthritic cases; in 60% of the 40 arthrosis cases; in 62.5% of 6 synovitis cases; in 25% of osteochrondrosis cases, and 85.7% of 14 articular fractures. The results obtained for degenerative and inflammatory diseases were better than those obtained for osteochondrosis.

**Dose response effect of hyaluronic acid on experimentally induced carpal synovitis in the horse.**

Twenty-four quarterhorses were divided into 4 groups (6 horses/group) and experimental acute synovitis was induced in carpal joints by injection of Freund's Complete Adjuvant. Groups II, III, and IV were treated i.a. in affected joints with a single dose of 5, 20, or 40 mg of hyaluronic acid, respectively, after lameness was confirmed. Group I was dosed intra-articularly with saline. Animals were evaluated prior to treatment and weekly for 4 weeks following treatment.

No signs of systemic or local toxicity were reported. Horses receiving saline or 5 mg hyaluronic acid demonstrated little or no clinical response and remained lame. Animals in Groups III and IV showed constant clinical improvement throughout the duration of the study. Significant decreases in carpal heat ($p<0.005$), pain ($p<0.005$), resting angle flexion ($p<0.0001$), circumference, maximal carpal flexion allowed by manual force ($p<0.0001$), lameness scores ($p<0.0001$), and synovial protein levels, as well as longer stride length, as compared to Groups I and II, were observed in Groups III and IV. Over the 4-week post-treatment observation period, carpal volumes decreased in both the Group III and IV animals, while an increase was seen in the Group I and II animals.

**Modifications in the synovial fluid of horse joints indued by the intra-articular administration of sodium hyaluronate[4].**

Up to 60 mg of Hyalovet® was injected i.a. into joints affected by various diseases up to 3 times (15–30 days and 60–90 days after initial treatment). Synovial fluid was withdrawn prior to all injections and evaluated. Synovial fluid from healthy, untreated horses served as controls.

A consistent improvement in color, quantity, transparency, and viscosity of removed synovial fluid was observed. Hyaluronic acid concentration and A/G ratios were normalized in treated animals, as compared to untreated controls.

## XI. SUMARY OF THE CLINICAL INVESTIGATION

A multicenter clinical investigation was conducted in the United States under an approved Investigational Device Exemptions (IDE), G900169.

### A. OBJECTIVE

The objective of this study was to determine the safety and effectiveness of intra-articular injections of Hyalgan® in relieving pain in patients with osteoarthritis of the knee.

B. INCLUSION AND EXCLUSION CRITERIA

The study population consisted of subjects who met the following inclusion criteria: diagnosis of idiopathic osteoarthritis of the knee (as defined by American College of Rheumatology criteria, 1986), grades 2 or 3 osteophytes as defined by Kellgren's criteria, moderate or marked pain as assessed by the masked observer, a minimal score of 20 mm on at least one of the five WOMAC pain subscale assessments, age $\geq$ 40, OA symptoms in the knee for at least one year, knee pain on greater than one-half of the days during the preceding month, no changes in physiotherapy regimens during the entire study, absence of knee trauma, evidence of adequate contraceptive methods in women of child-bearing age potential, and willingness to discontinue NSAIDS 14 days prior to study onset.

A subject was considered ineligible for enrollment into the study if they met at least one of the following exclusion criteria: diagnosis of inflammatory joint disease, secondary arthritis of the knee, inability to perform the 50-foot walk test; grade 0, 1, or 4 osteoarthritis by Kellgren's criteria; large axial (medio-lateral) deviations; peripheral neuropathy; intraarticular injections in the preceding three months, prior hyaluronic acid injections, chronic active fibromyalgia, osteonecrosis of either knee, instability, gout, joint infection, acute pseudogout flare in the preceding three years, use of prohibited analgesics, NSAIDS, steroids, or opiates, sensitivity to any study medications, vascular insufficiency, active anticoagulation, clinically significant hepatic or renal disease, women using inadequate contraceptive methods or who are pregnant or lactating, and poor general health.

C. PATIENT POPULATION AND DEMOGRAPHICS

A total of 495 subjects (206 males and 289 females) with moderate to severe pain were randomized into three treatment groups in a ratio of 1:1:1 receiving Hyalgan®, placebo, or naproxen.

The demographics of trial participants were comparable across treatment groups with regard to age, gender, race, height, weight, medically relevant characteristics and abnormalities, history of osteoarthritis, prior NSAID use, physiotherapy history; and weight bearing/use of assistive devices.

The mean age of the study participants was 63.7±9.8 years (range: 40–90 years); 58.4% of the participants were female and 41.6% were male; 81.8% were Caucasian, 16.2% were black, and 2.0% were classified as "other"; the mean height was 168±10.52 cm (range: 102–198 cm); and the mean weight was 88.7±18.2 kg (range: 45–170 kg).

D. EVALUATION SCHEDULE

After meeting initial screening requirements, all subjects discontinued all non-steroidal anti-inflammatory drug (NSAID) therapy for a period of two weeks but were allowed up to 4 grams per day of acetaminophen as needed for pain relief. After two weeks, all subjects returned for baseline evaluations. Subjects who were eligible to participate in the study were randomized within center on the basis of strata for moderate vs. marked pain, as determined by the masked observer at the baseline evaluation.

All subjects who tolerated the NSAID washout and met all entry requirements received their first injection immediately after stratification/randomization. Intra-articular injections (Hyalgan®,

placebo, or sham) were administered weekly for a total of 5 injections (Weeks 0–4). Subsequent visits and evaluations took place at weeks 5, 9, 12, 16, 21, and 26.

E. STUDY DESIGN

This study was a double-masked (masked-evaluator), placebo- and naproxen-controlled, multi-center prospective clinical trial. A total of 495 subjects (206 males and 289 females) with moderate to severe pain were randomized into three treatment groups in a ratio of 1:1:1 for treatment with Hyalgan®, placebo, or naproxen as follows:

| Routes of Administration | Hyalgan® | Placebo | Naproxen |
|---|---|---|---|
| s.c. | Lidocaine (1%) | Lidocaine (1%) | Lidocaine (1%) |
| i.a.* | Hyalgan® (20 mg/2 mL) | Phosphate-Buffered Saline (2 mL) | Sham Injection |
| p.o./b.i.d. | Placebo for naproxen capsules | Placebo for naproxen capsules | Naproxen capsules (500 mg) |
| p.o./p.r.n. (not to exceed 4 grams/day) | Acetaminophen | Acetaminophen | Acetaminophen |

* Synovial fluid was aspirated (when present) in the Hyalgan® and placebo groups.

F. SUCCESS CRITERIA

1. Primary Effectiveness

For this trial, overall success for effectiveness was defined as meeting all the following four success criteria using scores from week 26:

a. *Analysis of a Visual Analog Scale (VAS) for pain during the 50-foot walk test.* A positive outcome for this variable was a statistically significantly (alpha = 0.05) greater reduction on the mean VAS for Hyalgan®-treated subjects when compared to placebo-treated subjects at week 26. This difference was also to exceed one-fourth of a standard deviation of the change from baseline.

b. *A categorical assessment (0 = none to 5 = marked) of pain, as assessed by a masked evaluator*, during the 48 hours preceding visits, was to be concordant with the VAS results for the trial to be considered successful. That is, improvement for the Hyalgan®-treated subjects when compared to placebo-treated subjects at week 26 was to be in the same direction, but, not required to be statistically significant.

c. *A categorical assessment (0 = none to 5 = marked) of pain, as assessed by the subject*, during the 48 hours preceding visits, was to be concordant with the VAS results for the trial to be considered successful. That is, improvement for the Hyalgan®-treated subjects

when compared to placebo-treated subjects at week 26 was to be in the same direction, but, not required to be statistically significant.

d. *The magnitudes of the observed effect for Hyalgan® versus placebo for the VAS and both of the categorical pain assessments* were to be at least 50% of those observed for naproxen versus placebo. That is, the mean change in VAS from baseline of the Hyalgan®-treated subjects minus the mean change from baseline of the placebo-treated subjects was to be at least 50% of the comparable difference between naproxen-treated and placebo-treated subjects. For the categorical scale, the difference between Hyalgan® and placebo in percent of patients showing improvement at week 26 was to be at least 50% of the difference between naproxen and placebo in percent of patients showing improvement at week 26.

2. Safety

*In order for the product to be considered safe, the incidence of severe swelling and pain consequent to intra-articular injection of Hyalgan® in this study should be less than 5%.*

3. Secondary Effectiveness Parameters

Secondary effectiveness measures were the WOMAC osteoarthritis index; time to perform the 50-foot walk test; knee range of motion (extension and flexion); heel to buttock distance measurement; effusion (patellar ballottement, bulge sign, and synovial fluid aspiration [if aspirated]); knee circumference; acetaminophen consumption; and subject and masked observer global assessment of treatment effectiveness. These effects were to be evaluated with two-sided tests, and alpha was to be 0.05.

G. EFFECTIVENESS ANALYSES

For the purpose of effectiveness, the analysis performed was based on those subjects who completed the study. An intent-to-treat analysis performed on all randomized subjects revealed numerical improvement in the effectiveness parameters for those subjects treated with Hyalgan® over placebo-treated subjects, but, no statistical significance was shown. The effectiveness analyses that follows is, therefore, based on data obtained from those subjects who completed the study.

1. Patient Accountability

Of the 495 subjects enrolled in the study, 162 discontinued prior to study completion, resulting in a final study cohort of 333 subjects (Hyalgan® = 105, placebo = 115, and naproxen = 113) who completed the entire 26 weeks. The number and time course of discontinuations were comparable across treatment groups (Hyalgan® = 59, placebo = 53, and naproxen = 50; see Table 2), and the differences in proportions discontinued were not significant ($p = 0.5$, Fisher's Exact Test).

2. Primary Effectiveness Parameters:

a. *VAS for Pain — 50-Foot Walk Test*: Among study completers, the Hyalgan®-treated group exhibited statistically significantly greater improvement in this parameter as compared to the placebo group at Weeks 4, 5, 12, 21, and 26 ($p<0.05$). At Week 26,

the difference between the Hyalgan®-treated group and the placebo group adjusted means was 8.85 mm (p = 0.004 by ANCOVA), which is a difference of approximately one-third of a standard deviation.

b. *Categorical Assessment of Pain – Masked Evaluator*: The masked evaluator's assessment of pain indicated a shift towards less pain at Week 26 in all groups with a higher percentage of Hyalgan®-treated (82/105, 78%) as compared to both placebo-treated (80/115, 70%) and naproxen-treated (82/112, 73%) subjects improved. Although these proportions were not statistically significantly different, the results of the categorical assessment by the masked evaluator are in concordant with those obtained in the VAS 50-foot walk test pain assessment.

c. *Categorical Assessment of Pain – Subjects'*: The subjects' assessment of pain generally agreed with the masked evaluator's assessment of pain and indicated a shift towards less pain at week 26 in all groups with a higher percentage of Hyalgan®-treated (77/105, 73%) as compared to placebo-treated (72/115, 63%) and naproxen-treated (76/113, 67%) subjects improved. Although these proportions were not statistically significantly different, the results of the categorical assessment by the subjects are in accordance with those obtained in the VAS 50-foot walk test pain assessment.

d. *The magnitude of the observed effect for Hyalgan®-placebo comparison:* The improvement in pain in the VAS for the 50-foot walk test exhibited by the Hyalgan®-placebo comaprison at week 26 was 3.99 mm greater than the improvement in pain exhibited by the naproxen-placebo comparison at week 26. Although this difference was not statistically significant it did exceed 50% of the improvement exhibited by the naproxen-placebo comparison. The results of the catagorical pain assessments by the masked evaluator and the subjects indicated the percentage of subjects showing improvement in the Hyalgan®-treated group relative to the placebo-treated group at week 26 exceeded the percentage of subjects showing improvement in the naproxen-treated group relative to the placebo-treated group at week 26, although this difference was not statistically significant.

3. Safety:

Severe swelling and pain occurred in 2/164 (1.2%) of Hyalgan-treated subjects. Therefore, the incidence of severe swelling and pain consequent to intra-articular injection of Hyalgan® in this study was less than 5%.

4. Secondary Effectiveness Parameters:

a. *WOMAC Scale*: On each major section of the WOMAC scale (pain, stiffness, and physical function), the Hyalgan®-treated subjects had the lowest mean VAS among the three treatment groups at week 26, although the differences were not statistically significant, these findings were concordant with the results of the primary efficacy analysis of pain on the 50-foot walk test. On both the WOMAC A scale (pain, p = 0.041) and the WOMAC C scale (physical function, p = 0.047), Hyalgan®-treated subjects exhibited statistically significantly greater improvement than placebo-treated patients.

b. *Time to Perform 50-Foot Walk Test*: The Hyalgan® and placebo groups exhibited an initial increase in this parameter between the screening and baseline evaluations, followed by decreases that persisted throughout the study period. The decreases in time to perform the 50-foot walk test observed at Week 26 were not statistically significant ($p = 0.285$, by ANCOVA, Hyalgan® vs. placebo). The decreases for each group were: 11.9% (placebo), 15.7% (Hyalgan®), and 12.6% (naproxen).

c. *Knee Range of Motion – Extension*: Small changes in knee extension, which were not statistically significant ($p = 0.8$, by ANCOVA, Hyalgan® vs. placebo), were observed throughout the trial in all groups (change from baseline at Week 26 = -0.65°, -0.47°, and -1.57° for the Hyalgan®-treated, placebo-treated, and naproxen-treated groups, respectively).

d. *Knee Range of Motion – Flexion*: Small changes in maximum flexion, which were not statistically significant ($p = 0.8$, by ANCOVA, Hyalgan® vs. placebo), were observed throughout the trial in all groups (change from baseline at Week 26 = 6.5°, 4.7°, and 3.6° for the Hyalgan®-treated, placebo-treated, and naproxen-treated groups, respectively)

e. *Heel to Buttock Distance Measurement*: Each treatment group exhibited a small mean increase in this parameter from the screening to baseline evaluations, followed by an irregular decline over the first 5 weeks and a less variable time course from Weeks 9–26. At Week 26 the mean reduction in heel to buttock distance was 2.71 cm less than the baseline evaluation for the Hyalgan®-treated group and the placebo-treated group mean at week 26 was 2.41 cm lower than at the baseline evaluation. These differences were not statistically significant ($p = 0.8$ by ANCOVA, Hyalgan® vs. placebo). The mean for the naproxen-treated groups was 2.69 cm less than at baseline. .

f. *Effusion – Patellar Ballottement*: Each treatment group had a lower proportion of subjects with patellar ballottement at week 26 than at baseline. The percentage of subjects with patellar ballottement in the Hyalgan®-treated group and the placebo-treated group was comparable at baseline and week 26, while the percentage in the naproxen-treated group was lower at these two time periods. These differences were not statistically significant ($p = 0.5$ by ANCOVA, Hyalgan® vs. placebo).

g. *Effusion – Bulge Sign*: The number of subjects exhibiting a decrease in bulge signs at study termination was comparable across all treatments at week 26 (approximately 16%).

h. *Effusion – Synovial Fluid Aspiration*: The numbers of subjects who had fluid aspirated were higher in the Hyalgan® and placebo groups compared to the naproxen group; however, the naproxen knees were not routinely aspirated at each treatment. The proportions of Hyalgan®-treated and placebo-treated subjects with aspirates were not statistically different at baseline (Hyalgan® 48/164 = 29.3%; placebo 60/168 = 35.7%; $p = 0.2$, two-tailed Fisher's Exact Test) or at Week 3 (Hyalgan® 31/148 = 20.9%; placebo 45/160 = 28.1%; $p = 0.2$, two-tailed Fisher's Exact Test). The frequency of subsequent aspiration among subjects presenting with effusion at baseline and, separately, among those without effusion at baseline was assessed. There were no significant differences between the treatment groups with respect to the frequency of subsequent aspirations. There were 33 subjects (18 from the Hyalgan® group and 15

from the placebo group) who reported at least one white cell count greater than 2,000. There did not appear to be any noteworthy differences between Hyalgan®- and placebo-treated subjects with respect to these analyses.

i. *Knee Circumference*: There were no treatment-related changes in this parameter through the course of the study, and all groups were comparable.

j. *Acetaminophen Consumption*: For study completers, at week 26, there was no statistically significant difference in the average number of acetaminophen tablets consumed per day in Hyalgan®-treated vs. placebo-treated subjects but each group consumed more acetaminophen than the naproxen group (Hyalgan®-naproxen: 3.68-3.08, p = 0.1, two-tailed t-test, placebo-naproxen: 3.76-3.08, p = 0.055, two-tailed t-test). Comparisons within each category of pain at week 26 did not yield any statistically significant differences. The naproxen-treated subjects tended to consume approximately one tablet less per day than either the Hyalgan®- or placebo-treated subjects. Additional analyses indicated no significant differences between the Hyalgan®- and placebo-treated subjects within severity groups.

k. *Subject and Masked Evaluator Assessment of Treatment Effectiveness*: A higher percentage of completers in the Hyalgan®-treated group (34%) rated their treatment "very effective" in comparison to the completers in the placebo-treated group (23%). The same held true for the masked observer assessments. In addition, the percentage of "not effective" ratings was lower in the Hyalgan® group (8%) than in the placebo group (20%). However, none of these differences were statistically significant.

5. Additional Analyses Requested by FDA:

   a. *Clinical improvement greater than or equal to a 20 mm decrease in VAS as compared to baseline.* Clinical improvement greater than or equal to a 20 mm decrease in VAS as compared to baseline was assessed at each time point for the following four measures: VAS for pain while walking 50 feet, and the WOMAC A, B, and C scales. The results of these analyses are summarized in Table 3.

   Greater proportions of Hyalgan®-treated subjects than either placebo- or naproxen-treated subjects were "successful" under this definition of success for the VAS measure; and, for Hyalgan® versus placebo, p-values were less than 0.05 for VAS during a 50-foot walk test, the Patient Categorical Assessment, and the WOMAC A regardless of whether or not the analyses were adjusted for severity strata.

   b. *Clinical improvement on five-point categorical scales as being greater than or equal to one point.* Hyalgan®-treated subjects had a higher proportion of subjects who improved as assessed by either patients or masked evaluator using these categorical scale criteria than either placebo- or naproxen-treated subjects. The p-value for the Patient Categorical Assessment at week 26 was less than 0.05 (52.4% vs. 37.4% and 38.1%, respectively, see Table 3).

   c. *Differences between groups in the percentages of patients who meet these criteria.* To avoid the multiple hypothese testing implicit in presentation of these p-values and also to perform a "sustained success" analysis that includes all of the time points simultaneously, the definition of success was extended as follows: Success for each

of the relevant variates across the follow-up period is defined as having attained success by week 5 and having maintained success at all visits through week 26. Using this definition of sustained success over time, higher proportions of Hyalgan® than placebo subjects attained improvement and maintained it to week 26 for all six measures shown in Table 3; for example, 20 mm or greater improvement on the VAS: 56% of the Hyalgan®-treated subjects as compared to 41% of the placebo-treated subjects (p = 0.03, two-tailed Fisher's Exact Test); at least a 1 grade improvement in the patient categorical assessment: 52% of the Hyalgan®-treated subjects as compared to 37% of the placebo-treated subjects (p = 0.030, two-tailed Fisher's Exact Test); and WOMAC A: 39% of the Hyalgan®-treated subjects as compared to 21% of the placebo-treated subjects (p = 0.005, two-tailed Fisher's Exact Test) from week 5 continuously through week 26.

d. *Repeated Measures (longitudinal analyses)* A repeated measures (RM) analysis of patients who completed treatment was performed for the following variables:
- Pain on the 50 foot walk test by VAS;
- WOMAC Sections A,B, and C
- Knee circumference
- Heel to buttock distance
- Range of motion (maximum flexion and maximum extension.

The results of these analyses reaffirm the results presented in the pre-planned analyses. The p-values for the 50-foot walk test, WOMAC A, and WOMAC C are each less than 0.05 (see Table 4).

e. *Breakdown of Response by Baseline Severity.* Subjects with less severe pain have less room to improve. The population in this trial was, therefore, divided at the median (54 mm) of the baseline VAS and it was observed that the mean effect size (adjusted mean difference between Hyalgan® and placebo) in the "less severe" group was 3.67 mm, but was 14.34 mm in the "more severe" half of the population.

f. *Additional ANCOVAs.* The sponsor performed a series of ANCOVAs using a model that included levels for treatment (Hyalgan®, placebo); gender (female, male); center (1–15); severity strata (moderate, marked); effusion at baseline (present, absent); and Kellgren grade (2,3); and the following covariates: age (years); duration of disease (years); baseline VAS for 50-Foot walk (mm); baseline VAS for WOMAC A (pain, mm); baseline VAS for WOMAC B (stiffness, mm); and baseline VAS for WOMAC C (function, mm). Regardless of the variations in the models, the Hyalgan®-treated subjects attained lower model-adjusted pain scores than the placebo-treated subjects and all p-values were less than 0.05. Results of a more limited model that was restricted to particular subpopulations and concentrated on Kellgren Grades demonstrated a statistically significant gender effect among Kellgren Grade 2 subjects. This result is neither contrary to an overall improvement for Hyalgan® over placebo, nor explains the overall effect of Hyalgan®. Furthermore, this finding may be explained in part by the small cell size. Finally, when the full model was reduced to those levels (treatment, center, severity stratum, and Kellgren grade) and covariates (baseline VAS for the 50-foot walk test and for the WOMAC A) that had p-values <0.10, the overall results were quite similar to the original analysis.

g. *Gender Analysis* In the overall analysis both sexes showed a trend towards improvement. Overall analyses were performed including gender as a factor and no statistically significant gender or gender-by-treatment interaction effects were noted.

h. *Differences Between Groups in the Percentages of Subjects who Reported Pain.*. A higher percentage of Hyalgan®-treated than placebo-treated subjects reported no or slight pain at week 26.

- *Catagorical Assesment of Pain - Masked Observer:* The percentage of subjects with no or slight pain in the Hyalgan®-treated group (26%) was statistically significantly greater than the placebo-treated group (13%, p = 0.025, Fisher's Exact Test).

- *Catagorical Assesment of Pain - Subjects:* The percentage of subjects with no or slight pain in the Hyalgan®-treated group (48%) was statistically significantly greater than the corresponding percentage in the placebo-treated group (33%, p = 0.039, Fisher's Exact Test).

The additional analyses are concordant with the principal effectiveness analysis. The absence of significant interactions and the general equivalence of effect sizes across models suggest that the result of this trial is not the consequence of choice of analysis, unbalanced randomization or interactions of distributions of subjects' characteristics. While there may was a statistically significant gender-by-Kellgren grade interaction, larger sample sizes would be needed in order to determine the full extent of this effect.

H. SAFETY ANALYSES

1. *Deaths*: One subject, an 80-year-old Caucasian woman from the Hyalgan®-treated group died of a myocardial infarction after completing 53 days of the study. The death was considered not to be treatment related, but "probably" related to concomitant illness.

2. *Discontinuations*: As noted above, 162 subjects did not complete the study. The distribution of discontinuers and the reasons for discontinuation are shown in Table 2. The only statistically significant difference among treatment groups with respect to reasons for discontinuation was the higher number of discontinuations resulting from gastrointestinal complaints in the naproxen group (p = 0.0001, Fisher's Exact Test). The number of subjects who terminated because of injection site pain was higher in the Hyalgan®-treated group, compared to the other groups, but the difference was not statistically significant (p = 0.06, Fisher's Exact Test).

3. *Adverse Events*: For the 495 subjects entered in to the study, there was a total of 2,201 adverse event reports, of which 1,114 were repeated reports of the same event in the same subject and 1,087 were unduplicated reports. The frequency, severity, and relation to treatment of adverse events recorded in each treatment group are summarized in Table 5.

   The frequency of adverse events reported across treatment groups was comparable. The adverse event maximal severity distribution observed in the Hyalgan®- treated and placebo-treated groups was comparable, while fewer naproxen-treated subjects reported "severe" adverse events, although the difference was not statistically significant.

The distribution of the seven most frequently-reported adverse events, which comprise 75% of all adverse events, is summarized in Table 1. These events were gastrointestinal complaints, injection site pain, headache, local joint pain and knee swelling/effusion, rash, pruritus, and ecchymosis. Hyalgan®-treated subjects had the highest incidence of pain at injection site; the difference in incidence of this adverse event between the Hyalgan® group and either the naproxen or the placebo groups was statistically significant ($p = 0.0003$ and $p = 0.02$, respectively, two-tailed Fisher's Exact Test). Gastrointestinal adverse events were recorded more frequently for the subjects in the naproxen-treated group ($p = 0.02$, naproxen vs. Hyalgan®, two-tailed Fisher's Exact Test, Table 1). There were no statistically significant differences between groups with respect to the incidence of headache, local joint pain and knee swelling/effusion, rash, pruritus, and ecchymosis.

a. *Knee Swelling:* There were four reports of severe knee swelling and/or effusion, one each in placebo-treated and naproxen-treated subjects and two in Hyalgan®-treated subjects. One Hyalgan®-treated subject was reported to experience "severe pain at injection" and "severe" "effusion left knee (study knee)" on the date of the first injection. This patient was discontinued from the study. The other Hyalgan®-treated subject reported "severe pain in left knee (study knee)" at week 26. This patient was treated with "study Tylenol and rest" and completed the study.

b. *Infection:* Positive bacterial cultures were obtained from synovial fluid aspirated from the treated knee in 2/164 (1%) Hyalgan®-treated subjects and 3/168 (2%) placebo-treated subjects. The two Hyalgan® treated subjects and two of the placebo-treated subjects did not exhibit evidence of infection clinically or subsequently and were not treated with antibiotics. One of the placebo-treated subjects was hospitalized and received presumptive treatment for septic arthritis.

c. *Severe Events*: As indicated in Table 5, a total of 31 adverse event reports were classified as "severe". The distribution of maximal severities indicates nearly identical distributions for Hyalgan®-treated and placebo-treated subjects while fewer naproxen-treated subjects had a maximal severity of "severe." These proportions were not statistically significantly different.

   Medical review of the adverse event reports submitted by the injecting physicians did not indicate any evidence of anaphylactic or anaphylactoid reactions in this trial.

5. *Laboratory Findings*: A number of laboratory parameters exhibited statistically significant variations in each treatment group between the baseline, Week 9, and Week 26 evaluations. None were considered to be clinically significant.

In summary, the total frequencies and reported severities of adverse events were comparable across treatment groups. The type of adverse events differed across groups: more gastrointestinal events were recorded in the naproxen-treated group, whereas more injection-related events occurred in the placebo- and Hyalgan®-treated subjects.

**TABLE 1**
**DISTRIBUTION OF SELECTED ADVERSE EVENTS**

| | TREATMENT | | | |
|---|---|---|---|---|
| ADVERSE EVENT | HYALGAN® NUMBER(%)* | PLACEBO NUMBER(%)* | NAPROXEN NUMBER(%)* | TOTAL NUMBER(%)* |
| Gastrointestinal complaints | 48(29) | 59(36) | 68(41)[2] | 152(31) |
| Pain at injection site | 38(23)[1] | 22(13) | 14(9) | 74(15) |
| Headache | 30(18) | 29(17) | 17(10) | 76(15) |
| Local joint pain and swelling | 21(13) | 22(13) | 10(6) | 53(10) |
| Rash (local) | 12(7) | 16(4) | 13(8) | 41(8) |
| Pruritus (local) | 12(7) | 7(4) | 7(4) | 26(5) |
| Ecchymosis (local) | 11(7) | 10(6) | 16(10) | 37(8) |

* Number(%) = number of subjects and percentage of treatment group

1 p = 0.02, Hyalgan® vs. placebo, p = 0.0003, Hyalgan® vs. naproxen, Two-tailed Fisher's Exact test; p <0.0009, Three-way Fisher's Exact test
2 p = 0.02, naproxen vs. Hyalgan®, p = 0.3 Hyalgan® vs. placebo, Two-tailed Fisher's Exact test; p = 0.3; p = 0.06, Three-way Fisher's Exact test

**TABLE 2**
**SUBJECTS DISCONTINUED AND REASONS FOR DISCONTINUATION**

| | HYALGAN® | PLACEBO | NAPROXEN | TOTAL |
|---|---|---|---|---|
| Death | 1 | 0 | 0 | 1 |
| Gastrointestinal Adverse Events | 4 | 4 | 14* | 22 |
| Injection Site Pain | 6** | 1 | 1 | 8 |
| Lost to Follow-up | 7 | 8 | 3 | 18 |
| Lack of Efficacy | 17 | 16 | 10 | 43 |
| Non-compliant/ Protocol Violator | 3 | 5 | 1 | 9 |
| Other Medical Problems | 12 | 11 | 12 | 35 |
| Other Musculo-skeletal Pain | 9 | 8 | 9 | 26 |
| Total Discontinuations | 59 | 53 | 50 | 162 |
| Number of Completers | 105 | 115 | 113 | 333 |

* $p<0.0001$; Fisher's Exact Test, 3-way comparison
** $p = 0.063$, Fisher's Exact Test, 3-way comparison

**TABLE 3**
**Individual Criteria for Success (Number and Percent of Success [1] in Each Category)**

| | Hyalgan | | Placebo | | Naproxen | | Hyalgan® vs. Placebo p-values | Hyalgan® vs. Naproxen p-values |
|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | Fisher exact / Adjusted by stratum | Fisher exact / Adjusted by stratum |
| VAS for Pain at 50 foot Walk (>=20 mm improvement) | 59 | 56.2 | 47 | 40.9 | 51 | 45.1 | 0.03<br>0.03 | 0.1<br>0.1 |
| Patient Categorical Assessment (at least 1 grade) | 55 | 52.4 | 43 | 37.4 | 43 | 38.1 | 0.03<br>0.03 | 0.04<br>0.03 |
| MO Categorical Assessment (at least 1 grade) | 52 | 49.5 | 46 | 40.0 | 49 | 43.4 | 0.2<br>0.2 | 0.4<br>0.4 |
| WOMAC A Pain (>=20 mm improvement) | 41 | 39.1 | 24 | 20.9 | 33 | 29.2 | 0.005<br>0.004 | 0.2<br>0.1 |
| WOMAC B Stiffness (>=20 mm improvement) | 34 | 32.4 | 30 | 26.1 | 39 | 34.5 | 0.4<br>0.3 | 0.8<br>0.8 |
| WOMAC C Function (>=20 mm improvement) | 29 | 27.6 | 22 | 19.1 | 29 | 25.7 | 0.2<br>0.2 | 0.8<br>0.7 |

[1] Definition of success: an improvement of at least 20 mm for continuous variables in the VAS or at least 1 grade for the categorical variables, observed at Week 5 and maintained until Week 26.
Legend: MO = Masked Observer.

**TABLE 4**
**Summary of Repeated Measures Analyses by SAS PROC Mixed (1)**
**Principal Efficacy Measures, Overall Effects, and Interactions**
**Completed Subjects, Hyalgan® vs. Placebo Comparisons**

| Effectiveness Measures | Hyalgan® Mean (SE) | Placebo Mean (SE) | H - PL Mean (SE) p-value | Time by Treatment Interaction p-value |
|---|---|---|---|---|
| VAS for Pain on 50 ft Walk Test (mm) | 23.1 (1.9) | 29 (1.8) | -5.9 (2.3) p=0.01 | p=0.5 |
| WOMAC A (mm) | 29.88 (1.6) | 34.6 (1.6) | -4.7 (2) p=0.02 | p=0.3 |
| WOMAC B (mm) | 37.6 (1.8) | 41.1 (1.8) | -3.5 (2.3) p=0.1 | p=0.1 |
| WOMAC C (mm) | 35.6 (1.5) | 39.6 (1.5) | -4.0 (1.9) p=0.03 | p=0.2 |
| Knee Circumference (2) (cm) | 42.2 (0.1) | 42.1 (0.1) | 0.04 (0.2) p=0.8 | p=0.9 |
| Heel to Buttock Distance (2) (cm) | 33.2 (0.5) | 33.4 (0.5) | -0.2 (0.7) p=0.8 | p>0.9 |
| Range of Motion: Flexion (degrees) | 113.1 (0.7) | 113.4 (0.7) | -0.2 (0.9) p=0.8 | p=0.8 |
| Range of Motion: Extension (degrees) | 3.7 (0.2) | 3.8 (0.2) | -0.1 (0.3) p=0.6 | p=0.5 |

(1) Model includes: treatment (2 levels), severity stratum (2 levels), center (15 levels), time (10 levels, from Week 1 to Week 26), and baseline values as covariate; the TYPE = CS was selected as covariance structure. Reported means are those adjusted by the model of analysis.
(2) Based on data updated after an error in the procedure to load data in the SAS data set was found.
Legend: SD = Standard Deviation; SE = Standard Error; H = Hyalgan; PL = Placebo

**TABLE 5**
**SUMMARY OF ADVERSE EVENT REPORTS**

| | TREATMENT | | |
|---|---|---|---|
| | **Hyalgan®** | **Placebo** | **Naproxen** |
| | **Number (%)** | **Number (%)** | **Number (%)** |
| No AE Reports | 49 (29.9) | 49 (29.3) | 45 (27.6) |
| 1-5 AE Reports | 65 (39.6) | 69 (41.1) | 75 (46.0) |
| 6 or more AE Reports | 50 (30.5) | 50 (30.5) | 43 (26.4) |
| **TOTAL** | **164 (100.0)** | **168 (100.0)** | **163 (100.0)** |
| No. with ≥1 Severe AE Reports | 31 (18.9) | 33 (19.6) | 19 (11.7) |

* "Number"(%)" = number of subjects and percentage of treatment group



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## XII. SUMMARY OF OTHER CLINICAL INVESTIGATIONS

An unpublished non-U.S. study was submitted to support the results of the U.S. multicenter clinical trial. This study was a double-blind, placebo-controlled single-center prospective clinical trial conducted by Huskisson[5]. The design of the Huskisson study is comparable to the U.S. multicenter clinical trial. Due to difficulties in data auditing, this study was only reviewed for safety purposes. The adverse events reported during the study were consistent with those reported for the U.S. study and are included in the discussion in section XIII.

## XIII SAFETY ANALYSIS DERIVED FROM OTHER DATA OR INFORMATION DESCRIBED IN PMA

Forty non-U.S. clinical trials with Hyalgan®, involving a total of approximately 6,000 patients have been conducted. Due to differences in study designs and incomplete data these studies were only reviewed for safety purposes. Of these 40 clinical studies, 26 were controlled and 14 were uncontrolled. In the controlled studies, Hyalgan® was compared with either placebo, an active reference treatment (steroids, sulfated mucopolysaccharides, superoxide dismutase), or no treatment. Follow-up periods ranged from 2 months to greater than 12 months.

Adverse events were reported in 244/997 (25%) of the Hyalgan®-treated patients and 81/335 (24%) of the placebo-treated patients in the controlled non-U.S clinical trials and in 291/2314 (7%) of the patients treated with Hyalgan® in the uncontrolled trials.

Local events, such as injection site reaction and injection site pain were the most frequently reported adverse events associated with intra-articular Hyalgan® administration in the controlled non-U.S. trials. In these studies, 107/997 (11%) of the Hyalgan®-treated and 35/335 (11%) of the placebo-treated subjects reported a moderate or severe adverse local event.

One syncopal episode (occurring postprandially) was reported in a Hyalgan® patient, the patient continued the course of treatment uninterrupted.

One episode of shock was reported in a Hyalgan treated patient. The event was moderate in severity and resolved without leading to withdrawal. The investigator deemed it unrelated to the treatment.

Two allergic reactions were reported. One event was described by the physician on the patient report form as, "Allergic reaction? Generalized erythema, joint effusion, heat." The event resolved spontaneously after the patient withdrew from the study. The investigator deemed this event related to treatment. The other event described in the licensee's final report as, "Allergy, temperature, local reaction," which occurred after the first injection of Hyalgan®. This event was judged to be related to the treatment and lead to premature discontinuation by the physician.

One "pseudo-allergic" reaction[6] was reported occurring two hours following injection of Hyalgan®. The reaction consisted of a rise in temperature, local pain, effusion, and redness. The symptoms disappeared two hours after aspiration of the synovial fluid and application of an ice pack. The event was judged to be related to the treatment and lead to early withdrawal from the study.

Hyalgan® has been in clinical use in Europe since 1987. The applicant received 77 spontaneous reports of adverse events in 59 patients (17 in Italy, 24 in Germany, and 18 in Austria) . Analysis of the adverse events that have been reported with the use of Hyalgan® in Europe reveals that most of the events (50/77) are related to local symptoms such as pain, swelling/effusion, and warmth or redness at the injection site. In the two events reported as anaphylactoid reactions, Hyalgan® treatment was discontinued and both had favorable outcomes. Three cases of allergic reactions were reported in which the patients were discontinued from Hyalgan® treatment and the incidents resolved. Seven cases of fever were reported in which three of the cases were reported to be associated with local reactions; pyogenic arthritis was reported to be ruled out in these three cases. All the fever patients were discontinued from Hyalgan® treatment and all incidents resolved. One incident of shock (which was described as a "hypotensive crisis") was reported. The incident resolved and Hyalgan® treatment was continued.

In November of 1995, 18 patients in Austria experiencing adverse events were reported in less than a one month time period. Adverse events reported for the involved patients included: turbid yellow effusion from the treated knee, redness, pain and swelling of the treated knee, and raised temperature and sweating at night. The local licensee chose to recall a batch of Hyalgan® (lot #39300), on the basis of an "alleged" association between the use of this specific lot and the development of the adverse events. The applicant reports that the lot met manufacturing quality specifications both at the time of release and at re-test which was performed on specimens collected from the manufacturer's archives.

## XIV CONCLUSIONS DRAWN FROM THE STUDIES

### A. Conclusion

The preclinical and clinical data provide reasonable assurance of the safety and effectiveness of Hyalgan® for treatment of pain in osteoarthritis of the knee in patients who have failed to respond adequately to conservative nonpharmacologic therapy, and simple analgesics.

## XV. PANEL RECOMMENDATIONS

The Orthopedic and Rehabilitation Devices Panel met to discuss the application on November 21, 1996. The Panel recommended that the application be approved pending submission to and approval by the Center for Devices and Radiological Health (CDRH) of revised labeling which specifies that: (1) any reference to sustained relief of physical impairment will be removed; (2) the effectiveness of Hyalgan® has been established for reduction in pain; (3) Hyalgan® is intended for those patients who have failed conservative therapy, i.e., exercise and simple analgesics; (4) the safety and effectiveness of Hyalgan® has only been established for a single treatment regimen of a series of three to five injections, that relief of pain may last up to six months and may not occur until after the fifth injection; (5) data regarding potential long term effects of repeated treatment cycles of Hyalgan® do not suggest harm, but are incomplete; and (6) that there is a small risk of anaphylactoid events.

The Panel also recommended that if the applicant wishes to extend the labeling to specify that Hyalgan® is indicated for repeat injections, then a study would need be conducted to support both safety and effectiveness for that purpose.

## XVI. CDRH DECISION

CDRH concurred with the Orthopedic and Rehabilitation Devices Panel recommendations with the following exceptions:

1. CDRH believes the safety and effectiveness of Hyalgan® has been established for a single treatment regimen of a series of five injections not three to five injections as suggested by the Panel. The clinical data submitted to support the safety and effectiveness of Hyalgan® (the U.S. multicenter clinical trial and the Huskisson study) are based on a single treatment regimen of five injections. During the Panel Meeting the applicant referenced several non-U.S. clinical trials which were referenced in the application and reported use of a single treatment regimen of three injections. Due to differences in study designs and incomplete data these studies were analyzed for safety purposes only. CDRH believes complete effectiveness data is required to document the effectiveness of a single treatment regimen consisting of three injections.

2. The panel recommended that the labeling be modified to include a statement that data regarding potential long term effects of repeated treatment cycles of Hyalgan® do not suggest harm, but are incomplete. CDRH agrees that the data supporting the long-term effects of repeat treatment cycles is incomplete, and based on this, does not believe sufficient evidence is available to support the safety and effectiveness of repeat treatment cycles at this time. CDRH does not agree that a statement should be included in the labeling specifying that data do not suggest harm with repeat treatment cycles.

FDA inspections completed on December 12, 1996 determined the manufacturing facilities to be in compliance with the Good Manufacturing Practices (GMP) regulations.

CDRH issued an approval order on May 28, 1997.

## XVII. APPROVAL SPECIFICATIONS

Directions for Use: See labeling.

Hazards to Health from use of the device: See indications, contraindications, warnings, precautions, and adverse events in the labeling.

Post-approval Requirements and Restrictions: See Approval Order.

## REFERENCES CITED


1. Abatangelo, G., Botti, P., Del Bue, M., Gei ,G., Samson, J., Cortivo, R., De Galateo, A., and Martelli, M.1989 Intra-articular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. I. Biochemical results. Clin. Orthopaedics Related Res. 241:278-285,.

2. Schiavinato, A., Lini, E., Guidolin, D., Pezzoli, G., Botti, P., Martelli, M., Cortivo, R., de Galateo, A., and Abatangelo, G. 1989. Intraarticular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. II. Morphological Findings. Clin. Orthopaedics Related Res. 241:286-299.

3. Pezzoli, G., Botti, P., and Peri, P. 1983. Topical use of hyaluronic acid in the treatment of equine arthropathy. Proceedings of the 6th National Convention of the Italian Hippology Society, 8-9 June, 1983, Salsomaggiore Terme, Italy, Pp 2-15.

4. Muttini, A. and Busetto, R. 1985. Modifications in the synovial fluid of horse joints indued by the intra-articular administration of sodium hyaluronate. Proceedings of the International Congress of hyaluronic acid in the treatment of equine arthritis/arthrosis. Parma, 19 May, 1985, Pp. 64-73.

5. Huskisson, E.C. 1995. Randomized, placebo-controlled study to compare the efficacy of, and the patient satisfaction with intra-articular HYALGAN® in the treatment of osteoarthritis of the knee. Final report dated 12 June, 1995. (Unpublished)

6. Punzi, L., Schiavon, F., Ramonda, R., Malatesta, V., Gambari, P.F., and Todesco, S. 1988. Intra-articular hyaluronic acid in the treatment of inflammatory and non-inflammatory knee infusions. Curr. Ther. Res. 43:643-647.

# LABELING

## HYALGAN®
## (Sodium Hyaluronate)

### CAUTION

Federal law restricts this device to sale by or on the order of a physician.

### DESCRIPTION

Hyalgan® is a viscous solution consisting of a high molecular weight (500,000–730,000 daltons) fraction of purified natural sodium hyaluronate in buffered physiological sodium chloride, having a pH of 6.8–7.5. The sodium hyaluronate is extracted from rooster combs. Hyaluronic acid is a natural complex sugar of the glycosaminoglycan family and is a long-chain polymer containing repeating disaccharide units of Na-glucuronate-N-acetylglucosamine.

### INDICATIONS

Hyalgan® is indicated for the treatment of pain in osteoarthritis (OA) of the knee in patients who have failed to respond adequately to conservative nonpharmacologic therapy, and to simple analgesics, *e.g.*, acetaminophen.

### CONTRAINDICATIONS

- Do not administer to patients with known hypersensitivity to hyaluronate preparations.
- Intra-articular injections are contraindicated in cases of past and present infections or skin diseases in the area of the injection site.

### WARNINGS

- Do not concomitantly use disinfectants containing quaternary ammonium salts for skin preparation because hyaluronic acid can precipitate in their presence.
- Anaphylactoid and allergic reactions have been reported with this product. See Adverse Events Section for more detail.

- Transient increases in inflammation in the injected knee following Hyalgan® injection in some patients with inflammatory arthritis such as rheumatoid arthritis or gouty arthritis have been reported.

## PRECAUTIONS

### General

- The effectiveness of a single treatment cycle of less than 5 injections has not been established. Pain relief may not be seen until after the fifth injection.
- The safety and effectiveness of the use of Hyalgan® in joints other than the knee have not been established.
- The safety and effectiveness of use of Hyalgan® concomitantly with other intra-articular injectables has not been established.
- Use caution when injecting Hyalgan® into patients who are allergic to avian proteins, feathers, and egg products.
- Strict aseptic administration technique must be followed.
- STERILE CONTENTS. The vial/syringe is intended for single use. The contents of the vial must be used immediately once the container has been opened. Discard any unused Hyalgan®.
- Do not use Hyalgan® if package is opened or damaged. Store in original packaging (protected from light) below 77°F (25°C). DO NOT FREEZE.
- Remove joint effusion, if present, before injecting Hyalgan®.

### Information for Patients

- Provide patients with a copy of the Patient Labeling prior to use.
- Transient pain and/or swelling of the injected joint may occur after intra-articular injection of Hyalgan®.

- As with any invasive joint procedure, it is recommended that the patient avoid any strenuous activities or prolonged (i.e., more than 1 hour) weight-bearing activities such as jogging or tennis within 48 hours following intra-articular injection.

- The safety and effectiveness of repeat treatment cycles of Hyalgan® have not been established.

**Use in Specific Populations**

- **Pregnancy:** *Teratogenic Effects*- Reproductive toxicity studies, including multigeneration studies, have been performed in rats, and rabbits at doses up to 11 times the anticipated human dose (1.43 mg/kg per treatment cycle) and have revealed no evidence of impaired fertility or harm to the experimental animal fetus due to intra-articular injections of Hyalgan®. Animal reproduction studies are not always predictive of human response. The safety and effectiveness of Hyalgan® have not been established in pregnant women.

- **Nursing Mothers:** It is not known if Hyalgan® is excreted in human milk. The safety and effectiveness of Hyalgan® have not been established in lactating women.

- The safety and effectiveness of Hyalgan® have not been demonstrated in children.

**ADVERSE EVENTS**

Hyalgan® was investigated in a single U.S. clinical investigation in which there were three treatment arms (164 subjects treated with Hyalgan®; 168 with placebo; and 163 with naproxen) (refer to Table 1). Common adverse events reported for the Hyalgan®-treated subjects were: gastrointestinal complaints, injection site pain, knee swelling/effusion, local skin reactions (rash, ecchymosis), pruritus, and headache. Swelling and effusion, local skin reactions (ecchymosis and rash), and headache occurred at equal frequency in the Hyalgan® and placebo-treated groups. Hyalgan®-treated subjects had 48/164 (29%) incidents of gastrointestinal complaints that were not statistically different from the placebo-treated group. A statistically significant difference in the occurrence of pain at the injection site was noted in the Hyalgan®-treated subjects: 38/164 (23%) in comparison to 22/168 (13%) in the placebo-treated patients ($p=0.022$) There were 6/164 (4%) premature discontinuations in Hyalgan®-treated subjects due to injection site pain in comparison to 1/168 (<1%) in the placebo-treated subjects. These differences were not statistically significant.

Two 2/164 (1.2%) Hyalgan®-treated subjects and 3/168 (1.8%) placebo-treated subjects were reported to have positive bacterial cultures of effusion aspirated from the treated knee. The two Hyalgan®-treated subjects and two of the placebo-treated subjects did not exhibit evidence of infection clinically or subsequently and were not treated with antibiotics. One of the placebo-treated subjects was hospitalized and received presumptive treatment for septic arthritis.

Hyalgan® has been in clinical use in Europe since 1987. Analysis of the adverse events that have been reported with the use of Hyalgan® in Europe reveals that most of the events are related to local symptoms such as pain, swelling/effusion, and warmth or redness at the injection site. In the two events reported as anaphylactoid reactions, Hyalgan® treatment was discontinued and both had favorable outcomes. Three cases of allergic reactions were reported in which the patients were discontinued from Hyalgan® treatment and the incidents resolved. Seven cases of fever were reported in which three of the cases were reported to be associated with local reactions; pyogenic arthritis was reported to be ruled out in these three cases. All the fever patients were discontinued from Hyalgan® treatment and all incidents resolved. One incident of shock (which was described as a "hypotensive crisis") was reported. The incident resolved and Hyalgan® treatment was continued.

## CLINICAL STUDY

The use of Hyalgan® as a treatment for pain in OA of the knee was investigated in a multicenter clinical trial conducted in the United States.

### Study Design

This study was a double-masked, placebo and naproxen-controlled, multi-center prospective clinical trial with three treatment arms, as summarized in Table 2. A total of 495 subjects with moderate to severe pain were randomized (at baseline evaluation) into three treatment groups in a ratio of 1:1:1 Hyalgan®, placebo, or naproxen.

**TABLE 2**
**Study Design**

| Routes of Administration | Hyalgan® | Placebo | Naproxen |
|---|---|---|---|
| s.c. | Lidocaine (1%) | Lidocaine (1%) | Lidocaine (1%) |
| i.a.* | Hyalgan® (20 mg/2 mL) | Phosphate-Buffered Saline (2 mL) | none |
| p.o./b.i.d. | Placebo for naproxen capsules | Placebo for naproxen capsules | Naproxen capsules (500 mg) |
| p.o./p.r.n. (not to exceed 4 grams/day) | Acetaminophen | Acetaminophen | Acetaminophen |

Legend: s.c. = subcutaneous; i.a. = intra-articular; p.o. = by mouth; b.i.d. = twice a day; p.r.n. = as needed
* Synovial fluid was aspirated (when present) in the Hyalgan® and placebo groups.

## Patient Population and Demographics

The demographics of trial participants were comparable across treatment groups with regard to age, sex, race, height, weight, history of osteoarthritis, prior use of NSAIDs, prior physical therapy, use of assistive devices (refer to Table 3).

## Evaluation Schedule

After meeting initial screening requirements NSAID therapy was discontinued. After two weeks, all subjects returned for baseline evaluations. The baseline evaluation included assessment of three primary effectiveness criteria; measurement of pain during a 50 foot walk test using a 100 mm Visual Analog Scale (VAS), a categorical assessment (0 = none to 5 = disabled) of pain, as assessed by a masked evaluator, during the 48 hours preceding the visit, and a categorical assessment (0 = none to 5 = disabled) of pain, as assessed by the subject, during the 48 hours preceding the visit.

All subjects who completed the NSAID washout period and met all entry requirements received their first injection after randomization. All subjects received subcutaneous lidocaine injections.

Intra-articular injections (Hyalgan®, placebo) were administered weekly for a total of 5 injections (Weeks 0–4). The Naproxen group received 500 mg of Naproxen to be taken b.i.d. for 26 weeks.

Subsequent visits and evaluations took place at Weeks 5, 9, 12, 16, 21, and 26. Effectiveness criteria were assessed and recorded at these time periods.

**Clinical Results**

For this trial, overall success for effectiveness was defined as meeting all four of the success criteria listed in Table 4 using scores from week 26. The criteria were met (refer to Tables 4 through 8.)

**Additional Analyses**

a. An analysis of study completers was performed as following: Success was defined as 1) achieving a 20 mm decrease in the VAS for the 50-foot walk test by Week 5, and 2) maintaining this improvement through Week 26. In this analysis greater proportions of Hyalgan®-treated subjects 59/105 (56% ) than either placebo 47/115 (41%) or naproxen-treated subjects 51/113 (45%) were successful under this definition. The Hyalgan®-placebo comparison was statistically significant (p = 0.031, Fisher's Exact Test).

b. *Categorical Assessment of Pain – Subjects:* A longitudinal analysis of categorical assessment by the subject, which analyzed the percentage of subjects who attained success revealed that a significantly higher percentage of Hyalgan®-treated subjects as compared to the placebo-treated subjects 55/105 (52%) vs. 43/115 (37%), p = 0.030, Fisher's Exact Test) achieved success (an improvement of greater than or equal to one point on the five-point scale) and maintenaned this success from week 5 until week 26.

**Safety**

In order for the product to be considered safe, the incidence of severe swelling and pain consequent to intra-articular injection should be less than 5%. This criteria was met as indicated in Table 1. See the Adverse Events Section.

## DETAILED DEVICE DESCRIPTION

Each vial or syringe contains:

| | |
|---|---|
| Sodium Hyaluronate | 20.0 mg |
| Sodium chloride | 17.0 mg |
| Monobasic sodium phosphate · $2H_2O$ | 0.1 mg |
| Dibasic sodium phosphate · $12H_2O$ | 1.2 mg |
| Water for injection | q.s.* to 2.0 mL |

*q.s. = up to

## HOW SUPPLIED

Hyalgan® is supplied as a sterile, non-pyrogenic solution in 2 mL vials or 2 mL pre-filled syringes.

## DIRECTIONS FOR USE

Hyalgan® is administered by intra-articular injection once a week (one week apart), for a total of five injections.

**Precaution:** Do not use Hyalgan® if the package is opened or damaged. Store in the original packaging (protected from light) below 77°F (25°C). DO NOT FREEZE.

**Precaution:** Strict aseptic administration technique must be followed.

**Precaution:** Do not concomitantly use disinfectants containing quaternary ammonium salts for skin preparation because hyaluronic acid can precipitate in their presence.

Inject subcutaneous lidocaine or similar local anesthetic prior to injection of Hyalgan®.

**Precaution:** Remove joint effusion, if present, before injecting Hyalgan®.

Do not use the same syringe for removing joint effusion and for injecting Hyalgan®.

Take care to remove the tip cap of the syringe and needle aseptically.

Inject Hyalgan® into the joint through a 20 gauge needle.

**Precaution:** The vial/syringe is intended for single use. The contents of the vial must be used immediately once the container has been opened. Discard any unused Hyalgan®. Inject the full 2mL in one knee only. If treatment is bilateral a separate vial should be used for each knee.

**MANUFACTURED BY**
FIDIA S.p.A.
Via Ponte della Fabbrica 3/A
35031 Abano Terme, Padua (PD)
Italy

**DISTRIBUTED BY**

Sanofi Pharmaceuticals, Inc.
90 Park Avenue, New York, N.Y. 10016

TABLE 1

Incidence[1] of Adverse Events Occurring in More Than 5% of All Subjects

| Adverse Event | Hyalgan® N=164 | Placebo N=168 |
|---|---|---|
| Gastrointestinal Complaints[2] | 48 (29%) | 59 (36%) |
| Injection site pain[3] | 38 (23%)[4] | 22 (13%) |
| Headache | 30 (18.3%) | 29 (17.3%) |
| Local skin[5] | 23 (14%) | 17 (10%) |
| Local joint pain and swelling[6] | 21 (13%) | 22 (13%) |
| Pruritus (local) | 12 (7.3%) | 7 (4.2%) |

Notes:

[1] Number and % of patients.

[2] Severe in 4 Hyalgan®-treated subjects, and 4 placebo-treated subjects.

[3] Severe in 5 Hyalgan®-treated subjects and 2 placebo-treated subjects

[4] Statistically significant (p=0.02)

[5] Includes ecchymosis and rash.

[6] Severe in 2 Hyalgan®-treated subjects (1.2%), and 1 placebo-treated subject.

**TABLE 3**
**Demographic Characteristics of All Randomized Subjects**

| DEMOGRAPHIC VARIABLE | TREATMENT | | | |
|---|---|---|---|---|
| | **Hyalgan®** **N = 164** | **Placebo** **N = 168** | **Naproxen** **N = 163** | **TOTAL** **N = 495** |
| AGE (years): | | | | |
| Mean | 63.5 | 64.3 | 63.2 | 63.7 |
| SD | 10.1 | 10.0 | 9.2 | 9.8 |
| Range | 41–90 | 44–85 | 40–80 | 40–90 |
| Gender [N (%)]: | | | | |
| Female | 99 (60.3) | 91 (54.1) | 99 (60.7) | 289 (58.4) |
| Male | 65 (39.6) | 77 (45.8) | 64 (39.3) | 206 (41.6) |
| Race [N (%)]: | | | | |
| Caucasian | 137 (83.6) | 135 (80.4) | 133 (81.6) | 405 (81.8) |
| Black | 23 (14.0) | 32 (19.0) | 25 (15.3) | 80 (16.2) |
| Other | 4 (4.24) | 1 (1.0) | 5 (3.1) | 10 (2.0) |
| Height (cm): | | | | |
| Mean | 167.8 | 168.6 | 167.6 | 168.0 |
| SD | 8.8 | 10.7 | 11.9 | 10.5 |
| Range | 145–190 | 142–193 | 102–198 | 102–198 |
| Weight (kg): | | | | |
| Mean | 88.4 | 88.1 | 89.7 | 88.7 |
| SD | 18.0 | 18.2 | 18.4 | 18.2 |
| Range | 46–139 | 49–170 | 45–150 | 45–170 |
| NSAIDs Use (N, %) | 107 (65.2) | 117 (69.6) | 113 (69.3) | 337 (68.1) |
| Use of Assisted Devices (N, %) | 35 (21.3) | 34 (20.2) | 32 (19.6) | 101 (20.4) |
| Physical Therapy (N, %) | 20 (12.2) | 17 (10.1) | 25 (15.3) | 62 (12.5) |

## TABLE 4

## Clinical Results

| Evaluation | Success Criteria | Results |
|---|---|---|
| 100 mm VAS for pain during 50 foot walk. | A statistically significant (alpha = 0.05) reduction on mean VAS for Hyalgan® when compared to placebo at week 26.<br>This difference was also to exceed 1/4 Std. Dev. of mean change from baseline. | At Week 26, the difference between the Hyalgan®-treated group and the placebo group adjusted means was 8.85 mm (p = 0.0043), which is a difference of approximately one-third of a standard deviation (Table 5). |
| Masked Evaluator Categorical Assessment of patient pain (0=none to 5=disabled) during the 48 hours preceding visits. | The number of Hyalgan ® subjects showing improvement at week 26 was to be concordant with the VAS results, however, not required to be independently statistically significant.. | At week 26 the masked evaluators' categorical assessment of pain indicated that the Hyalgan® subjects experienced less pain than the placebo subjects (Table 6). |
| Subjects' Categorical Assessment of pain (0=none to 5=disabled) during the 48 hours preceding visits. | The number of Hyalgan ® subjects showing improvement at week 26 was to be concordant with the VAS results, however, not required to be independently statistically significant. | At week 26 the subjects' categorical assessment of pain indicated that the Hyalgan® subjects experienced less pain than the placebo subjects (Table 7 ). |
| Magnitude of the observed effect for Hyalgan® versus placebo on both the VAS and the categorical pain assessments. | At week 26 the magnitude of the observed effect for Hyalgan® versus placebo on both the VAS and the categorical pain assessments were to be at least 50% of those observed for the naproxen group. | The improvement in pain on the VAS exhibited by the Hyalgan®-treated group relative to the placebo-treated group were at least 50% of the benefits exhibited by the naproxen-treated group relative to the placebo-treated group.<br>The results of the categorical assessments by the masked evaluator and the subject indicated improvement of the Hyalgan®-treated group relative to the placebo-treated group was at least 50% of the benefits exhibited by the naproxen-treated group relative to the placebo-treated group (Table 8). |

TABLE 5
ANCOVA OF 50-FOOT WALK TEST (mm) VAS
BY WEEK FOR ALL COMPLETED SUBJECTS

| | Week | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 9 | 12 | 16 | 21 | 26 |
| Adjusted Means Hyalgan® | 27.23 | 21.54 | 19.29 | 20.04 | 20.26 | 20.83 | 18.44 | 17.88 |
| Placebo | 32.35 | 28.57 | 25.67 | 24.28 | 26.66 | 25.44 | 24.77 | 26.73 |
| Hyalgan® versus Placebo | 5.13 | 7.03 | 6.39 | 4.24 | 6.40 | 4.61 | 6.33 | 8.846 |
| p-value | 0.057 | 0.0106 | 0.0147 | 0.114 | 0.027 | 0.111 | 0.0217 | 0.0043 |

TABLE 6
MASKED OBSERVERS' CATEGORICAL ASSESSMENTS OF PAIN
FOR COMPLETED SUBJECTS IN PRIOR 48 HOURS:
LEVEL OF PAIN BY TREATMENT GROUP
AT BASELINE AND WEEK 26

| | NUMBER (%) OF SUBJECTS IN CATEGORY | | | | | |
|---|---|---|---|---|---|---|
| | Hyalgan® | | Placebo | | Naproxen | |
| | Baseline | Week 26 | Baseline | Week 26 | Baseline | Week 26 |
| None (0) | 0 (0.0) | 27 (25.7) | 0 (0.0) | 15 (13.0) | 0 (0.0) | 17 (15.0) |
| Slight (1) | 1 (1.0) | 23 (21.9) | 0 (0.0) | 27 (23.5) | 0 (0.0) | 32 (28.3) |
| Mild (2) | 2 (1.9) | 24 (22.9) | 2 (1.7) | 29 (25.2) | 2 (1.8) | 27 (23.9) |
| Moderate (3) | 69 (65.7) | 26 (24.8) | 85 (73.9) | 34 (29.6) | 79 (70.5) | 28 (24.8) |
| Marked (4) | 33 (31.4) | 5 (4.8) | 28 (24.3) | 10 (8.7) | 31 (27.7) | 9 (8.0) |
| TOTAL | 105 (100.0) | 105 (100.0) | 115 (100.0) | 115 (100.0) | 112* (100.0) | 113 (100.0) |

*One Naproxen-treated subject was missing a Baseline assessment

TABLE 7
SUBJECTS' CATEGORICAL ASSESSMENTS OF PAIN
IN PRIOR 48 HOURS: LEVEL OF PAIN
BY TREATMENT GROUP AT BASELINE AND WEEK 26

| | NUMBER (%) OF SUBJECTS IN CATEGORY | | | | | |
|---|---|---|---|---|---|---|
| | Hyalgan® | | Placebo | | Naproxen | |
| | Baseline | Week 26 | Baseline | Week 26 | Baseline | Week 26 |
| None (0) | 1 (1.0) | 23 (21.9) | 0 (0.0) | 14 (12.2) | 0 (0.0) | 13 (11.5) |
| Slight (1) | 2 (1.9) | 27 (25.7) | 0 (0.0) | 24 (20.9) | 1 (0.9) | 31 (27.4) |
| Mild (2) | 6 (5.7) | 19 (18.1) | 8 (7.0) | 24 (20.9) | 7 (6.2) | 26 (23.0) |
| Moderate (3) | 62 (59.0) | 26 (24.8) | 78 (67.8) | 40 (34.8) | 72 (63.7) | 31 (27.4) |
| Marked (4) | 34 (32.4) | 10 (9.5) | 29 (25.2) | 13 (11.3) | 33 (29.2) | 12 (10.6) |
| TOTAL | 105 (100.0) | 105 (100.0) | 115 (100.0) | 115 (100.0) | 113 (100.0) | 113 (100.0) |

**TABLE 8**
**Hyalgan® Effect as a Percentage of the Naproxen - Placebo Difference**

| Assessment | Hyalgan® (HYL) | Placebo (PLA) | Naproxen (NAP) | HYL-PLA | NAP-HYL | NAP-PLA | (HYL-PLA) % of (NAP-PLA) |
|---|---|---|---|---|---|---|---|
| VAS for 50' Walk Baseline Adjusted Mean Effect Sizes From ANCOVA | | | | -8.85 mm on a 100 mm VAS | 4.12 mm on a 100 mm VAS | -4.73* mm on a 100 mm VAS | 187% |
| % of Subjects Improved by Masked Evaluators | 78.1 | 69.6 | 73.2 | 8.5 | -4.9 | 3.6 | 236% |
| % of Subjects Improved by Subjects | 73.3 | 62.6 | 67.3 | 10.7 | -6.0 | 4.7 | 228% |

*Imputed as (NAP-HYL)+(HYL-PLA).

Note that Effectiveness Success Criterion D is satisfied since ((HYL-PLA) % of (NAP-PLA)) > 50% for all three of the above pain assessments.

PATIENT INFORMATION

# HYALGAN®

(Sodium Hyaluronate)

## WHAT IS HYALGAN®?

Hyalgan® is a sterile mixture that is made up mostly of a natural, highly purified sodium hyaluronate that comes from rooster combs. Hyaluronate is a natural chemical found in the body and it is present in a particularly high amount in joint tissues and in the fluid that fills the joints. The body's own hyaluronate acts like a lubricant and a shock absorber in the joint, and it is needed for the joint to work properly. In osteoarthritis, there may not be enough hyaluronate, and there may be a change in the quality of the hyaluronate in joint fluid and tissues.

Hyalgan® is available in either 2 mL glass containers or 2 mL pre-filled syringes. Hyalgan® is given in a shot directly into your knee.

## WHAT IS HYALGAN® USED FOR?

Hyalgan® is used to relieve knee pain due to osteoarthritis. It is used for patients who do not get adequate relief from simple painkillers or from exercise and physical therapy.

## WHAT ARE THE BENEFITS OF HYALGAN®?

A study involving 495 patients with knee pain due to osteoarthritis was performed in the United States. This study investigated the safety and effectiveness of Hyalgan®. The patients were placed in one of three groups. One group was given an injection of Hyalgan® into one knee joint once a week for 5 weeks. The second group was given an injection of saltwater into one knee joint once a week for 5 weeks. The third group received two naproxen tablets every day for 6 months. Joint pain was measured in all patients throughout the 6 months. Patients with osteoarthritic knee joint pain, who did not get relief from simple painkillers or from exercise and physical therapy, got pain relief from the Hyalgan® injections into the knee joint. Pain relief was not seen in some patients until after the fifth injection.

## WHAT OTHER TREATMENTS ARE AVAILABLE FOR OSTEOARTHRITIS?

If you have osteoarthritis, there are several things you can do that do not involve Hyalgan® injections. These include the following:

Non-drug treatments

- avoiding activities that cause excess pain in your joints
- exercise
- physical therapy

*Drug therapy*

- painkillers such as acetaminophen and narcotics
- drugs that reduce inflammation such as aspirin, and other nonsteroidal anti-inflammatory agents (NSAIDs) such as ibuprofen and naproxen
- corticosteroids that are injected directly into the joint

## ARE THERE ANY REASONS WHY I SHOULD NOT TAKE HYALGAN®?

- You should not take this product if you have had any previous allergic reaction to Hyalgan® or similar material, i.e., hyaluronate products.
- You should not have an injection into the knee if you have infections or skin diseases around the injection site.

## THINGS YOU SHOULD KNOW ABOUT HYALGAN®

- Hyalgan® is only for injection into the knee, performed by a qualified physician.
- Consult your physician if you are allergic to products from birds such as feathers, eggs, and poultry.
- Immediately after you have the injection and for the next 48 hours, you may need to avoid activities such as jogging, tennis, heavy lifting, or standing on your feet for a long time.
- The safety and effectiveness of repeat treatment cycles of Hyalgan® have not been established.
- Although results of studies in rats and rabbits with Hyalgan® did not suggest that it could affect your ability to have children or cause harm to your child if you are pregnant or nursing, Hyalgan® has not been tested in pregnant women, or women who are nursing. You should tell your doctor if you think you are pregnant, or if you are nursing a child.
- The safety and effectiveness of Hyalgan® has not been shown in children.

## POSSIBLE COMPLICATIONS

- Hyalgan® has been used in a number of countries in Europe, South America, and Asia since 1987. During this time, two cases of serious, allergic-type events were reported right after the patients received the injection. For example, one patient experienced sweating, paleness, and a feeling of pressure in the chest and stomach. The patient's skin turned slightly blue, and blood pressure dropped. You should tell your doctor before you are given Hyalgan® if something like this has ever happened to you after receiving an injection of Hyalgan® or a similar material, i.e., hyaluronate products.
- Six cases of allergic reactions were reported in people outside of the United States after they had been given Hyalgan®. These people reported that their pulse became quicker, they had a heavy feeling, there were changes in their blood pressure and circulation, and they shivered, had a fever, and sweated. You should tell your doctor before you are given Hyalgan® if something like this has ever happened to you after receiving an injection of Hyalgan® or a similar material, i.e., hyaluronate products.
- Seven cases of fever were reported.
- One case of abnormally low blood pressure was reported.
- One episode of fainting occurred.
- Side effects sometimes seen when Hyalgan® is injected into the knee are pain, swelling, heat, and/or redness of the joint as well as rash, itching, or bruising where Hyalgan® is injected. These reactions were generally mild and did not last long.
- If any of the above symptoms or signs appear after you are given Hyalgan®, or if you have any other problems, you should call your doctor.

## HOW IS HYALGAN® GIVEN?

Your doctor will give you an injection of Hyalgan® (20 mg/2 mL) into your knee once a week, for a total of five injections.


**MANUFACTURED BY**
FIDIA S.p.A.
Via Ponte della Fabbrica 3/A
35031 Abano Terme, Padua (PD), Italy
**DISTRIBUTED BY**
Sanofi Pharmaceuticals, Inc.
90 Park Avenue, New York, NY 10016

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