Study Protocol Title:
A Prospective Pilot Study to Evaluate Efficacy and Safety of Euflexxa for the Treatment of Osteoarthritis

Principal Investigator: Carlos Higuera, MD **Phone**: 216-636-1136

Funding Sponsor:
Ferring Pharmaceuticals, Parsippany, NJ

Project Contact: Peter Shaw
Senior Director, Medical Affairs – Orthopaedics
Ferring Pharmaceuticals Inc.
100 Interpace Parkway, Parsippany, NJ 07054
Phone: 862-286-5188 Cell: 908-323-3663
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Regulatory Sponsor:
This study is an Investigator Initiated research trial. Each study site will be considered its own regulatory sponsor and is responsible for internal data monitoring and any study reporting required by ClinicalTrials.gov.

Introduction

Knee osteoarthritis is one of the most common joint disorders which affects a large portion of aging population[1]. One of the treatment options is viscosupplementation, which is intra-articular injection of hyaluronic acid IA-HA. Multiple studies have been conducted on the safety and efficacy of IA-HA, but none of the studies, to our knowledge, have evaluated the synovial fluid markers. Therefore, it becomes essential to investigate the effect of IA-HA on the concentration of various synovial fluid markers.

Background and Significance

The FLEXX trial[2] was the first well-controlled, randomized, double-blind, multicenter study evaluating the efficacy of Euflexxa (1% sodium hyaluronate) therapy for knee osteoarthritis. Five-hundred-and-eighty patients were randomized to receive either Euflexxa or normal saline. There were significant improvements in the Visual analogue scale score Osteoarthritis Research Society International responder index, HRQoL, and function at 26-week follow-up[2]. However, the FLEXX trial did not evaluate changes in synovial fluid bio-marker levels.
The hypothesis of this study is that Euflexxa therapy for knee osteoarthritis will improve levels of inflammatory and degenerative synovial fluid bio-markers in patients with knee osteoarthritis. In addition, we also hypothesize that Euflexxa therapy will improve pain, clinical outcomes, and time to total knee arthroplasty.

Study Design

Methods

- A prospective pilot study

Sample

- 25 subjects for a pilot study

Inclusion criteria:

1) OA of the knee by American College of Rheumatology criteria
2) Those who failed non-pharmacological measures or simple analgesics
3) moderate to severe pain score of 41 to 90 mm recorded on 100-mm visual analog scale (VAS) immediately following a 50-foot walk
4) bilateral standing anterior-posterior radiograph demonstrating Kellgren and Lawrence grade 2 or 3 OA of the target knee
5) ability and willingness to use only acetaminophen as the analgesic (rescue) study medication
6) unassisted walking 50 feet on a flat surface and going up and down stairs
7) willingness and ability to complete efficacy and safety questionnaires

Exclusion criteria:

1. Age less than 18 years
2. any major injury to the target knee within the prior 12 months
3. any surgery to the target knee within the prior 12 months
4. surgery to the contralateral knee or other weight-bearing
5. inflammatory arthropathies
6. gout or pseudogout within the previous 6 months
7. radiographic acute fracture, severe loss of bone density, avascular necrosis, and/or severe bone or joint deformity in the target knee

8. osteonecrosis of either knee
9. fibromyalgia, pes anserine bursitis, lumbar radiculopathy, and/or neurogenic or vascular claudication
10. target knee joint infection or skin disorder/ infection within the previous 6 months
11. symptomatic OA of the hips, spine, or ankle; known hypersensitivity to acetaminophen, IA-BioHA, or phosphate-buffered saline solution
12. Women of childbearing potential who are pregnant, nursing, or planning to become pregnant, and those who do not agree to remain on an acceptable method of birth control throughout the study
13. history of immune disorders; vascular insufficiency of lower limbs or peripheral neuropathy
14. current treatment or treatment of cancer within the previous 2 years (excluding basal cell or squamous cell carcinoma of the skin)
15. active liver or renal disease
16. any clinically significant abnormal laboratory value [to be defined in detail at a later point]
17. any intercurrent chronic disease or condition that might interfere with the completion of the study
18. participation in any experimental device study within the prior 6 months or any experimental drug study within the prior month

Research Procedures – Define the data to be collected to answer the hypothesis/research question(s)

Injection:

Twenty five patients will be assigned to receive Euflexxa (n=10) (1% sodium hyaluronate). Three consecutive injections (2 sets) will be performed with 1 weeks' time in-between each injection at the index visit (Index visit, Week 1±3 days and Week 2±3 days) and at six months (6 month, Week 7, and Week 8).

Aspiration (primary outcome)

Knee joint aspiration will be performed prior to the initial injection (at visit 1), and at visit 4 (6 weeks ± 1 week) and visit 6 (6-months ±2 weeks) as outlined in previous studies which measured synovial fluid bio-markers.[3,4] Following aspiration, the synovial fluid will be placed on ice as soon as possible, and transported to the lab. In the lab it will be centrifuged at 1,500 rpm for 10 minutes at a temperature of 4°C. Subsequently, the supernatant fluid will be removed and 1mL of Trizol will be added to the cell pellet (sediment). The pellet will be stored at -20°C and shipped to Rush University Medical Center for future RNA sequencing analyses (cell markers and TLR/Nfk-b Pathway genes). The results of these tests (RNA sequencing) will not be shared with the patients, and will not be documented in the medical record.

The supernatant fluid will be used for biomarker analysis. Remaining fluid will be frozen and stored for future research. The purpose of the aspiration is to measure the following synovial fluid bio-markers[3,4]:

Biomarker	Measurement Method
1. Chondroitin 6-sulfate (C6S)	FACE[1] and sizing
2. Chondroitin 4-sulfate (C4S)	FACE and sizing
3. Keratan sulfate (KS)	FACE and sizing
4. Hyaluronate (HA)	FACE and sizing
5. Matrix metalloproteinase-3 (MMP-3)	ELISA[2]
6. Tissue inhibitor of MMP-1 (TIMP-1)	ELISA
7. Tissue Necrosis Factor-alpha (TNF-α)	ELISA
8. IL-6	ELISA
9. Tumor necrosis factor-stimulated gene 6 (TSG-6)	ELISA
10. Inter alpha inhibitor (IαI)	Western Blot

[1] FACE- Fluorophore-assisted carbohydrate electrophoresis
[2] ELISA – Enzyme-linked immunosorbent assay

Secondary outcome measurements[5]

- The effect of Euflexxa on pain, using the Visual Analougue Scale (VAS)
- The effect of Euflexxa on Clinical outcomes using the following standardized scales and questionnaires:
    1. Western Ontario and McMaster Universities Arthritis Index (WOMAC)
    2. Knee Society Score (KSS)
    3. Veterans Rand-12 item form (VR-12)
    4. UCLA activity score (UCLA)
- Time to total knee arthroplasty, which will be collected by asking questions regarding whether or not the patient received a total knee arthroplasty will be asked at each visit (Knee surgery?)(**Table 1**).

Visual analogue scale (VAS), Western Ontario and McMaster Universities Arthritis Index (WOMAC), Knee Society Score (KSS), Short Form-12 (SF-12), UCLA activity score (UCLA), and questions regarding whether or not the patient received a total knee arthroplasty will be asked at each visit (**Table 1**).

	Index visit /Visit 1	**Visit 2 (Week 1 ± 3 days)**	**Visit 3 (Week 2 ± 3 days)**	**Visit 4 (Week-6±2 weeks) (visit 4)**	**Visit 5 (3-month±2 weeks)**	**Visit 6 (6-month±4 weeks)**	**Visit 7 (One week from visit 6 ± 3 days)**	**Visit 8 (Two weeks from visit 6 ± 3 days)**	**Visit 9 (1-year±2 months)**	**Visit 10 (2-year±2 months)**
Aspiration	X			X		X				
Injection	X	X	X			X	X	X		
VAS	X	X	X	X	X	X			X	X
WOMAC	X	X	X	X	X	X			X	X
KSS	X	X	X	X	X	X			X	X
SF-12	X	X	X	X	X	X			X	X
UCLA	X	X	X	X	X	X			X	X
Knee Surgery?	X	X	X	X	X	X			X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X

The subjects will be paid a stipend of 40 dollars for each visit (visit 1 to visit 10). Thus, a total of 400$ will be paid to the patient if all the visits are completed. Additionally, the subjects will be provided free parking for all the visits.

Data Analysis

Unless otherwise indicated, all testing of statistical significance will be two-sided, and a difference resulting in a p-value of less than or equal to 0.05 will be considered statistically significant. Also, after each analysis, General Linear Models (GLM) will be used to control for possible confounders, including BMI, gender, age and ethnicity.

Adverse Events and Data Monitoring Committee(DMC)

Procedural safety will be documented in this study through patient and surgeon reported adverse events. AEs will be documented for all cases in this study.

An Unanticipated Problem Involving Risks to Participants or Others is any event that (1) is unforeseen, (2) caused harm or placed a person at increased risk of harm, and (3) is related to the research procedures.

An Adverse Event (AE) is any untoward or unfavorable medical occurrence, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptoms, or disease. Adverse events can encompass both physical and psychological harms.

An Internal Adverse Event (AE) is an untoward medical occurrence, which occurs to participants in research conducted by Cleveland Clinic and/or Cleveland Clinic is the IRB of record.

External Adverse Event (AE) is an untoward medical occurrence experienced by subjects enrolled at other institutions for the same study approved at Cleveland Clinic or a different study using the same study drug/device.

A Serious Adverse Event (SAE) is any adverse experience that results in any of the following outcomes:

- death
- a life-threatening experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity

a congenital anomaly/birth defect

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An Unexpected Adverse Event means any AE not previously known or included in the current Investigator's Brochure, consent form or other risk information.

Related/Possibly Related means there must be reasonable evidence to suggest the event was caused by the drug, device or investigational intervention.

1. Internal Serious Adverse Events (events that occur to participants enrolled in research being conducted by Cleveland Clinic or when Cleveland Clinic is the IRB of record) must be promptly reported to the IRB using the IRB AE Report Form within 10 working days from discovery/awareness which meet any of the following criteria as assessed by the PI/Co-I:

a) Serious, Unexpected and Related/Possibly Related.

b) AE's determined to be occurring at a significantly higher frequency or severity than expected.

c) Other Unexpected AE's, regardless of severity, that changes the risk benefit ratio of the study and results in changes to the Research Protocol or Informed Consent process/document.

All Internal SAEs are also reported at continuing review using the AE Summary Log.

2. External Serious Adverse Events (events experienced by subjects enrolled at other institutions for the same study approved at Cleveland Clinic or a different study using the same study device/drug) are reportable to the IRB using the IRB AE Report Form within 10 working days from discovery/awareness when:

a. The External SAE report includes reasonable evidence as assessed by a central monitoring entity [Coordinating or Statistical Center, or a Data Safety Monitoring Board (DSMB) or Data Monitoring

Committee (DMC)] that the event is Serious, Unexpected, and Related/Possibly Related AND places the subjects or others at a greater risk of physical or psychological harm than was previously known or recognized. This will require a change in the protocol and/or consent document.

b. External SAE reports provided by the Sponsor to the investigator indicating the event is Serious, Unexpected and Related/Possibly related but without reasonable evidence or DSMB/DMC determination of greater risk are not reportable to the IRB within the

10 day window. Without Sponsor evidence or assessment the implications of the event cannot be determined by the research team and therefore need not be reviewed. These SAE' shall be placed on the AE Summary log to be submitted at the annual continuing renewal.

3. DEATHS are to be reported to the IRB using the IRB AE Report Form according to the following guidelines:

a) Internal Death

• Related/possibly related whether expected or unexpected– within 5 working days from discovery/awareness

• not related and expected – at time of continuing review

• Not related and unexpected – at time of continuing review except cancer studies

 Cancer: Not related and unexpected within 10 working days from discovery/awareness

b) External Death

Related/possibly related and unexpected – within 5 working days from discovery/awareness not related whether expected or unexpected – at time of continuing review related/possibly related and expected – at time of continuing review

c) ALL Deaths are also reported at time of continuing review using the AE summary log.

4. Non-serious Adverse events (Internal and External) that are both Related/Possibly related and unexpected are reported on the AE Summary Log at time of continuing review to assess trends.

5. An IRB staff (a qualified, licensed practitioner assigned to this function by the IRB chair and IRB Executive Director) reviews Adverse Event Reports to determine whether they represent Unanticipated Problem Involving Risks to Participants or

Others. Events that are assessed, by either the IRB Staff or Investigator, to place subjects or others at a greater risk of harm than was previously known or recognized, or changes the risk/benefit ratio of the study, or requires a change in the protocol and/or consent document are referred to Full Board for review under Policy #70.

Events that do not involve risk to Participants or Others or changes to the informed consent or protocol do not require further review. Investigators are informed of the determination and the IRB file is updated.

6. The AE Summary Log is reviewed by the IRB at the time of continuing review to identify trends in frequency and severity which may impact subject safety.

This study is an Investigator Initiated research trial. Each study site will be considered its own regulatory sponsor and is responsible for internal data monitoring and any study reporting required by ClinicalTrials.gov.

Consent

A research personnel will approach the potential subject during the office visit time once he/she is identified from the screening process. Then the consenting will be conducted if the subject agrees to participate at a private exam room. After the explanation of the study, and questions being answered, the subject and the research personnel will both sign the consent form.

References

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3. Shimizu M, Higuchi H, Takagishi K, Shinozaki T, Kobayashi T. Clinical and biochemical characteristics after intra-articular injection for the treatment of osteoarthritis of the knee: prospective randomized study of sodium hyaluronate and corticosteroid. *Journal of Orthopaedic Science.* Jan 2010;15(1):51-56.
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5. Jauregui JJ, Banerjee S, Cherian JJ, Elmallah RD, Mont MA. Rating Systems to Assess the Outcomes After Total Knee Arthroplasty. *Surgical Technology International.* May 2015;26:289-294.