HiLow - Visco-suppletive joint device

3.2% - 16 mg (H-HA) + 16 mg (L-HA)/1 ml
3.2% - 32 mg (H-HA) + 32 mg (L-HA)/2 ml

for intra-articular use

With the following brand names:
Sinovial HL
Intragel HL
Yaral HL

CLINICAL EVALUATION REPORT
Based on MEDDEV 2.7.1:2016 and MEDDEV 2.12-2:2012
In accordance with Directive 93/42/EEC as amended by 2007/47/EC

IBSA Farmaceutici Italia srl – Via Martiri di Cefalonia 2, 26900 Lodi - Italy

SIGNATURE PAGE

CLINICAL EVALUATION TEAM

ROLE	NAME	DATE	SIGNATURE
Evaluator	Ivano Oliveri	07/02/2017	
Co-Evaluator	Sara Fosforani	07/02/2017	
Co-Evaluator	Chiara Rossi	07/02/2017	

MANUFACTURER APPROVAL

ROLE	NAME	DATE	SIGNATURE
SCIENTIFIC DIRECTOR	G. Zitto	07.02.2017	

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ABBREVIATIONS

HA: Hyaluronic Acid
IA: intra-articular
OA: osteoarthritis
VS: viscosupplementation
LMW: Low Molecular Weight
HMW: High Molecular Weight
SF: Sinovial fluid

1 SUMMARY

Osteoarthritis (OA), the most common musculoskeletal condition, is a long-term chronic disease involving the thinning of cartilage in joints which results in bones rubbing together, creating stiffness, pain, and impaired movement. OA is related with age, but is associated with a variety of both modifiable and non-modifiable risk factors, including obesity, lack of exercise, genetic predisposition, bone density, occupational injury, trauma, and gender.

Osteoarthritis is a major cause of disability in elderly populations around the globe, especially in developed countries. The prevalence of OA is increasing and will continue to do so as the population increases, ages, and is subject to risk factors such as the obesity epidemic. As OA causes pain and impairs functionality of the patient, it places a major burden on individuals, communities, health systems, and social care systems.

The current control strategy mainly consists of palliative pain treatment, as there are several medicines on the market that alleviate pain and improve function in OA patients. In severe cases, joint replacement surgery has been proven effective in relieving the painful and debilitating effects of the disease, though the high cost and use of advanced resources mean these procedures are not available in many countries around the world. There are currently no therapies available that can reverse or halt the progression of osteoarthritis; larger studies are needed to evaluate the clinical and cost effectiveness of the few therapies that have shown promise in animal trials.

Another principal aspect of osteoarthritis care that requires further research is diagnostic techniques. The current methods of clinical diagnosis and X-rays are not precise enough to effectively measure status and progression of the condition, which presents serious difficulties in evaluating both the impact of risk factors and the effectiveness of potential therapies. The lack of valid biomarkers limits pharmaceutical development and clinical monitoring.

A non-surgical approach for the management of osteoarthritis symptoms is the use of lubrication injections. Intra-articular hyaluronic acid injections, also known as viscosupplementation, are widely used by orthopedic surgeons to treat osteoarthritis and, according to several clinical studies, they are effective. The two most common types of knee injection for OA are corticosteroids and hyaluronic acid. HA is a naturally occurring glycosaminoglycan and a component of synovial fluid (SF) and cartilage matrix. The intra-articular injection of HA is thought to restore normal viscoelastic properties of the pathologically altered SF, which explains the term of the approach: “viscosupplementation”. It is also thought that HA temporarily restores the lubricating and shock-absorbing effects of SF. Moreover, several studies suggest that viscosupplements also have effects, such as protection against cartilage erosion, and promotion of intra-articular HA production.

"HiLow - Visco-Suppletive Joint device" is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, *"HiLow - Visco-Suppletive Joint device"* reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action. The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight

Hyaluronic Acid chains (L-HA) contained in this medical device, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity. High and Low Molecular Weight Hyaluronic Acid contained in this device is produced through the biosynthesis of a natural substrate, without further chemical transformations, thus having excellent biocompatibility and allowing the natural re-establishment of the viscoelastic properties of the synovial fluid when injected in the joints.

The clinical studies commented in this Clinical Evaluation report support efficacy and safety of HA-based viscosupplementations such as *"HiLow - Visco-Suppletive Joint device".*

Filardo *et al.* demonstrated that Platelet-Rich Plasma (PRP) does not provide a superior clinical improvement with respect to HA, and therefore it should not be preferred to viscosupplementation as injective treatment of patients affected by knee cartilage degeneration and OA.

Giarratana *et al.* proved that Condrotide was as effective as Hyalubrix in reducing knee OA symptoms but showed an earlier response on pain reduction and can therefore be considered a valid alternative to the use of HA in the treatment of OA, avoiding the adverse events of NSAIDs and of intra-articular corticosteroids.

Zoboli *et al.* concluded that no statistical difference between the single application of 6 ml of sodium hyaluronate and classic application with three weekly injections. However, only the classical regime showed statistically significant improvement in baseline pain (WOMAC pain and VAS).

Jüni *et al.* compared hylan and hyaluronic acid viscosupplementations, obtaining slight differences between the two groups regarding both safety and efficacy.

Petrella *et al.* proved that combining a range of MW hyaluronic acid may be advantageous long term, particularly among active osteoarthritis patients.

Roux *et al.* showed that intra-articular sodium hyaluronate injections into the carpometacarpal joint in OA can be efficacious on pain and functionality against osteoarthritis.

Berenbaum *et al.* concluded that treatment with 3-weekly injections of intermediate MW HA may be superior to low MW HA on knee OA symptoms over 6 months, with a similar safety profile.

Atay *et al.* found no differences between two HA viscosupplementations, one composed of low molecular weight HA and the other one with high molecular weight HA.

Lucas et al. investigated the efficacy of viscosupplementations, finding a significant positive effect in treated patients.

Kon *et al.* found that PRP injections showed more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function.

Diracoglu *et al.* demonstraeted that intra-articular injection of hyaluronan in patients with knee OA led to a short-term increase in proprioception and isokinetic muscle force, and also significant improvements in the functional conditions of patients.

Carpenter *et al.* found that viscosupplementation combined with arthroscopy may be more beneficial than arthroscopy alone.

Conrozier *et al.* concluded that a single 6 mL injection of hylan G-F 20 may be as efficacious, and as well tolerated, as 3 x 2 mL one week apart.

Borras-Verdera *et al.* evaluated safety and efficacy of a single intra-articular injection of 2% HA + mannitol. A significant reduction in joint pain, stiffness and functional disability compared with baseline was observed at every follow-up visit.

Palmieri *et al.* demonstrated that Hyaluronic acid - alone and in combination with sodium clodronate or diclofenac sodium - produced a significant improvement in mean VAS pain score at 3 and 6-month follow-up. In addition, no serious adverse events were observed.

Strand *et al.* demonstrated that a single injection of Gel-200, a new cross-linked hyaluronic acid product, was well tolerated and relieved pain associated with symptomatic OA of the knee over 13 weeks.

Navarro-Sarabia *et al.* compared against placebo the efficacy and safety of repeated injections of hyaluronic acid and its effect on disease progression over 40 months. Significantly more patients responded to hyaluronic acid compared with placebo (p=0.004). The number of responders to HA increased through the study, whereas those to placebo did not change. No safety problems were recorded in this study.

Munteanu *et al.* found no statistically significant differences in foot pain between the groups at 3 months. There were few statistically significant differences in the secondary outcome measures. Overall, the incidence of adverse effects was not significantly different between treatment group and placebo (saline solution) group.

Chevalier *et al.* demonstrated that, in patients with knee osteoarthritis, a single 6 ml intra-articular injection of hylan G-F 20 is safe and effective in providing statistically significant, clinically relevant pain relief over 26 weeks, with a modest difference versus placebo.

Lundsgaard *et al.* found that the effects of hyaluronate 2 mL, physiological saline 20 mL, and physiological saline 2 mL did not differ significantly in reducing knee pain, knee function, or consumption of analgesics. The VAS and KOOS - Osteoarthritis Outcome Score - outcomes all improved significantly over time ($p<0.0005$), regardless of intervention group. No adverse events were reported.

Waddell *et al.* concluded by the authory that the presence of an effusion at onset of viscosupplementation requiring aspiration does not negatively impact efficacy of hylan G-F 20 or increase adverse event rates.

Karalezli *et al.* suggested that HA injection in the carpometacarpal joint is a tolerable procedure, but the patients complained of pain and discomfort during the injections. The pain in group A was much greater than in group B. Viscosupplementation for the treatment of trapeziometacarpal osteoarthritis is a viable treatment option for stages 3 and 4 patients when they do not want to be operated on. It is a tolerable but not a painless procedure especially when it is done without fluoroscopy control.

Di Sante *et al.* concluded that intra-articular PRP had an immediate effect on pain that was not maintained at longer term follow-up when, on the contrary, the effects of intra-articular HA were evident.

Trueba *et al.* compared HA and corticosteorids injections for knee OA. At 12 months, they found a significant reduction of pain and function improvement in the HA group compared to bethamethasone (BM) patients.

De Campos *et al.* found that the addition of triamcinolone to HA viscosupplementations does not improve clinical outcomes in patients with OA.

Vanelli *et al.* proved that the reduction in pain was statistically significant for patients treated either with intra-articular polynucleotides or with hyaluronan. No significant adverse events were reported. The authors concluded that intra-articular polynucleotides may be a valid alternative to traditional hyaluronan supplementation for the treatment of knee OA.

Raman *et al.* concluded that the clinical effectiveness and general patient satisfaction were better amongst patients who received Hylan G-F 20.

Iannitti *et al.* demonstrated that treatment of knee OA with a cross-linked HA and a HA viscosupplementations resulted in a significant improvement vs baseline in all endpoints at 3 and 6 months. Treatment with Variofill resulted in a high percentage improvement in Visual Analogue Scale pain, Western Ontario McMaster universities Osteoarthritis Index score pain and physical activity, when compared to Synvisc viscosupplementation, at 6 months.

Rat *et al.* found that both joint effusion and prior viscosupplementation could be associated with a more modest improvement in QoL.

Di Martino *et al.* found no statistically relevant differences between HA viscosupplementation and placebo in the clinical scores regarding a single injection of HA performed the day after anterior cruciate ligament (ACL) reconstruction.

Panuccio *et al.* demonstrated that the treatment group HA + IA showed a positive trend compared to the group treated with HA only for all the efficacy variables observed, in particular regarding the VAS and the analgesic consumption.

Van Den Bekerom *et al.* reported that there was no significant difference in duration of the effect of the first infiltration between the three groups. The positive effect was still ongoing at the end point of the study in 46 hips: 51% of the patients did not undergo total hip arthroplasty, 3 years after viscosupplementation.

Altogether, the Clinical Evaluation based on literature route of the medical device *"HiLow - Visco-suppletive joint device"* resulted in a positive risk/benefit ratio for the application of the product after assessment of the risks/benefit with regard to the intended purpose. All risks addressed in the risk analysis were considered within an acceptable range or as far as possible by the Risk Management Team.

Moreover, a critical assessment of data collected from the literature demonstrates that *HiLow - Visco-suppletive joint device"* achieves its intended purpose and claims made in relation to safety and performance, in compliance with Annex X of EC-Directive 93/42/EEC and the European guideline: MEDDEV 2.7.1 of June 2016.

2 SCOPE OF THE CLINICAL EVALUATION

Clinical evaluation is a methodologically sound ongoing procedure to collect, appraise and analyse clinical data pertaining to a medical device and to analyse whether there is sufficient clinical evidence to confirm compliance with relevant essential requirements for safety and performance when using the device according to the manufacturer's instructions for use.

Clinical evaluation is an ongoing process conducted throughout the life cycle of a medical device. It is first performed during the conformity assessment process leading to the marketing of a medical device and then repeated periodically as new clinical safety and performance information about the device is obtained during its use. This information is fed into the ongoing risk analysis and may result in changes to the Instructions for Use.

Therefore, this Clinical evaluation is intended:

- To demonstrate that the medical device *"HiLow - Visco-suppletive joint device"* reaches the prefixed scope regarding performance and safety during normal conditions of use, in accordance with Annex X of Medical Device Directive 93/42/EEC as amended by Directive 2007/47/EC, and that any claims made about the devices' performance and safety (e.g. product labelling and instructions for use) are supported by suitable evidence.
- To verify that the known and foreseeable risks, and any adverse events, are minimized and acceptable when weighed against the benefits of the intended performance.
- To review the state of the art of clinical data based on performance and safety criteria of *"HiLow - Visco-suppletive joint device"*.

3 CLINICAL BACKGROUND, CURRENT KNOWLEDGE, STATE OF THE ART

3.1 *IDENTIFICATION OF THE MEDICAL FIELD*

Viscosupplementation

"HiLow - Visco-Suppletive Joint device" is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, *"HiLow - Visco-Suppletive Joint device"* reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action.

3.2 *APPLICABLE STANDARDS AND GUIDANCE DOCUMENTS*

A list of applicable standards and guidance documents is reported below:

- MEDDEV 2.7.1:2016 rev. 4 - Clinical evaluation: a guide for Manufacturers and Notified Bodies under Directive 93/42/EEC and 90/385/EEC;
- EN ISO 14971:2012 - Medical devices – Application of Risk Management to medical devices;
- EN 1041:2008 - Information supplied by the manufacturer with medical devices;
- EN ISO 10993-1:2009 - Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process;
- EN ISO 10993-3:2014 - Biological evaluation of medical devices - Part 3: tests for genotoxicity, carcinogenicity and reproductive toxicity;
- EN ISO 10993-4: 2009- Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood;
- EN ISO 10993-5:2009 - Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity;
- EN ISO 10993-10:2010 - Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization;
- EN ISO 10993-11:2009 – Biological assessment of medical devices –Part 11: Systemic toxicity tests;
- EN ISO 10993-12:2012 - Biological evaluation of medical devices - Part 12: Sample preparation and reference materials;
- OECD Guideline for testing of chemicals 471 21st July 1997. Genetic Toxicology: *Salmonella Typhimurium*, Reversion Mutation Assay;
- EN 10993-6:2009 – Biological evaluation of medical devices - Part 6: Test for local effects after implantation;
- EN ISO 14155:2011 - Clinical investigation of medical devices for human subjects — Good Clinical Practice;
- IEC 62366-1:2015- Medical devices - Application of usability engineering to medical devices.

3.3 *ANATOMICAL BACKGROUND*

A joint, also known as an *articulation* or *articular surface*, is a connection that occurs between bones in the skeletal system. Joints can be structurally and functionally classified.

The structural classification divide joints into fibrous, cartilaginous, and synovial joints depending on the material composing the joint and the presence or absence of a cavity in the joint [1]:

- **Fibrous joints**: the bones of fibrous joints are held together by fibrous connective tissue. There is no cavity, or space, present between the bones, so most fibrous joints do not move at all. There are three types of fibrous joints: *sutures (skull)*, *syndesmoses (e.g. joint of the tibia and fibula in the ankle)*, and *gomphoses (e.g. joint between the teeh and their sockets)* (Figure 1).

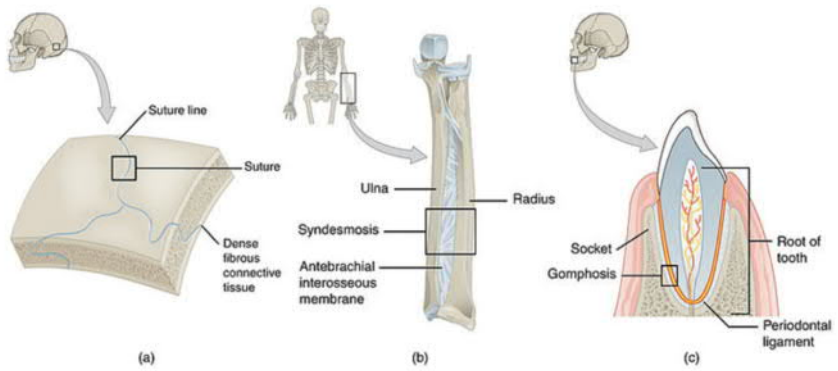


Figure 1. Three types of fibrous joints. a) Sutures b) Syndesmosis c) Gomphosis [1].

- **Cartilaginous joint**s: cartilaginous joints are those in which the bones are connected by cartilage. There are two types of cartilaginous joints: *synchondroses* and *symphyses*. In a synchondrosis, the bones are joined by hyaline cartilage (e*.g. synchondroses are found in the epiphyseal plates of growing bones in children*). In symphyses, hyaline cartilage covers the end of the bone, but the connection between bones occurs through fibrocartilage (*e.g. joints between vertebrae and between the pubic bones*) (Figure 2).

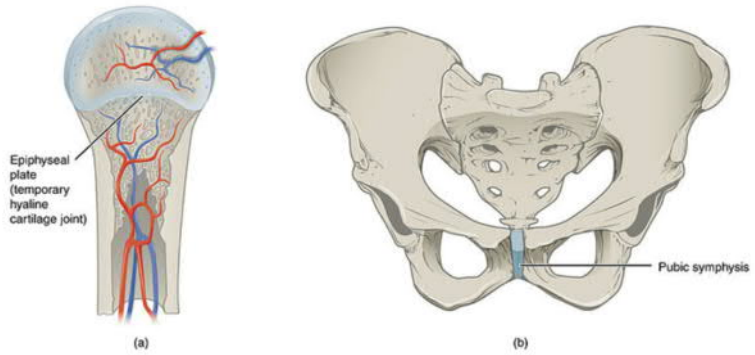


Figure 2. Synchondrosis joint with epiphyseal plate (temporary hyaline cartilage joint) indicated (a) and a symphysis (b) [1].

- **Synovial joints:** these joints not directly joined, and are the only joints that have a space between the adjoining bones**.** This space, referred to as the synovial (or joint) cavity, is filled with synovial fluid. Synovial fluid lubricates the joint, reducing friction between the bones and allowing for greater movement. The ends of the bones are covered with articular cartilage, a hyaline cartilage. The entire joint is surrounded by an articular capsule composed of connective tissue. This allows movement of the joint as well as resistance to dislocation. Articular capsules may also possess ligaments that hold the bones together. Synovial joints are capable of the greatest movement of the three structural joint types; however, the more mobile a joint, the weaker the joint. Knees, elbows, and shoulders are examples of synovial joints (Figure 3).

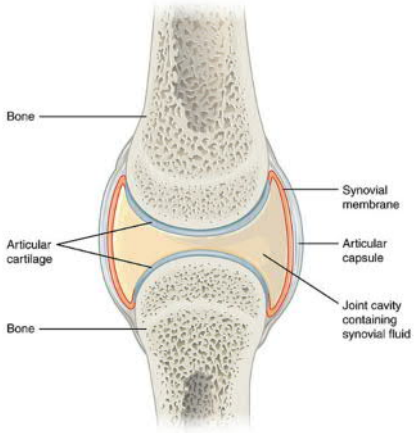


Figure 3. Synovial joint delineates the articular cartilage, articular capsule, bone, synovial membrane and joint cavity containing synovial fluid [1].

Joints can also be classified functionally according to the type and degree of movement they allow **[**2**]**:

- **Synarthroses (immovable articulations):** these include all those articulations in which the surfaces of the bones are in almost direct contact, fastened together by intervening connective tissue or hyaline cartilage, and in which there is no appreciable motion, as in the joints between the bones of the skull, excepting those of the mandible. There are four varieties of synarthrosis: sutura, schindylesis, gomphosis, and synchondrosis. A sutura is a form of articulation where the contiguous margins of the bones are united by a thin layer of fibrous tissue; it is met with only in the skull.
- **Amphiarthroses (slightly movable articulations):** in these articulations the contiguous bony surfaces are either connected by broad flattened disks of fibrocartilage, of a more or less complex structure, as in the articulations between the bodies of the vertebræ; or are united by an interosseous ligament, as in the inferior tibiofibular articulation. The first form is termed a symphysis, the second a syndesmosis.
- **Diarthroses (freely movable articulations):** this class includes the greater number of the joints in the body. In a diarthrodial joint, the contiguous bony surfaces are covered with articular cartilage, and connected by ligaments lined by synovial membrane. The joint may be divided, completely or incompletely, by an articular disk or meniscus, the periphery of which is continuous with the fibrous capsule while its free surfaces are covered by synovial membrane. Since they allow for free movement, synovial joints (e.g. knee or ankle joints) are classified as diarthroses.

Knee joints

The knee joint is one of the strongest and most important joint in the human body. It allows the lower leg to move relative to the thigh while supporting the body's weight. Movements at the knee joint are essential to many activities, including sitting, standing, walking and running [3]. The knee, also known as the tibiofemoral joint, is a synovial hinge joint formed between three bones: the femur, tibia, and patella (Figure 4). Two rounded, convex processes (known as condyles) on the distal end of the femur meet two rounded, concave condyles at the proximal end of the tibia. The patella lies in front of the femur on the anterior surface of the knee with its smooth joint-forming processes on its posterior surface facing the femur [3].

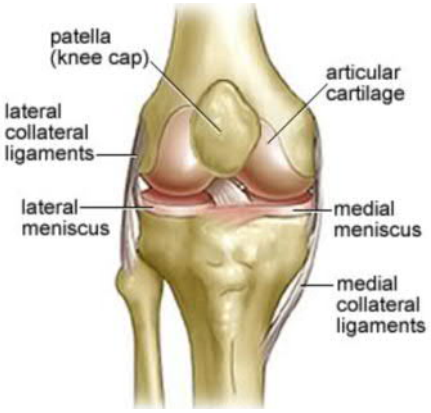


Figure 4. Right knee anatomy [1].

The joint-forming surfaces of each bone are covered in a thin layer of hyaline cartilage that gives them a smooth surface and protects the underlying bone from damages. Between the femur and tibia is a rubbery fibrocartilage known as the meniscus. The meniscus acts as a shock absorber inside the knee to prevent the collision of the leg bones during activities such as running and jumping [3].

Many strong ligaments surround the joint capsule of the knee to reinforce its structure and hold its bones in the proper alignment. In addition to the joint capsule and ligaments that support the knee, there are also several structures surrounding the knee that help and protect the joint from friction and outside forces. Small pockets of synovial fluid, known as bursae, surround the knee to reduce the friction from movement of tendons across the surface of the joint [3].

Hip Joint

The hip joint, or coxofemoral joint, is the articulation of the acetabulum of the pelvis and the head of the femur. These two segments form a diarthrodial ball-and-socket joint. The primary function of the hip joint is to support the weight of the head, arms, and trunk both in static posture and in dynamic postures such as ambulation, running, and stair climbing [4].

The acetabulum is formed by the merging of the ossification centers of ilium, ischium and pubis bones of pelvis. Hyaline cartilage lines both the acetabulum and the head of the femur, providing a smooth surface for the moving bones to glide past each other. Hyaline cartilage also acts as a flexible shock absorber to prevent the collision of the bones during movement. Between the layers of hyaline cartilage, synovial membranes secrete watery synovial fluid to lubricate the joint capsule. Surrounding the hip joint are many tough ligaments that prevent the dislocation of the joint. The strong muscles of the hip region also help to hold the hip joint together and prevent dislocation [5,6].

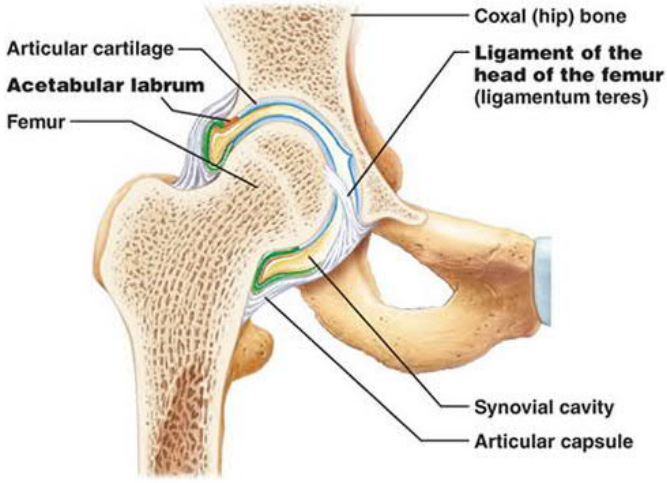


Figure 5. Frontal section of a hip joint [7].

3.3.1 Histological characteristics of the sinovial cavity and its physiology

"HiLow - Visco-Suppletive Joint device" is intended for the treatment for osteoarthritis, and is particularly indicated for pain or reduced mobility due to degenerative diseases (arthrosis), post-traumatic diseases and tendinopathy associated with joint disabilities. *"HiLow - Visco-Suppletive Joint device"* is injected into the synovial cavity.

The sinovial cavity is the space found between bone segments and articular capsule; it is delimited by a fibrous wrap internally covered by a synovial membrane and contains a slight film of synovial fluid. The synovial cavity consists, depending on where it is found, of the **joint cavity**, the **bursae** and the **tendon sheaths** [8] (Figure 6).
The synovial fluid has a variable volume according to the dimension of the articular cavity and it represents, physiologically, a thin veil to protect the cartilage surface; it acts as a lubricant and it has nourishing functions for the cartilage itself. The synovial fluid is filtered from the blood plasma and it contains a maximum of 200 cell/cc. It also contains electrolytes, glucose, enzymes, immunoglobulins and proteins mainly originating from blood, with the addition of mucin - mostly hyaluronic acid - that makes the synovial fluid viscous, elastic and plastic [8].

The articular capsule consists of intertwisted bundles of connective fibrous tissue, whose insertion onto bone occurs as a continuous line. At some points the capsule is strengthened by the intrinsic capsular ligaments, represented by local thickenings (made of fibrous or fibro-elastic tissue) of the capsule itself, where the fiber bundles become parallel. The articular capsule is internally covered by the **synovial membrane**. The synovial membrane is a connective tissue of mesenchymal origin, covering any exposed osseous surface, the synovial bursae in communication with the joint cavity and the intracapsular ligament and tendons; it is

not present on meniscal and discal surfaces and it stops right before the edge of joint cartilage, the peripheral area of which, only a few millimeters thick, constitutes a zone of transition from synovial membrane to cartilage.
In the synovial cavities of some joints, adipose tissue is stored in specific regions, forming mobile and elastic pads that fill in the spaces of the articular cavity. Such adipose stores, when the joint moves, adapt to the changes of shape and volume of the synovial cavity, supporting the lubrication of the joint surfaces.
The synovial membrane is made of a cellular intima lying on a fibrovascular subintimal lamina consisting of abundant loose areolar tissue, collagen and elastic fibers. When the synovial membrane covers the intracapsular tendons or ligaments, the subintima is hardly identifiable as a separate layer, being fused together with the capsule, the ligament or the adjacent tendon [8].

The synovial intima is made of cells, called synoviocytes A and B, whose function is to remove the debris found in the joint cavity and to synthesize some molecules for the synovial fluid. The synoviocytes do not actively proliferate under basal conditions, while the speed of cellular division is considerably increased after trauma and acute hemarthrosis [8].

The bursae are virtual spaces localized in specific regions of the joint where high friction between closely opposing structures occurs. The bursae can be visualized almost solely in pathologic conditions, because they physiologically contain a slight film of synovial fluid. As above, the bursae are covered by the synovial membrane that continues from the synovial membrane of the articular cavity, so that it constitutes communicating bursae where the synovial fluid is freely circulating. The communicating bursae have a further biomechanical function: they decrease the endoarticular pressure when there is a fluid collection in the joint cavity [8].

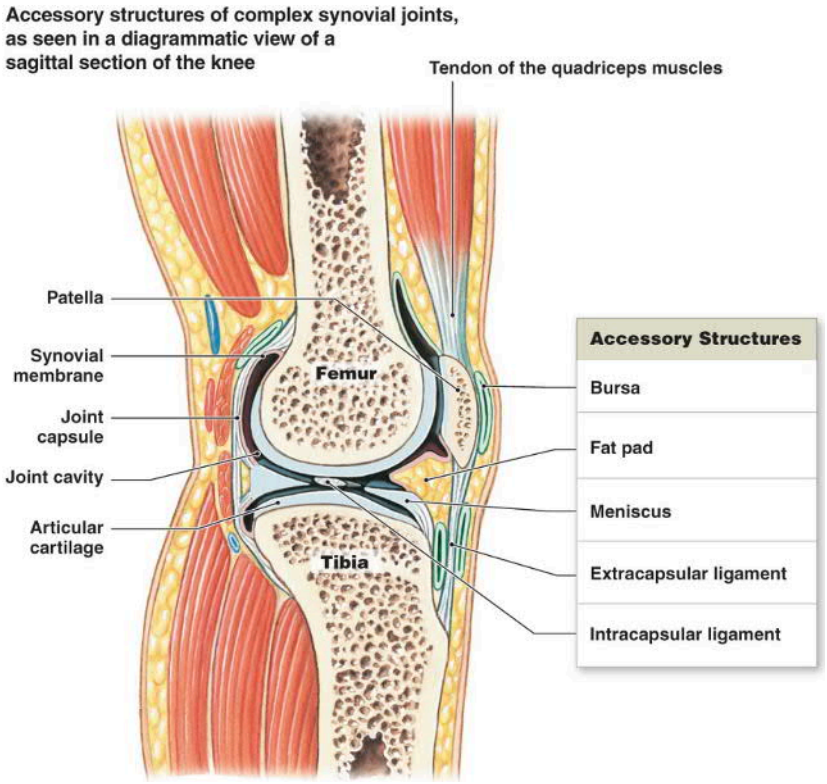


Figure 6. Structure of a synovial joint and joint cavity - the knee [9].

3.4 *GENERAL DESCRIPTION OF INTERESTED MEDICAL CONDITION*

"HiLow - Visco-suppletive joint device" is intended for the treatment for osteoarthritis, and is particularly indicated for pain or reduced mobility due to degenerative diseases (arthrosis), post-traumatic diseases and tendinopathy associated with joint disabilities. Tendinopathy is a broad term encompassing painful conditions occurring in and around tendons in response to overuse [10].

Among degenerative/traumatic diseases, osteoarthritis (OA) is one of the most common conditions. OA refers to a clinical syndrome of joint pain accompanied by varying degrees of functional limitation and reduced quality of life. It is the most common form of arthritis, and one of the leading causes of pain and disability worldwide. The most commonly affected peripheral joints are the knees and the hips, but also small hand joints [11].

OA is characterized by multiple risk factors, which can be genetic factors (heritability estimates for hand, knee and hip osteoarthritis are high at 40–60%), constitutional factors (for example, ageing, female sex, obesity, high bone density) and biomechanical risk factors (for example, joint injury, occupational/recreational usage, reduced muscle strength, joint laxity, joint malalignment*)* [12,13,14].

OA is characterised pathologically by localised loss of cartilage, remodelling of adjacent bone and associated inflammation. OA includes a slow but efficient repair process that often compensates for the initial trauma, resulting in a structurally altered but symptom-free joint. In some people, because of either overwhelming trauma or compromised repair, the process cannot compensate, resulting in eventual presentation with symptomatic osteoarthritis; this might be thought of as 'joint failure'. This in part explains the extreme variability in clinical presentation and outcome that can be observed between people, and also at different joints in the same person [15,16]. The main signs and symptoms of OA are pain, stiffness and loss of movement and function. As no cure exists for osteoarthritis, current treatments are mainly aimed at reducing pain and improving joint function [18].

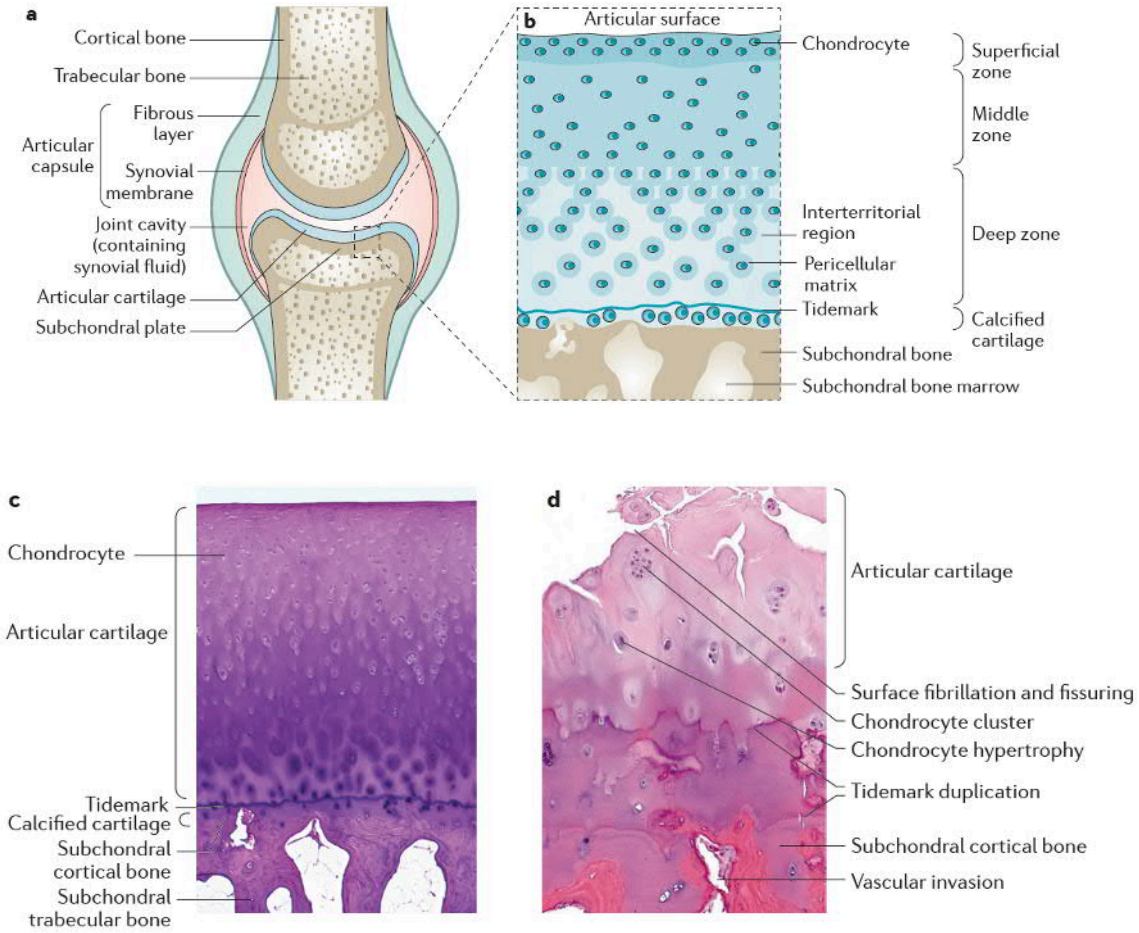


Figure 7. a) Diarthrodial joints join two adjacent bones nthat are covered by specialized articular cartilage and are encased in a connective tissue capsule lined by a synovial membrane, consisting of a thin cell layer of macrophages and fibroblasts. b,c) Cross-section of the articular surface of a diathrodial joint illustrating schematically (part b) and histologically (part c) the main structural elements, including the articular cartilage (with chondrocytes), tidemark (separating the calcified and articular cartilage), calcified cartilage, and subchondral cortical and trabecular bone. d) Histopathological cross-section of the articular surface showing advanced osteoarthritic changes characterized by fissuring and fragmentation of the articular cartilage, chondrocyte proliferation and hypertrophy, duplication and advancement of the tidemark, expansion of the zone of calcified cartilage, thickening of the subchondral cortical plate and vascular invasion of the bone and calcified cartilage [19].

3.5 *PREVALENCE OF OSTEOARTHRITIS*

The incidence and prevalence of OA are difficult to determine because clinical syndrome of osteoarthritis (joint pain and stiffness) does not always correspond with the structural changes of osteoarthritis (usually defined as abnormal changes in the appearance of joints identified by radiographs) [18].

This area is becoming more complex with sensitive imaging techniques such as magnetic resonance imaging, which demonstrate more frequent structural abnormalities than detected

by radiographs [18].

OA at individual joint sites (notably knee, hip and hand) demonstrates consistent age-related increases in prevalence. However symptomatic osteoarthritis is not an inevitable consequence of ageing [20]. Although prevalence of osteoarthritis rises in frequency with age, it does affect substantial numbers of people of working age. The number of people with osteoarthritis in the UK is increasing as the population ages, and as the prevalence of risk factors such as obesity and poor levels of physical fitness also continues to rise [18].

3.6 *RISK FACTORS*

The main risk factors for OA are advancing age, genetic predisposition, mechanical stress and a sedentary lifestyle. However, there are factors that directly interfere in its prevalence, such as sex, trauma, ethnicity, inflammatory diseases, obesity (which accelerates the degradation process), primary changes in cartilage, heredity (woman), mechanical, hormonal and metabolic factors, and infections [21]. It is believed that the etiology of OA is related to a lack of adaptation to the functional demands of the body, i.e. surges, macro- or micro-traumas [22,23,24,2,5,26,27].

3.7 *PATOPHYSIOLOGY*

Osteoarthritis (OA) is a degenerative joint disease, chronic and progressive, affecting synovial joints [28,29].
These processes result in different interactions between the joint cartilage and adjacent tissues in response to injury or chondrocyte extracellular matrix [30,31].
From the lesion starts matrix degradation by proteolytic enzymes such as Matrix Metalloproteinase (MMPs). The specific collagenases include MMP-1 (collagenase up-1), MMP-8 (collagenase-2) and MMP-13 (collagenase-3). These enzymes are distinguished by the ability to degrade other regions of the triple helical helix of type I collagen, II and III. The gelatinases MMP-2 (gelatinase A) and MMP-9 (gelatinase B) is another group of enzymes that degrade collagen types IV, V, VII and XI. This group acts synergistically with collagenase in cleavage of collagen. In addition, degrade elastin, agrecans and cartilage link protein. Other enzymes are also able to degrade extracellular matrix, such as cathepsin D, degrade agrecans; cathepsins B and L cleave telopeptides regions of collagen types I and II resulting in depolymerized collagen fibrils, agrecans and helical regions of the collagen IX and XI. There are still serine proteases, such as plasmin, which directly degrade extracellular matrix, or by activating metalloproteinase precursors [32].
At the same time, the cartilage components are organized to control progression degeneration [33]. The decomposition of proteoglycan and collagen bundles triggers increased amount of water, the space between the fibrils followed by a superficial necrosis of chondrocytes and reduced density of these cells. Consequently, the joint surface will change affecting the joint capsule, subchondral bone, ligaments, muscles and tendons, including the synovial fluid.

Increased hydration of cartilage and proteoglycans, promotes changes in mechanical properties of the tissue, triggering the loss of integrity of the articular surface and the presence of vertical cracks progressing to deep erosions with the consequent exposure of the subchondral bone [33,34]. These conditions cause pain, swelling and loss of joint mobility in osteoarthritis.

Acute pain of early osteoarthritis usually has a tendency to disappear within one year after having emerged, but may return and become chronic if no maintenance. Thus, immediate and proper treatment of osteoarthritic pain is crucial to maintain mobility and quality of life of the individual [35].

3.8 *CLINICAL PRESENTATION*

Osteoarthritis (OA) is as degenerative joint disease, a chronic condition characterized by the breakdown of joint cartilage, which becomes roguher and thinner. The bone underneath thickens and the joint becomes inflamed (Figure 8). The tissues around the joints, such as ligaments and the joint capsule, may thicken and become tighter, too.

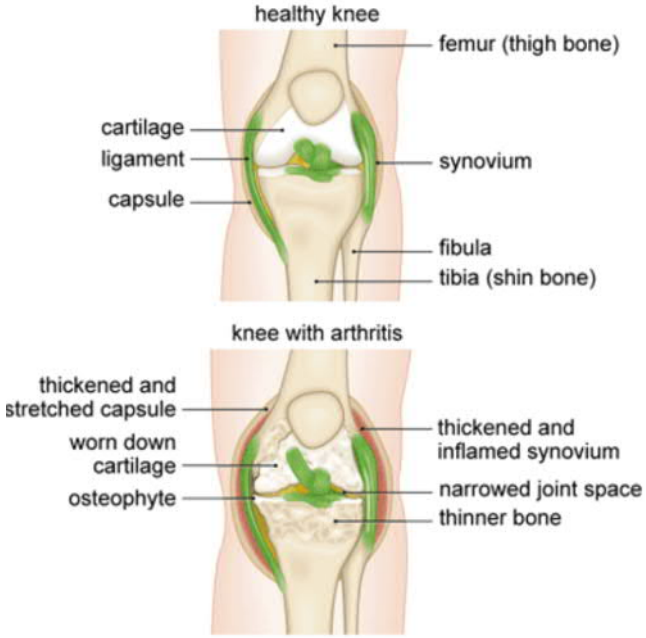


Figure 8. Normal knee compared with knee with osteoarthritis [36].

OA has many symptoms, which generally comprise those caused by mechanical or chemical stimulation. The main symptoms are pain and stiffness in the affected joint, but many other signs and symptoms may develop over time.
Pain is triggered by degenerative changes (bone remodeling, subchondral micro fractures, periostitis, nerve compression by osteophytes). In contrast to inflammatory arthritides - such as rheumatoid arthritis, with their prolonged morning stiffness and worsened pain in the

morning - OA tends to worsen as the day progresses. The stiffness in OA is termed "inactivity stiffness" and contrasts with the prolonged "morning stiffness" of rheumatoid arthritis. Inactivity stiffness in osteoarthritic lower limb joints lasts about 5 to 10 minutes and occurs when the patient gets up and bears weight after prolonged immobility [37].
Pain may arise from the nociceptive fibers and mechanoreceptors in the synovium, subchondral bone, periosteum, capsule, tendons, or ligaments. Pain in large joint OA (such as knee or hip) is also thought to arise from bone marrow lesions, and synovitis/effusion by stimulation of nociceptive fibers and intra-articular hypertension, respectively, and a similar mechanism may also operate in the small joints. However, hyaline cartilage is aneural, and is not a source of pain in OA. Whatever its source, both central and peripheral sensitization perpetuate and amplify pain in OA [38,39].

Crepitus is a coarse crunching sensation or sound caused by friction between damaged articular cartilage and/or the bone. It may be more prominent during active movement than during passive movement during physical examination. It is often present throughout the range of movement. Crepitus may be exacerbated by stressing the joint surfaces. Transmitted crepitus (felt on the adjacent periarticular bone) suggests a full-thickness cartilage defect on the affected side [40].

Tenderness in and around the joint is common in OA. Joint-line tenderness suggests an articular disorder, whereas tenderness away from the joint line suggests a periarticular soft tissue disorder [43].

Other common clinical manifestations of OA include [41,42]:

- Inelasticity;
- Paresthesia sensation of upper / lower limbs;
- Deformities. Malalignment with a bony enlargement may occur. Most cases of osteoarthritis do not involve erythema or warmth over the affected joint(s); however, a bland effusion may be present 44];
- Disease progression cause movement limitation associated with muscle spasm, contraction of the capsule and osteophytes or intra-articular bodies [43].

Other manifestations in patients with OA include sequelae such as muscle weakness, poor balance and comorbidities like fibromyalgia [45].

3.8.1 Osteoarthritis classification

OA can be classified as primary (idiopathic) when its etiology is not well defined and secondary when there is a specific disease-causing process. The primary is localized or widespread, more common in women, in middle age and progresses slowly as an accentuation of the normal aging process of the joint. The secondary OA is the result of genetic factors, trauma, more common in men at any age, inflammatory, neuropathic,

metabolic or endocrine diseases result from congenital abnormality of the joint, joint infection, inflammatory disorders, metabolic arthritis, hemochromatosis repeated, traumatic injuries and deformities, acquired articular incongruity, joint misalignment or instability of the joint. Therefore, most of the cases are secondary to osteoarthritis another condition. The most commonly affected joints are the knees, hips, hands, neck, and lumbar spine [41,46].

One of the most accepted hypotheses would be a defect in the articular cartilage or collagen metabolism. Among the genes potentially involved in the disease are: Insulin-like Growth (IGF-I and IGF-II) factor of the Vitamin D Receptor (VDR), oligomer proteins of cartilage matrix and regions of the Human Leukocyte Antigen (HLA). There seem loci linked to osteoarthritis in areas of chromosomes 2q and 11q. Although the hypothesis of defects in structural proteins such as collagen type II and IX have been proposed, there is no concrete evidence of their involvement in disease occurrence. Osteoarthritis results from an imbalance in the metabolic processes mediated by chondrocytes and is characterized by a gradual degradation of extracellular matrix components of fibrocartilage, with or without secondary inflammatory factor [23,47,48].

3.9 *DIAGNOSTIC OPTIONS*

A diagnosis of osteoarthritis may be suspected after a medical history and physical examination is done. Blood tests are usually not helpful in making a diagnosis.

The current gold standard for morphological assessment of knee osteoarthritis is plain radiography [49], which can be used to establish the severity of joint damage and monitor disease progression [50,51]. However, there is a great deal of conflicting evidence about the relationship between radiographic findings and clinical symptoms [52,53].

Computed tomography (CT), ultrasound and magnetic resonance imaging (MRI) are used to assess the soft tissues and fluid-filled spaces or to exclude other diseases and conditions. Arthrocentesis should be performed to analyse synovial fluid for evidence of crystals or joint deterioration: joint aspiration may help rule out other medical conditions or other forms of arthritis [54].

3.10 *TREATMENT OPTIONS*

There is no cure for osteoarthritis, however treatments are available to manage symptoms. Treatment choices fall into four main categories: nonpharmacologic, pharmacologic, complementary and alternative, and surgical. Surgical management should be reserved for those who do not improve with behavioral and pharmacologic therapy, and who have intractable pain and loss of function [55].

Non-pharmacologic and physical therapy

Nonpharmacologic therapy often starts with exercise. While it may be hard to think of exercise when the joints hurt, moving is considered an important part of the treatment plan. Studies show that simple activities like walking around the neighborhood can reduce pain and help maintain (or attain) a healthy weight.

Strengthening exercises build muscles around OA-affected joints, easing the burden on those joints and reducing pain. Range-of-motion exercise helps maintain and improve joint flexibility and reduce stiffness. Aerobic exercise helps to improve stamina and energy levels and also help to reduce excess weight. A randomized clinical trial compared supervised home-based exercise with no exercise in 786 patients with osteoarthritis of the knee. The exercise program consisted of muscle strengthening and range-of-motion exercises. The researchers found statistically significant improvements in a validated arthritis symptom score at six, 12, 18, and 24 months [56].

The U.S. Department of Health and Human Services recommends that everyone, including those with arthritis, get 150 minutes of moderate exercise per week.

Therapeutic ultrasound is a physical therapy modality often used in OA treatment. A Cochrane review of this modality concluded that, although statistically significant improvements were noted in visual analog pain scales following therapeutic ultrasound for knee OA, the clinical significance of these changes is questionable. The authors found that the studies were underpowered to properly determine the effectiveness of therapeutic ultrasound for knee or hip osteoarthritis [57].

A Cochrane review on transcutaneous electrical nerve stimulation found no clinically significant improvement in knee osteoarthritis pain [57].

Pharmacological approach

OA symptoms, primarily pain, may be helped by certain medications, including [58]:

- Acetaminophen. Acetaminophen (Tylenol, others) is an OTC (over-the-counter) analgesic that has been shown to be effective for people with osteoarthritis who have mild to moderate pain. A 2006 Cochrane review concluded that acetaminophen is better than placebo for treating mild osteoarthritis, and equal to nonsteroidal anti-inflammatory drugs (NSAIDs), but with fewer gastrointestinal adverse effects [59].
- Nonsteroidal anti-inflammatory drugs (NSAIDs). When acetaminophen fails to control symptoms, or if symptoms are moderate to severe, NSAID therapy is recommended. Over-the-counter NSAIDs, including ibuprofen (Advil, Motrin IB, others) and naproxen sodium (Aleve, others), taken at the recommended doses, typically relieve osteoarthritis pain. Stronger NSAIDs, available by prescription, may also slightly reduce inflammation along with relieving pain.
- Opioids. These drugs are often used to treat pain and are an option for osteoarthritis pain. Because of the potential for abuse, opioids should be an option only if the

patient has not responded to acetaminophen or NSAID therapy, or cannot tolerate them because of adverse effects [60].

- Topical analgesics. These include Diclofenac sodium gel and solution, only available as a prescription, Lidocaine patches, Methyl salicylate and menthol (Bengay) and Trolamine (Aspercreme), topical cream containing an aspirin-like drug that relieves inflammation and pain [61].

Surgical approach

Surgery should be reserved for patients whose symptoms have not responded to other treatments. The well-accepted indication for surgery is continued pain and disability despite conservative treatment. [60,62]:

- Cortisone injections. Injections of corticosteroid medications may relieve pain in the joint. During this procedure the physician numb the area around the joint, then places a needle into the space within the joint and injects medication. The use of intra-articular corticosteroids primarily provides short-term relief lasting four to eight weeks. It has proven effectiveness in osteoarthritis of the knee, but may not be as effective for osteoarthritis of the shoulder or hand [63,64]. Many physicians inject a corticosteroid and a local anesthetic, such as lidocaine (Xylocaine). The lidocaine can provide some immediate relief, which confirms that the medication was injected into the correct area. Patients should be warned of a potential flare-up of symptoms within the first 24 hours, followed by an improvement from baseline at 48 hours. Repeat injections are possible in the same joint, but usual practice is limited to four injections annually [65].
- Realigning bones**.** If osteoarthritis has damaged one side of the knee more than the other, an osteotomy might be helpful. In a knee osteotomy, a surgeon cuts across the bone either above or below the knee, and then removes or adds a wedge of bone. This shifts the body weight away from the worn-out part of the knee (Figure 9).

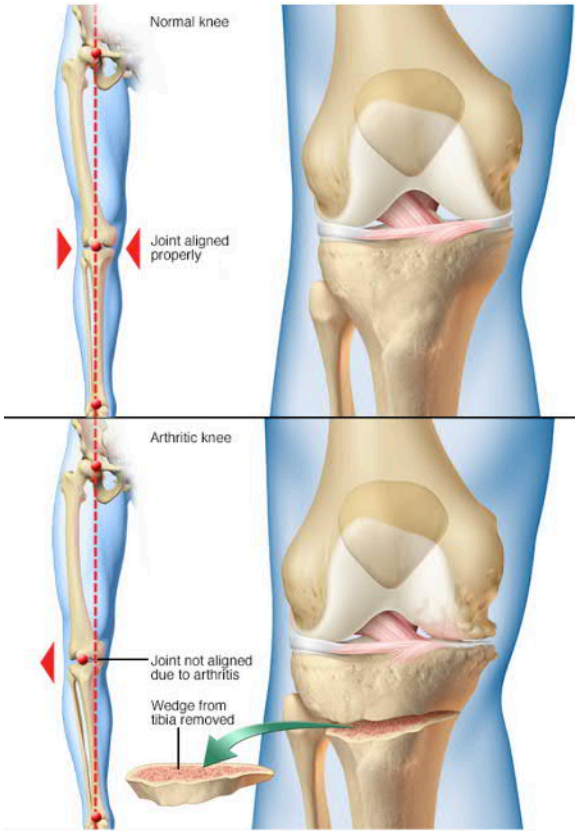


Figure 9. Knee osteotomy [65].

- Joint replacement. In joint replacement surgery (arthroplasty), the surgeon removes the damaged joint surfaces and replaces them with plastic and metal parts (Figure 10). Joint replacement is the most effective surgical intervention, with excellent patient outcomes following total joint replacement of the hip, knee, and shoulder [66].

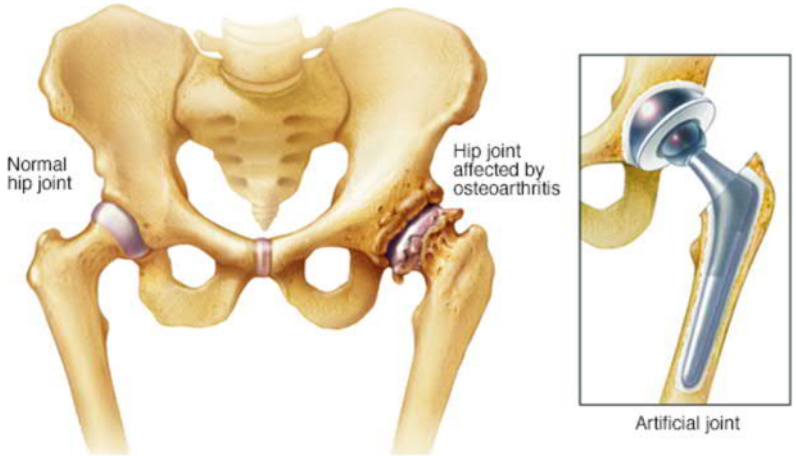


Figure 10. Hip prostheses are designed to mimic the ball-and-socket action of the hip joint. During hip replacement surgery, the surgeon removes the diseased or damaged parts of the hip joint and inserts the artificial joint [62].

- Lubrication injections. Intra-articular hyaluronic acid injections, also known as viscosupplementation, are widely used by orthopedic surgeons to treat osteoarthritis of the knee. A 2006 Cochrane review of 76 clinical trials concluded that viscosupplementation was effective for treating knee osteoarthritis. The treatment effect often lasted for up to 4 months and led to improvements in pain and function [67]. There have been trials comparing corticosteroid injections and hyaluronic acid injections. A meta-analysis of knee injections found that corticosteroids had a better short-term response rate and were equal to hyaluronic acid in the intermediate four- to eight-week range, but were inferior to hyaluronic acid after eight weeks from the time of injection [68]. Therefore, in stable patients with an acute flare-up of osteoarthritis symptoms, corticosteroids may be preferred. For patients experiencing chronic osteoarthritis pain, hyaluronic acid should be considered.

Complementary and alternative medicine

A meta-analysis on the effectiveness of acupuncture for osteoarthritis of the knee found only short-term benefit, which the authors described as clinically irrelevant [69].

The most widely used supplements for osteoarthritis are glucosamine and chondroitin. The literature consisted of small clinical trials until the release of the Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT), which included more than 1,500 patients. The trial had five arms comparing glucosamine alone, chondroitin alone, a combination of glucosamine and chondroitin, celecoxib, and placebo. The results were favorable only for the combination of glucosamine and chondroitin, which appeared to be effective for moderate to severe osteoarthritis of the knee [70]. Chondroitin alone did not show benefit for osteoarthritis of the knee or hip in a meta-analysis [71].

There also is evidence supporting the use of the supplement S-adenosylmethionine (SAM-e) to reduce functional limitation, but not compared with placebo in patients with osteoarthritis pain. The effectiveness of SAM-e is comparable to that of NSAIDs in some studies but with fewer adverse effects [72].

Balneotherapy is a heterogeneous group of treatments also known as spa therapy or mineral baths. A Cochrane review concluded that mineral baths were of some benefit to patients with osteoarthritis, but the authors addressed methodologic flaws in the studies and urged caution in interpreting the findings [73].

Capsaicin cream is a topical analgesic derived from chili peppers, which has been found to be superior to placebo in treating osteoarthritis pain [74].

3.11 *IDENTIFIED DISADVANTAGES AND HAZARDS DUE TO SUBSTANCES/MATERIALS AND TECHNOLOGIES*

The main risk in OA treatment is related to the pharmacological approach (medications) and surgical approach.

Medications' side effects

Acetaminophen is an OTC analgesic used in suffering from OA who experience mild to moderate pain. The *Arthritis Foundation* recommends taking no more than 3,000 mg of acetaminophen per day, because taking higher doses for a long time can lead to liver damage or failure. This can also be fatal [75].

Systemic nonsteroidal anti-inflammatory drugs (NSAIDs) can cause stomach irritation that may result in bleeding, ulcers, or perforation of the stomach or intestines. NSAIDs can also cause cardiovascular problems, bleeding problems, liver and kidney damage, constipation, diarrhea, gas, heartburn, nausea, vomiting and dizziness. Topical NSAIDs have fewer side effects and may relieve pain just as well.

Corticosteroids have many risks if used for long-term treatment; like NSAIDs, they reduce inflammation but are hard on the stomach. Unlike NSAIDs, they do not cause kidney problems. Other side effects of steroids include stomach ulcers, high blood pressure, irritability, depression, osteoporosis and high blood sugar levels [76].

Opioids should be prescribed first at low dosages and carefully monitored to evaluate for potential dependence. Opioids also make the patient sleepy or impair balance, and cause chronic constipation and can place older patients at risk of falls [77,78].

Surgery side effects

As mentioned before, joint replacement is the most effective surgical intervention, with excellent patient outcomes following total joint replacement of the hip, knee, and shoulder [66]. This technique, however, is not free from risks: surgical risks include infections and blood clots. Moreover, artificial joints can wear out or come loose and may need to eventually be replaced [79].

The two most common types of knee injection for OA are corticosteroids and hyaluronic acid. Corticosteroid injections are useful for treating flare-ups of OA pain and swelling with fluid buildup in the knee. However, the number of injections each year is generally limited to 3or 3 injections, because the medication can worsen joint damage over time. In some people who receive cortisone injections, the cartilage softens and the tendons weaken in the joint that is being treated. Infection at the site of your injection is a rare, but still serious potential side effect of cortisone shots [80].

Viscosupplementation with Hyaluronic acid (HA) injections_works differently, by helping

cushion and lubricate the moving parts within the joint area. However, generally HA require more than 1 (up to 5) injections, usually within a 5-week period. Viscosupplementation is considered a safe procedure, but like any medical procedure it does carry some risks and side effects. Patients who undergo viscosupplementation may have mild discomfort immediately after the procedure. Typical side effects at the injection site include: localized swelling, skin warmth and/or redness, soreness and joint stifness. It has been estimated that 1% to 3% of patients experience localized swelling and skin changes. However, side effects aree usually mild and go away in 1 to 2 days [81].

4 MEDICAL DEVICE UNDER EVALUATION

4.1 *GENERAL DETAILS*

Table 1. Description of the medical device.

Device Name	*"HiLow - Visco-Suppletive Joint device"*
Trade Name	HiLow - Visco-Suppletive Joint device Sinovial HL Intragel HL Yaral HL Sinovial HL Intragel HL Yaral HL
Manufacturer name and address	**IBSA Farmaceutici Italia srl** Via Martiri di Cefalonia 2 26900 Lodi Italy
Intended Purpose in accordance with device's IFU and indications	*"HiLow - Visco-Suppletive Joint device"* is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, *"HiLow - Visco-Suppletive Joint device"* reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action.
Indications	*"HiLow - Visco-Suppletive Joint device"* is intended for the treatment for osteoarthritis, and is particularly indicated for pain or reduced mobility due to degenerative diseases (arthrosis), post-traumatic diseases and tendinopathy associated with joint disabilities.

Product Description (physico-chemical, mechanical and technical specifications)	"*HiLow - Visco-Suppletive Joint Device*" is an intra-articular visco-supplementation product that allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. "*HiLow - Visco-Suppletive Joint Device*" appears in the form of: • A 1.25 ml glass syringe containing 1 ml of solution; • A 2.25 ml glass syringe containing 2 ml of solution. The content of the syringe is sterile and pyrogen-free. "*HiLow - Visco-Suppletive Joint Device"* consists of a buffered saline solution of hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The other components of the product are: sodium chloride, sodium phosphate and water for injections. The sodium salt of hyaluronic acid is formed of repeated chains of disaccharide units of N-acetylglucosamine and sodium glucuronate, and is a fundamentally important component of the synovial fluid to which it gives its visco-elastic properties. The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA) contained in "this medical device, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity. High and Low Molecular Weight Hyaluronic Acid contained in this device is produced through the biosynthesis of a natural substrate, without further chemical transformations, thus having excellent biocompatibility and allowing the natural re-establishment of the viscoelastic properties of the synovial fluid when injected in the joints. Moreover, the results of the studies carried out on cultured human mesenchymal stem cells (MSC) differentiated in chondrocytes demonstrate that the Platelet-rich Plasma (PRP) therapy, used for the treatment of the intra-articular infiltrative osteoarthritis, doesn't modify the rheological structure of sodium hyaluronate, which therefore retains its viscosuppletive function.
Size(s)/Packaging	Pack with 1 pre-filled syringe in the following available volumes: • 1ml pre-filled syringe (16 mg (H-HA) + 16 mg (L-HA) of hyaluronic acid sodium salt in 1 ml of sodium chloride buffered physiological solution); • 2 ml pre-filled syringe (32 mg (H-HA) + 32 mg (L-HA) of hyaluronic acid sodium salt in 2 ml of sodium chloride buffered physiological solution).

Ingredients/materials in contact with the patient/user	"*HiLow - Visco-Suppletive Joint Device*" consists of a pre-filled syringe containing hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The other components are: sodium chloride, sodium phosphate and water for injections.
Directions of use	As reported on product's leaflet: • Aspirate any joint effusion before proceeding with the injection of "*HiLow - Visco-Suppletive Joint Device*"; • Unscrew the cap of the tip of the syringe, being particularly careful to avoid contact with the opening;  • Insert the needle, of 18 - 22 G diameter; • Gently grip the needle guard and mount the needle on the luer-lock mount, screwing it tight until a slight counter-pressure is felt in order to ensure an airtight grip and prevent leakage of the liquid during administration;  • Inject "*HiLow - Visco-Suppletive Joint Device*" at room temperature and with strict asepsis conditions. • Inject "*HiLow - Visco-Suppletive Joint Device*" only into the synovial space.
Posology	The treatment can be carried out up to three injections depending on the severity of joint degeneration. It is the doctor's responsibility to evaluate the appropriateness of repeating the treatment and its frequency for each patient, taking into consideration the risk/benefit ratio of the treatment in each case.
Invasiveness (MD Directive)	"*HiLow - Visco-Suppletive Joint Device*" is applied in the joint cavity. According to the classification criteria set out by Italian Legislative Decree no. 46/97, amended by Italian Legislative Decree no. 37/2010, Annex IX at paragraph 2.4, Rule 8 the product is defined as "long-term surgically invasive device" intended to be absorbed. In accordance with Directive 93/42/EEC definition, an invasive device is: "*A device which, in whole or in part, penetrates inside the body, either through a body orifice or through the surface of the body*". A body orifice is defined as "*Any natural opening in the body, as well as the external surface of the eyeball, or any permanent artificial opening such as a stoma*".

Parts of the body contacted by the device (ISO 10993-1)	The medical device is in contact with the joint cavity as substitute of the synovial fluid.
Duration of use or contact with the body (ISO 10993-1)	"*HiLow - Visco-Suppletive Joint Device*" is categorized according to the duration of contact as "permanent" device, i.e. medical device whose cumulative single, multiple or repeated use or contact is > 30 days according to ISO 10993-1:2009 definition. It is a long-term use device according to Directive 93/42/EEC.
Primary Mechanism of Action, Principle of operation	"*HiLow - Visco-Suppletive Joint Device*" is a medical device that integrates the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, the device reduces the pain quickly and re-establishes joint and tendon mobility. "*HiLow - Visco-Suppletive Joint Device*" acts only at the level of the joint into which it is injected, without exercising any systemic action.
Sterility (including sterilization method)	The pre-filled syringe has been sterilised by moist heat.
Single use/reusable device	The medical device is disposable.
Warnings	"*HiLow - Visco-Suppletive Joint Device*" warnings are specified on product's leaflet: • *The content of the pre-filled syringe is sterile. The syringe is packaged in a sealed blister pack.* • *The external surface of the syringe is not sterile.* • *Do not use the device after the expiry date shown on the pack.* • *Do not use the device if the packaging is open or damaged.* • *The injection site must be on healthy skin.* • *Do not inject intravenously. Do not inject outside the joint cavity, into the synovial tissue or into the articular capsule.* • *Do not administer the device in the presence of heavy intra-articular effusion.* • *Do not sterilize again. The device was foreseen as a throwaway device only.* • *Do not reuse to avoid any risk of contamination.* • *Store between 0 - 25° C away from heat sources. Do not freeze.* • *Once opened, the device must be used immediately and discarded after use.* • *Keep out of reach and sight of children.* • *After the intra-articular injection advise the patient to avoid any intense physical activity and to resume his or her normal activities only after several days.* • *The presence of an air bubble does not alter in any way the quality of the product.*

Precautions	As reported on product's leaflet: *"Do not mix the device with disinfectants containing quaternary ammonium salts or chlorhexidine, as hyaluronic acid can precipitate in their presence".*
Contraindications	As reported on product's leaflet, the device must not be injected in the presence of an infected or seriously inflamed joint or if the patient has a cutaneous disease or an infection in the area of the injection site.
Identified side effects	As reported on product's leaflet: Extra-articular seepage of the product may cause undesired effects locally. During the use of the product, symptoms such as pain, the sensation of heat, reddening or swelling may appear at the injection site. These secondary emergences can be relieved by applying ice to the treated joint. They generally disappear in a short space of time. Doctors must ensure that patients notify them of any undesired effects which occur after the treatment.
Main claims	The therapeutic action of the medical device is carried out by the particular characteristics of the Hyaluronic Acid used. The hyaluronic acid contained in this product is a combination of high- and low- molecular weight hyaluronic acid and it is produced by fermentation and without any chemical modification, so it can reach an excellent tolerability. The chains of HA with different molecular weight contained, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device, thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.
Residual risk(s), relevant risks identified in the Risk Analysis	All risks associated with the design, manufacturing and use of the medical device were taken into consideration, and all risks were judged as acceptable after the application of risk control measures. All risks, regardless of their dimension, were reduced as much as possible and were balanced against the benefit of the device. The risk/benefit ratio was acceptable in each caseAll the measurement controls implemented during the development phase were proven effective. The Risk Management Team declared that no residual risks were identified that required further actions. The Manufacturer, IBSA Farmaceutici srl, declared that it activates and maintains a Post Market Surveillance System of the medical device placed on the market with the aim of gathering all useful data concerning the adequacy and the safety in use of the medical device, and activating - if necessary - further corrective / preventive actions to reduce the risks connected to its use.
Regulatory status	The product is CE marked.

Device/documentation modifications from pre-market Clinical Evaluation/PMCF (e.g. design, manufacturing process, labelling, IFU, advertising material, ...)	The instruction for use have been updated as a consequence of the direct assembling of the backstop on the pre-filled syringe during manufacture, which will be no more assembled by the doctor before use.
Reference documentation	• PIL HiLow Intra-articular 3,2 % (Leaflet, Last patient information leaflet review is of September 2014); • Technical File (TF-22.04.18) current edition; • Risk Management File (RMFI 22.04.18) current edition and Risk Management Report (RGR-22.04.18c) current edition; • Clinical Evaluation Report (IAHiLow_CLE_RES) rev.04 of September 2014; • Post market Clinical Follow-up (HiLow_PMCFP and HiLow_PMCFR of March 2016).

4.2 *DEVICE DESCRIPTION*

4.2.1 The concept

IBSA Farmaceutici developed "*HiLow - Visco-Suppletive Joint Device*", a medical device intended to be used on human beings for alleviation of a disease (degenerative diseases, post-traumatic diseases or joint and tendon alterations), whose main action is to substitute the synovial fluid and to allow the re-establishment of the physiological and rheological properties of joints affected by arthrosis.

4.2.2 Device description and composition

The medical device "*HiLow - Visco-Suppletive Joint Device*" consists of a pre-filled syringe, containing a buffered saline solution of hyaluronic acid (HA) with visco-elastic properties.

"*HiLow - Visco-Suppletive Joint Device*" contains 3,2% highly purified sodium hyaluronate with high and low molecular weight. The other components of the product are: sodium chloride, sodium phosphate and water for injections.
The sodium salt of hyaluronic acid is formed of repeated chains of disaccharide units of N-acetylglucosamine and sodium glucuronate, and is a fundamentally important component of the synovial fluid to which it gives its visco-elastic properties.
The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA) contained in "*HiLow - Visco-Suppletive Joint Device*", thanks to a specific and patented treatment of the solution, interact each other providing unique

rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.

High and Low Molecular Weight Hyaluronic Acid contained in "*HiLow - Visco-Suppletive Joint Device*" is produced through the biosynthesis of a natural substrate, without further chemical transformations, thus having excellent biocompatibility and allowing the natural re-establishment of the viscoelastic properties of the synovial fluid when injected in the joints.
Moreover, the results of the studies carried out on cultured human mesenchymal stem cells (MSCs) differentiated in chondrocytes demonstrate that the Platelet-rich Plasma (PRP) therapy, used for the treatment of the intra-articular infiltrative osteoarthritis, does not modify the rheological structure of sodium hyaluronate, which therefore retains its visco-suppletive function.

"*HiLow - Visco-Suppletive Joint Device*" is available in the following packages:

- 3.2% - 16 mg High Molecular Weight (H-HA) + 16 mg Low Molecular Weight (L-HA)/1 ml Hyaluronic acid sodium salt (1,25 ml glass syringe);
- 3.2% - 32 mg High Molecular Weight (H-HA) + 32 mg Low Molecular Weight (L-HA)/2 ml Hyaluronic acid sodium salt (2,25 ml glass syringe).

The medical device is for single use only and the content of the syringe is sterile and pyrogen-free.
As indicated on the box and in the instructions for use, "*HiLow - Visco-Suppletive Joint Device*" may only be sold by prescription.

4.2.3 Chemico-physical controls

The analytical controls carried out on the bulk solution are: appearance, pH and dynamic viscosity.
The analytical controls carried out on the finished product are: appearance, extractable volume, pH, osmolality, dynamic viscosity, sodium hyaluronate HMW and LMW identification and total sodium hyaluronate assay. For more details, please refer to product's Technical File (TF).

4.2.4 Microbiological controls

Both the sterility test and the determination of bacterial endotoxins are carried out on the finished product. For more details, please refer product's Technical File (TF).

4.2.5 Device specifications

"*HiLow - Visco-Suppletive Joint Device*" finished product specifications are represented in the following table (Table 2):

Test	Specification
Appearance	Syringes containing a clear, colourless, homogeneous gel
Extractable Volume	≥ 1.0 ml (1 ml syringe) ≥ 2.0 ml (2 ml syringe)
pH	6.5 – 7.5
Dynamic Viscosity (25°C, 167.6 s^{-1})	500 - 2000 mPa.s
Osmolality	250 – 400 mOsm/Kg
Sodium Hyaluronate HMW – High Molecular Weight and LMW – Low Molecular Weight Identification (HPLC)	Presence of two peaks corresponding to Sodium Hyaluronate low and high molecular weight
Total Sodium Hyaluronate assay (UV-vis method)	90.0 – 110.0% of the theoretical value
Sterility	Sterile
Bacterial Endotoxins	< 11,2 IU/ml (1 ml syringe) < 5,63 IU/ml (2 ml syringe)

Table 2. "*HiLow - Visco-Suppletive Joint Device*" specifications.

4.2.6 Packaging

The medical device "*HiLow - Visco-Suppletive Joint Device*" is packed with 1 pre-filled syringe in the following available volumes:

- 1ml pre-filled syringe (16 mg (H-HA) + 16 mg (L-HA) of hyaluronic acid sodium salt in 1 ml of sodium chloride buffered physiological solution);
- 2 ml pre-filled syringe (32 mg (H-HA) + 32 mg (L-HA) of hyaluronic acid sodium salt in 2 ml of sodium chloride buffered physiological solution).

According to Manufacturer's declaration, primary packaging is phthalates-free.

In order to verify that the closure system of the pre-filled syringe (pre-filled syringe-cone cover-piston) is intact and does not allow the inward penetration of any external microbial agent, periodical tests for control are performed. The test provides that 500 pre-filled syringes are filled with a culture medium and then subjected to the normal sterilisation cycle foreseen for the manufacturing process. At the end of the sterilization cycle in the autoclave, the syringes are placed in a sterile container and are submerged in culture medium inoculated with a suitable microbial agent with a concentration of 10^6 CFU/ml at minimum. The pre-filled syringes are kept in contact with the culture medium for at least 24

hours at room temperature, then the culture medium is removed and the pre-filled syringes are put to incubate at room temperature for 14 days. Lastly, no microbial growth must have occurred in any pre-filled syringe used for the execution of the negative control conducting using 500 syringes filled with cultural medium, sterilised and kept to incubate for 14 days at room temperature, without being placed in contact with the microbial agent. For further details, please refer to Enclosure 17 of product's Technical File (TF).

4.3 *RATIONALE FOR THE USE OF "HILOW - VISCO-SUPPLETIVE JOINT DEVICE"*

4.3.1 Introduction

Several pharmaceutical approaches for OA, such as analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) and steroids have been proposed, with the aim of reducing pain and maintaining and/or improving joint function. However, none of these options has shown to delay the progression of osteoarthritis or reverse joint damage [82].

Viscosupplementation was first used in Europe and Asia, and was approved by the U.S. Food and Drug Administration (FDA) in 1997: it consists in the administration of hyaluronan and/or hyaluronic acid preparations to joint synovial fluid, in order to restore the rheological and biological properties of normal HA [83].

Sodium hyaluronate (also referred to as hyaluronic acid, or HA) is the largest molecular component of synovial fluid and contributes both viscous (lubrificating) and elastic (shock-absorbing) properties that are important in the lubrication and protection of cartilage. Hyaluronate is a polymer found in all parts of the body but is of particular importance in articular joints [84]. Several clinical trials have shown that HA is more active than saline in reducing arthritic pain in osteoarthritis of the knee with significant improvements in pain and physical function [85,86] and an excellent tolerability profile with a low incidence of complications at local level and absence of systemic effects [87].

Intra-articular HA is relatively well established as a treatment option for knee OA in some patients and is recommended as a treatment option by many organizations, including the American College of Rheumatology and the Osteoarthritis Research Society International. The American College of Rheumatology recommends intra-articular HA as a treatment option for patients with knee OA who are at increased risk for gastrointestinal tract adverse events as an alternative for oral agents [88].

At present, preparations with different molecular weight are available (Low and High Molecular Weight), which display distinct effects. The enhanced penetration of low molecular weight preparations (0.1 - 1.1 millions Dalton) through the extracellular matrix of the synovium is thought to maximize the concentration. However, because of the low elastoviscosity of these hyaluronan compounds, compared to native hyaluronan in the synovial fluid, interests were shifted to a visco-supplementation fluid similar to the native hyaluronic acid (High Molecular weight) [89].

Regarding the safety, several factors may contribute to the occurrence of side effects with the use of intra-articular HA: among them, the characteristics and amount of HA

preparation injected, the number of injections, the skill of the operator, the technique used, the local and systemic tissues reactions [90].

4.3.2 Role of HA in viscosupplementation

While endogenous HA provides adequate viscoelastic and lubricating properties to maintain joint homeostasis in a healthy joint, during OA, the properties of HA are diminished and contribute to further cartilage destruction. Intra-articular injection of HA has been shown to inhibit cartilage degradation. It is important to note that the effect of HA is dependent on its molecular weight (MW): currently, there are two types of hyaluronic acid preparations: Low MW (LMW) and High MW (HMW).

HA as only cross-linked or high MW HA is effective in mitigating inflammation. Indeed, a 2012 report of a clinical trial using HA therapy indicated that intermediate MW HA was more superior as compared to low MW HA in alleviating knee OA symptoms [91]. Similar to HA, intra-articular administration of low dose corticosteroids has shown to reduce both the expression of proinflammatory mediators and the permeability in the inflamed area by lessening vascular dilation, as well as decrease inflammation and swelling in OA joints, thereby managing pain and enhancing joint mobility [92,93]. For both HA and corticosteroid treatment, rates of adverse side effects are low; however, it is worth noting that corticosteroids, particularly at a higher level, may have a damaging effect toward bone formation [94].

4.3.3 Safety of HA in viscosupplementation

General tolerance for HA injection is excellent and local tolerance satisfactory despite usually minor reactions that can be limited by good injection technique. Rare cases of allergy to avian derivatives (such as HYALGAN® or SYNVISC®) and some cases of transient asthenia have been reported [95].

Post-injection infection is exceptional with HA, with only a few reported cases although this is probably an underestimation; but this severe complication is not to be overlooked and mandates prevention in the form of perfect asepsis and patient information on signs of infection [95].

The main adverse effect is painful or inflammatory local reaction. Frequency is low, at 2-6% in the knee, a little more in other joints, and treatment efficacy does not seem to be impaired. Pain is mainly at the injection site and inflammation is early, moderate and transient; the patient should be alerted to such risk [95].

4.3.4 New HA derivatives in viscosupplementation

The field of HA derivatives evolved in the last few years, with the development of combined forms. In this context applies IBSA Farmaceutici *"HiLow - Visco-Suppletive Joint device"*. This medical device consists of a buffered saline solution of hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA), thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.

It is now known that hybrid cooperative HA complexes, produced through a patented technology, represent a new and valuable alternative, permitting to deliver the double of the HA amount in the same volume with a contained and even reduced dynamic viscosity. In particular, D'Agostino *et al*. in 2015 reported the efficiency of hybrid complexes (H-HA; MW 1200 ± 200 kDa and L-HA: Mw = 100 ± 5 kDa) molecular weight HA on a scratch *in vitro* model. It was found that H-HA/L-HA hybrid complexes improved the reparation processes compared to control and even H-HA alone. These hybrid cooperative hyaluronan complexes - due to their outstanding biochemical and biophysical features, and to the remarkable biological action - could represent a valuable alternative to cross-linked HA for different biomedical device applications [96].

4.4 *PRECLINICAL STUDIES CARRIED OUT ON "HILOW - VISCO-SUPPLETIVE JOINT DEVICE"*

According to ISO 10993-1 Current Edition, "*HiLow - Visco-suppletive Joint device*" is defined implantable, in permanent contact with tissue/bone.

Specifically, the following tests were performed, and are described in the following suparagraphs:

1. Cytotoxicity by direct contact (ISO 10993-5);
2. Intracutaneous reactivity (ISO 10993-10);
3. Subcutaneous implant (ISO 10993-6);
4. Systemic toxicity (ISO 10993-11);
5. Salmonella typhimurium – reverse mutation assay (Ames test) (ISO 10993-3);
6. Delayed hypersensitivity test (ISO 10993-10);

4.4.1 Test for in vitro cytotoxicity according with ISO 10993-5:2009 on "HiLow - Visco-Suppletive Joint Device" [Report Ref. 2011/1745 AMi]

This test was conducted in order to evaluate any cytotoxic effect of the test product. The test was carried out using BALB 3T3 cellular line (fibroblasts from mouse embryo). The test product was directly applied on filter paper in the middle of each well containing cell monolayer and was incubated at 37°C ± 1°C in CO_2 atmosphere for 24 hours. The negative control was represented by filter paper placed in the middle of each well; the positive control was represented by 30 mm^2 of latex placed in the middle of each well.

After 24 hours, the cells were observed with microscope (qualitative evaluation) in order to evaluate the biological reaction. A quantitative evaluation was also performed using the Neutral Red Uptake Method (NRU): after qualitative evaluations, the cells were treated for 3 hours with the medium containing the cell vitality dye and then with a Desorb solution allowing to obtain a cell lysate. Then, optic density was calculated after a 540 nm soectrophotometric reading.

Interpretation and acceptability criteria

Qualitative evaluation

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules, no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached, and without

		intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of cells are round and devoid of intracytoplasmic granules. No extensive cell lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed.
4	Severe	Nearly complete destruction of the cell layers.

Table 3. Interpretation criteria for biological reactivity evaluation.

The achievement of a numerical grade greater than 2 is considered a cytotoxic effect. Negative control must be ≤ 1, while positivde control must be ≥ 3.

Quantitative evaluation

The results were interpreted according to the following criteria (Table 4):

Non-cytotoxic sample	Cellular vitality reduction ≤ 30%
Cytotoxic sample	Cellular vitality > 30%

Table 4. Quantitative results' interpretation criteria.

Standard deviation of each group must be ≤ 18%; the positive control % cellular vitality must be y 70%.

Results

Qualitative evaluation

After 24 hours of contact, in the wells treated with the test product, some malformed or degenerated cells under specimen were observed. The reactivity grade was 1.

Quantitative evaluation

Cells treated with the test sample showed a cell vitality reduction of 16,56%.

Cells treated with the positive control showed a cell vitality reduction of 36,76%.

On the basis of the results obtained, and interpreted according to ISO 10993-5, the product was considered NOT CYTOTOXIC.

4.4.2 Intracutaneous reactivity test on "HiLow - Visco-Suppletive Joint Device" according to ISO 10993-10: 2010 [Report Ref. 2011/1746 AMi]

This test was carried out to investigate the intracutaneous reactivity of the medical device *"HiLow - Visco-Suppletive Joint Device*".

The test was performed by repeated applications of the test sample on a group of three male New Zealand White rabbits. For this purpose, 0,2 ml of the test sample was injected intracutaneously in five sites of 3 albino rabbits.

Another group of three rabbits was treated with the same procedure, but using sodium chloride injection (control group).

All animals were observed at 24, 48 and 72 hours for injection and evaluated for each toxic symptom. Injection sites were examined for evidence of any tissue reaction such as erythema, edema and eschar. Test and control sites were scored according to the following table (Table 5):

Reaction	**Numerical Grading**
Erythema and Eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (edges raised approximately 1mm)	3
Severe oedema (raised more than 1 mm and extended beyond area of exposure)	4

Table 5. Numerical grading for erythema - macroscopic examination.

Interpretation of results

After the 72 ± 2 h grading, all erythema grades plus oedema grades (24 ± 2) h, 48 ± 21) h and (72 ± 2) h are totalled separately for each test sample or blank for each individual animal. To calculate the score of a test sample or blank on each individual animal, divide each of the totals by 15 (3

scoring time points x 5 test or blank sample injection sites). To determine the overall mean score for each test sample and each corresponding blank, add the scores for the three animals and divide by three. The final test sample score can be obtained by subtracting the score of the blank from the test sample score. The requirements of the test are met if the final test sample score is 1,0 or less. If at any observation period the average reaction to the test sample is questionably greater than the average reaction to the blank, repeat the test using three additional rabbits.

Results

In the treated group, all sites treated with the test sample showed no sign of erythema or sign of oedema. The same results were observed in the control group.

The primary irritation index was 0,00.

One the basis of the results obtained, and interpreted according to ISO 10993-10, the test product satisfied the requirements of the test.

4.4.3 Systemic toxicity test on "HiLow - Visco-Suppletive Joint Device" [Report Ref. 2011/1746 AMi]

Systemic toxicity has been tested in mice, through intraperitoneal injections, either with 50 mg/kg of the test product or sodium chloride (control). All animals were observed after injection and after 4, 24, 48 and 72 hours. Toxic symptoms and/or mortality were recorded.

Interpretation of results

The test conditions were satisfied if none of the animals treated with the extract of the sample showed significant difference in behaviour compared to the control animals. If one of the animals treated with the sample showed slight signs of biological reactivity, an no more than one animal showed gross symptoms of biological reaqctivity or died, the test must be repeated using groups of 10 mice.

The conditions of the repeated test are satisfied if during observation period none of the animals treated with the extract of the sample will show a biological reactivity greater than the animals treated with the control. It two or more mice died, if two or more mice showed abnormal symptoms - such as convulsion or weakness - or if the weight loss was greater than 10% in 3 ore more animals, the test substance does did not satisfy the requirements of the test.

Results

None of the test or control animals exhibited overt signs of toxicity at any of the observation points.

On the basis of the results obtained, and interpreted according to ISO 10993-11, the test product did not cause toxic symptoms and satisfied the requirements of the test.

4.4.4 Delayed hypersensitivity test (GPMT) on "HiLow - Visco-Suppletive Joint Device" [Report Ref. 2011/1746 AMi]

The Guinea Pig Maximisation Test (GPMT) was carried out in order to identify the potential sensitising effects of the test product "*HiLow - Visco-Suppletive Joint Device".*

The maximization test consisted of a preliminary test, an induction phase and a challenge phase. Fifteen Albino guinea pigs were used, 10 of which were treated with the test item, and 5 were used as a control group.

During the induction phase, guinea pigs were treated with 3 pairs of intradermal injections (each of 0.1 ml) subdivided:

- 1st - stable emulsion of Freund complete adjuvant (FCA) in Sodium Chloride injection 50:50 (v:v)
- 2nd test sample for treated animals, Sodium Chloride Injection for control animals;
- 3rd test sample diluted 50:50 (v:v) with stable emulsion of FCA and Sodium Chloride Injection (50%) for treated animals, Sodium Chloride Injection diluted 50:50 (v:v) with stable emulsion of FCA and Sodium Chloride Injection (50%) for control animals.

Six days after performing the intradermal injections, treated and control ones, a local application was performed on all the animals by massaging 1 ml of Sodium Lauryl Sulfate at 10% in vaseline.

Seven days after performing the intradermal injections, 0.5 ml/animal of test sample was applied to the skin in 10 treated animals and left in place with an occlusive patch for 48 hours. The same treated was performed on control group with Sodium Chloride Injection.

In the challenge phase - 21 days after the beginning of the treatment on all animals - the challenge phase was carried out applying 0.5 mL of test sample on the right flank of all 15 guinea pigs, while Sodium Chloride Injection was applied on the left side. The dressing was left in place for 24 hours.

24 and 48 hours after the removal of the patches, the skin reactions of both treated and control animals were evaluated. The intensity of erythema and/or oedema were evaluated according to the following scale:

Reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 6. Intensity of erythema and/or oedema interpreted according to ISO 10993-10.

Interpretation of results

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals. If grades of 1 or higher are noted in the control animals, then the reactions of the test animal which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge. The outcome of the test is presented as the frequency of positive challenge results in the test and control animals.

Results

No abnormalities were observed in the animals treated with the test sample (and same result was obtained for control animals).

Animal N.	Time after removal of the patch	
	24 hours	48 hours
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Table 7. Skin reactions in treated animals.

Animal N.	Time after removal of the patch	
	24 hours	48 hours
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0

Table 8. Skin reactions in control animals.

On the basis of the results obtained, interpreted according to ISO 10993-10, it was concluded that the test product was NOT SENSITIZING.

4.4.5 Subcutaneous implantation test on "HiLow - Visco-Suppletive Joint Device" [Report Ref. 2011/1747 AMi]

This test was conducted in order to determine the local effect after subcutaneous implantation. 0,5 ml of the test product were injected in 4 sites in the right paralumbar region of 9 albino rabbits divided in 3 groups. In the left site of the paralumbar region of the same rabbits was injected 0,5 ml subcutaneous in four sites of sodium chloride injection used as control.

Three explantation times were scheduled, at 4, 12 and 26 weeks post-implant.

After that animal were sacrificed and a macroscopic evaluation of all implanted sited was conducted,

All sites implanted with the test sample and with the negative control were also subjected to the histological exam.

It was found that - after 4 and after 12 weeks - the treated sites with the test sample did not show any local effects. The same results were obtained for the control sites. The macroscopic evaluation did not showed any abnormality in all implantation sites.

Results at 26 weeks confirmed those previously obtaned.

The results of the histological exam after 4, 12 and 26 weeks are resumed in the following tables:

	Test sample			Control sample		
Animal N.	1293	1294	1295	1293	1294	1295
Inflammation						
Polymorphonuclear	0	0	0	0	0	1
Lymphocytes	0	0	0	0	0	1
Plasma cells	0	0	0	0	0	1
Macrophages	0	0	0	0	0	1
Giant cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
Neovascularisation	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	0
Fatty infiltrate	0	0	0	0	0	0

Table 9. Results of semi-quantitative evaluation of study samples, week 4.

In one sample, the presence of serocellular crust associated with minimal focal epidermal hyperplasia was obserbeved, together with inflammatory infiltrate oin the dermis.

	Test sample			Control sample		
Animal N.	1297	1299	1301	1297	1299	1301
Inflammation						

Polymorphonuclear	0	0	0	0	0	0
Lymphocytes	1	0	0	1	0	0
Plasma cells	0	0	0	0	0	0
Macrophages	1	0	0	1	0	0
Giant cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
Neovascularisation	0	0	0	0	0	0
Fibrosis	2	1	2	2	2	2
Fatty infiltrate	0	0	0	0	0	0

Table 10. Results of semi-quantitative evaluation of study samples, week 12.

The occurrence of hemorrages, graded as minimal, were observed in the sample T4 from animal N. 1299 and T3 from animal 1301. In addition, in all the sample, the presence of hair shaft fragment was noted.

	Test sample			Control sample		
Animal N.	1296	1298	1300	1296	1298	1300
Inflammation						
Polymorphonuclear	0	0	0	0	0	0
Lymphocytes	0	0	0	0	0	0
Plasma cells	0	0	0	0	0	0
Macrophages	0	0	0	0	0	0
Giant cells	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
Neovascularisation	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	0
Fatty infiltrate	0	0	0	0	0	0

Table 11. Results of semi-quantitative evaluation of study samples, week 26.

The occurrence of hemorrage, graded as light, were observed in the sample C4 from animal 1296 and in the sample C3 from animal 1300. In the superficial dermis from sample T4 from the animal 1296 and in the samples T1, T2 from the animal 1300, the presence of subacute dermatitis graded as slight to moderate and characterized by infiltration of neutrophils, lymphocytes and plasmacells, was noted. The following changes were observed in the skeletal muscle: focal mineralization, graded as minimal focal infiltration of neutrophils (sample T3 from animal 1298) and lymphocytes (animal 1296 treated with control and test item).

On the basis of the results obtained, and interpreted according to ISO 10993-6, it was concluded that the test product did not cause local toxic effects significantly different with respect to the control sample.

4.4.6 Salmonella typhimurium – reverse mutation assay (Ames test) on "HiLow - Visco-Suppletive Joint Device" [Report Ref. 2011/1744 AMi]

To perform the Bacterial reverse mutation assay five mutant strains of Salmonella typhimurium (TA1535, TA1537, TA98, TA100, TA102) were used. The presumed mutagenlc activity of the test substance was determined by comparing number of reverting colonies in treated cultures with the number of the reverting organisms In the control cultures. The direct incorporation method in a plate was used both in the presence of, and without, an enzymatic system for metabolic activation. The test material was tested neat.

Plate test without metabolic activation

0.1 ml of assay sample, 0.1 ml of the bacterial suspensions and 0.5 ml of PBS was added to aliquotted top agar in tube, then briefly stirred and poured Into minimal glucose agar plates.

Al the sarne time, negative controls, solvent controls and positive controls were also prepared,

The plates were then Incubated at 37'C±1 °C for 48hours.

After Incubation, the reverted colonies of the assay sample at the different concentrations, as well as those of the negative controls and positive controls, were counted In each plate.

Three replications were performed with the assay sample, negative and positive controls.

Plate test with metabolic activation

0.1 ml of assay sample, 0.1 ml of the bacterial suspensions and 0.5 ml of the enzymatic system for metabolism activation was added to allquotted top agar in tube, then briefly stirred and poured Into minimal glucose agar plates.

At the same time, negative controls, solvent controls and positive controls were also prepared.

The plates were then Incubated at 37°C±1 °C for 48 hours. After incubation, the reverted colonies of the assay sample at the different concentrations, as well as those of the negative controls and positive controls, were counted in each plate.

Three replications were performed with the assay sample, negative and positive controls.

Neither a concentration-related increase over the range tested nor a reproducible Increase, at each concentration and for each test strain, in the number of revertants colonies per plate in any strain with or without metabolic activation system was detected. Besides, the statistical test applied (t-test) showed no significant difference between the number of revertants colonies at the different dilutions of the test substance vs. negative control.

The verification of the genetic characteristics showed that the test strains maintained the required genetic properties in both the assay repetitions.

The test substance did not show toxic effects either In the presence or absence of the enzymatic system for metabolism activation.

The number of spontaneously reverting colonies in the negative control plates did not exceed the established limits and all the positive controls caused a significant increase of number of reverting colonies.

The mlcroblological control performed didn't show any contamination.

On the basis of the results, interpreted according to OECD 471:1997 and ISO 10993-3, the test substance proved to be NOT MUTAGENIC for all the test strains, either in the presence or absence of metabolic activation.

4.4.7 Report 2011/2104 (Genotoxicity evaluation)

A genotoxicity evaluation based on literature was conducted, on hyaluronic acid and on the other components of "*HiLow - Visco-Suppletive Joint Device*". All ingredients were not genotoxic. For sodium phosphate monobasic dehydrate, no biliographical data related to its genotoxic potential were found.

It was concluded that, on the basis of the results of the bibliographical search pertaining to product's ingredients, and also on the basis of the test conducted oin the finished product (according to ISO 10993-3), "*HiLow - Visco-Suppletive Joint Device*" was judged as NOT MUTAGENIC. For more details, please refer to full report on genotoxicity evaluation.

5 METHOD

5.1 *LITERATURE REVIEW PROCESS*

According to MEDDEV 2.7.1:2016 Guidelines, the review process has been conducted on the basis of a customized Clinical Evaluation Plan in which is defined the search strategy and the method for conducting the Clinical Evaluation of *"HiLow - Visco-Suppletive Joint Device".*

5.2 *TYPES OF STUDIES*

Randomised controlled (RCT) and non-randomized controlled studies (non-RCTs) have been included, with a minimum follow-up of 3 months.

Randomized controlled trials (RCTs) have an advantage over non-randomized trials for the evaluation of therapeutic procedures, as randomization renders the groups of patients comparable not only in respect of known prognostic factors, but also with regard to unknown factors (e.g. genetic) that might affect the outcome. Theoretically, RCTs have a good internal validity but may have poor external validity whilst the converse may be true with non-randomized designs. A well-designed non-randomized controlled study may, in fact, be preferable to a small, poorly designed RCT. Although the inclusion of non-RCTs increases the susceptibility for bias, non-RCTs have been included because a wide variety of approaches and designs have been used in the management of osteoarthritis symptoms and a limited number of RCTs have been conducted.

5.3 *TYPES OF INTERVENTIONS*

"*HiLow - Visco-Suppletive Joint Device*" is viscosuppletive treatment, a hyaluronic acid-based medical device indicated in the management of pain or reduced joints' mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations.

Therefore, only data related to viscosuppletive devices containing hyaluronic acid have been taken in consideration. Other therapies for the management of joint diseases or tendons diseasesand related signs and symptoms, such as pharmacological treatment, cortisone injections, surgical joint replacement etc., have been excluded (see Table 7 of product's Clinical Evaluation Plan).

5.4 *TYPES OF OUTCOME MEASURES*

The following outcomes have been defined for inclusion: positive physicians' global assessment, reduction of pain, reduction of stiffness, reduction of disability, increase of functionbal capacity and reduction of analgesics' use. Personal patients' performance and

tolerability evaluation have been excluded, but only if included in the study as the only outcome measure.

5.5 *SEARCH STRATEGY*

Search strategy has been developed using PubMed and key words selected in the Clinical Evaluation Plan for "*HiLow - Visco-Suppletive Joint Device*", in order to identify the articles for grading. The search has been carried out also on the sites of manufacturers of equivalent/similar devices for "*HiLow - Visco-Suppletive Joint Device*".

5.6 *ARTICLES' SELECTION*

Based on the pre-defined inclusion and exclusion selection criteria (see Clinical Evaluation Plan CEP_IAHiLow), relevant papers have been selected from the database search for the review. A preliminary selection of articles has been carried out accordingly with the inclusion/exclusion criteria defined in the Clinical Evaluation Plan. Grey literatures such as dissertation and non-peer review have been excluded. Full texts of the selected articles have been examined in order to avoid duplication of data.

5.7 *DATA EXTRACTION*

Data extracted has been compared in order to avoid consistency loss and, when possible, these inconsistencies have been corrected. Data extracted from pivotal articles has been discussed and inserted in comparison tables, including relevant information about study design, methods and results.

5.8 *STUDIES SUITABILITY*

Studies suitability has been evaluated through analysis of the characteristics of the trials and of the data contribution of the articles selected using the criteria defined in the Clinical Evaluation Plan for "*HiLow - Visco-Suppletive Joint Device*".

6 DATA ANALYSIS

6.1 *LITERATURE SEARCH RESULTS*

As shown in Figure 11, the electronic searches yielded 314 hits. Following the removal of duplicates, 176 remained. Following the review of full text, 35 articles were identified as meeting the inclusion criteria. After the weighting for data contribution and the selection on the basis of a compounded weight, four pivotal articles have been retrieved. Additional articles have been included as indirect supportive data: a total of 32 articles were included in the Clinical Evaluation supporting safety and performance of "*HiLow - Visco-Suppletive Joint Device*" as pivotal and indirect supportive data. The full texts of these articles can be found in **Appendix 3**.

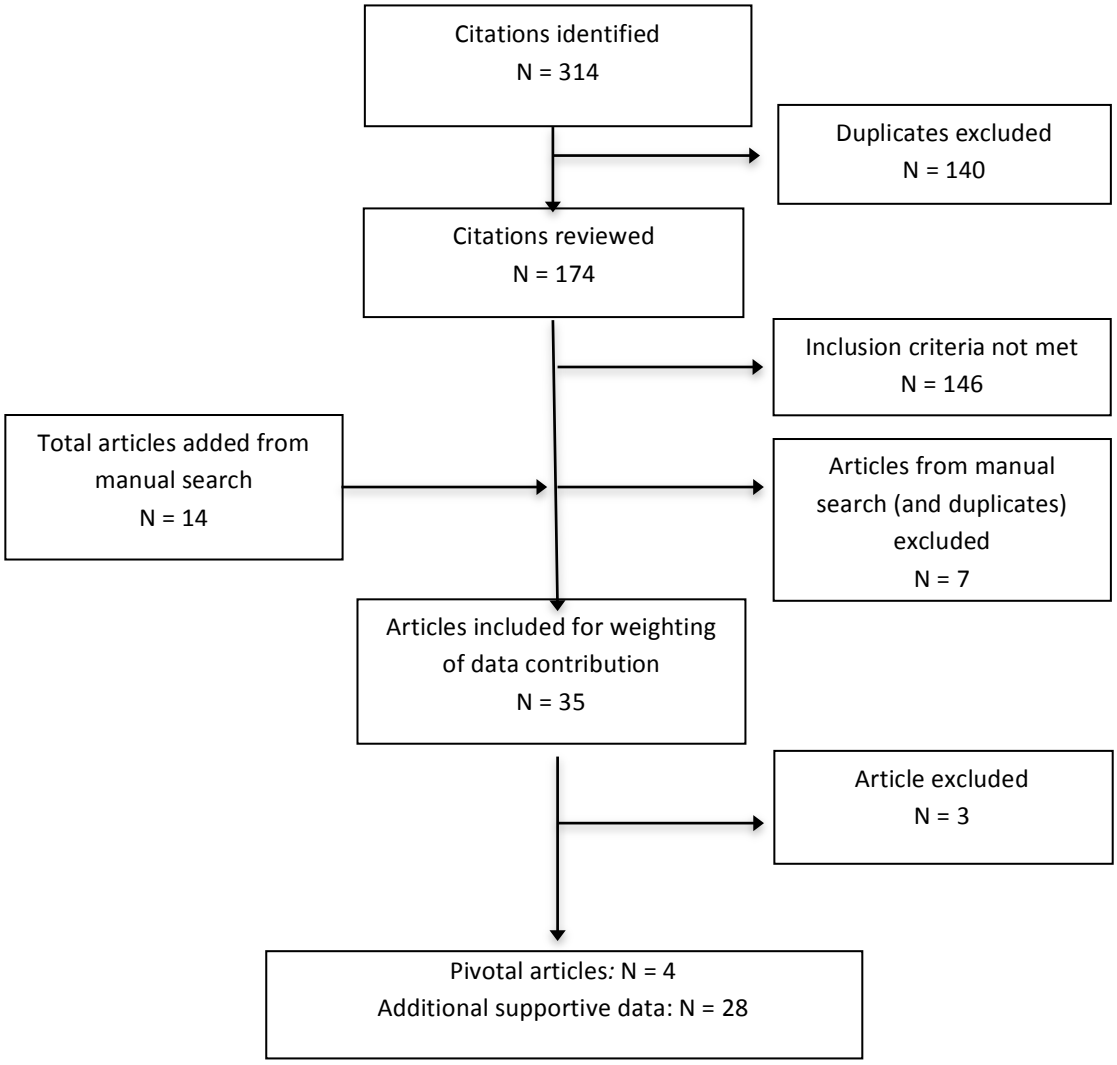


Figure 11. Flowchart: articles' selection.

7 SUMMARY AND APPRAISAL OF CLINICAL DATA

7.1 *SUITABILITY FOR APPRAISAL*

Table 12. Evaluation of suitability for appraisal.

ID	Article	Brief description	Compounded Weight
PIVOTAL ARTICLES			
1.	Filardo G, Di Matteo B, Di Martino A, Merli ML, Cenacchi A, Fornasari P, Marcacci M, Kon E. **Platelet-Rich Plasma Intra-articular Knee Injections Show No Superiority Versus Viscosupplementation: A Randomized Controlled Trial.** *Am J Sports Med.* 2015 Jul;43(7):1575-82.	The aim of this study was to evaluate the benefit provided by platelet-rich plasma (PRP) injections to treat knee joint degeneration in comparison with hyaluronic acid (HA). Two patients reported severe pain and swelling after HA injections, while no major adverse events were noted in the PRP group. However, PRP presented overall significantly more postinjection swelling and pain. Both treatments proved to be effective in improving knee functional status and reducing symptoms. The comparative analysis of the two treatments showed no significant intergroup difference at any follow-up evaluation in any of the clinical scores adopted. It was concluded by the authors that PRP does not provide a superior clinical improvement with respect to HA, and therefore it should not be preferred to viscosupplementation as injective treatment of patients affected by knee cartilage degeneration and OA.	0.25
2.	Giarratana LS, Marelli BM, Crapanzano C, De Martinis SE, Gala L, Ferraro M, Marelli N, Albisetti W. **A randomized double-blind clinical trial on the treatment of knee osteoarthritis: the efficacy of polynucleotides compared**	This randomized, double-blind, parallel-group clinical trial aims to assess the equivalence of intra-articular polynucleotides compared to standard hyaluronic acid (HA) viscosupplementation in the treatment of knee osteoarthritis (OA).	0.25

	to standard hyaluronian viscosupplementation. *Knee*. 2014 Jun;21(3):661-8.	Condrotide was as effective as Hyalubrix in reducing knee OA symptoms but showed an earlier response on pain reduction and can therefore be considered a valid alternative to the use of HA in the treatment of OA, avoiding the adverse events of NSAIDs and of intra-articular corticosteroids.	
3.	Zóboli AA, de Rezende MU, de Campos GC, Pasqualin T, Frucchi R, de Camargo OP. **Prospective randomized clinical trial: single and weekly viscosupplementation.** *Acta Ortop Bras.* 2013;21(5):271-5.	The objective of this study was to compare two different dosages of an intermediate molecular weight sodium hyaluronate (HA) (Osteonil®-TRB Pharma) assessing whether a single 6 ml application of this HA has the same effectiveness as the classical three-weekly 2 ml dose. It was concluded that there was no statistical difference between the single application of 6 ml of sodium hyaluronate and classic application with three weekly injections. However, only the classical regime showed statistically significant improvement in baseline pain (WOMAC pain and VAS).	0.25
4.	Jüni P, Reichenbach S, Trelle S, Tschannen B, Wandel S, Jordi B, Züllig M, Guetg R, Häuselmann HJ, Schwarz H, Theiler R, Ziswiler HR, Dieppe PA, Villiger PM, Egger M; Swiss Viscosupplementation Trial Group. **Efficacy and safety of intraarticular hylan or hyaluronic acids for osteoarthritis of the knee: a randomized controlled trial.** *Arthritis Rheum*. 2007 Nov;56(11):3610-9.	The objective of this study was to compare the efficacy and safety of intraarticular hylan and 2 hyaluronic acids (HAs) in osteoarthritis (OA) of the knee. Pain relief was similar in all groups. The difference in changes between baseline and 6 months between hylan and the combined HAs was 0.1 on the WOMAC pain score. There was a trend toward more local adverse events in the hylan group than in the HA groups during the first cycle, and this trend became more pronounced during the second cycle.	0.125
INDIRECT SUPPORTIVE DATA			
5.	Petrella RJ, Decaria J, Petrella MJ **Long-term efficacy and safety of a combined low and high molecular weight hyaluronic acid in the treatment of**	The aim of this study was to evaluate the long-term efficacy and safety of a combined HA of low and high molecular weight and different concentrations (DMW) in comparison to low molecular weight (LMW	-

	osteoarthritis of the knee. *Rheumatology Reports* 2011; 3: e4	500-730 KDa) or high molecular weight (HMW 6000 KDa) HA products in reducing pain at rest and pain at walking associated with knee osteoarthritis, as compared to placebo. Intra-articular hyaluronic acid injections using any of low, high or combined MW were highly effective in improving resting and more so, walking pain in patients with osteoarthritis of the knee. Greater improvement in both rest and activity outcomes in patients who received the DMW product, with concomitantly greater patient satisfaction and fewer use of concomitant therapeutic modalities at 16, 52 and 104 weeks suggest that combining a range of MW hyaluronic acid may be advantageous long term, particularly among active osteoarthritis patients.	
6.	Roux C, Fontas E, Breuil V, Brocq O, Albert C, Euller-Ziegler L **Injection of intra-articular sodium hyaluronidate (Sinovial) into the carpometacarpal joint of the thumb (CMC1) in osteoarthritis. A prospective evaluation of efficacy.** *Joint Bone Spine* 2007; 74: 368-372	The aim of this study was to compare the efficacy on pain relief and function of one, two or three injections of intra-articular hyaluronic acid in symptomatic osteoarthritis (OA) of the carpometacarpal joint of the thumb (CMCJ). Each subject was randomly allocated to receive, at weekly intervals, 1 (group 1) or 2 (group 2) or 3 injections (group 3) of 1ml Sodium Hyaluronate (Sinovial). No significant differences were found between each group over the study period for pain relief and function. But the intra groups analysis results show that intra-articular sodium hyaluronate injections into the CMCJ in OA can be efficacious on pain and fuctionality.	-
7.	Berenbaum F, Grifka J, Cazzaniga S, D'Amato M, Giacovelli G, Chevalier X, Rannou F, C Rovati L, Maheu E **A randomised, double-blind, controlled trial comparing two intra-articular hyaluronic acid preparations differing by their molecular weight in symptomatic knee osteoarthritis.** *Ann Rheum Dis 2012*	The objective of the study was to compare the effects of an intermediate molecular weight (MW) intra-articular hyaluronic acid (HA) with a low MW product on knee osteoarthritis (OA) symptoms. It was concluded that treatment with 3-weekly injections of intermediate MW HA may be superior to low MW HA on knee OA symptoms over 6 months, with a similar safety profile.	-

8.	Atay T, Aslan A,Baydar ML, Ceylan B, Baykal B, Kiridemir V **The efficacy of low- and high-molecular-weight hyaluronic acid applications after arthroscopic debridement in patients with osteoarthritis of the knee.** *Acta Orthop Traumatol Turc,* 2008; 42(4): 228-233	The authors evaluated the efficacy of viscosupplementation with low- or high-molecular-weight hyaluronic acid (HA) preparations following arthroscopic debridement (AD) in patients with osteoarthritis of the knee. Patients were randomized to receive 1. three intra-articular injections of 2 ml hylan G-F 20 (Synvisc), 2. five intra-articular injections of 2 ml sodium hyaluronate (Hyalgan), and 3. No injections (controls). Injections were administered at one-week intervals. WOMAC scores showed significant decreases in all the groups at 6 and 12 months. Compared to the control group, differences between pre- and post-treatment scores at 12 months were significantly greater in the Synvisc (p=0.004) and Hyalgan (p=0.003) groups, with no significant difference between the two HA groups (p>0.05).	-
9.	Lucas Y Hernandez J, Darcel V, Chauveaux D, Laffenêtre O. **Viscosupplementation of the ankle: a prospective study with an average follow-up of 45.5 months.** *Orthop Traumatol Surg Res*. 2013 Sep;99(5):593-9	The goals of this study were to evaluate the efficacy of viscosupplementation, explore which factors better predict Patient's response and propose an injection protocol. This study showed that viscosupplementation had a significant positive effect (p<0.05) in patients with ankle OA when a 3-injection protocol was used every two years on average. Neither etiology nor severity of the OA was predictive of the response. The authors concluded that fluoroscopy-guidance is essential for these injections.	-
10.	Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, Fornasari PM, Giannini S, Marcacci M. **Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis.**	The aim of our study was to compare the efficacy of autologous platelet-rich plasma (PRP) and viscosupplementation (hyaluronic acid) intra-articular injections for the treatment of knee cartilage degenerative lesions and OA. PRP injections showed more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function. Better results were achieved in younger and more active patients with a	-

	Arthroscopy. 2011 Nov;27(11):1490-501	low degree of cartilage degeneration, whereas a worse outcome was obtained in more degenerated joints and in older patients, in whom results similar to those of viscosupplementation have been observed.	
11.	Diracoglu D, Vural M, Baskent A, Dikici F, Aksoy C. **The effect of viscosupplementation on neuromuscular control of the knee in patients with osteoarthritis.** *J Back Musculoskelet Rehabil*. 2009;22(1):1-9.	The aim of this study was to investigate the short-term effects of intra-articular injection of hyaluronan (Hylan G-F 20) on proprioception, isokinetic muscle force, self reported pain and functional condition in patients with knee OA. In this study, it was demonstrated that intra-articular injection of hyaluronan in patients with knee OA led to a short-term increase in proprioception and isokinetic muscle force, and also significant improvements in the functional conditions of patients.	-
12.	Carpenter B, Motley T. **The role of viscosupplementation in the ankle using hylan G-F 20.** *J Foot Ankle Surg.* 2008 Sep-Oct;47(5):377-84	The goal of this study was to compare pain reduction following ankle arthroscopy versus that following ankle arthroscopy combined with weekly intra-articular instillation of hylan G-F 20 during the first 3 post-operative weeks. Both treatment groups experienced statistically significantly decreased pain following the intervention (p=0.002 and p=0.0009 for the arthroscopy alone and arthroscopy plus hylan groups, respectively), and that those who received 3 intra-articular injections of hylan G-F 20 following ankle arthroscopy improved statistically significantly (p=0.0014) more than did those who underwent arthroscopy as a sole therapy. These results suggest that viscosupplementation combined with arthroscopy may be more beneficial than arthroscopy alone.	-
13.	Conrozier T, Jerosch J, Beks P, Kemper F, Euller-Ziegler L, Bailleul F, Chevalier X. **Prospective, multi-centre, randomised evaluation of the safety and efficacy of five dosing regimens of viscosupplementation with hylan G-F 20 in patients with symptomatic tibio-femoral osteoarthritis: a pilot study.**	The aim of this study was to assess different dosing regimens of hylan G-F 20 in the treatment of pain due to knee OA. This study suggests that a single 6 mL injection of hylan G-F 20 may be as efficacious, and as well tolerated, as 3 x 2 mL one week apart.	-

	Arch Orthop Trauma Surg. 2009 Mar;129(3):417-23		
14.	Borrás-Verdera A, Calcedo-Bernal V, Ojeda-Levenfeld J, Clavel-Sainz C. **[Efficacy and safety of a single intra-articular injection of 2% hyaluronic acid plus mannitol in knee osteoarthritis over a 6-month period].** *Rev Esp Cir Ortop Traumatol.* 2012 Jul-Aug;56(4):274-80	The aim of this study was to evaluate the safety and efficacy of a single intra-articular injection of 2.0% hyaluronic acid (HA)+mannitol in symptomatic knee OA. A significant reduction in joint pain, stiffness and functional disability compared with baseline was observed at every follow-up visit ($p<0.001$). Joint function improved by 38.7% on Day 30, reaching 47.5% on Day 180. Rescue medication use decreased from 58.2% at baseline to 2.5% on Day 90, increasing in the last visits. Efficacy and safety were positively evaluated, both by investigators and by patients.	-
15.	Palmieri B, Rottigni V, Iannitti T. **Preliminary study of highly cross-linked hyaluronic acid-based combination therapy for management of knee osteoarthritis-related pain.** *Drug Des Devel Ther*. 2013;7:7-12	The aim of this trial was to investigate, for the first time, the effects of a highly cross-linked hyaluronic acid, Variofill®, alone or in combination with diclofenac sodium or sodium clodronate, for the management of bilateral knee OA-related pain. Hyaluronic acid - alone and in combination with sodium clodronate or diclofenac sodium - produced a significant improvement in mean VAS pain score at 3 and 6-month follow-up. No serious adverse events were observed.	-
16.	Strand V, Baraf HS, Lavin PT, Lim S, Hosokawa H. **A multicenter, randomized controlled trial comparing a single intra-articular injection of Gel-200, a new cross-linked formulation of hyaluronic acid, to phosphate buffered saline for treatment of osteoarthritis of the knee.** *Osteoarthritis Cartilage.* 2012 May;20(5):350-6	The objective of this study was to compare the safety and efficacy of a single intra-articular (IA) injection of a new cross-linked hyaluronic acid product, Gel-200, with PBS in patients with symptomatic knee OA. Effectiveness of Gel-200 by WOMAC pain subscores was statistically significant at week 13 ($p=0.037$). Mean improvements from baseline in WOMAC pain subscores consistently favored Gel-200 at each visit. Effectiveness of Gel-200 treatment was statistically significant over weeks 3-13 by WOMAC total score, physical function, and physician global evaluations ($p<0.05$). Adverse events were not significantly different between treatment groups.	-

		This trial demonstrated that a single injection of Gel-200 was well tolerated and relieved pain associated with symptomatic OA of the knee over 13 weeks.	
17.	Navarro-Sarabia F, Coronel P, Collantes E, Navarro FJ, de la Serna AR, Naranjo A, Gimeno M, Herrero-Beaumont G; AMELIA study group. **A 40-month multicentre, randomised placebo-controlled study to assess the efficacy and carry-over effect of repeated intra-articular injections of hyaluronic acid in knee osteoarthritis: the AMELIA project.** *Ann Rheum Dis.* 2011 Nov;70(11):1957-62.	This clinical trial was designed to compare against placebo the efficacy and safety of repeated injections of hyaluronic acid and its effect on disease progression over 40 months. At the 40-month visit, significantly more patients responded to hyaluronic acid compared with placebo (p=0.004). The number of responders to HA increased through the study, whereas those to placebo did not change. No safety problems were recorded in this study.	-
18.	Munteanu SE, Zammit GV, Menz HB, Landorf KB, Handley CJ, Elzarka A, Deluca J. **Effectiveness of intra-articular hyaluronan (Synvisc, hylan G-F 20) for the treatment of first metatarsophalangeal joint osteoarthritis: a randomised placebo-controlled trial.** *Ann Rheum Dis*. 2011 Oct;70(10):1838-41	The aim was to evaluate the effectiveness of a single intra-articular injection of hylan G-F 20 (Synvisc) for symptomatic first metatarsophalangeal joint (MTPJ) OA. Subjects with symptomatic first MTPJ OA were randomly allocated to receive up to 1 ml intra-articular injection of either hylan G-F 20 or placebo (saline). No statistically significant differences in foot pain were found between the groups at 3 months. There were few statistically significant differences in the secondary outcome measures. Overall, the incidence of adverse effects was not significantly different between groups.	-
19.	Chevalier X, Jerosch J, Goupille P, van Dijk N, Luyten FP, Scott DL, Bailleul F, Pavelka K. **Single, intra-articular treatment with 6 ml hylan G-F 20 in patients with symptomatic primary osteoarthritis of the knee: a randomised, multicentre, double-blind, placebo controlled trial.** *Ann Rheum Dis*. 2010 Jan;69(1):113-9.	The primary objective was to compare a single, 6 ml, intra-articular injection of hylan G-F 20 with placebo in patients with symptomatic knee OA. The safety of a repeat injection of hylan G-F 20 was also assessed. This study demonstrated that, in patients with knee osteoarthritis, a single 6 ml intra-articular injection of hylan G-F 20 is safe and effective in providing statistically significant, clinically relevant pain relief over 26 weeks, with a modest difference versus placebo.	-

20.	Lundsgaard C, Dufour N, Fallentin E, Winkel P, Gluud C. **Intra-articular sodium hyaluronate 2 mL versus physiological saline 20 mL versus physiological saline 2 mL for painful knee osteoarthritis: a randomized clinical trial.** *Scand J Rheumatol*. 2008 Mar-Apr;37(2):142-50	The aim of this study was to evaluate intra-articular viscosupplementation i in patients with painful knee OA. Patients were randomized to receive four weekly intra-articular injections of sodium hyaluronate 2 mL (Hyalgan 10.3 mg/mL) versus physiological saline 20 mL (distention) versus physiological saline 2 mL (placebo); they were followed up for 26 weeks. It was found that the effects of hyaluronate 2 mL, physiological saline 20 mL, and physiological saline 2 mL did not differ significantly in reducing knee pain, knee function, or consumption of analgesics. The VAS and KOOS - Osteoarthritis Outcome Score - outcomes all improved significantly over time ($p<0.0005$), regardless of intervention group. No adverse events were reported.	-
21.	Waddell BS, Waddell WH, Waddell DD. **Comparison of Efficacy and Tolerability of Hylan G-F 20 in Patients with and without Effusions at the Time of Initial Injection.** *J Knee Surg.* 2015 Jun;28(3):213-22.	This study compared efficacy of hylan G-F 20 in patients with and without an effusion. Patients with knee OA received three weekly injections of hylan G-F 20. Patients were followed for 26 weeks. Both effusion and control group VAS was significantly lowered at all time points. WOMAC scores improved ($p < 0.025$) at all visits in the effusion group except for WOMAC A-1 week 14. Control WOMAC scores also significantly improved at all visits ($p < 0.027$), except for full WOMAC and WOMAC A-1 at week 1. Neither group experienced an adverse event. Its was concluded by the authory that the presence of an effusion at onset of viscosupplementation requiring aspiration does not negatively impact efficacy of hylan G-F 20 or increase adverse event rates.	-
22.	Karalezli N, Ogun TC, Kartal S, Saracgil SN, Yel M, Tuncay I. **The pain associated with intraarticular hyaluronic acid injections for trapeziometacarpal osteoarthritis.** *Clin Rheumatol.* 2007 Apr;26(4):569-71	The purpose of this study was to evaluate pain and tolerability of viscosupplementation therapy with hyaluronic acid (HA) for trapeziometacarpal OA. Groups A and B consisted of eight patients who underwent one cycle of three injections of (one per week sodium	-

		hyaluronate. The injections for group A were under fluoroscopy control, while fluoroscopy was not used in group B. The results suggested that HA injection in the carpometacarpal joint is a tolerable procedure, but the patients complained of pain and discomfort during the injections. The pain in group A was much greater than in group B. Viscosupplementation for the treatment of trapeziometacarpal osteoarthritis is a viable treatment option for stages 3 and 4 patients when they do not want to be operated on. It is a tolerable but not a painless procedure especially when it is done without fluoroscopy control.	
23.	Di Sante L, Villani C, Santilli V, Valeo M, Bologna E, Imparato L, Paoloni M, Iagnocco A. **Intra-articular hyaluronic acid vs platelet-rich plasma in the treatment of hip osteoarthritis.** *Med Ultrason*. 2016 Dec 5;18(4):463-468	The aim of this study was to compare the efficacy of ultrasound-guided intra-articular (IA) treatment with platelet-rich plasma (PRP) versus viscosupplementation (hyaluronic acid [HA]) in hip OA. Data analysis revealed that, compared to T0, in the PRP-treated group VAS scores significantly decreased at T1 but not at T2, thereby indicating an early effect on pain which was not maintained at a longer term follow-up. In the HA group a significant decrease of both VAS and WOMAC values was registered only between T0 and T2. It was concluded that intra-articular PRP had an immediate effect on pain that was not maintained at longer term follow-up when, on the contrary, the effects of intra-articular HA were evident.	-
24.	Trueba Davalillo CÁ, Trueba Vasavilbaso C, Navarrete Álvarez JM, Coronel Granado P, García Jiménez OA, Gimeno Del Sol M, Gil Orbezo F. **Clinical efficacy of intra-articular injections in knee osteoarthritis: a prospective randomized study comparing hyaluronic acid and betamethasone.** *Open Access Rheumatol.* 2015 Jan 9;7:9-18.	The objective of the study was to evaluate HA and CS (corticosteroid) in patients with knee OA in terms of clinical efficacy over 12 months. Pain was significantly reduced in both groups at the first follow-ups. At 12 months, the mean pain reduction in the HA group was 33.6% compared to 8.2% in bethamethasone (BM) ($p<0.0001$). Function improvement was higher in HA through every visit, and mean improvement at 12 months was 47.5% in HA patients vs 13.2%	-

		in the BM group (p<0.0001). All patients from both groups achieved the Minimal Clinically Important Improvement (MCII) for both pain and function up to 6 months. At 9 months and 12 months, the MCII figures were higher in HA group with ≥80% compared to ≤10% in BM group (p<0.0001). Adverse reactions were rare and related to the administration procedure.	
25.	de Campos GC, Rezende MU, Pailo AF, Frucchi R, Camargo OP. **Adding triamcinolone improves viscosupplementation: a randomized clinical trial.** *Clin Orthop Relat Res*. 2013 Feb;471(2):613-20	The addition of triamcinolone hexacetonide to viscosupplementation improved first-week symptom and functional scores of viscosupplementation, but not beyond. It did not seem to increase the likelihood of adverse effects.	-
26.	Vanelli R, Costa P, Rossi SM, Benazzo F. **Efficacy of intra-articular polynucleotides in the treatment of knee osteoarthritis: a randomized, double-blind clinical trial.** *Knee Surg Sports Traumatol Arthrosc*. 2010 Jul;18(7):901-7	This trial was conducted over 16 weeks to assess the efficacy and safety profile of intra-articular polynucleotides gel injections in the treatment of knee OA associated with persistent pain. Patients were enrolled and randomized to receive intra-articular polynucleotides or hyaluronan; patients received five weekly intra-articular knee injections and the follow-up period was 3 months after the end of treatment. The mean global VAS pain decreased from 5.7 + or - 1.9 cm (T0) to 1.9 + or - 1.5 cm (T16) in polynucleotide group and from 4.9 + or - 2.0 cm (T0) to 2.1 + or - 1.4 cm (T16) in hyaluronan group. The reduction in pain was statistically significant for both groups. No significant adverse events were reported. The authors concluded that intra-articular polynucleotides can be a valid alternative to traditional hyaluronan supplementation for the treatment of knee OA.	-
27.	Raman R, Dutta A, Day N, Sharma HK, Shaw CJ, Johnson GV. **Efficacy of Hylan G-F 20 and Sodium Hyaluronate in the treatment of osteoarthritis of the knee -- a prospective randomized clinical trial.**	In this clinical trial, the authors compared the clinical effectiveness, functional outcome and patient satisfaction following intra articular injection with two viscosupplementation agents - Hylan G-F-20 and Sodium Hyaluronate in patients with	-

	Knee. 2008 Aug;15(4):318-24.	OA of the knee. Although both treatments offered significant pain reduction, it was achieved earlier and sustained for a longer period with Hylan G-F 20. From this study, it appeared that the clinical effectiveness and general patient satisfaction were better amongst patients who received Hylan G-F 20.	
28.	Iannitti T, Rottigni V, Palmieri B. **A pilot study to compare two different hyaluronic acid compounds for treatment of knee osteoarthritis.** *Int J Immunopathol Pharmacol.* 2012 Oct-Dec;25(4):1093-8.	The aim of this study was to investigate the clinical effectiveness of viscosupplementation with a new highly cross-linked hyaluronic acid, Variofill, in patients affected by bilateral knee OA in comparison with the widely used Synvisc. Both treatments resulted in a significant improvement vs baseline in all endpoints at 3 and 6 months ($p<0.001$). Treatment with Variofill resulted in a high percentage improvement in Visual Analogue Scale pain, Western Ontario McMaster universities Osteoarthritis Index score pain and physical activity, when compared to Synvisc viscosupplementation, at 6 months ($p < 0.05$).	-
29.	Rat AC, Baumann C, Guillemin F. **National, multicentre, prospective study of quality of life in patients with osteoarthritis of the knee treated with hylane G-F 20.** *Clin Rheumatol*. 2011 Oct;30(10):1285-93	The aim of this study was to describe the changes in QoL in patients receiving hylane G-F 20 in routine practice for the treatment of knee osteoarthritis and to determine the factors associated with changes in QoL (quality of life). It was found that both joint effusion and prior viscosupplementation could be associated with a more modest improvement in QoL.	-
30.	Di Martino A, Tentoni F, Di Matteo B, Cavicchioli A, Lo Presti M, Filardo G, Zaffagnini S, Marcacci M, Kon E. **Early Viscosupplementation After Anterior Cruciate Ligament Reconstruction: A Randomized Controlled Trial.** *Am J Sports Med*. 2016 Oct;44(10):2572-	The aim of the present double-blind, randomized controlled trial was to evaluate pain control and functional recovery provided by a single injection of HA performed the day after anterior cruciate ligament (ACL) reconstruction. No severe adverse events were documented after early viscosupplementation. A significant	-

	2578. Epub 2016 Jul 27.	improvement was documented in both treatment groups. Significant differences were documented in the transpatellar circumference at 60 days and in active ROM at 30 days postoperatively; patients who received HA had better values compared with the placebo group (p=0.022 and 0.027, respectively). No statistically relevant intergroup differences were found in the clinical scores.	
31.	Panuccio E, Memeo A, Richetta S. **[Evaluation of the combined treatment of oral viscosupplementation with hyaluronic acid intra-articular injection on symptomatic knee osteoarthritis].** Clin Ter. 2015;166(5):e321-6	The goal of this trial was to evaluate whether combined treatment with intra - articular injection of HA and AI is more effective than treatment with HA only for the symptomatic treatment of knee OA. AI combined a hydrolyzed low molecular weight collagen matrix providing high content of depolymerised HA and corticostreroid, with methylsulfonylmethane (MSM), Manganese and a milk glycoprotein. The treatment group HA + IA showed a positive trend compared to the group treated with HA only for all the efficacy variables observed, in particular regarding the VAS and the analgesic consumption.	-
32.	van den Bekerom MP, Rys B, Mulier M. **Viscosupplementation in the hip: evaluation of hyaluronic acid formulations.** *Arch Orthop Trauma Surg*. 2008 Mar;128(3):275-80	This prospective clinical study aimed to compare three different hyaluronate formulations and evaluates functionality, time of satisfactory pain relief and also the delay in performing a total hip arthroplasty. One hundred and twenty patients (126 hips) received viscosupplementation with one of the three hyaluronate formulations. All patients were candidate for surgical treatment with a total hip arthroplasty. Three different products were consecutively used: Adant® , Synocrom® or Synvisc®. Results reported that there was no significant difference in duration of the effect of the first infiltration between the three groups. The positive effect was still ongoing at the end point of the study in 46 hips: 51% of the patients did not undergo total hip arthroplasty, 3 years after	-

		viscosupplementation.	

7.2 *CLINICAL DATA FROM LITERATURE*

7.2.1 Pivotal data

Citation 1

Title	Filardo G, Di Matteo B, Di Martino A, Merli ML, Cenacchi A, Fornasari P, Marcacci M, Kon E. **Platelet-Rich Plasma Intra-articular Knee Injections Show No Superiority Versus Viscosupplementation: A Randomized Controlled Trial.** *Am J Sports Med.* 2015 Jul;43(7):1575-82.
Aim of the study	The objective of the study was to evaluate the benefit provided by platelet-rich plasma (PRP) injections to treat knee joint degeneration in comparison with hyaluronic acid (HA), the most common injective treatment currently adopted for this condition.
Relevance of the study	New options are currently being proposed to treat earlier stages of joint degeneration. Among these options, a novel biological treatment approach, platelet-rich plasma (PRP), has been introduced into clinical practice as a minimally invasive solution to improve the status of the joint surface and allow a fast return to full activity. However, despite the widespread application of PRP, there is no solid evidence in the literature to back up its real usefulness for the management of chondropathy and osteoarthritis (OA). Thus, the aim of this study was to evaluate the benefit provided by PRP to treat early stages of joint degeneration in comparison with hyaluronic acid (HA) injection. This study might be useful to sustain both the safety and efficacy of HA used as a viscosuppletive agent.
Equivalent Device	Two different treatment groups: those receiving intra-articular injections of **PRP - platelet rich plasma** - versus those receiving **high-molecular-weight HA (Hyalubrix** 30 mg/2 mL, molecular weight .1500 kDa; Fidia SpA).
Study Design	Randomized double blind trial.
Study period	This trial lasted 4 years (2009-2013).
Sample size	A total of 443 patients were screened, and 192 of them were enrolled. Sample size calcunation: a power analysis was performed for the primary endpoint of the IKDC subjective score improvement at the 12-month follow-up. From a pilot study, a standard deviation of 15.2 points was found. With an alpha error of .05, a beta error of .2, and a minimal clinically significant difference of 6.7 points corresponding to one-third of the documented mean improvement, the minimum sample size was 83 for each group. Considering a possible dropout rate of 15%, 96

	patients per group were required, for a total of 192 patients who were effectively enrolled.
Inclusion Criteria	Inclusion criteria were the following: (1) Unilateral symptomatic knee with history of chronic pain (at least 4 months) or swelling and (2) imaging findings of cartilage degeneration, that is, chondrop-athy (Kellgren-Lawrence score of 0, detected by magnetic resonance imaging [MRI]) or osteoarthritis (Kellgren-Lawrence score of 1-3).
Exclusion Criteria	The exclusion criteria were age greater than 80 years, Kellgren-Lawrence score more than 3, major axial deviation (varus > 5°, valgus > 5°), focal chondral or osteochondral lesion, presence of any concomi-tant knee lesion causing pain or swelling (ie, ligamentous or meniscal injury), inflammatory arthropathy, hematolog-ical diseases, severe cardiovascular diseases, infections, immunodepression, therapy with anticoagulants or antiag-gregants, use of nonsteroidal anti-inflammatory drugs in the 5 days before blood donation, and hemoglobin count lower than 11 g/dL and platelet count lower than 150,000/mm^3.
Intervention	Patients were randomly divided into 2 dif-ferent treatment groups: those receiving 3 weekly intra-articular injections of PRP versus those receiving 3 weekly administrations of high-molecular-weight HA (Hyalubrix 30 mg/2 mL, molecular weight >1500 kDa; Fidia SpA). To keep the patients blinded, all of them underwent blood harvesting to obtain autologous PRP, which was used only in half of them. Before the injection, the syringe was appropriately covered to prevent patients from discovering the substance they were receiving. After the injection, they were sent home with instructions to restrict the use of the leg for at least 24 hours and to use ice or other cold therapy on the affected area to relieve pain. The treatment consisted of 3 injections at 1-week intervals. During the treatment period, rest or mild activities were permitted, and subse-quently a gradual resumption of normal sport or recrea-tional activities was allowed as tolerated.
Outcomes	<u>Outcomes</u> International Knee Documentation Committee (IKDC) subjective score (main outcome), Knee injury and Osteoarthritis Outcome Score, EuroQol visual analog scale and Tegner score. <u>Measures and timepoints</u> Patients were prospectively evaluated at baseline and then at 2, 6, and 12 months after the last injection; evaluation included the International Knee Documentation Committee (IKDC*[1]) subjective measure, Knee injury and Osteoarthritis Outcome Score (KOOS*[2]), EuroQol visual analog scale (EQ-VAS), and Tegner score. Range of motion and the transpatellar circumference of both the index knee and the contra-lateral knee were measured. Patient satisfaction and adverse events were also recorded. To guarantee the double-blinding of the trial, all the clinical evaluations were performed by an independent physician not involved in the injection procedure.
Study Results	**In the PRP group**, a statistically significant improvement in all clinical scores was

Performance	documented. In particular, the IKDC subjective score increased from 52.4 ± 14.1 to 63.2 6 16.6 at 2 months (p<0.0005) and remained stable for up to 12 months (66.2 ± 16.7; p=nonsignificant vs 2 months). Similarly, an increase was recorded in all KOOS subscales. The evaluation of sport activity level through the Tegner score showed a significant improvement from pretreatment (2.9 ± 1.3) to 2 months (3.6 6 1.4; p < 0.0005) and then values were stable up to the final follow-up (3.7 ± 1.3; p = nonsignificant), although it was not pos-sible to regain the same preinjury level (5.2 ± 1.9). The EQ-VAS score for general health revealed a significant increase from baseline to the 12-month follow-up (73.2 ± 12.0 vs 77.6 ± 11.1; p = 0.006). A significant reduction in transpatellar circumference was also observed from the baseline evaluation to 12-month follow-up (410 ± 34 vs 402 ± 33 mm; p = 0.001), whereas no significant changes occurred in knee ROM at any follow-up. **In the HA group,** two patients reported severe pain and swelling after the first HA injection, which led them to withdraw from the injective treatment. A statistically significant improvement in all clinical scores was found. In particular, the IKDC subjective score increased from 49.6 ± 13.0 to 63.6 ± 15.2 at 2 months (P < 0.0005) and remained stable for up to 12 months (64.2 ± 18.0; p = nonsignificant vs 2 months). Similarly, an increase was recorded in all KOOS subscales. The Tegner score showed a significant improvement from pretreatment level (2.8 ± 1.3) to 2 months (3.3 ± 1.5; p<0.0005) and then remained stable up to the final follow-up (3.4 ± 1.5; p = nonsignificant) but without reaching the preinjury value (4.9 ± 1.7). No significant variation was reported in the EQ-VAS score. A statistically significant reduction in transpatellar circumference was observed from the baseline evaluation to the final follow-up (415 ± 35 vs 406 ± 34 mm; p = 0.002), whereas no significant changes occurred in the knee ROM at any follow-up. **PRP vs HA** Both treatments proved to be effective in improving knee functional status and reducing symptoms, but the comparative analysis showed no significant inter-group difference at any follow-up in any of the clinical scores adopted. The objective evaluation of the transpatellar circumference and knee ROM with respect to the contralateral joint and in terms of changes over time did not show any difference when the measurements of the two treatment groups were compared. The satisfaction rate was 88.3% in the PRP group and 89.9% in the HA group.
Study Results **Safety**	No severe adverse events were reported. **PRP vs HA** PRP injections produced significantly more post-injection swelling and pain with respect to HA. However, these reactions were self-limiting and lasted for just a few days, requiring no medical intervention.
Limits of the study	Not retrieved.

Discussion	Although a significant clinical improvement was observed after treatment, no significant difference was found with respect to viscosupplementation in any evaluation performed at any of the follow-up times. Overall, the clinical benefit provided by injections was quite modest (i.e. swelling reduction associated with a little symptomatic and functional improvement) for both PRP and HA administration. In any case, the results of the present study are in contrast with a previous series of patients evaluated after PRP treatment and reported in the literature.
Conclusions of the authors	This study shows that leukocyte-rich PRP offers a modest clinical benefit at short term and cannot provide a greater improvement with respect to HA; therefore, PRP should not be preferred to viscosupplementation as injective treatment for patients affected by cartilage degeneration and OA.

[3] **IKDC**

The IKDC - International Knee Documentation Committe - Standard Knee Evaluation Form has three domains: 1) symptoms, including pain, stiffness, swelling, locking/catching, and giving way; 2) sports and daily activities; and 3) current knee function and knee function prior to knee injury (not included in the total score). The items are a total of 18 (7 items for symptoms, 1 item for sport participation, 9 items for daily activities, and 1 item for current knee function).

[*2] **KOOS**

The Knee injury and Osteoarthritis Outcome Score (KOOS) was developed as an extension of the WOMAC Osteoarthritis Index with the purpose of evaluating short-term and long-term symptoms and function in subjects with knee injury and osteoarthritis. The KOOS holds five separately scored subscales: Pain, other Symptoms, Function in daily living (ADL), Function in Sport and Recreation (Sport/Rec), and knee-related Quality of Life (QOL). The KOOS is a validated for several orthopaedic interventions, such as anterior cruciate ligament reconstruction, meniscectomy and total knee replacement.

Citation 2

Title	Giarratana LS, Marelli BM, Crapanzano C, De Martinis SE, Gala L, Ferraro M, Marelli N, Albisetti W. **A randomized double-blind clinical trial on the treatment of knee osteoarthritis: the efficacy of polynucleotides compared to standard hyaluronian viscosupplementation.** *Knee*. 2014 Jun;21(3):661-8.
Aim of the study	This clinical trial aims to assess the equivalence of intra-articular polynucleotides compared to standard hyaluronic acid (HA) viscosupplementation in the treatment of knee osteoarthritis (OA).
Relevance of the study	The treatment of OA is still an open issue. During the last few years, the use of hyaluronian viscosupplementation has grown as a treatment of moderate-degree OA: the goal of this treatment method is to replace the quantity of intra-articular HA, that is reduced in patients affected by osteoarthritis, in order to restore the natural viscosity of the synovial fluid and therefore protect cartilage, relieving patient's pain. Different results have been obtained with the use of intra-articular HA in patients affected by OA but, according to the currently available evidence, the long-term clinical efficacy of intra-articular HA was not yet been proven at that time.
Equivalent Device	The product under study is a gel composed by polynucleotides, derived from natural sources (brood trout), whose trade name is **Condrotide**. It appears colorless, transparent, viscoelastic and it is pro-vided in pre-filled glass sterile disposable syringes containing a solution of 2 ml (the concentration of polynucleotides is 20 mg/ml). Standard hyaluronian viscosupplementation was perfomed using **Hyalubrix** (pre-filled glass sterile disposable syringes containing 30 mg of hyaluronic acid in 2 ml of buffered physiological saline solution).
Study Design	Randomized, double-blind clinical trial.
Study period	This trial has been carried out from 2009 to 2012.
Sample size	Seventy-five patients all affected by knee OA (diagnosis based on the ACR—American College of Rheumatology Classification) were assessed for eligibility. Three of 75 recruited patients were not declared as eligible since two had suspended steroid infiltration therapy since less than three months and one declined to participate.
Inclusion Criteria	Following the main inclusion criteria, patients had to be between 18 and 80 years, having followed at least five years of undergraduate school, having developed persistent pain for at least two months, having stated a VAS level less than or equal to four at the first clinical evaluation.
Exclusion Criteria	Exclusion criteria included alcohol or drug abuse, pregnancy or breastfeeding, hypersensibility to polynucleotides or hyaluronic acid, OA due to metabolic disorders, presence of severe pathologies at the first clinical evaluation, hyaluronic acid or steroid infiltration therapy ongoing or suspended since less than three months, systemic

	treatment with steroids ongoing or suspended since less than one month, fractures or severe traumatic episodes that affected the knee, presence of rheumatoid arthritis or other articular inflammatory pathologies and relevant hematological diseases.
Intervention	Among the enrolled and randomized 72 patients, 36 were treated with Condrotide (Group C) and 36 were treated with Hyalubrix (Group H). Group C included 20 females and 16 males with a mean age of 64.92 years (range 31–80 years); group H included 21 females and 15 males with a mean age of 64.14 years (range 43–76 years). Since 3 patients from group C and one patient from group H were excluded, the efficacy set was composed by 33 patients for group C and 35 patients for group H. All patients underwent three intra-articular injections of Condrotide or Hyalubrix with an interval of one week between each injection: the first one was performed at the beginning of the treatment (T0 = baseline time), the second one after one week (T1), and the third one after two weeks (T2); then patients returned for a clinical follow-up after six weeks (T6), ten weeks (T10), 18 weeks (T18), and 26 weeks (T26) since the beginning of the treatment. Serum levels of COMP were determined at T0, T6, and T26. Injections were performed by highly skilled medical staff, following all standard rules and all principles of asepsis for the administration of intra-articular injections. To respect the double-blind condition, injections were performed by a different physician from the one who dealt with the following clinical evaluations.
Outcomes	The first primary outcome of this study was represented by the evaluation of the results of KOOS (Knee Injury and Osteoarthritis Outcome Score). The other primary outcome of this study was the modification of pain level at rest, at weight-bearing and during physical activity, evalu-ated through the Visual Analogue Scale (VAS) at T1, T2, T6, T10, T18 and T26. As regards primary outcomes, two analyses have been performed: the first one consists of the evaluation of KOOS and VAS values at differ-ent time-points with respect to their baseline values considering the two treatments separately; the second one considers the KOOS and VAS values as a comparison between the effects of the two treatments at different time-points from T0 up to T26. Secondary outcome measurements included the determination of COMP (Cartilage Oligomeric Matrix Protein) serum levels at T0, T6, and T26, NSAIDs consumption, crackling during movement, articular mobility limitation (LMA), and articular edema.
Study Results Performance	In the first analysis, the KOOS parameters in Group C and Group H were considered separately, observing the trend of their values at different time-points with respect to their baselines. The most remarkable re-sult was achieved for the parameter “Symptoms”: in fact the outcome obtained with the treatment with Condrotide was statistically significant already after 2 weeks since the beginning of the treatment (at T2 $p = 0.003$), while the results achieved with Hyalubrix became significant only after 18 weeks (at T18 $p = 0.010$). Another important result concerns the parameters “pain” and “Function in sports and recreation”: Condrotide showed statistically significant results after 6 weeks (for KOOS “pain”: at T6 $p = 0.03$; for KOOS “Function in sports and recrea-tion”: at T6 $p = 0.012$) since the beginning of the treatment, while Hyalubrix outcome

	became significant only after 18 weeks (for KOOS "pain": at T18 p = 0.0001; for KOOS "Function in sports and recreation": at T18 p = 0.003). Finally, considering the parameters "Function in daily living" and "Quality of life", the results of both treatments be-came statistically significant after 6 weeks. Concerning the comparison between Condrotide and Hyalubrix at different time-points a statistically significant difference in favour of Condrotide was observed at T10 for parameters "Pain", "Function in daily living", and "Function in sports and recreation". In all the other cases the efficacy of both treatments can be considered equal. As regards parameters "Symptoms" and "Pain", the linear fit of group C is clearly steeper, showing that Condrotide has a faster effect on their reduction if compared to Hyalubrix. Concerning the other parameters, the slopes of groups C and H are similar, illustrating that both treatments almost perform in the same way. As regards VAS "at rest" since T2 both groups C (at T2 p = 0.043) and H (at T2 p=0.043) showed a statistically significant difference, that was also later maintained. Analyzing VAS values "standing" and "walking", Condrotide showed a statistically significant difference earlier than Hyalubrix (T1 for group C vs T2 for group H). The evaluation of COMP showed a statistically significant reduction of its serum levels since the beginning to the end of the treatment (T26-T0) in group H (p = 0.001), while the treatment with Condrotide caused a mild increase of COMP levels at T6 with a new successive reduction. Besides, the comparison between the two treatments did not show any statistical significance.
Study Results **Safety**	No safety results provided.
Limits of the study	Not retrieved.
Discussion	The results of this work showed how the use of both treatments (Condrotide vs Hyalubrix) determined a favorable effect on the analyzed parameters, in particular pointing out a reduction of pain and an improve-ment of the activities of daily living and, therefore, of the quality of life. The outcome of this study did not show any statistically significant difference between the two treatments; nevertheless, an earlier clinical efficacy of Condrotide with respect to Hyalubrix was observed.
Conclusions of the authors	The intra-articular use of Condrotide might therefore represent a favorable alternative to the use of HA in the treatment of OA connected to persistent pain, avoiding the adverse events due to the use of NSAIDs and intra-articular corticosteroids.

Citation 3

Title	Zóboli AA, de Rezende MU, de Campos GC, Pasqualin T, Frucchi R, de Camargo OP. **Prospective randomized clinical trial: single and weekly viscosupplementation.** *Acta Ortop Bras.* 2013;21(5):271-5.
Aim of the study	The aim of this study was to compare two different dosages of an intermediate molecular weight sodium hyaluronate (HA) (Osteonil®-TRB Pharma) assessing whether a single 6 ml application of this HA has the same effectiveness as the classical three-weekly 2 ml dose.
Relevance of the study	Viscosupplementation (VS) is a relatively new intervention in the treatment of osteoarthritis (OA), currently recommended by the main therapeutic guidelines.
Equivalent Device	Two different dosages of Ostenil (TRB Pharma) - intermediate molecular weight HA.
Study Design	Prospective randomized trial.
Study period	Not available.
Sample size	108 patients diagnosed with OA of the knee.
Inclusion Criteria	Inclusion criteria were as follows: 1. Fulfill the diagnostic criteria for OA of the American College of Rheumatology; 2. Understand, agree to and sign the informed consent form; 3. Absence of history of previous fracture of the knee to be studied; 4. Absence of history of allergy to any of the substances used; 5. Not have performed any infiltration in the studied knee in the last 6 months; 6. Be in treatment in the group for at least six months; 7. Not have made use of non-hortmonal anti-inflammatory agents in the last seve days.
Exclusion Criteria	Exclusion criteria were as follows: 1. Submit to surgery on the studied knee during the follow-up period; 2. Require further infiltration in the studied knee during the folow-up period - severe reaction to the procedure; 3. Development of active infection in the studied joint during the study; 4. Use non-hormonal anti-inflammatory agents at any time.
Intervention	The patients were randomized in two groups of 54 patients each. The groups were designated “single” (S) and “weekly” (W). The patients from group S underwent a viscosupplementation procedure through a single application of 6 ml of HA and 1 ml triamcinolone hexacetonide in the arthritic knee. The patients from group W underwent a viscosupplementation procedure through three applications with 2 ml of HA in the arthritic knee, with a one-week interval between them, and the first application also involved the infiltration of 1 ml (20 mg) of triamcinolone hexacetonide.

	The knee infiltration was performed with the patient seated with their knees at a 90-degree angle and legs off the gurney. The approach chosen for the articular injection was anterolateral. The procedures were executed by three investigators with experience in viscosupplementation. Soon after the procedure the patients were discharged without restrictions, with instructions to take 500 mg paracetamol every 6/6 hours for three days.
Outcomes	Both study groups were assessed before, at one month and three months after treatment application, by responding to the WOMAC[*1], Lequesne*[2], IKDC and VAS questionnaires.
Study Results **Performance**	An increase (improvement) in IKDC after one month in both groups and a small reduction after three months. Only the group that received the weekly application (W Group) presented an improvement in the WOMAC questionnaires and their pain subscale (WOMAC pain) over the course of treatment, particularly during the first month.
Study Results **Safety**	No safety results presented.
Limits of the study	Despite limiting the use of NSAIDs, the investigators did not limit the use of analgesics or any other non-pharmacological treatments, as they believed that viscosupplementation should not be the sole treatment in OA. Another limit is in the fact that clinical questionnaires such as WOMAC and Lequesne do not distinguish one knee from the other when the patient presents bilateral arthrosis. Lastly, the study did not have a placebo-controlled group. This is a non-inferiority study, where the goal was to evaluate whether they would find the same results obtained with the classical application regime through the single application regime.
Discussion	Both study groups exhibited an improvement after viscosupplementation, particularly in the first month. There was no statistical difference between the groups at any time. However, only the group submitted to the classical application regime (Group W) presented statistically significant improvement in the WOMAC, WOMAC pain and VAS questionnaires. The weekly application regime therefore exhibited superior analgesia in comparison to the single application regime. This was probably due to the ength of time the drug remains in the joint. The symptomatic and structural benefit promoted by viscosupplementation is obtained with a single treatment cycle, either composed of three to five weekly applications, as in the majority of hyaluronates, or through a single application, in the case of hylan. Therefore, the joint-drug contact duration will be able to define the magnitude of the changes promoted by this drug. It is known that the half-life of hyaluronate in the joint is 13 hours, which leads to a length of permanence of around seven days in the joint. Thus three to five weekly applications produce a joint-drug contact time between 21 and 35 days. Today the only drug whose single application is recommended presents intra-articular half-life of eight days, with continued presence in the joint for around 40 days. It is probably due to this reason that the use of intermediate molecular weight hyaluronate in a

	single dosage cannot promote sufficient time of contact of the drug with the joint. Accordingly, it is possible to speculate that a treatment with five weekly applications could produce an even better result.
Conclusions of the authors	The results of this study suggest that both application regimes improve function, but the regime of 3 weekly applications of 2 ml was more efficient at improving pain.

[*1] **WOMAC SCORE**

The WOMAC score (Western Ontario and McMaster Universities Osteoarthritis Index) is a validated score frequently used to assess pain, stiffness, and physical function in patients with hip and / or knee osteoarthritis (OA). The WOMAC consists of 24 items divided into 3 subscales:

- Pain (5 items): during walking, using stairs, in bed, sitting or lying, and standing;
- Stiffness (2 items): after first waking and later in the day;
- Physical Function (17 items): stair use, rising from sitting, standing, bending, walking, getting in / out of a car, shopping, putting on / taking off socks, rising from bed, lying in bed, getting in / out of bath, sitting, getting on / off toilet, heavy household duties, light household duties.

[*2] **Lequesne Score**

Lequesne *et al.* developed an index of severity for osteoarthritis for the hip (ISH). This can be used to assess the effectiveness of therapeutic interventions. The sections for index are: (1) pain or discomfort (2) maximum distance walked (3) activities of daily living.

Citation 4

Title	Jüni P, Reichenbach S, Trelle S, Tschannen B, Wandel S, Jordi B, Züllig M, Guetg R, Häuselmann HJ, Schwarz H, Theiler R, Ziswiler HR, Dieppe PA, Villiger PM, Egger M; Swiss Viscosupplementation Trial Group. **Efficacy and safety of intraarticular hylan or hyaluronic acids for osteoarthritis of the knee: a randomized controlled trial.** *Arthritis Rheum*. 2007 Nov;56(11):3610-9.
Aim of the study	The aim of this study was to compare the efficacy and safety of intraarticular hylan and 2 hyaluronic acids (HAs) in osteoarthritis (OA) of the knee.
Relevance of the study	Until recently, three viscosupplementation preparations were available in Switzerland, differing in terms of their origin, structure, molecular weight, and costs. These hyaluronic acid-based product, Synvisc, Orthovisc and Ostenil were compared and evaluated for their efficacy and safety. This article might be important to support the safety and the efficacy of viscosupplementation with hyaluronic acid.
Equivalent Device	High molecular weight cross-linked hylan derived from rooster combs (**Synvisc**; Genzyme, Cambridge, MA), 2) a non–cross-linked medium molecular weight HA derived from rooster combs (avian HA) (**Orthovisc**; Anika Therapeutics, Woburn, MA), or 3) a non–cross-linked low molecular weight HA obtained through bacterial fermentation (bacterial HA) (**Ostenil**; TRB Chemedica, Geneva, Switzerland).
Study Design	Multicenter, patient-blind, randomized controlled trial.
Study period	Between June 2003 and April 2004, patients were included in the trial.
Sample size	Two hundred twenty-two patients were allocated to receive hylan, 219 to receive avian HA, and 219 to receive bacterial HA. The sample size was calculated to detect a difference between groups of 0.8 units in standardized WOMAC pain scores for the pairwise comparisons of the hylan with each of the 2 conventional HAs, using Bonferroni correction and assuming an SD of 2. The difference of 0.8 units in standardized WOMAC pain scores corresponds to a difference in effect sizes of 0.4 SD units between hylan and HA that was expected from indirect comparisons derived from the meta-analysis by Lo *et al*. The authors calculated that a sample size of 200 patients per trial arm would provide >96% power to detect this difference with P set at 0.025.
Inclusion Criteria	Men and nonpregnant women with radiographically confirmed knee OA (Kellgren/Lawrence grade >=2 who were symptomatic for at least 6 months and reported pain on most days for the previous 3 months were eligible. Patients had an American College of Rheumatology functional class rating of II to IV and had not responded sufficiently to, or could not tolerate, acetaminophen or nonsteroidal antiinflammatory drugs (NSAIDs) taken regularly in adequate dosages.

Exclusion Criteria	Patients were excluded if they had inflammatory joint disease, chondrocalcinosis (evidence from radiographs or synovial fluid analysis), infection in or around the study knee, relevant skin disease in the area of the injection site, a history of allergy or intolerance to experimental preparations, or previous replacement surgery in the study knee, or if they were currently receiving anticoagulant therapy or had received previous viscosupplementation treatment within 6 months.
Intervention	Patients were randomly allocated to receive 1 cycle of 3 intraarticular injections of 2 ml per treated knee of 1 of the following 3 preparations: 1) a high molecular weight cross-linked hylan derived from rooster combs (Synvisc), a non–cross-linked medium molecular weight HA derived from rooster combs (avian HA) (Orthovisc), or 3) a non–cross-linked low molecular weight HA obtained through bacterial fermentation (Ostenil). Injections were administered at weekly intervals. The decision about whether bilateral knee OA required injections in both knees and the designation of the study knee remained at the discretion of the treating physician. One cycle per knee was allowed during the first 6 months of the trial. Intraarticular corticosteroid injections concurrent with the injection of viscosupplementation preparations were not permitted. It was originally planned to offer patients a maximum of 2 additional treatment cycles during months 7–18. Due to resource limitations, patients were offered only 1 additional treatment cycle of 3 injections per knee during months 7–12.
Outcomes	The primary outcome measure was the change in the pain score of the WOMAC, version 3.1, between baseline and 6 months, with individual items graded on a 5-point Likert scale from 0 to 4. Secondary outcome measures were the WOMAC global score and subscores on stiffness and disability; health-related quality of life based on the 5 dimensions and visual analog scale (VAS) of the European Quality of Life (EuroQol) questionnaire; self-reported health care utilization for knee disease; the frequency of local adverse events, defined as the occurrence of an effusion (evidence from clinical examination or arthrocentesis) or a flare (hot, painful, swollen knee occurring within 48 hours of injection of the study preparation); corticosteroid injections or treatment interruptions due to local adverse events; and the frequency of serious adverse events (adverse events leading to serious disability, hospital admission, or prolongation of hospitalization; life-threatening events; or death). All efficacy outcomes were assessed at 6 months using patient-administered mailed questionnaires and, if necessary, telephone calls by blinded interviewers. For exploratory purposes, the investigatorsperformed an interim followup at 3 months, which was restricted to the prespecified 50% random sample. After completion of each treatment cycle, information on serious and local adverse events was actively gathered from patients and physicians using mailed questionnaires or telephone calls by blinded interviewers.
Study Results	It could not be detected a difference in the WOMAC pain score between the hylan

Performance

group and the HA groups at 3 and 6 months. No differences were observed in the number of patients receiving intraarticular steroid injections in the 4 weeks before the 6-month assessment.

Results of the stratified analyses of the primary outcome are reported in the figure below.

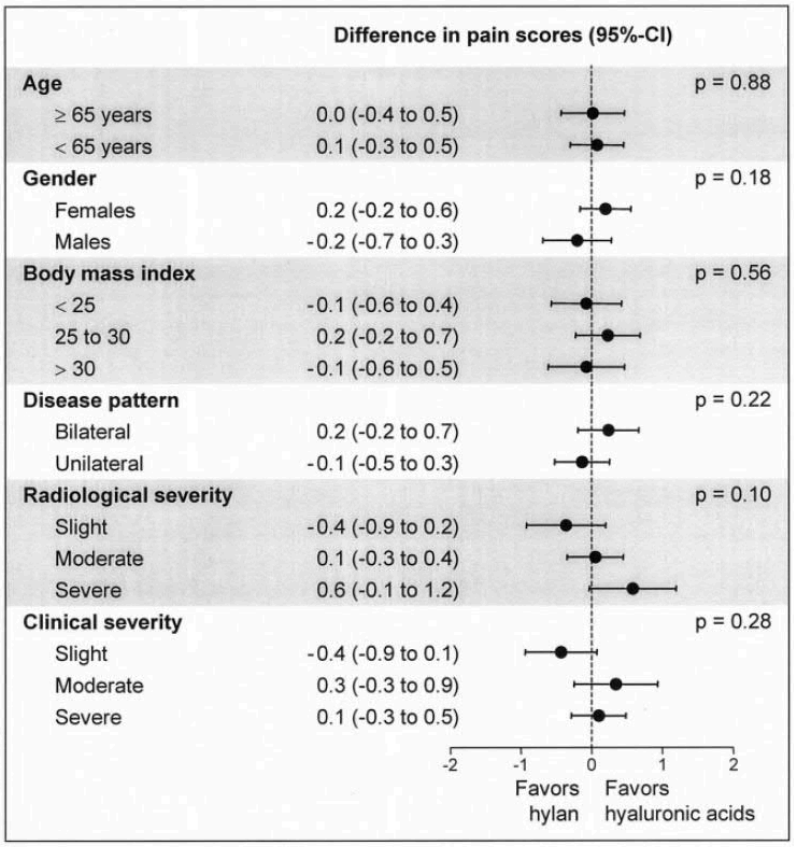


Figure 12. Results of the stratified analyses of the primary outcome according to the indicated characteristics. Values are differences in mean changes between hylan and the hyaluronic acids at 6 months, accoimpanied by 95% confidence intervals. p values are from tests of interaction between allocated treatment and stratum. Body mass index values are kg/m^2.

The difference in changes between baseline and 6 months between hylan and the HAs was 0.1 (95% CI -0.2, 0.4) for the WOMAC overall score, 0.1 (95% CI -0.3, 0.4) for the WOMAC stiffness score, and 0.1 (95% CI -0.2, 0.4) for the WOMAC disability score. There was little evidence for a difference between groups on the Euro-Qol VAS (0.1 [95% CI -0.2, 0.4]) and health state index (0.2 [95% CI -0.1, 0.4]).

There was no statistical evidence for differences between groups in the use of pain medication or other disease-specific treatments, including surgical interventions.

Study Results

Safety

Serious adverse events during the first cycle, which occurred in 15 of 222 patients allocated to receive hylan and in 25 of 438 patients allocated to receive HAs are shown in the following table.

	Hylan (n = 222)	Avian HA (n = 219)	Bacterial HA (n = 219)
Serious adverse events	15 (6.8)	12 (5.5)	13 (5.9)
ICD class (code)			
Neoplasms (C or D)	1 (0.5)	0 (0.0)	2 (0.9)
Endocrine and metabolic disorders (E)	1 (0.5)	0 (0.0)	0 (0.0)
Mental and behavioral disorders (F)	1 (0.5)	1 (0.5)	0 (0.0)
Disorders of the nervous system (G)	1 (0.5)	0 (0.0)	0 (0.0)
Disorders of the circulatory system (I)	2 (0.9)	3 (1.4)	2 (0.9)
Disorders of the respiratory system (J)	0 (0.0)	0 (0.0)	2 (0.9)
Disorders of the digestive system (K)	0 (0.0)	2 (0.9)	1 (0.5)
Disorders of the musculoskeletal system and connective tissue (M)	3 (1.4)	1 (0.5)	2 (0.9)
Disorders of the genitourinary system (N)	1 (0.5)	0 (0.0)	2 (0.9)
Symptoms, signs, and other disorders, not classified elsewhere (R)	1 (0.5)	2 (0.9)	0 (0.0)
Injuries and complications of health care, not classified elsewhere (S or T)	4 (1.8)	3 (1.4)	2 (0.9)

* Values are the number (%) of patients. HA = hyaluronic acid; ICD = International Classification of Diseases.

Table 13. Patients experiencing serious adverse eventsa during the first cycle (months 0-6).

There was little evidence for a difference between groups. Two serious adverse events were judged to be probably related to the evaluated intervention. These included 1 episode of septic arthritis, which occurred after injection of the avian HA, and 1 episode of anaphylactic shock, which occurred after injection of the hylan.

Three hundred thirty patients were randomly allocated to receive a second cycle of treatment with the originally assigned preparations, 110 in the hylan group and 220 in the HA groups.

Local adverse events occurred more frequently in the hylan group than in the HA groups (difference 6.4% [95% CI 0.6, 12.2]). This difference was most pronounced for flares (difference 6.4% [95% CI 1.8, 10.9]), but was apparent for all outcome measures (Table 14).

	First cycle			Second cycle		
	Hylan (n = 222)	HAs (n = 438)	Difference (95% CI)	Hylan (n = 110)	HAs (n = 220)	Difference (95% CI)
Local adverse event	21 (9.5)	32 (7.3)	2.2 (−2.4, 6.7)	10 (9.1)	6 (2.7)	6.4 (0.6, 12.2)
Type of local adverse event						
Effusion	7 (3.2)	14 (3.2)	0.0 (−2.9, 2.8)	8 (7.3)	6 (2.7)	4.6 (−0.8, 9.9)
Flare	19 (8.6)	23 (5.3)	3.3 (−0.9, 7.5)	7 (6.4)	0 (0.0)	6.4 (1.8, 10.9)
Corticosteroid injections because of local adverse event	5 (2.3)	5 (1.1)	1.2 (−1.1, 3.3)	4 (3.6)	0 (0.0)	3.6 (0.1, 7.1)
Treatment stopped because of local adverse event	2 (0.9)	6 (1.4)	−0.5 (−2.1, 1.2)	5 (4.5)	0 (0.0)	4.5 (0.7, 8.4)

* Values are the number (%) of patients. HAs = hyaluronic acids; 95% CI = 95% confidence interval.

	Table 14. Patients experiencing local adverse events during the first cycle (months 0–6) and the second cycle (months 7–12).
Limits of the study	This trial lacked a placebo control. Because of limited resources, the authors evaluated only 50% of patients at 3 months.
Discussion	We found no evidence for clinically relevant differences in efficacy between any of the 3 evaluated viscosupplementation preparations, either in the analysis of WOMAC pain scores or in analyses of secondary outcomes or the stratified analyses. The difference in the WOMAC pain score between hylan and the HAs corresponds to a difference in pain decrease of <1 mm on a VAS ranging from 0 to 100 mm. However, the most expensive, cross-linked, high molecular weight hylan was associated with a trend toward more local adverse events, particularly during the second cycle.
Conclusions of the authors	The authors found no evidence for a difference in efficacy between hylan and HAs. In view of its higher costs and potential for more local adverse events, we see no rationale for the continued use of hylan in patients with knee OA.

7.2.2 Indirect supportive data

Citation 5

Title	Petrella RJ, Decaria J, Petrella MJ **Long-term efficacy and safety of a combined low and high molecular weight hyaluronic acid in the treatment of osteoarthritis of the knee.** *Rheumatology Reports* 2011; 3: e4
Aim of the study	The aim of the study was to evaluate the long term efficacy and safety of a combined HA of low and high molecular weight and different concentrations (DMW) in comparison to low molecular weight (LMW 500-730 KDa) or high molecular weight (HMW 6000 KDa) HA products in reducing pain at rest and pain at walking associated with knee OA, as compared to placebo.
Relevance of the study	While a given HA product has a limited range of molecular weight typically low, medium or high, no product has been designed to provide a complement of composition that mimics the needs of active OA of the knee joint. These attributes may promote more beneficial rheological environment in the oesteoarthritic joint.
Equivalent Device	Lower (500-730 kDa - LMW), higher (6 million Da - HMW) or combined lower and higher MW (DMW) Sodium Hyaluronate. No further specification.
Study Design	Randomized, double-blind, placebo-controlled study.
Study period	Not available.
Sample size	A total of 225 patients were enrolled in the study. 25 were excluded, as they were not meeting the inclusion criteria (n=13) or they refused to participate in the study (n=12). A total of 200 were therefore randomized in the four groups (treatment 1: DMW, treatment 2: LMW, treatment 3: HMW, placebo: saline). The sample size was determined to allow the detection of a 20-mm difference in weight-bearing VAS at W16 assuming a standard deviation 10 mm of the mean distribution, and alpha of 5% and alpha beta level of 10%, giving a statistical power of 90%. With a potential dropout rate of 20%, the authors estimated a sample size of 225 patients.
Inclusion Criteria	Not available.
Exclusion Criteria	Not available.
Intervention	Two hundred eligible consented patients were randomized into four cohorts - active treatment 1 (DMW), active treatment 2 (LMW), active treatment 3 (HMW) and placebo (saline). Patients received intra-articular injection once weekly for three weeks and were followed up at week 16, 52 and 104.
Outcomes	Assessments were done at baseline, weeks 2, 3, 16, 52 and 104. Efficacy measures

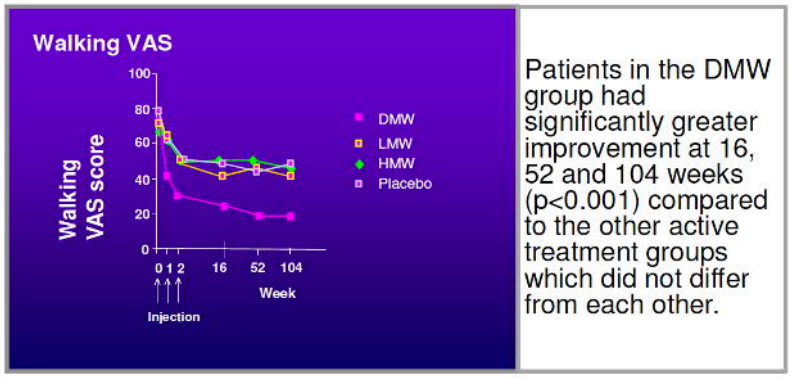
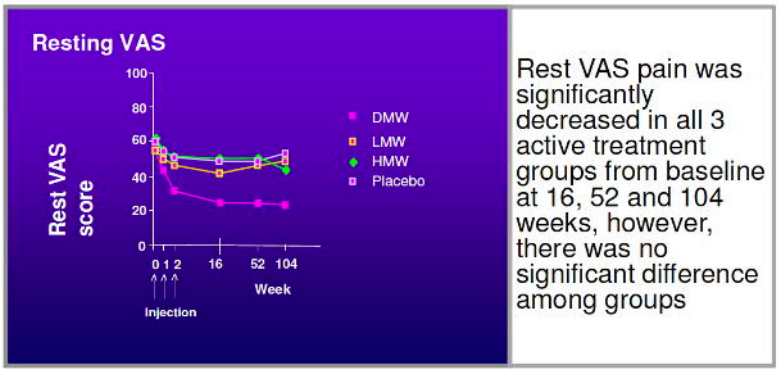
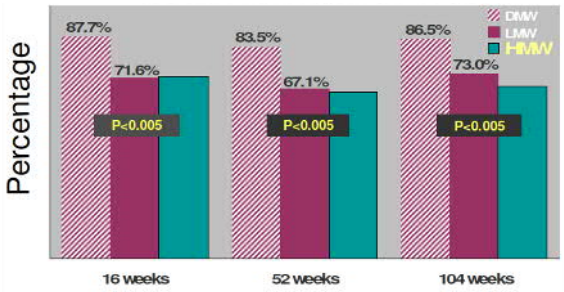
	included patient's visual analogue scale (VAS) of pain when seated (0-100 mm) and VAS of self-paced 40m walking pain. Other efficacy measures incuded the use of concomitant medications between groups, the review of adverse events, patient global satisfaction of knee osteoarthritis. At week 52, repeat intra articular injections were given to patients with walking VAS pain >45 mm.
Study Results Performance	At 16, 52 and 104 weeks respectively, walking VAS pain was significantly improved in all treatment groups vs. placebo: - DMW (89.3%, p< 0.001; 87.4%, p < 0.001; 88.1%, p < 0.001); - LMW (81.3%, p < 0.001; 78.2%, p < 0.001; 77%, p < 0.001); - HMW (78.1%, p < 0.001; 81.1%, p < 0.001; 79.4%, p < 0.001) At 52 weeks, 8 patients in DMW group has resting VAS < 45 mm. No patient in the LMW or HMW groups has VAS at rest. Similar differences were observed for walking VAS (77 mm vs 89 mm vs 91 mm, respectively). 39, 41 and 43 (DMW, LMW, HMW) received repeat injections. At 104 weeks, these differences were similar.  **Figure 13**. Walking VAS. 

	Figure 14. Resting VAS. There were no differences among the active treatments for concomitant OA medications. There was no significant change in concomitant medications at any of the study timepoints. Global patient satisfaction was significantly higher for the DMW group compared to the others, at 16, 52 and 104 weeks (p< 0.005).  **Figure 15.** Global patients' satisfaction.
Study Results Safety	There were no serious adverse events. DMW and LMW had no reported side effects. HMW had two local reactions at 52 weeks and 1 at 104 weeks. DMW and LMW had no reported adverse events; HMW had 2 local reactions at 52 weeks and 1 at 104 weeks. Non-serious adverse events included pain and local swelling at the injection site (21%), erythema at the injection site (12%) and stiffness in the index knee (7%).
Limits of the study	Not available.
Discussion	Greater improvement in patients who received the DMW product was achieved by the second injection persistent to 104 weeks. Combination of sodium hyaluronate of lower and higher ranges of molecular weight with low and high concentrations, may provide patients with a more physiologically dynamic HA viscosupplementation and hence a more responsive synovial rheology that improves pain and function in their osteoarthritic knee.
Conclusions of the authors	Intra-articular hyaluronan injections using any of low, high or combined molecular weight hyaluronic acid, were highly effectuive in improving resting and more so, walking pain in patients with OA of the knee. Greater improvement in both rest and activity outcomes in patzients receivcing the DMW, with concomitantly greater patient satisfation and fewer use of concomitant therapeutic modalities at 16, 52 and 104 weeks suggest that combining a range of molecular wright hyaluronic acid may be advantageous long-term, particularly among active OA patients.

Citation 6

Title	Roux C, Fontas E, Breuil V, Brocq O, Albert C, Euller-Ziegler L **Injection of intra-articular sodium hyaluronidate (Sinovial) into the carpometacarpal joint of the thumb (CMC1) in osteoarthritis. A prospective evaluation of efficacy.** *Joint Bone Spine* 2007; 74: 368-372
Aim of the study	The goal of the present prospective study was to investigate a difference of efficacy in pain relief of one, two or three injections of hyaluronic acid in OA of the CMC1 (carpometacarpal joint of the thumb). The authors studied the effect of injections all the study long and looked for a difference in efficacy at three months on pain and functionality.
Relevance of the study	CMC1 intraarticular hyaluronic acid injections literature is poor. Viscosupplementation with hyaluronic acid injections seems to relieve pain and improve function in the management of OA in various joints, principally the knee, so the authors investigated the role of hyaluronan injections in the management of OA of the CMC1.
Equivalent Device	**Sinovial**
Study Design	Prospective randomized study.
Study period	October 2003-June 2005.
Sample size	Fourty-four subjects were enrolled. Five patients were lost to follow up between one and three month (two each in groups 1 and 3, one in group 2). All five exhibited X-ray grade 3. The reasons for drop out were lack of efficacy (n = 3) and non-attendance to scheduled visits (n = 2). Each of the three treatment groups comprised 14 patients.
Inclusion Criteria	Patients with symptomatic OA of the CMC1 joint (visual analogue scale [VAS] <40) and refractory to other therapeutic interventions were enrolled. All subjects had CMC1 OA grade 2-4 according to Kellgren and Lawrence on standard X-ray within 6 months of inclusion, and met ACR criteria for hand OA.
Exclusion Criteria	Exclusion criteria included symptomatic OA in any other digit, use of steroid injection in the previous 6 months and previous use of sodium hyaluronidate injection. Subjects with blood coagulation abnormalities, infection, and hand trauma were also excluded.
Intervention	Subjects were randomly assigned to receive one, two or three injections. Group 1 received a single injection; group 2 received a second injection after one week; and group 3 received three injections, at weekly intervals. To receive the treatment, patients sat with the affected hand in a semi-prone position on a table. The intercarpometacarpal space was identified by palpation, the needle tip inserted lateral to the abductor pollicis longus tendon and the injection carried out under radioscopic control. Needle tip position was confirmed using an image intensifier. One ml was injected to each patient. Standard X-ray was

	systematically performed.
Outcomes	The primary efficacy endpoint was the comparison of time course changes in VAS score. The secondary efficacy parameter was the comparison of time course changes in Dreiser score. Also, within each group, differences in VAS and in Dreiser score were assessed between baseline, M1 and M3 using a Wilcoxon's test.
Study Results **Performance**	In group 1, the mean VAS was 58.4 (16.2) at baseline, 46.2 (21.9) at month 1, and 43.1 (22.8) at month 3. Dreiser test results were 12.1 (5.2), 9.0 (5.1), and 9.7 (4.9), respectively. The reduction in pain (VAS) between baseline and different evaluations times did not reach statistical significance (Baseline-3 months: p = 0.18; baseline-1 month: p = 0.09). In group 2, the mean VAS was 54.6 (18.9) at baseline, 48.1 (27.9) at month 1, and 39.5 (28.6) at month 3. Dreiser values were 13.4 (5.9); 10.7 (9.7), and 10.1 (7.9), respectively. Pain reduction (VAS) between baseline and different evaluation times was statistically significant (Baseline-3 months: p =0.01; baseline-1 month: p = 0.01). In group 3, the mean VAS value was 60.1 (17.0) at baseline, 28.4 (20.8) at month 1, and 29.8 (21.9) at month 3. Dreiser test values were 11.9 (6.6), 5.9 (3.7), and 7.1 (4.6), respectively. Pain reduction (VAS) between baseline and 3 months was statistically significant (p = 0.002) as between baseline and 1 month (p = 0.001). No significant difference was found between 1 month and 3 month VAS (p = 0.5).

	Inclusion Mean (SD)	Month 1 Mean (SD)	Month 3 Mean (SD)	*p* value[a]
Group 1				
VAS	58.4 (16.2)	46.2 (21.9)	43.1 (22.8)	0.182
Dreiser	12.1 (5.2)	9.0 (5.1)	9.7 (4.9)	0.136
Group 2				
VAS	54.6 (18.9)	48.1 (27.9)	39.5 (28.6)	0.013
Dreiser	13.4 (5.9)	10.7 (9.7)	10.1 (7.9)	0.040
Group 3				
VAS	60.1 (17.0)	28.4 (20.8)	29.8 (21.9)	0.002
Dreiser	11.9 (6.6)	5.9 (3.7)	7.1 (4.6)	0.002

[a] Baseline *versus* month 3.

Table 15. Comparison of mean VAS and Dreiser test values between inclusion and months 1 and 3 in three groups.

Study Results **Safety**	Injections were well tolerated. Pain and/or swelling and/or heat and/or redness, always moderate happened equally in the 3 groups in about 30% of cases. When occurring they lasted less than 3 h in most cases, and always less than 2 days in few cases. No septic arthritis was observed.
Limits of the study	The authors performed an open study without control which did not permit to take into account placebo effect which is generally supposed to be great in osteoarthritis injection studies.

Discussion	The present pilot study did not show any difference in effect on pain and function depending on number of injections. Nevertheless, intragroup analyses suggest that an effect on VAS exists as significant differences between baseline and 3 months have been assessed for groups 2 and 3 with an efficacy as early as the first month. Such a difference is not seen in group 1. However, it is important to note that most radiological severe cases were in group 1, even if not statistically significant; furthermore, clinical response is probably dependent on the radiological lesions. One month after injection (baseline) with a shorter delay between injection and evaluation in group 3 is a possible reason of stronger re-sponse at one month in this group. The efficacy however persist at three months.
Conclusions of the authors	In this small prospective evaluation study no significant differences were found between the groups all the study long for pain relief and function, so the authors did conclude a dose-effect. However, the results show that intra-articular sodium hyaluronidate injections into the carpometacarpal joint of the thumb in OAcan be efficacious on pain and functionality as early as the first month with persistant effects at 3 months.

Citation 7

Title	Berenbaum F, Grifka J, Cazzaniga S, D'Amato M, Giacovelli G, Chevalier X, Rannou F, C Rovati L, Maheu E **A randomised, double-blind, controlled trial comparing two intra-articular hyaluronic acid preparations differing by their molecular weight in symptomatic knee osteoarthritis.** *Ann Rheum Dis 2012*
Aim of the study	The objective was to compare the effects of an intermediate molecular weight (MW) intra-articular hyaluronic acid (HA) with a low MW product on knee osteoarthritis (OA) symptoms.
Relevance of the study	Low MW HA thus often remains the preferred option when using HA in knee osteoarthritis. However, there is a paucity of appropriately sized, high-quality trials comparing the effects of different MW preparations, with particular regard to potential differences between low and intermediate MW products, given the worse safety profi le of high MW formulations. This study was therefore designed to compare the effects of the reference low MW HA product (Hyalgan) with a well-characterised intermediate MW preparation (GO-ON) on knee osteoarthritis symptoms.
Equivalent Device	**GO-ON** (Rottapharm|Madaus, Monza, Italy) is a preparation of sodium hyaluronate obtained by fermentation from *Streptococcus equi*, with an intermediate MW (range 800.000-1.500.000 Daltons), presented in 2.5 ml prefilled syringes and a concentration of 10 mg/ml. **Hyalgan** (Fidia Abano Terme, Italy) is sodium hyaluronate derived from rooster combs, with a low MW (range 500.000-730.000) and a concentration of 10 mg/ml in 2 ml. Both preparations are recommended for cycles of 3–5-weekly injections, but studies have shown no apparent difference between the two regimens with Hyalgan.
Study Design	Multicentre (50 orthopaedics and/or rheumatology practice sites in France and Germany), prospective, randomised, double-blind, controlled, parallel-group trial.
Study period	The first patient was enrolled in November 2008 and the last patient was completed in November 2009.
Sample size	The sample size was calculated assuming a SD of 23 mm, based on a previous study with Hyalgan and conservatively increased by 15%, resulting in 144 patients per group in the PP population to achieve a power of 90% at a signifi cance level of 5%. Assuming a 30% discontinuation rate, this was increased to 200 patients per group in the ITT population, which consisted of all randomly assigned patients with at least one injection and one post-injection assessment of the primary endpoint. For drop-outs and other exclusions from the PP population, missing values were replaced by the baseline value, according to the baseline observation carried-forward approach. Out of 437 patients randomly assigned, 217 and 209 in the GO-ON and Hyalgan

	groups, respectively, were included in the ITT population, thus excluding only 11 patients according to the predefined criteria.
Inclusion Criteria	Patients of either sex, aged 50–80 years, fulfilling the American College of Rheumatology clinical and radiological criteria for knee osteoarthritis, were enrolled if they had a history of symptoms for at least 6 months and insufficient/failed response to analgesics and/or regular non-steroidal anti-inflammatory drugs (NSAID), or were intolerant to regular NSAID or weak opioids. Current symptoms (after ≥ 2 days wash-out from NSAID, including topical agents, or 1 day from non-narcotic analgesics) had to include global knee pain of 40 mm or greater on a 100 mm visual analogue scale (VAS), Western Ontario and McMaster Universities (WOMAC) pain subscale score of 25 or greater on the 0–100 normalised scale and Lequesne index of 4 or greater. x-Rays (past 12 months) had to show Kellgren and Lawrence stage II or III; radiological evidence of bilateral knee osteoarthri-tis was accepted if global pain VAS in the contralateral knee was less than 30 mm.
Exclusion Criteria	Main exclusions were: isolated/predominantly patellofemoral symptomatic osteoarthritis, secondary knee osteoarthritis, symptomatic hip osteoarthritis homolateral to the target knee, infl ammatory or other rheumatic diseases, clinical joint effusion, excessive (≥ 8°) varus or valgus knee deformity (at physical examination, as confirmed by standard radiograph).
Intervention	Patients received 3-weekly injections of the test or comparator preparations (1:1 allocation ratio) and were then seen at weeks 6, 14, 20 and 26, ie, 4, 12, 18 and 24 weeks following the end of treatment.
Outcomes	The pain subscale of the WOMAC osteoarthritis index (VAS version VA3.1) was the study primary endpoint. Results were normalised on a 0–100 scale for each domain, with the total index, physical function and stiffness subscales being assessed as secondary endpoints. Other secondary efficacy endpoints included: global knee pain during the past 48 h on a 0–100 mm VAS; the Lequesne algofunctional index; the intermittent and constant osteoarthritis pain (ICOAP) index on the 0–100 score transformation recommended by the OARSI and outcome measures in rheuma-tology (OMERACT); patient global assessment (PGA) on a 100 mm VAS (see supplementary material 2, available online only, for the exact wording of this and the global knee pain VAS ques-tion); the proportion of OARSI/OMERACT responders. The proportion of patients achieving the minimum clinically important improvement (MCII) and patient acceptable symptom state (PASS) was also calculated for global pain VAS, WOMAC function subscale and PGA. Consumption of the rescue medica-tion was another effi cacy endpoint. With regards to safety, particular attention was paid to local painful reactions at the injection site, postinjection reactions (e.g. effusions) and acute pseudoseptic arthritis.
Study Results **Performance**	Patients in both groups improved markedly during the first month after treatment and the effect was maintained for the duration of the study, with GO-ON exhibiting

an overall better trend, that was particularly consistent between 12 and 24 weeks after the end of treatment. After 6 months from the end of treatment (week 26), patients who had received GO-ON had decreased their WOMAC pain score by 22.9±1.4 mm (mean±SE), compared with 18.4±1.5 mm with Hyalgan in the ITT population.

It was concluded a statistical superiority of GO-ON versus Hyalgan (p=0.021).

Global knee pain VAS decreased by over 50% with GO-ON at week 26, but less with Hyalgan (effect size 0.26). A similar degree of effi cacy was detected for all WOMAC scales and the Lequesne index underwent an over 4-point decrease with GO-ON versus 3 points with Hyalgan (effect size 0.34). The degree of improvement was similar for the ICOAP index, but the difference between groups was barely significant only for constant pain, while the two preparations behaved similarly on intermittent pain. Patients had also improved their global assessment VAS by almost 20 mm with GO-ON, but the better trend versus Hyalgan was not significant in ITT (p=0.068), but only in the PP analysis (p=0.044).

There were 73% OARSI/OMERACT responders 6 months after the end of treatment with GO-ON, versus 58% with Hyalgan (difference 14.9%, p=0.001). The proportion of patients achieving MCII and PASS for global knee pain, function and PGA was also high with both treatments but significantly higher with GO-ON than with Hyalgan except for global pain PASS and MCII for PGA.

All superiority trends were similar at the 12-week endpoint (data not shown), with a signifi cant difference in the proportion of OARSI/OMERACT responders, 69.6% with GO-ON versus 60.3% with Hyalgan (p=0.044).

Patients used the rescue medications in a similar proportion: 166 out of 217 on GO-ON (77%) and 154 out of 209 (74%) with Hyalgan (p=0.50), with a low paracetamol daily mean consumption (218 and 223 mg/day, respectively, p=0.60).

Study Results

Safety

GO-ON and Hyalgan were equally well tolerated at the injection site. The proportion of patients reporting any AE in the safety population was similar: 74 out of 223 (33.2%) and 75 out of 213 (35.2%) with GO-ON and Hyalgan, respectively, most AE being unrelated to treatment.

Table 16 shows number - and proportion - of patients with local adverse events at the injection site in the safety population.

	GO-ON (n=223)	Hyalgan (n=213)	p Value
Joint effusion/swelling	1 (0.4%)	4 (1.9%)	
Joint pain	3 (1.4%)	2 (0.9%)	
Injection site haematoma	0 (0.0%)	2 (0.9%)	
Injection site warmth	0 (0.0%)	1 (0.5%)	
Total number of patients with any of the above local AE	4 (1.8%)	8 (3.8%)	0.17

AE, adverse event.

	Table 16. Number and proportion of patients with local AEs at the injection site.
Limits of the study	There was no placebo comparison. This might have been an issue in the case of results limited to non-inferiority, given the debated efficacy of HA in general. Moreover, ethics review boards might have raised ethical concerns in using intra-articular saline as a placebo when HA injections are now widely prescribed in knee OA. Moreover, it was not possible to provide identically appearing test and comparator preparations: the commercial preparations had to be used for obvious reasons, after appropriate packaging, and their effects may also differ given the different injected volumes. On the other hand, double-blind conditions were ensured by nominating at each site an 'injector' and a blinded 'assessor' investigator, while avoiding the patient's visual access to the injection field. Another limiutation was in the fact that his was a regulatory trial that was therefore industry-funded. However, an independent steering committee supervised the trial design and study conduct, participated in blind data review meetings before database lock and provided binding recommendations for data management, finally accessing all results. A fourth limitation was that the present trial only compared the intermediate HA product GO-ON with the reference low MW preparation, but not with a higher MW, cross-linked, formulation.
Discussion	Joint function improved to a similar extent and there were over 65% treatment responders on average with the two preparations used. The intermediate MW HA formulation GO-ON was not inferior to the reference low MW preparation Hyalgan on the WOMAC pain subscale score, but was also statistically superior on this primary and on most of the secondary outcomes as predetermined by the statistical analysis plan. While GO-ON tended to exhibit a trend for a better pattern of response throughout the study, both treatments behaved similarly well over the first month, when most of the therapeutic gain was observed, and during the first 3 months following the injection course. Afterwards, the benefit obtained with the low MW product tended to plateau, as acknowledged with most HA preparations, while there was a slight continuous improvement with GO-ON, i.e. a more pronounced carry-over effect resulting in a statistically significant superiority on most outcomes after 6 months.
Conclusions of the authors	This trial showed that the intermediate MW HA preparation GO-ON is effective on knee osteoarthritis symptoms over 6 months after a 3-weekly injection course, and may be more effective than the reference low MW formulation.

Citation 8

Title	Atay T, Aslan A, Baydar ML, Ceylan B, Baykal B, Kiridemir V **The efficacy of low- and high-molecular-weight hyaluronic acid applications after arthroscopic debridement in patients with osteoarthritis of the knee.** *Acta Orthop Traumatol Turc,* 2008; 42(4): 228-233
Aim of the study	The authors evaluated the efficacy of viscosupplementation with low- or high-molecular-weight hyaluronic acid (HA) preparations following arthroscopic debridement (AD) in patients with osteoarthritis of the knee.
Relevance of the study	It has been reported that intra-articular hyaluronic acid treatment resulted in positive outcome following 3 to 12 months after the treatment. Good results were reported from studies conducted on the effects of intra-articular hyaluronic acid following arthroscopic debridement. However, before to this study, no controlled study existed that was aimed to compare the viscosupplementation results established from the patients treated with various hyaluronic acid preparations of different molecular weights.
Equivalent Device	Synvisc and Hyalgan.
Study Design	Designed in a blinded, randomized and controlled fashion.
Study period	Not available.
Sample size	The study included 45 patients.
Inclusion Criteria	Patients diagnosed as having knee osteoarthritis according to the criteria of ACR (American College of Rheumatology), who still remained untreated even after three months of conservative treatment were included in this study. In all patients, there was an osteoarthritis manifestation at stage II or III.
Exclusion Criteria	Exclusion criteria were as follows: Patients with allergic disorder, oral or intramuscular corticosteroid agent administration history in last two months, individuals who have severe systemic disorders, intra-articular therapy history to the evaluated knee in last three months or arthroscopic intervention history in last three years.
Intervention	All patients were undergone AD intervention under general anesthesia. Following the surgical operation, the patients were seperated into three groups: Synvisc group (n=16), Hyalgan group (n=14) and controls (n=15). Each patient was only given an envelope which includes a number showing a number representing the treatment type and none of the participants was told which kind of treatment was they were administered. After the AD operation, Hylan G-F 20 at 2 ml dose was administered once a week for three weeks to Synvisc group, whereas sodium hyaluronate at 2 ml dose was given to Hyalgan group once a week for five weeks. Control group did not receive any injection treatment following AD. After the surgery, all patients were applied compressive elastic bandage, active quadriceps exercise and continuous passive motion program. Also, tiaprofenic acid (Surgam Aventis, Turkey) treatment was initiated for all subjects. Patients were discharged

	from the hospital after three days from the operation with a home exercise programme.
Outcomes	All participants were evaluated before and 6 / 12 months after the operation by means of pain, joint stiffness and physical function via using WOMAC (western Ontario and McMaster Universities) Index For Osteoarthritis.
Study Results **Performance**	Post-operative WOMAC scores were prominently lower in whole groups, when compared with the scores established prior to the operation. There was a statistically significant difference in WOMAC scores measured before the operation and 6 / 12 months after the operation (p=0.000, p=0.001 and p=0.001, respectively). As there was a significant difference in preoperative WOMAC scores, improvemet rates in WOMAC values were calculated for each group in order to evaluate the efficiency of AD operation applied in combination with viscocupplementation treatment. There was a significant decrease especially in WOMAC scores recorded at 12th month and this difference was present for both Synvisc and Hyalgan groups, when compared with the controls (p=0.004 and p=0.003. respectively). However, there was no significant difference between tyhe Synvisc and Hyalgan groups (p=0.616).
Study Results **Safety**	Not available.
Limits of the study	Not available.
Discussion	The results of this study indicated that hyaluronic acid preparations with low and high molecular weight have no superiority to each other in ameliorating gonarthrosis symptoms. However, when it is taken into consideration that comparison studies are generally focused on gonarthrosis patients who have not been undergone surgical intervention yet, it should be kept in mind that AD operation may lead to some alterations in pathogenetical mechanisms which underlies the symptoms of gonrathrosis, thereby cause a difference in outcomes of hyaluronic acid treatment approaches between operated and unoperated joints. For this reason, some issues such as whether hyaluronic acid treatment is necessary following an arthroscopic intervention to knee and if so, which hyaluronic acid type should be preffered requie more comprehensive clinical, radiological and biological studies in order to be elucidated clearly.
Conclusions of the authors	The authors concluded that AD is beneficial in osteoarthritis of the knee in patients with appropriate indications, viscosupplementation increases the efficacy of treatment, and that low- and high-molecular-weight HA preparations have similar efficacy.

Citation 9

Title	Lucas Y Hernandez J, Darcel V, Chauveaux D, Laffenêtre O. **Viscosupplementation of the ankle: a prospective study with an average follow-up of 45.5 months.** *Orthop Traumatol Surg Res*. 2013 Sep;99(5):593-9
Aim of the study	This study had three objectives: validate a three-injection treatment protocol, verify the efficacy of HA in the ankle and look for factors that are predictive of the response.
Relevance of the study	Viscosupplementation, which consists of the intra-articular injection of hyaluronic acid (HA) in order to correctquantitative and qualitative changes in endogenous HA, seems to relieve the symptoms of osteoarthritis. Multiplestudies have looked at the efficacy of viscosupplementa-tion for knee osteoarthritis and have found mostly positiveresults. HA has a visco-inductive effect in vitro: the addition ofexogenous HA induces the synthesis of HA. This property ismore apparent with high-molecular weight hyaluronic acid. It contributes to its regeneration and limits interleukin-1related inflammation. It also has an analgesic effect and a chondroprotective effect. The HA concentration is 2—3 mg/ml in normal joints and is reducedto 0.8—2 mg/ml in joints of arthritic patients. From aqualitative point of view, pathological HA molecules are 0.5and 4.0 MDa (millions of Dalton) in size, versus 5.0 in normalcases. In its altered state, HA contributes to inflam-mation and no longer has lubricating and hydrophilicproperties. Viscosupplementation is an accepted treatment modality for knee osteoarthritis.
Equivalent Device	High-molecular weight HA (Synvisc® 6000 kD, 2 ml)
Study Design	Prospective
Study period	January 2003-December 2009.
Sample size	Thirty-three patients met the inclusion criteria. Patients with an associated surgery were excluded to obtain a case series of patients only treated by viscosupplementation. At the end, 18 patients (26 ankles) corresponded to study criteria were the subject of the study.
Inclusion Criteria	Inclusion criteria were: patients presenting with Grade 1 or 2 talocrural osteoarthritis based on the Morrey and Wiedeman classification (Grade 0: normal ankle, Grade 1: small osteophytes and minimal joint narrowing, Grade 2: moderate osteo-phytes and moderate joint narrowing, Grade 3: significant narrowing with joint deformation or fusion), that had been progressing for at least one year and that was resistant to traditional conservative analgesic treatment.
Exclusion Criteria	Exclusion criteria consisted of a corticosteroid injection within the last month, systemic or local infection, coagulation problems, history of open fracture, known allergy to hyaluronic acid or associated conservative surgery proce-dure

	(arthroscopy, calcaneal osteotomy).
Intervention	The treatment consisted of multiple injections from an ampule of high-molecular weight HA (Synvisc® 6000 kD, 2 ml) using a standardized technique. The protocol always comprised three injections, 15 days apart; this was considered as one series. The injections were always performed on an outpatient basis in the operating room using fluoroscopy system. No contrast product was used. The fluoroscopy allowed us to verify the needle position. After mobilization of the ankle, full weight-bearing was allowed immediately, while advising the patient to get rest during the following days. A new series of three injections could be repeated after the effect had worn off after a period of 12 months. Each series was considered as an independent parameter from a statistical point of view. In cases of treatment failure or no response to treatment, which was defined as a significant drop in the AOFAS score (below 40/100), a radical surgery procedure was proposed to the patient.
Outcomes	The treatment efficacy was evaluated at 4 months, 12 months and then every year thereafter using the ankle functional score in the AOFAS and a four-level patient satisfaction scale (very satisfied, satisfied, disappointed or dissatisfied). The evaluation was performed by an independent evaluator. All adverse effects were evaluated.
Study Results **Performance**	The average follow-up was 45.5 months (range 22.5-71.8), with no patients being lost to follow-up. With regard to subjective effect of the injections, nineteen of the 26 injection series were evaluated was being satisfactory. With regard to the AOFAS score, the average score went from 61.8 ± 15.0 before the viscosupplementation to 73.7 ± 16.6 at 12 months after, with variations seen depending on the initial AOFAS grouping (Table 17). **Table 17**. Change in the overall AOFAS score and by stage. Of the 18 study patients, three failures were noted after the first series and two after the second series; the initial positive effect lasted for 24 months on average. Ankle joint replacement was proposed in three cases and tibio-talocalcaneal fusion in two cases. For these five patients, the average time between the first injection and this radical surgery was 27 months (range 10-43).
Study Results **Safety**	During all study, no adverse effects were reported.
Limits of the study	The main limitations of the current study are the small sample size, lack of a control

	Before	4 months	12 months
AOFAS overall	61.8 ± 15	74.4 ± 14.5	73.7 ± 16.6
AOFAS stage I (*n* = 5)	40.2 (24–48)	56.2 (49–73)	60.8 (46–76)
AOFAS stage II (*n* = 17)	63.2 (51–74)	76.0 (47–98)	73.6 (43–97)
AOFAS stage III (*n* = 4)	83.2 (79–89)	90.2 (89–92)	90.2 (89–92)

	group and lack of control over the oral analgesics taken by the patients. Nevertheless, the inclusion criteria were ankle osteoarthritis pain that had not been alleviated with common analgesics for at least one year.
Discussion	According to investigators' opinion, the use of this three-injection protocol is essential, as is fluoroscopy. The study confirmed the level I studies showing superiority of viscosupplementation over placebo for the ankle. The current study showed that viscosupplementation was effective against pain. This effect was not correlated to the initial condition of the ankle. The change in the AOFAS score showed that the treatment efficacy was extended significantly during the entire year after the injection series. In this series, the treatment efficacy extended to an aver-age of 27.8 months, which is greater than the accepted protocol for the knee (repeated yearly). In authors' opinion, one of the basic requirements is that injections be performed under fluoroscopy. This procedure avoids the injection site complications found in other studies (inflammation and pain at the injection site) and ensures that good results are achieved.
Conclusions of the authors	This study confirmed the efficacy of viscosupplementation using a protocol of three consecutive injections, 15 days apart for all patients with ankle osteoarthritis, no matter the etiology, having Grade 1 or Grade 2 disease according to the Morrey and Wiedeman classification. This effect was apparent at four months and was maintained out to 12 months; it became less marked after about 28 months on average. Also, since a certain number of patients eventually fail with this treatment, our study showed that this option delayed radical surgery by an average of 27 months.

Citation 10

Title	Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, Fornasari PM, Giannini S, Marcacci M. **Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis.** *Arthroscopy*. 2011 Nov;27(11):1490-501
Aim of the study	The aim of our study was to compare the efficacy of platelet-rich plasma (PRP) and viscosupplementation (hyaluronic acid [HA]) intra-articular injections for the treatment of knee cartilage degenerative lesions and osteoarthritis (OA).
Relevance of the study	The current clinical solutions from OA suffer from significant limitations, such as safety and effectiveness, and they are not able to completely restore the patient's mobility and quality of life. Research is studying innovative approaches of stimulating repair or replacing damaged cartilage, and studies regarding tissue biology have highlighted a complex regulation of growth factors (GFs) for the normal tissue structure and the reaction to tissue lesions. In fact, the role of GFs in chondral repair is now widely investigated in vitro and in vivo. Platelet-rich plasma (PRP) is a simple, low-cost, and minimally invasive method that allows one to obtain from the blood a natural concentrate of autologous GFs. The hypothesis of the investigators in this case was that PRP would improve symptoms and function, possibly through the release of GFs and bioactive molecules, in patients affected by knee degeneration.
Equivalent Device	Platelet-rich plasma (PRP) and viscosupplementation (hyaluronic acid [HA]), i.e. igh–molecular weight (HW) HA (30 mg/2 mL of HA with molecular weight 1,000 to 2,900 kDa) and the other with low–molecular weight (LW) HA (20 mg/2 mL of HA with molecular weight 500 to 730 kDa).
Study Design	Prospective comparative study.
Study period	Not available.
Sample size	A total of 150 patients were enrolled.
Inclusion Criteria	The following diagnostic criteria for patient selec-tion were used: patients affected by a unilateral lesion with a history of chronic (>=4 months) pain or swelling of the knee and imaging findings (radiog-raphy or magnetic resonance imaging [MRI]) of degenerative changes of the joint.
Exclusion Criteria	Exclusion criteria included systemic disorders such as diabetes, rheumatic diseases, hematologic diseases (coagulopathies), severe cardiovascular diseases, infections, immunosuppression, patients receiving therapy with anticoagulants-antiaggregants, use of non-steroidal anti-inflammatory drugs in the 5 days before blood donation (for reasons of caution, because disagreement exists on the use of concomitant

	non-steroidal anti-inflammatory drugs before the PRP treatment), and patients with hemoglobin (g/dl) values of less than 11 and platelet values of less than 150,000/cubic mm.
Intervention	For this study, 150 consecutive patients affected by cartilage degenerative lesions (Kellgren grade 0) (Fig 1), early OA (Kellgren grade I to III), and severe OA (Kellgren grade IV) were enrolled and treated with intra-articular knee injections. One-third of the patients underwent previous knee surgery, but surgery was performed at least 1 year before the injectable treatment. Among these patients, 50 were treated with 3 autologous PRP intra-articular injections, whereas 2 homogeneous groups of patients were treated with HA injections, 1 with high–molecular weight (HW) HA (30 mg/2 mL of HA with molecular weight 1,000 to 2,900 kDa) and the other with low–molecular weight (LW) HA (20 mg/2 mL of HA with molecular weight 500 to 730 kDa). After the injection, the patients were sent home with instruc-tions on limiting the use of the leg and to not use nonsteroidal medication but to use cold therapy for pain for at least 24 hours. During the injection cycle, rest or mild activities (such as exercise bike or mild exercises in a pool) were indicated, and subsequently, a gradual resumption of normal sport or recreational activities was allowed as tolerated in all the treatment groups.
Outcomes	All the patients were prospectively evaluated at 2- and 6-month follow-up visits. Subjective International Knee Documentation Committee (IKDC) and EQ VAS scores (as recommended by the International Cartilage Repair Society evaluation package) were used for clinical evaluation. Adverse events and patient satisfaction were also recorded.
Study Results **Performance**	A statistically significant improvement in all clinical scores from basal evaluation to the 2- and 6-month follow-up visits was observed in all treatment groups. In the PRP group a higher IKDC improvement at 6 months was observed in patients affected by cartilage degeneration compared with patients affected by early OA ($P = 0.004$) or advanced OA ($P < 0.0005$). In the LW HA group patients affected by advanced OA showed worse IKDC results at 2 months compared with patients affected by cartilage degeneration ($P = 0.001$) or early OA ($P = 0.002$). In the HW HA group higher EQ VAS results were found at 2 months in patients affected by cartilage degeneration compared with patients af-fected by early OA ($P = 0.003$) or advanced OA ($P = 0.05$). Comparison of the satisfaction level obtained in the 3 groups showed a significant difference, with a higher number of satisfied patients in the PRP group (82% [41 of 50] v 64% [32 of 50] in the LW HA group and 66% [33 of 50] in the HW HA group; $P = 0.04$). At the 2-month evaluation, the same results were found in the PRP and LW

	HA groups, whereas lower IKDC (P = 0.009) and EQ VAS (P = 0.001) scores were observed in the patients treated with HW HA. The analysis at the 6-month follow-up, the primary outcome of our study, showed better IKDC results in the PRP group compared with the LW HA group (P = 0.003), as well as compared with patients treated with HW HA (P = 0.005), and the same results were found with the EQ VAS (PRP v LW HA, P = 0.001; PRP v HW HA, P = 0.002). After the 2-month follow-up (at which the same results were obtained from the PRP and LW HA groups), a significant difference was documented over time (P = 0.001), with a further improvement in the PRP group and a worsening of the results obtained in the patients treated with LW HA injections.
Study Results **Safety**	No complications related to the infiltrations were observed during the treatment and follow-up period.
Limits of the study	Not available.
Discussion	The results of this study showed positive effects of PRP in patients affected by knee degeneration, with an improvement of symptoms and function.
Conclusions of the authors	The clinical results of this comparative study suggested that this procedure may be useful for the treatment of degenerative articular pathology of the knee. Autologous PRP injections showed more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function, in particular in more active patients with a low degree of cartilage degeneration. In patients aged 50 years or younger, LW HA and PRP were more effective than HW HA at 2 months and PRP was more effective than LW HA or HW HA at 6 months, whereas in patients older than 50 years, results were equivalent at both 2 and 6 months.

Citation 11

Title	Diracoglu D, Vural M, Baskent A, Dikici F, Aksoy C. **The effect of viscosupplementation on neuromuscular control of the knee in patients with osteoarthritis.** *J Back Musculoskelet Rehabil*. 2009;22(1):1-9.
Aim of the study	The aim of this study was to investigate the short-term effects of intra-articular injection of hyaluronan (Hylan G-F 20) on proprioception, isokinetic muscle force, self reported pain, and functional condition in patients with knee osteoarthritis (OA). Here, the investigators tested this hypothesis: "*One of the mode of actions of intra-articular hyaluronan in knee OA is the increase of proprioception*."
Relevance of the study	When patients with knee OA are compared to healthy individuals at the same age, there is much more loss of the sense of proprioception. Proprioception can be defined as the conscious or unconscious perception of extremity position in space and the awareness of movement and position of the joints.
Equivalent Device	Hylan G-F 20 (Synvisc; Wyeth-Ayerst Pharmaceuticals, Philadelphia, Pennsylvania) and sterile physiological saline (0.9% sodium chloride).
Study Design	Prospective, randomized, placebo controlled, double-blind (blinded patient/blinded evaluator) study.
Study period	Not available.
Sample size	60 patients were included in the study. Subjects were randomized, with 42 of them into the treatment group and 21 of them into the placebo group.
Inclusion Criteria	Enrolled patients were diagnosed with bilateral knee OA according to the criteria of the American College of Rheumatology, and were at stage II and III according to the Kellgren-Lawrence scale. They also had minimum of 50 points from the VAS-pain scale of 100 mm during motion on both knees.
Exclusion Criteria	Patients with septic arthritis, Paget's disease, gout and pseudogout, major dysplasia or congenital abnormalities, ochronosis, acromegaly, hemochromatosis, Wilson's disease, primary osteochondromatosis, Ehlers-Danlos syndrome, neuropathic arthropathy (Charcot joints), hyperparathyroidism, hypothyroidism, or active synovitis, patients who have had serious knee trauma or surgical operation, or undergone arthroscopy of the knee joint in the last one year, patients who have received intra-articular steroids or HA injection in the knee joint in the last 6 months, patients with concomitant rheumatoid disease, and pregnant patients were not included in the study.
Intervention	Hylan G-F 20 (Synvisc) was intraarticularly injected into both knees of the subjects which were in the treatment group, whereas sterile physiological saline (0.9% sodium chloride) was intraarticularly injected to the subjects which were in the placebo group. Injections were repeated in both groups three times after every

	one-week. Patients did not use any analgesic medication during the study and were not prescribed any exercise program.
Outcomes	Measurement of proprioception was made before and immediately after every injection. It was also repeated one week after the last injection. Measurement of the isokinetic muscle force, 0–100 mm visual analogue scale (VAS), and the Western Ontario and Mc-Master Universities Osteoarthritis Index (WOMAC-5-point likert 3.0) were performed before the injection and one week after the last injection.
Study Results **Performance**	The AAAE values of the treatment group were detected to be significantly lower at the measurements performed following the 3rd injection and one week after. 120 knees of 60 patients were evaluated at the isokinetic measurements. With respect to 60◦/sec angular speed, the post-injection differences were determined to be significantly higher in the treatment group com-pared to placebo group ($p<0.05$). However there was no significant difference between the differences obtained in 180 and 240◦/sec angular speed ($p>0.05$). Before the injections, there was no significant differ-ence between the treatment and placebo groups regard ing the VAS and WOMAC parameters ($p>0.05$). After the injections, activity and resting VAS-pain values, all WOMAC parameters (except the WOMAC stiffness) were detected to be significantly lower in the treatment group ($p<0.05$). There was no significant difference between the groups in WOMAC-stiffness values.
Study Results **Safety**	No pseudoseptic reaction or adverse event was determined. Local adverse events were not reported in any patient.
Limits of the study	Limitation of our study is that it shows the short-term effect of the injection on the proprioception. The long-term nature of this effect is not well known.
Discussion	Following three intra-articular injections of HA into the knee joint, the proprioception was significantly improved. The HA, when injected intra-articularly three times, was shown to increase maximal isokinetic muscle force in a short term. However, this increase was significant only at the speed of 60◦/sec and 240◦/sec in the right extremity. A significant increase in muscle force at an angular speed of 240◦/sec in the right extremity was also observed in the placebo group. The reason for this condition may be based on the fact that the right extremity is the dominant extremity in most of the patients, and due to this, right side is more sensitive to the treatment. Besides, the ratio of agonist/antagonist did not change and this demonstrates that injection does not have a negative effect on the flexor-extensor balance. The most important effect of the treatment is on proprioception, which has an im-portant role in the pathogenesis of OA. The proprioceptive improvement obtained from HA injection may indirectly result in increase in muscle force or reduction in

	pain, or the proprioceptive progress may have affected the pain and functional condition directly.
Conclusions of the authors	In this study, it was demonstrated that intraarticular injection of hyaluronan in patients with knee OA led to a short-term increase in proprioception and isokinetic muscle force, and also significant improvements in the functional conditions of patients. Howevert, long-term studies are needed.

Citation 12

Title	Carpenter B, Motley T. **The role of viscosupplementation in the ankle using hylan G-F 20.** *J Foot Ankle Surg.* 2008 Sep-Oct;47(5):377-84
Aim of the study	The goal of this clinical trial was to compare pain reduction following ankle arthroscopy versus that following ankle arthroscopy combined with weekly intra-articular instillation of hylan G-F 20 during the first 3 postoperative weeks.
Relevance of the study	To the best of authors' knowledge, at the time there were no long-term ankle viscosupplementation studies published in the peer-reviewed biomedical literature.
Equivalent Device	Hylan G-F 20 (**Synvisc**, Genzyme Corporation, Cambridge, MA) is a mixture of two hylan polymers, 80% by volume hylan A fluid and 20% hylan B gel. The average molecular weight of hylan G-F 20 is 6 x 10^6 daltons.
Study Design	Prospective, non randomized
Study period	September 2002 - June 2004
Sample size	A total of 26 patients were treated.
Inclusion Criteria	To be included in the investigation, patients had to have a diagnosis of ankle osteoarthritis; display radiographic evidence of joint space narrowing with or without subchondral sclerosis, osteophytosis, or subchondral cyst formation; failed to satisfactorily respond to a course of conservative therapy consisting of exercise and at least 6 weeks of nonsteroidal anti-inflammatory drug (NSAID) administration and 3 or fewer intra-articular corticosteroid injections at bi-weekly intervals; and be suitable candidates for, and consent to, either ankle arthroscopy alone or in combination with intra-articular hylan instillation.
Exclusion Criteria	Not available.
Intervention	The interventions of interest in this investigation included ankle arthroscopy alone (AAA), and ankle arthroscopy plus hylan (AA+H) instillation. After obtaining consent to surgery, and to participation in the investigation, all of the patients underwent ankle arthroscopy. At the end of the procedure, the ankle was lavaged with approximately 200 mL of normal sterile saline. For the patients in the AA+H intervention group, beginning 1 week post arthroscopy, a 2-mL (unit dose) intra-articular injection of hylan G-F 20 (Synvisc) was instilled into the same ankle. Before the articular injection, joint fluid was aspirated. This process of aspiration followed by instillation of hylan G-F 20 was repeated again at 2 and 3 weeks post arthroscopy, for a total of 3 intra-articular instillations of hylan following the arthroscopic procedure.

Outcomes	Baseline outcomes included the age, gender, side of ankle involvement, and the subjective measurement of pain using a 10-point categorical pain scale that is commonly used in the clinical setting. Specifically, each patient was asked to rate their ankle pain from 0 to 10, with 0 representing no pain and 10 representing the worst pain that the patient could imagine. Postoperative pain score data were obtained at approximately 3 months following the intervention in all of the patients in the series. The patients were followed for at least 12 months also to determine whether or not any adverse events developed.
Study Results **Performance**	Overall, the median and interquartile range for the pre-intervention and post-intervention pain scores was 8.5 (8, 9) and 2 (1, 3), respectively, and this difference was statistically significant (P <0.0001). Overall, the median and interquartile range for the reduction in pain (the difference between the pre- and post-intervention pain scores) was 6 (5, 8). For the AAA group, the median and interquartile range for the pre-intervention pain score was 8 (7.5, 9.5), whereas that for the post-intervention score was 3 (2, 3.5), and this difference was statistically significant (P <0.002). For the AA+H group, the median and interquartile range for the pre-intervention pain score was 9 (8, 9), and that for the post-intervention pain score was 1 (0, 2), and this difference was highly statistically significant (P <0.0009). The median and interquartile range for the pre-intervention pain score for the AAA group was 8 (7.5, 9.5); whereas that for the AA+H group was 9 (8, 9), and this difference was not statistically significant (P <0 .6525). The median and interquartile range for the post-intervention pain score for the AAA group was 3 (2, 3.5); whereas that for the AA+H group was 1 (0, 2), and this difference was statistically significant (P <0.0002). The median and interquartile range for the reduction in pain for the AAA group was 5.5 (5, 6); whereas that for the AA+H group was 7.5 (6, 9), and this difference was statistically significant (P <0.0014).
Study Results **Safety**	None of the patients in this series suffered with any type of postoperative complication, and none of those receiving hylan G-F 20 injections dis-played any type of local or systemic adverse reaction to the agent.

Variable	AAA, median (interquartile range) or count (%)	AA+H, median (interquartile range) or count (%)	*P* value*
Age, y	55.0 (44.0, 61.5)	59.0 (51.0, 66.0)	.4396
Male gender	6 (50%)	6 (42.86%)	.7211
Right ankle	3 (25%)	7 (50%)	.2002
Pre-intervention pain†	8.0 (7.5, 9.5)	9.0 (8.0, 9.0)	.6525
Post-intervention pain†	3.0 (2.0, 3.5)	1.0 (0, 2.0)	.0002
Pain reduction‡	5.5 (5.0, 6.0)	7.5 (6.0, 9.0)	.0014

AAA, ankle arthroscopy alone (n = 12); AA+H, ankle arthroscopy plus hylan (n = 14).
*Wilcoxon rank-sum (Mann-Whitney) test.
†10-point categorical pain scale (0 = no pain, 10 = the most severe pain the patient can imagine).
‡Decrease in pain scores between the pre-intervention and post-intervention periods.

Table 18. Comparison of different clinical variables by treatment group.

Limits of the study	The small number of patients is the main limit of the study. Another limitation is in the fact the fact that treatment allocation was not randomized, and patients were enrolled consecutively as they presented for treatment of their ankle arthritis. The decision as to which intervention would be used was determined at the discretion of the patient after discussion with the surgeon. Moreover, the investigators did not use a visual analog pain scale to determine the pre- and post-intervention pain scores. Instead, they used a 10-point categorical scale that is commonly used in the clinical realm. It is likely that this form of pain measurement is not as valid as a visual analog scale.
Discussion	In this study, it was found that both treatment groups experienced statistically significantly decreased pain following the intervention and that those who received 3 intra-articular injections of hylan G-F 20 following ankle arthroscopy improved statistically significantly more than did those who underwent arthroscopy as a sole therapy. Although these results are preliminary in nature, they provide some evidence as to the beneficial effects that viscosupplementation, combined with arthroscopy, has in regard to pain relief in the treatment of osteoarthritis of the ankle.
Conclusions of the authors	These preliminary results suggest that viscosupplementation combined with arthroscopy may be more beneficial than arthroscopy alone. The authors concluded that the results of this investigation may be useful in the development of future investigations into the treatment of ankle osteoarthritis by means of viscosupplementation.

Citation 13

Title	Conrozier T, Jerosch J, Beks P, Kemper F, Euller-Ziegler L, Bailleul F, Chevalier X. **Prospective, multi-centre, randomised evaluation of the safety and efficacy of five dosing regimens of viscosupplementation with hylan G-F 20 in patients with symptomatic tibio-femoral osteoarthritis: a pilot study.** *Arch Orthop Trauma Surg*. 2009 Mar;129(3):417-23
Aim of the study	The aim of this study was to assess different dosing regimens of hylan G-F 20, a high molecular-weight cross-linked derivative of HA, in the treatment of pain due to knee OA.
Relevance of the study	A 6 mL single injection could be particularly useful for patients undergoing concomitant anti-thrombotic therapy (i.e. vitamin K antagonists, aspirin, clopidogrel). In those patients with active and busy lifestyles, or who have travelling challenges due to distance or schedule, a single dose treatment regimen may improve patient compliance. Additionally, a single dose regimen would reduce the risk of procedure-related local AE's, particularly infectious arthritis and oVer medico-economic benefits. Furthermore, not only a single injection allows a major compliance from the patients and reduces risks connected to intra-articular injection, but also has its importance in pharmaco-economics: a minor number of injections shortens medical costs connected to hospital visits, medications, work time of physicians and nurses and patients absenteeism.
Equivalent Device	Hylan G-F 20 (**Synvisc**, Genzyme Corporation, Cambridge, MA) is a mixture of two hylan polymers, 80% by volume hylan A fluid and 20% hylan B gel. The average molecular weight of hylan G-F 20 is 6 x 10^6 daltons.
Study Design	Prospective, multi-centre, randomised, open, five-arm trial.
Study period	Not available.
Sample size	The ITT population consisted of 100 patients.
Inclusion Criteria	The main inclusion criteria were: Male or female patient aged 40 years or older with an active lifestyle, consulting for OA pain in one knee and scoring >=50 and <=80 mm on a 100 mm OA pain visual analogue scale (VAS) where 0 mm = no pain and 100 mm = worst possible pain; tibio-femoral OA (ACR criteria); Kellgren–Lawrence grade II or III diagnosed by standard X-rays taken within 3 months prior to enrolment; no surgical intervention planned in the study knee in the next 6 months. If taking analgesics (except permitted doses of paracetamol <=3g/day for rescue analgesia), NSAIDs or cyclooxygenase-2 inhibitors, patient were required to comply with a washout period of 1–3 weeks depending on the half-life of the medication.
Exclusion Criteria	The main exclusion criteria were: Patients with bilateral symptomatic knee OA or predominantly patello-femoral involvement of the study knee; knee OA flare with obvious tense effusion, diagnosed by clinical examination, at the study knee; clinical

	symptoms of meniscal instability or significant valgus/varus that required corrective osteotomy; significant ligamentous instability; any prior viscosupple-mentation therapy or history of sepsis in the study knee; systemic or intra-articular injection of corticosteroids in any joint within 3 months of enrolment; chondrocalcinosis and microcrystals-mediated arthritis, concomitant inflammatory or other rheumatologic, neurological or cardiovascular diseases which could affect the evaluation of knee pain.
Intervention	Patients meeting the inclusion and exclusion criteria were randomised to 1 of 5 groups: Group 1: 1 intra-articular injection of 6 mL hylan G-F 20 Group 2: 1 intra-articular injection of 4 mL hylan G-F 20 Group 3: 2 intra-articular injections of 4 mL hylan G-F 20 administered 2 weeks apart Group 4: 3 intra-articular injections of 4 mL hylan G-F 20 administered 1 week apart Group 5: 3 intra-articular injections of 2 mL hylan G-F 20 administered 1 week apart Intra-articular injections were performed under strict aseptic technique by a trained physician using a lateral, medial mid patellar or antero-medial injection route (according to the injector's preference) after aspiration of any synovial fluid. Patients were followed-up 7 days after each injection then at 3, 8, 16 and 24 weeks after the Wrst injection. Safety and eYcacy were assessed at each patient visit. At week 24, patients who scored >=50 and <=80 mm on the pain VAS, or patients who experienced a worsening of pain (>15 mm on VAS) compared to week 16, were eligible to receive a second cycle of treatment (Extension Study). Patients undergoing a second cycle of treatment received the same dosing regimen as that dictated by their original randomisation.
Outcomes	The primary objective of this pilot study was therefore to assess the safety and efficacy profiles of new dosing regimens of hylan G-F 20 in patients with knee OA, using higher single dose volumes (4 and 6 mL) and reducing the number of injections (1 or 2) and to compare these results to the dosing regimen currently approved (3 x 2mL). The secondary objective was to assess the safety and efficacy profiles of a second cycle of these new dosing regimens. The possible relationship between clinical efficacy and total volume injected (6 mL currently, up to 12 mL in this study) was also examined. Target knee and systemic AE's were monitored throughout the study. Additionally, patients assessed overall safety using a four point side-effect rating scale.
Study Results **Performance**	Treatment with hylan G-F 20 resulted in a statistically significant improvement from baseline to week 24 in all end-points for all treatment regimens. The largest changes were observed in Group 5 (3 x 2 mL) with a mean change [SD] from baseline at week 24 in the patient-completed knee OA pain VAS score of -36.7 mm [26.9]. Groups 1 (1 x 6 mL) and 4 (3 x 4 mL) consistently showed similar mean improvement (respectively -34.9 mm [16.4] and -32.6 mm [25.3]). Smaller changes (-24.0 mm

	[22.9] and -24.3 mm [28.3]) were found in Group 3 (2 x 4mL) and Group 2 (1 x 4mL). The table summarises the rankings of mean response to treatment for all primary and secondary endpoints by treatment group. **Table 19.** Rankings of mean response at week 24 by treatment group. The group with the highest number of re-treated patients (n = 7) was Group 3 (2 x 4mL). Group 1 (1 x 6 mL) had the lowest number of patients qualifying for repeat treatment.
Study Results **Safety**	The treatment was well tolerated. There were no serious or severe, device-related AE's in any of the studied dosing regimens, nor were any new safety concerns identified following initial or repeat treatment. Group 4 (3 x 4mL) had the highest percentage of patients reporting device-related local AE's (30%) while Group 1 (1 x 6 mL) and Group 5 (3 x 2 mL) had only 10%. These device-related local AE's consisted mostly of mild or moderate post-injection pain (n = 12 patients) with local inflammation (described as synovitis by some investigators, n =3) or effusion (n = 1). Twenty-four patients (24%) were re-treated in the extension study; no safety concerns were raised by re-treatment with the same injection schedules. Four patients that were re-treated experienced five target knee AE's. No patients experienced AE's in Group 1, while one patient reported an AE in each of Groups 2–5. None of the target knee, treatment-emergent AE's was serious. One patient in Group 4 (3 x 4 mL) discontinued from the study due to synovitis with eVusion at the target knee. One case of synovitis in Group 5 (3 x 2 mL) was severe.
Limits of the study	Not available.
Discussion	The data presented here suggest that alternative protocols could be proposed to replace the current 3 x 2mL reg-imen of viscosupplementation with hylan G-F 20, offering similar clinical effiacy without a greater percentage of adverse events. In patients with hip, shoulder and ankle OA. A single intra-articular injection of 2 mL hylan G-F 20 has demonstrated significant immediate, and sustained, symptomatic effect for up to 6 months.
Conclusions of the	In summary, the risk/benefit profile of a single 6 mL injection appears to be good

Assessment	Group 1 [1 × 6 mL (*N* = 20)]	Group 2 [1 × 4 mL (*N* = 21)]	Group 3 [2 × 4 mL (*N* = 19)]	Group 4 [3 × 4 mL (*N* = 20)]	Group 5 [3 × 2 mL (*N* = 20)]
Patient knee OA Pain	2	4	5	3	1
WOMAC A	2	5	4	1	3
WOMAC B	4	5	3	2	1
WOMAC C	2	4	5	1	3
Patient global assessment	1	5	4	2	3
Physician global assessment	1	5	4	3	2
Total	2	5	4	1	3

authors	and this regimen could be developed as an alternative to the currently approved 3 x 2 mL regimen for the treatment of symptomatic knee OA.

Citation 14

Title	Borrás-Verdera A, Calcedo-Bernal V, Ojeda-Levenfeld J, Clavel-Sainz C. **[Efficacy and safety of a single intra-articular injection of 2% hyaluronic acid plus mannitol in knee osteoarthritis over a 6-month period].** *Rev Esp Cir Ortop Traumatol.* 2012 Jul-Aug;56(4):274-80
Aim of the study	The aim of this study was evaluate the safety and efficacy of a single intra-articular injection of 2% hyaluronic acid (HA) + mannitol in symptomatic knee osteoarthritis (KOA).
Relevance of the study	There is evidence that repeated intra-articular injections of HA improve symptoms in KOA. This article might be helpful in sustaining the safety of hyaluronic acid injection for viscosupplementation of the osteoarthritic knee.
Equivalent Device	Ostenil Plus
Study Design	Pilot, multicentre, open, non-comparative study.
Study period	Not available.
Sample size	Eighty patients with painful KOA, of whom 79 completed the study.
Inclusion Criteria	Not available.
Exclusion Criteria	Not available.
Intervention	Patients received one injection of 2 ml of 2% HA + 0.5% mannitol (Day 0) and were followed-up for 6 months.
Outcomes	On Days 0, 15, 30, 60, 90, 120, 150 and 180, pain and joint function were assessed using a visual analogue scale (VAS) and WOMAC index. Efficacy and safety by investigator and patient, and rescue medication, as an indirect measure of pain, were also recorded.
Study Results Performance	A significant reduction in joint pain, stiffness and functional disability compared with baseline was observed at every follow-up visit ($P<0.001$). Joint function improved by 38.7% on Day 30, reaching 47.5% on Day 180. Rescue medication use decreased from 58.2% at baseline to 2.5% on Day 90, increasing in the last visits. Efficacy was positively evaluated by investigators and patients.
Study Results Safety	Safety was positively evaluated by investigators and patients. No serious adverse events were observed. Mild side effects were reported in 4 patients (local pain and swelling in the infiltration area).
Limits of the study	In the study design, no control group was included.
Discussion	There is evidence that repeated intra-articular injections of HA improve symptoms in KOA. However, studies with a single injection of HA have shown mixed results. This study demonstrates that one single intra-articular injection of non-cross-linked HA reduces joint pain and increases function in patients with KOA over a period of

	at least 6 months.
Conclusions of the authors	This study was the first to demonstrate that a single i.a. of non-crosslinked 2% + 0.5% mannitol is an effective treatment in osteoarthritis of the knee, since it reduces pain and improves joint function for a minimum period of time of 6 months and, in addition, presents a low incidence of associated mild adverse events.

Citation 15

Title	Palmieri B, Rottigni V, Iannitti T. **Preliminary study of highly cross-linked hyaluronic acid-based combination therapy for management of knee osteoarthritis-related pain.** *Drug Des Devel Ther*. 2013;7:7-12
Aim of the study	The aim of the present study was to investigate, for the first time, the effect of a highly cross-linked hyaluronic acid, Variofill®, alone or in combination with diclofenac sodium or sodium clodronate, for the management of bilateral knee OA-related pain.
Relevance of the study	Before this study, to investigators' knowledge, no one had tested local intra-articular delivery of highly cross-linked hyaluronic acid combined with bisphosphonate or non-steroidal anti-inflammatory drugs for the management of knee OA-related pain in the clinical setting.
Equivalent Device	**Variofill®**, Adoderm, GmbH, Langenfeld, Germany
Study Design	Randomized double-blind study.
Study period	Not available.
Sample size	One hundred and twenty-two patients were screened. Sixty patients were not included in the study because they did not meet the inclusion criteria. Sixty-two patients signed the informed consent and participated in the present study.
Inclusion Criteria	The inclusion criteria were symptomatic bilateral medial tibiofemoral knee osteoarthritis (Kellgren–Lawrence grade II and III), as assessed by x-rays taken 2–3 months prior to the beginning of the study and pain in both knees corresponding to daily VAS >= 30 mm in the previous month.
Exclusion Criteria	Exclusion criteria were: unilateral knee osteoarthritis or unilateral/bilateral knee osteoarthritis concerning predominantly the patellofemoral region, meniscal-related or ligamentous-related instability as assessed by physical examination, any prior viscosupplementation or intra-articular injection of corticosteroids or any other drugs into the knee during the 5 months prior to the beginning of the study and presence of concomitant pathology affecting the knee.
Intervention	Patients were divided into three groups in a randomized fashion. **Group 1** (n = 20)

	was treated with **an injection of hyaluronic acid alone** (66 mg/2 mL [Variofill®, Adoderm, GmbH, Langenfeld, Germany]) into each knee; **group 2** (n = 21) was treated with **an injection of hyaluronic acid** (49.5 mg/1.5 mL [Variofill®, Adoderm, GmbH, Langenfeld, Germany]) **plus diclofenac sodium** (0.5 mL/5 mg [INFORCE, IBSA, Lugano, Switzerland]) into each knee; and **group 3** (n = 21) was treated with **an injection of hyaluronic acid** (49.5 mg/1.5 mL [Variofill®, Adoderm, GmbH, Langenfeld, Germany]) **plus sodium clodronate** (0.5 mL/5 mg [Clasteon®, Abiogen Pharma, Pisa, Italy]) into each knee. The overall volume injected was 2.5 mL because 0.5 mL of 1% lidocaine (Angelini, Rome, Italy) was added to every injection in order to relieve injection-related pain. Injections were prepared under aseptic conditions by the same operator throughout the study using an emulsification needle to obtain a complete physical mixture of the drugs before delivery. The study injections were given by a surgeon who was blinded for the duration of the study. The patients were also blinded to their treatment allocation. Following viscosupplementation, all patients were advised to avoid NSAIDs for 6 months, but paracetamol, at a maximum dose of 2000 mg/day, was allowed for pain management. All patients were advised to stop paracetamol for 24 hours before assess-ment at 3- and 6-month follow-up examination.
Outcomes	The primary outcome measurement was knee pain as assessed by VAS (0–100 mm, 0 = no pain, 100 = very severe pain) at baseline and at 3 and 6 months after treatment (VAS pain score was calculated for each knee and then values were averaged together). Secondary outcome measures were erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) assessed by blood tests performed immediately before and at 6-month follow-up.
Study Results **Performance**	Group 1 showed a decrease in VAS pain score from a mean baseline value of 67.5 ± 2.04 mm to 46.8 ± 2.09 mm at 3 months and to 31.3 ± 2.4 mm at 6 months. Group 2 showed a decrease in VAS pain score from a mean baseline value of 71.9 ± 1.1 mm to 48.86 ± 0.9 mm at 3 months and to 32.1 ± 1.1 mm at 6 months. Group 3 showed a decrease in VAS pain score from a mean baseline value of 76.9 ± 1.9 mm to 47.5 ± 1.05 mm at 3 months and to 26.8 ± 1.2 mm at 6 months. When comparing the percentage change in mean VAS pain score from baseline in the three treatment groups, the therapy including sodium clodronate was the most beneficial in terms of percentage improvement in VAS pain score. A significant decrease in ESR and CRP versus baseline was observed at 6 months after the procedure in each treatment group. In group 1, ESR decreased from 76.4 ± 2.6 mm/hr to 23.7 ± 1.5 mm/hr ($P <= 0.001$) and CRP decreased from 7.4 ± 0.3 mg/L to 1.5 ± 0.09 mg/L ($P <= 0.001$). In group 2, ESR decreased from 77.1 ± 2.5 mm/hr to 23.2 ± 1.1 mm/hr ($P <= 0.001$) and CRP decreased from 7.1 ± 0.3 mg/L to 1.8 ± 0.1 mg/L ($P <= 0.001$). In group 3, ESR decreased from 76.7 ± 2.5 mm/hr to 22.8 ± 1.2 mm/hr ($P <= 0.001$) and CRP decreased from 6.8 ± 0.3 mg/L to 1.5 ± 0.08 mg/L ($P <= 0.001$). No significant difference was observed when the percentage change from baseline related to

	these parameters was compared among the groups.
Study Results **Safety**	No serious adverse events were observed in any group. Some bruising at 4 hours after injection containing sodium clodronate was reported by four patients, but resolved without any further treatment. No pain was observed at the injection site.
Limits of the study	A limitation of the present study is the fact that the authors did not take into account functional outcome measures, such as the Western Ontario and McMaster Universities Osteoarthritis Index.
Discussion	This study shows that intra-articular hyaluronic acid, alone or in combination with sodium clodronate or diclofenac sodium, improved VAS pain score in patients affected by bilateral osteoarthritis of the knee. This was consistent with a significant decrease in ESR and CRP at 6 months after the procedure in each treatment group and without significant intergroup differences. According to these results, highly cross-linked hyaluronic acid is suitable for use in combination with other drugs, namely NSAIDs or bisphosphonates without complications.
Conclusions of the authors	The authors concluded that further studies are necessary to determine the effect of a therapy based on hyaluronic acid combined with diclofenac sodium or sodium clodronate in larger cohorts of patients affected by knee osteoarthritis and in longer-term follow-up.

Citation 16

Title	Strand V, Baraf HS, Lavin PT, Lim S, Hosokawa H. **A multicenter, randomized controlled trial comparing a single intra-articular injection of Gel-200, a new cross-linked formulation of hyaluronic acid, to phosphate buffered saline for treatment of osteoarthritis of the knee.** *Osteoarthritis Cartilage.* 2012 May;20(5):350-6
Aim of the study	The objective of this study was to compare the safety and efficacy of a single intra-articular (IA) injection of a new cross-linked hyaluronic acid product, Gel-200, with phosphate buffered saline (PBS, control) in a multi-center randomized controlled trial in patients with symptomatic osteoarthritis (OA) of the knee.
Relevance of the study	Gel-200 is a sterile, transparent, viscoelastic hydrogel composed of cross-linked hyaluronate, a derivative of a highly purified sodium hyaluronate product extracted from chicken combs. Non-cross-linked HA diffuses out of the synovial fluid rapidly after administration into the knee joint, while injected Gel-200 was found to persist in synovial fluid for up to 7 days and synovium for as long as 28 days in rabbits without intra-articular inflammation. Based on observations in pre-clinical animal studies, it was expected that a single injection of Gel-200 would provide more prolonged benefit than multiple injection IA-HA products.

	This study may be useful to sustain the safety of hyaluronic acid in OA viscosupplementation (worst case, as Gel-200 is composed of HA animal-derived).
Equivalent Device	Cross-linked IA-HA product (**Gel-One**, Seikagaku Corporation, Tokyo) vs PBS (control)
Study Design	Randomized, double-blind, multi-center controlled trial.
Study period	August 2006-December 2007
Sample size	Sample size calculations of 375 patients were determined based on the following assumptions: (1) two-sided t-test, (2) 90% power, (3) 5% significance level, (4) 2:1 randomization allocation in favor of Gel-200, (5) 10 mm detectable difference on a 100 mm VAS WOMAC pain subscore, (6) standard deviation (SD) of 25 mm for Gel-200 and 27 mm for PBS, and (7) an allowed 10% dropout rate per group.
Inclusion Criteria	Patients were 40-80 years of age, with knee OA, and pain in the affected knee of >=4 weeks in duration while standing or walking; KellgreneLawrence (KeL) grade 1-3 by X-ray; Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscores >=40 mm in affected knee and <=20 mm in contralateral knee by 100-mm Visual Analog Scale (VAS); and willing to discontinue current OA treatments other than allowed medications, stable for >=4 weeks prior to entry.
Exclusion Criteria	Patients were excluded from study participation for the following: KeL grade 4 of the treated knee, inflammatory diseases of the knee other than OA, severe knee joint effusion, severe malalignment of the knee, history of joint replacement of knee or hip within the previous 12 months, arthroscopy of either knee within 3 months, IA injections with corticosteroids within the past 4 weeks, IA-HA injections within the past 6 months, and/or serious systemic diseases or infectious/inflammatory skin diseases in the area of the affected knee.
Intervention	Following aspiration of synovial fluid if an effusion was present, patients received a single IA injection of Gel-200 (30 mg cross-linked HA in 3.0 mL) or PBS (3.0 mL) at week 0. Follow-up visits assessed safety and clinical benefit at weeks 1, 3, 6, 9 and 13 after injection. Acetaminophen up to 4,000 mg/day was provided as rescue medi-cation except within 24 h of a treatment evaluation. Non-steroidal anti-inflammatory drugs (NSAIDs), nonprescription herbal therapies and chondroprotective agents (e.g., oral HA, glucosamine, chondroitin sulfate, minocycline) were allowed if patients did not change their treatment regimen and continued regular administration at stable doses from 4 weeks prior to randomization throughout protocol participation. Intermittent use of short-acting oral opiates was also allowed. Use of any medications for symptomatic pain relief was prohibited within 24 h prior to each visit evaluation. Physical therapy was prohibited throughout the study.
Outcomes	The primary outcome measure of effectiveness was patient-reported WOMAC pain subscores by VAS in the affected knee at week 13. Secondary outcome measures included Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACTeOARSI) “strict” responses: defined by improvements from baseline in WOMAC pain or physical function subscores >=50% with absolute changes >=20 mm (termed “strict responders”) or >= 20% with

	absolute changes >=10 mm in two of three measures: WOMAC pain or physical function subscores; and/or patient global assessments of disease activity (termed "responders"). Mean changes from baseline in total WOMAC, physical function and stiffness subscores, patient and physician global assessments of disease activity by VAS, and acetaminophen consumption, were recorded at each visit. Medical Outcomes Survey Short-Form 36 (SF-36) for assessment of health related quality of life was collected at weeks 0 and 13. The percentage of patients reporting improvements meeting or exceeding minimum clinically important differences (MCID) e.g., >= 10 mm in WOMAC pain subscores, and/or "moderate" and "substantial" changes defined as >= 30% and >= 50%, respectively, by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) working group were defined in an exploratory analysis. Times to response post injection (1-13 weeks) were also assessed. Safety evaluations included adverse events (AEs) coded by Medical Dictionary for Regulatory Activities (MedDRA Ver. 10.0) and examination of the affected knee for swelling, redness or effusion at each visit following injection. Hematology and serum chemistries were assessed at screening and week 13. Any adverse signs and symptoms or clinically significant laboratory abnormalities were collected as AEs during the study. Blinded investigators evaluated the severity of reported AEs and their potential relationship with treatment.
Study Results **Performance**	Mean changes from baseline in WOMAC pain subscores demonstrated a statistically significant advantage of 6.39 mm for Gel-200 treatment over PBS at week 13 (P = 0.037). Treatment differences at weeks 3 and 6 exceeded 8 mm (P = 0.001 and P = 0.003, respectively), and the overall difference over weeks 3 through 13 was 7.10 mm (P = 0.005). Mean improvements from baseline in WOMAC pain subscores consis-tently favored Gel-200 at each visit, with improvements of 40.6% at week 3 and 44.1% at week 6. Effectiveness in the Gel-200 treated group was sustained over weeks 3-13 by WOMAC total score, physical function, and physician global evaluations with statistical significance (P < 0.05) in addition to WOMAC pain. In the ITT population, the odds ratio (OR) for "strict" OMERACTeOARSI responders was statistically significant for Gel-200 vs PBS from weeks 6 to 13 [OR = 1.59; P = 0.022] There were no statistically significant differences in SF-36 between weeks 0 and 13, although benefit was demonstrated in both treatment groups. In terms of clinically meaningful responses over weeks 3e13, 64.5-72.8% of patients reported improvements >=MCID in Gel-200; compared with 57.1-69.5% in PBS, moderate improvements >=30%in a maximum of 62.1% vs 54.0% at week 6 and substantial improvements >=50% in a maximum of 49.4% vs 37.9% at week 6.
Study Results **Safety**	The incidence of AEs was similar in both treatment groups; 182 treatment-related AEs were reported in 100 patients: 67 (26.9%) in Gel-200 and 33 patients (25.8%) in PBS groups, respectively. Most common treatment-related AEs included joint swelling, effusions and arthralgia, without significant differences between treatment groups. Serious adverse events (SAEs) were reported in eight patients, including five cases of cancer. None were judged by investigators to be related to study treatment, although all SAEs occurred in the Gel-200 group, including one death. No clinically notable changes in laboratory results were identified.

Limits of the study	Not available.
Discussion	When comparing differences in mean changes from baseline in WOMAC pain subscores between Gel-200 treatment and PBS, a statistically significant advantage of 8.12 mm was evident by week 3 and sustained through end of study week 13 at 6.39 mm. In contrast to other IA-HA injections, Gel-200 demonstrated earlier onset of benefit. Although few trials of IA-HA products have reported a statisti-cally significant effect on physical function, Gel-200 treatment resulted in absolute mean changes exceeding 20 mm in WOMAC physical function subscores over weeks 3e13, reflecting >=30%improvements at each post-injection visit. Strict OMERACTeOARSI responses requiring >=50% improvements in this trial were evident as soon as 6 weeks following injection as were clinically mean-ingful changes from baseline in both WOMAC pain and physical function subscores over weeks 3 through 13; 62% of patients reported >=30% pain relief, and approximately 50% of patients reported >=50% pain relief. On the other hand, there was no statistically significant difference between Gel-200 and PBS groups in patient global assessment of disease activity. However, the advantage of Gel-200 administration was evident by clinically meaningful improvements in WOMAC pain and physical function scores, and in statistically significantly more strict OMERACT/OARSI responders. Eight cases of SAEs were reported in the Gel-200 group; all judged unrelated to study treatment, including five cancers diagnosed soon after treatment administration. These are consistent with the age of the study population and neither their timing of occurrence nor pre-clinical data would suggest a plausible rela-tionship to administration of Gel-200. In pre-clinical studies, Gel-200 was not shown to be associated with carcinogenicity. AE rates were generally comparable between treatments. No unanticipated treatment-related AEs were reported. As might be expected, the most common treatment-related AEs were joint swelling, joint effusion and arthralgia, frequently reported in other IA-HA studies. Importantly, pseudosepsis, an AE associated with another cross-linked IA-HA product, hylan G-F 20, and allergic reactions were not reported in the 249 patients receiving Gel-200 in this trial.
Conclusions of the authors	Treatment with Gel-200 offered statistically significant and clinically meaningful improvements both in pain and physical function, of early onset, in patients with knee OA, thereby demonstrating the multi-dimensional effectiveness of this therapy. The absence of allergic reactions or ‘pseudosepsis’ and the low incidence of treatment associated AEs support a favorable safety profile for this cross-linked IA-HA product for treatment of symptomatic OA of the knee.

Citation 17

Title	Navarro-Sarabia F, Coronel P, Collantes E, Navarro FJ, de la Serna AR, Naranjo A, Gimeno M, Herrero-Beaumont G; AMELIA study group. **A 40-month multicentre, randomised placebo-controlled study to assess the efficacy and carry-over effect of repeated intra-articular injections of hyaluronic acid in knee osteoarthritis: the AMELIA project.** *Ann Rheum Dis.* 2011 Nov;70(11):1957-62
Aim of the study	AMELIA (OsteoArthritis Modifying Effects of Long-term Intra-articular Adant) was designed to compare against placebo the efficacy and safety of repeated injections of hyaluronic acid (HA) and its effect on disease progression over 40 months.
Relevance of the study	This study may be important to further support the efficacy and safety of repeated injections of hyaluronic acid (HA) for viscosupplementation.
Equivalent Device	Sodium hyaluronate with a mean molecular weight of 900.000 daltons, obtained through a fermen-tation process from strains of *Streptococcus zoopidemicus* (**Adant** - Tedec-Meiji Farma, Madrid, Spain).
Study Design	Randomised, patient and evaluator blinded, placebo-controlled study with parallel groups.
Study period	Recruiting started in October 2003 and the last follow-up was performed on July 2009.
Sample size	The participating centres screened a total of 446 patients, of whom 140 were screening failures. Five patients did not provide any efficacy data after randomisation and were not included in the analysis of efficacy, leaving a total of 301 patients in the ITT population. A total of 109 and 94 patients receiving HA or placebo, respectively, completed the study.
Inclusion Criteria	Eligible patients were men and women of at least 45 years of age with knee osteoarthritis in the medial tibiofemoral compartment according to the American College of Rheumatology with grade II to III radiographic stage osteoarthritis and minimum medial femorotibial joint space width of the target knee of 2 mm or greater. Patients were required to have pain of 55 mm or greater on a visual analogue scale (VAS) at any time during the week before inclusion.
Exclusion Criteria	Main exclusion criteria were body mass index greater than 32 kg/m^2, a history of trauma or surgery in the target knee, arthroscopy surgery during the year before inclusion, joint inflammatory diseases and/or microcrystalline arthropathies, coagulation/platelet disorders or any concomitant disease that could interfere with the evaluation. The administration of intra-articular steroids in the previous 3 months, HA injections during the past year or NSAID treatment during 2 weeks before inclusion were also reasons for exclusion.

Intervention	Patients with osteoarthritis of the knee were randomly assigned to receive intra-articular injections of 2.5 ml 1% sodium hyaluronate or placebo injections (2.5 ml of saline solution). The study consisted of four treatment cycles of fi ve weekly injections each one. The follow-up periods were 6 months long after the first and second cycles and 1 year long after the third and fourth cycles, resulting in a total study duration of 40 months. The repeated cycles were administered regardless of whether the patients had symptoms or not.
Outcomes	The primary efficacy outcome was the percentage of subjects with a clinical response according to Osteoarthritis Research Society International (OARSI) 2004 criteria at the end of follow-up. Patients were classified as responders if the pain or physical function score decreased at least 50% and at least 20mm on the VAS, or if two of the following three findings were recorded: a decrease in pain of at least 20% or at least 10 mm on the VAS, a decrease in physical function of at least 20% and at least 10 mm on the VAS, or an increase in the score of the patient's global assessment by at least 20% and at least 10 mm on the VAS. Secondary outcomes included the percentage of subjects with clinical response according to OMERACT–OARSI criteria at each follow-up visit; each component of OMERACT–OARSI (reduction in pain, improvement in function using the Western Ontario and McMaster Universities Osteoarthritis Index function subscale and in patients' global assessment (all of them measured using VAS) and consumption of rescue medication for osteoarthritis (paracetamol and NSAID) throughout the study). Treatment safety and tolerability was evaluated based on the incidence and type of adverse events (with special attention to allergic reactions such as skin rash, urticaria, pruritus, swelling and/or erythema) and the results of blood laboratory tests and physical examinations throughout the duration of the study. Safety analyses were performed in those patients who received at least one intra-articular injection (safety population).
Study Results **Performance**	At the end of follow-up (40 months) significantly more patients receiving HA responded to treatment in comparison with placebo according to OARSI 2004 criteria (p=0.004), the number of responders being 22% higher in HA group after the four treatment cycles (RR 1.22, 95% CI 1.07 to 1.41). The number of responders to HA injections progressively increased after each treatment cycle (from 71.1% to 80.5%), whereas responses to placebo remained fairly stable (from 67.8% to 65.8%). This progression gave results with strong statistical significance and differences between the two groups from the second until the last evaluation at 40 months. Among those non-responders after the first cycle, up to 54% of HA and 38% of placebo patients evolved positively over the study. At the 40-month visit the number of responders in this subgroup was 54% with HA versus 31% in the placebo group (p=0.026).

	All of the OARSI components (pain, function and patient global assessment) were analysed at the end of the study, showing that the degree of improvement in the HA group was signifi cantly higher compared with placebo (p values = 0.025, 0.023 and 0.002, respectively). A total of 26.8% of patients receiving HA did not complete the study compared with 38.2% in the placebo group. It is noteworthy that the number of losses due to lack of efficacy were significantly higher in the placebo group (p=0.027). The demographic and baseline characteristics of completers and dropouts were analysed, and no differences were found with the exception of age in the placebo group, with the completers being younger than the dropouts (p=0.047). Aspiration in the target knee was performed in 22.82% of patients in the HA group and 21.05% of the placebo group (p=0.712), with a median of two aspirations per patient in both groups during the overall study period. Overall, rescue medication (paracetamol/NSAID) was consumed during the study by 71.1% and 71.7% of the HA and placebo patients, respectively. Paracetamol was consumed by 48% of the patients and the mean daily dose during the study experienced a 27% reduction in the HA group compared with baseline versus only a 4% reduction in the placebo group. A logistic regression analysis was performed with no differences between the HA and placebo (p=0.9129) groups, concluding that rescue medication did not interfere with the clinical assessment of patients.
Study Results **Safety**	The number of patients who experienced at least one adverse event was the same in both treatment groups (Table 20). Twenty-two patients (11 in each group) experienced a total of 29 related adverse events. Most of them were related to the study intervention, such as local bleeding, pain of mild intensity or allergic reaction, none of them was serious.

	HA (n=153)	Placebo (n=153)
Related adverse events (n (%))	15 (9.8)	14 (9.1)
Mild	7 (4.6)	12 (7.8)
Moderate	8 (5.2)	2 (1.3)

	HA (n=153)		Placebo (n=153)	
	Mild	Moderate	Mild	Moderate
Allergic reaction	2 (1.3) (1 rash, 1 swelling)	1 (0.7) (1 rash)	3 (1.9) (3 rash)	0
Pain at injection site	2 (1.3)	4 (2.6)	2 (1.3)	0
Bleeding at injection site	2 (1.3)	0	6 (3.9)	0
Arthralgia	0	2 (1.3)	1 (0.7)	1 (0.7)
Others	1 (0.7)	1 (0.7)	0	1 (0.7)

HA, hyaluronic acid.

Table 20. Adverse events.

Limits of the study	Not available.
Discussion	The results obtained in this trial are in line with those reported previously, granting HA greater efficacy than NSAID and than steroids after 5–8 weeks post-treatment,

	even though previous studies do not provide long-term data.
Conclusions of the authors	The results of AMELIA reveal that repeated cycles of intra-articular injections of HA not only improve knee osteoarthritis symptoms during the in-between cycle period, but also exert a marked carry-over effect for at least 1 year after the last injections. In this regard, it is not possible to establish whether this carry-over effect reflects a true disease remission or just a modification of the natural course of the disease.

Citation 18

Title	Munteanu SE, Zammit GV, Menz HB, Landorf KB, Handley CJ, Elzarka A, Deluca J. **Effectiveness of intra-articular hyaluronan (Synvisc, hylan G-F 20) for the treatment of first metatarsophalangeal joint osteoarthritis: a randomised placebo-controlled trial.** *Ann Rheum Dis*. 2011 Oct;70(10):1838-41
Aim of the study	The aim of the study was to evaluate the effectiveness of a single intra-articular injection of hylan G-F 20 (Synvisc) for symptomatic first metatarsophalangeal joint (MTPJ) osteoarthritis (OA).
Relevance of the study	This study is the first reported randomised placebo-controlled trial investigating the efficacy and safety of intra-articular hyaluronan (Synvisc, hylan G-F 20) to reduce pain in people with symptomatic first MTPJ OA.
Equivalent Device	(**Synvisc**, hylan G-F 20)
Study Design	This was a parallel group, participant and assessor-blinded, randomised placebo-controlled study.
Study period	Enrollment occurred from June 2008 to January 2010. The trial was completed in July 2010.
Sample size	A total of 151 participants were recruited.
Inclusion Criteria	Not available.
Exclusion Criteria	Not available.
Intervention	Participants received a single intra-articular injection of up to 1 ml of hylan G-F 20 (Synvisc; Genzyme Corporation, Ridgefield, New Jersey, USA) or sterile saline (placebo, 0.9% w/v NaCl) into the first MTPJ. The injections were performed by an interventional radiologist (AE) guided by fluoroscopy. If the participant had bilateral symptoms, only the most painful side was treated and used for data collection. Participants were given the option of a second and final intra-articular injection (of hylan G-F 20 or sterile saline according to their allocated treatment group) at month 1 or 3 if there was no improvement in first MTPJ pain.
Outcomes	Outcomes were evaluated at 1, 3 and 6 months after injection. The primary outcome measurement was the foot pain domain of the Foot Health Status Questionnaire (FHSQ) at 3 months. Secondary outcome measurements were foot function assessed via the FHSQ, fi rst MTPJ pain and stiffness, magnitude of symptom change, global satisfaction, health-related quality of life (assessed using the Short-Form-36 version two), first MTPJ dorsiflexion range of motion, hallux

	plantar flexion strength, use of pain-relieving medication or co-interventions and changes in plantar pressures.
Study Results **Performance**	Both groups experienced improvements in foot pain compared with baseline, but there were no statistically significant differences between the hylan G-F 20 or placebo groups at any time point. No further data available.
Study Results **Safety**	The proportion of local adverse events at 1 month was significantly less in the hylan G-F 20 group (RR=0.602, 95% CI 0.378 to 0.960). There were no other statistically significant differences in the proportion of participants reporting adverse events or in the frequency of local adverse events for any time period. One participant (hylan G-F20 group) developed cellulitis at the injection site 2 days after injection that was defi nitely related to the study treatment and this resolved after treatment with antibiotics for 12 days. There were no serious adverse events.
Limits of the study	Not available.
Discussion	A single intra-articular injection of hylan G-F 20 was shown to be no more effective than a placebo for the treatment of painful first MTPJ OA. Although there were improvements within the hylan G-F 20 and placebo groups in the primary and secondary outcome measurements during the study, with few exceptions, there were no differences between the groups at any time point. No major safety issues occurred in this study. The most common adverse event was local transient pain (mean 7 days) arising after the injection, which is in agreement with previous studies. The risk of participants reporting at least one adverse event or frequency of self-reported adverse events did not differ significantly between the hylan G-F20 and placebo groups with the exception of the first month, where approximately 40% fewer participants in the hylan G-F 20 group reported at least one local adverse event. The reasons for this finding are unclear, particularly as they are in contrast to other studies, which have reported an increased risk of local adverse events in those receiving intra-articular hyaluronan injections.
Conclusions of the authors	A single intra-articular injection of hylan G-F 20 is no more effective than a placebo (saline) in reducing symptoms in people with symptomatic first MTPJ OA. Accordingly, hylan G-F 20, administered according to the protocol used in this trial, is not recommended for first MTPJ OA.

Citation 19

Title	Chevalier X, Jerosch J, Goupille P, van Dijk N, Luyten FP, Scott DL, Bailleul F, Pavelka K. **Single, intra-articular treatment with 6 ml hylan G-F 20 in patients with symptomatic primary osteoarthritis of the knee: a randomised, multicentre, double-blind, placebo controlled trial.** *Ann Rheum Dis*. 2010 Jan;69(1):113-9.
Aim of the study	The primary objective was to compare a single, 6 ml, intra-articular injection of hylan G-F 20 with placebo in patients with symptomatic knee osteoarthritis. The safety of a repeat injection of hylan G-F 20 was also assessed.
Relevance of the study	A single 6 ml injection may represent an attractive alternative to the current treatment regimen, reducing the number of intra-articular injections required and thereby offering potential comfort and safety benefits to patients.
Equivalent Device	Hylan G-F 20 (**Synvisc-One**, Genzyme Corporation, Ridgefield, New Jersey, USA), was supplied in 6 ml PBS. Placebo was 6 ml PBS.
Study Design	Randomized, multicentre, double-blind, placebo controlled trial.
Study period	Not available.
Sample size	A total of 329 patients enrolled; 76 patients (23.1%) were screening failures; 253 patients (73 men, 180 women) were randomly assigned and analysed: 124 to receive hylan G-F 20 and 129 to receive placebo. All 253 randomly assigned patients were included in the safety population. One patient was randomly assigned to the hylan G-F 20 group but received placebo in error and was therefore counted in the placebo group for safety and the hylan G-F 20 group for ITT efficacy. A total of 232 patients (91.7%) completed the study. Nine patients (7.3%) randomly assigned to hylan G-F 20 and 12 patients (9.2%) randomly assigned to placebo failed to complete the study schedule as planned. The sample size estimation was based on the mean intergroup difference in the WOMAC A pain subscale change from baseline over 26 weeks. The following assumptions were made to compute the sample size: anticipated overall treatment difference of 0.297; common SD of 0.725; dropout rate of 25%; two-sided significance level of 5%. A resulting sample size of approximately 250 patients (125 patients per group) provided greater than 80% power to detect a difference between the hylan G-F 20 and placebo groups over 26 weeks.
Inclusion Criteria	Patients were required to meet the American College of Rheumatology criteria for osteoarthritis (knee pain for most days of the previous month and osteophyte(s) at the joint margin visible on x ray).

	Main inclusion criteria were: age 40 years or greater; diagnosis of primary osteoarthritis of the target knee; radiographic evidence of osteoarthritis in the medial and/or lateral tibiofemoral compartment (one or more osteophyte(s) and a measurable joint space on a standard radiograph taken within 3 months before screening); continued osteoarthritis pain in the target knee despite conservative treatments. Patients were required to have a score of 2 or 3 (0 to 4 scale) on question 1 of the WOMAC (Likert version 3.1) pain (A) subscale (pain while walking on a flat surface) as this is the most commonly reported symptom in clinical practice and the protocol was designed to weight this symptom more heavily. Included patients required a mean score of 1.5–3.5 on the WOMAC A (total pain) subscore.
Exclusion Criteria	Main exclusion criteria were: secondary osteoarthritis in the target knee; grade IV radiographic stage osteoarthritis (Kellgren–Lawrence grading system); clinically apparent tense effusion of the target knee; significant valgus/varus deformities; viscosupplementation in any joint in the past 9 months; surgery in the knee within the past 6 months; symptomatic osteoarthritis of the contralateral knee or either hip unresponsive to paracetamol; systemic or intra-articular injection of corticosteroids in any joint within 3 months before screening.
Intervention	Before commencing the study, a washout period of prohibited pain and osteoarthritis medications (analgesics and non-steroidal anti-inflammatory drugs with half lives of > 5 h and systemic corticosteroids) was required. Patients were randomly assigned to receive arthrocentesis plus a 6 ml intra-articular injection of either hylan G-F 20 or buffered physiological sodium chloride solution (PBS) (placebo) on day 0. The injection approach was left to the unblinded injector's clinical discretion. Paracetamol ((4000 mg/day) was permitted as rescue medication for the target knee.
Outcomes	Patients were followed up 1, 4, 8, 12, 18 and 26 weeks after injection. The primary efficacy analysis was performed on the intent-to-treat (ITT) population (all randomly assigned patients), based on a repeated-measures analysis of covariance that was used to test for intergroup differences in the WOMAC A (pain) subscore over 26 weeks. The analysis of covariance model included terms for treatment, site, time and time-by-treatment interaction, as well as the baseline WOMAC A score as a covariate. Secondary efficacy outcomes were analysed using generalised estimating equations for a proportional odds logistic regression. The generalised estimating equations model was fitted to the observed data and included terms for baseline measure, site, visit, treatment group and a visit-by-treatment group interaction. These analyses included the difference between the groups from baseline at week 26 in WOMAC A and the differences from baseline over and at 26 weeks in WOMAC A1, WOMAC subscale C, PGA, COGA, and the responders to treatment per the Outcome Measures in Rheumatology, Osteoarthritis Research Society International

	(OMERACT–OARSI) responder criteria. For the WOMAC A1 responder analysis, patients were classified at each post-baseline visit into a responder category (yes/no). Those patients with at least a one-point category improvement from baseline who did not withdraw due to lack of efficacy were considered responders. To assess the safety of a repeat injection of 6 ml hylan G-F 20, patients from both groups were permitted to enter a 4-week openlabel repeat treatment phase 26 weeks after their initial injection if they had no major safety concerns during the first course of treatment and an average WOMAC A score of at least 1.
Study Results **Performance**	The treatment effect with hylan G-F 20 was statistically significantly superior to placebo for the primary endpoint, change in WOMAC A (pain) over 26 weeks. The estimated treatment difference between the hylan G-F 20 group and placebo group over the 26-week study was statistically significant (p=0.047). Some, but not all, of the secondary endpoints, including WOMAC A1 (walking pain), PGA and COGA, showed statistically significant differences between the two groups favouring hylan G-F 20 treatment. Seventy-one per cent (88/124) of the patients were WOMAC A1 (walking pain) responders at week 18 in the hylan G-F 20 group compared with 53% (69/129) in the placebo group (p=0.003). At week 26, 64% (79/124) of patients in the hylan G-F 20 group were WOMAC A1 responders compared with 50% (64/129) in the placebo group (p=0.028). The change in WOMAC C (function) scores did not reach statistical significance. Further exploratory analyses of predefined covariates were carried out to understand better the lack of effect of hylan G-F 20 on the WOMAC C endpoint. In patients without any other lower limb osteoarthritis (defined as hip or contralateral knee involvement), those treated with Synvisc experienced a greater change in WOMAC C than those treated with placebo (20.71 and 20.55, respectively). The OMERACT–OARSI responder analysis over 26 weeks approached statistical significance (p=0.059). At week 26, 73 patients (59%) in the hylan G-F 20 group and 66 patients (51%) in the placebo group were responders. Overall, patients consumed a mean daily dose of 0.26 g (SD 0.654 g) of paracetamol in the hylan G-F 20 group, and 0.28 g (SD 0.570 g) in the placebo group. Throughout the study there was no statistically significant difference in paracetamol consumption between the two groups (p=0.370).
Study Results **Safety**	There were no target knee serious AE and no serious AE that were related to the study treatment or the study procedure. The overall frequency of AE was comparable between the two treatment groups (hylan G-F 20, n = 70, 56.9%; placebo, n = 79, 60.8%). The most commonly reported AE were pain in the target knee (coded as ‘‘arthralgia’’), joint stiffness, joint effusion and joint swelling. The incidence of AE was slightly higher in the hylan G-F 20 group (n = 7, 5.7%) than in the placebo group (n = 4, 3.1%) but this was not statistically significant (p=0.366). In

	addition, there were no statistically significant differences between the groups in treatment-related (p=0.203) or procedure-related (p=0.531) target knee AE, all of which were of mild or moderate severity. A total of 160 patients was treated in the open, repeat treatment phase, of which 77 received a second injection of hylan G-F 20 and 83 received a first injection of hylan G-F 20, having received placebo during the initial treatment phase. There were no target knee serious AE. In the group receiving a second injection of hylan G-F 20 one patient (1.3%) experienced target knee AE related to the study treatment and four patients (5.2%) experienced target knee AE related to the study procedure. Patients who developed target knee AE during the initial phase of the study, and who subsequently received repeat treatment, did not experience target knee AE on repeat exposure to hylan G-F 20. All treatment-related and procedure-related target knee AE were of mild or moderate severity.
Limits of the study	Not available.
Discussion	This study demonstrates that a single intra-articular injection of hylan G-F 20 is safe and effective in providing statistically significant, clinically relevant pain relief, as measured by WOMAC A1 (walking pain) over 26 weeks, with a modest difference compared with placebo. Several secondary efficacy results also show the superiority of hylan G-F 20 over placebo. Pain while walking is particularly medically relevant for the assessment of symptomatic relief and has been selected as the primary efficacy measure in other studies of hylan G-F 20 or other hyaluronans. The OMERACT–OARSI responder analysis also favoured hylan G-F 20 although statistical significance was not reached (p=0.059). Evaluation of the safety profile for the higher injected volume (6 ml) of hylan G-F 20 was also a major objective of this study. The similarity in the safety profiles of hylan G-F 20 and placebo (PBS) is reassuring. The safety profile of hylan G-F 20 was confirmed during the repeat treatment phase of the study.
Conclusions of the authors	This placebo-controlled study demonstrated that, in patients with knee osteoarthritis, a single 6 ml intra-articular injection of hylan G-F 20 is safe and effective in providing statistically significant, clinically relevant pain relief over 26 weeks, with a modest difference versus placebo.

Citation 20

Title	Lundsgaard C, Dufour N, Fallentin E, Winkel P, Gluud C. **Intra-articular sodium hyaluronate 2 mL versus physiological saline 20 mL versus physiological saline 2 mL for painful knee osteoarthritis: a randomized clinical trial.** *Scand J Rheumatol*. 2008 Mar-Apr;37(2):142-50
Aim of the study	The aim of this trial was to compare hyaluronate 2 mL (HyalganH 10.3 mg/mL) versus physiological saline 20 mL (distention) versus physiological saline 2 mL (placebo) in elderly patients with osteoarthritic knee pain resistant to analgesics.
Relevance of the study	Saline washout, closed needle joint lavage, and saline injection without lavage have been reported to diminish knee osteoarthritis symptoms; lavage may remove debris from the joint, may dilute cytokines and degradative enzymes, and may reduce the distention of the joint capsule. The authors compared hyaluronate 2 mL (HyalganH 10.3 mg/mL) versus physiological saline in elderly patients with OA of the knee. This article might be important to sustain the efficacy and safety of hyaluronic acid in viscosupplementation of the knee affected by OA.
Equivalent Device	Hyaluronate 2 mL (**HyalganH** 10.3 mg/mL) versus physiological saline 20 mL (distention) versus physiological saline 2 mL (placebo).
Study Design	Randomized, patient- and observer-blind trial
Study period	Recruitment began in May 1999 and ended in November 2001.
Sample size	Two hundred and fifty-one patients were randomized to one of the three interventions. The primary outcome measure, pain on movement on a 100-mm VAS (VAS-movement), has a standard deviation of 26 mm. Based on α=0.05 and β=0.05 and a minimal relevant difference of 15 mm, it was estimated that at least 80 patients were needed in each group, making a total of 240 patients. After 251 patients were randomized, the inclusion was stopped.
Inclusion Criteria	Eligible patients were over 59 years of age with daily knee pain above 20 mm on a 100-mm visual analogue scale (VAS-movement) that did not respond satisfactorily to analgesics. Based on radiographic findings, OA patients were classified into mild (Kellgren–Lawrence grade 1 or 2) or severe (Kellgren–Lawrence grade 3 or 4).
Exclusion Criteria	Patients were excluded if they had rheumatoid arthritis or other inflammatory arthritis as diagnosed by the American College of Rheumatology, intra-articular steroid injections within the previous 2 months, invasive knee procedures within the past 6 months, contraindications to hyaluronate (e.g. allergy), contra-indications to injections into the knee (e.g. local dermatological disease), medications that could interfere with the planned interventions, or coexisting diseases (e.g. psychosis, dementia) that could inter-fere with the investigation.

Intervention	The injections were given to the knee joint that was causing the patient the most pain. The patients were positioned sitting with the legs flexed. The knee was disinfected with an iodine solution twice. A cannula 21 G (diameter 0.8 mm) was adapted to a 5 mL syringe and inserted into the knee joint through the lateral midpatellar portal. Before treatment, any accumulation in the knee was withdrawn through aspiration. The cannula was left in situ and the syringe removed. Then the allocated intervention added in a syringe was injected intra-articularly. The syringe and cannula were removed and the injection site covered with sterile gauze. All patients were permitted analgesics of the acetaminophen, aspirin, NSAID (inclusive COX-2 selective inhibitors), codeine and tramadol groups.
Outcomes	Baseline values of all outcome measures were measured just prior to the first injection. The patients were evaluated at weeks 1, 2, 3, 8, 12, 16, and 26 after randomization. These evaluations were conducted before any new intervention was administrated. The primary outcome measure was pain on movement on a 100-mm visual analogue scale (VAS-movement). Secondary outcome measures were pain at rest (VAS-rest) and during the night (VAS-night), the quadriceps circumference (cm), ability to bend (degrees flexion) and stretch (degrees extension), and the knee injury and osteoarthritis outcome score (KOOS) of symptoms, rigidity, pain, daily functions during sport and leisure time, quality of life (all questions from the Western Ontario and McMaster Universities Osteoarthritis Index, WOMAC and sport function. In the KOOS scale zero represents extreme knee problems and 100 no knee problems. The response according to the Osteoarthritis Research Society International (OARSI) criteria was measured in all but 41 patients. At each follow-up the global assessment of the patient's condition as compared to that of the previous visit was recorded by the patient and the physician independently, and scored as greatly improved, improved, unchanged, deteriorated, or much deteriorated. The results were coded as 2, 1, 0, 21, and 22, respectively. It was assumed that the results could be meaningfully analysed within patients, but not between patients. Consequently, the scores were added within each patient and the results recoded as 1, 0, and 21 if the sum was positive, zero, or negative, respectively. The consumption of analgesics was scored at each visit as increased, unchanged, or decreased as compared to that observed on the previous visit. The scores were added within patients, and positive, zero, or negative sums transformed to 1, 0, and 21, respectively. BMI and quadriceps circumference were only measured at the last follow-up visit. Adverse events and serious adverse events were assessed at each follow-up visit, or if the patient had complaints.
Study Results	No significant interaction between time and group was observed (the range of p-

Performance

values was 0.13–0.91). Thus, the time curves of the three intervention groups were parallel except for random variation. The model was therefore simplified to include only main effects of time and of group, that is only differences between mean levels.

Tables 21 and 22 show the mean difference from the reference group (physiological saline 2%) of the primary and secondary outcome measures in each intervention group during and after intervention. p-values of the statistical analyses are presented. Table 22 includes assessment by OARSI criteria.

The mean levels of the primary and secondary outcome measures did not differ significantly between the three intervention groups except for extension gap, where a difference in borderline significance was noted.

Pairwise comparisons revealed that only the difference between the 20 mL vs. the 2 mL physiological saline groups was significant (p=0.033).

	Hyaluronate 2 mL	Physiological saline 20 mL	Physiological saline 2 mL	p of group main effect	p of time main effect
Primary outcome					
VAS-pain at movement (mm)	5.46 (−0.08 to 11.0)	3.87 (−1.69 to 9.44)	0.0	0.37	<0.0005
Secondary outcomes					
VAS pain at rest (mm)	0.75 (−3.54 to 5.04)	1.25 (−3.06 to 5.55)	0.0	0.72	<0.0005
VAS pain at night (mm)	−1.80 (−7.36 to 3.76)	0.39 (−5.19 to 5.97)	0.0	0.42	0.011
KOOS symptoms	−3.12 (−6.14 to 1.79)	−1.25 (−4.29 to 1.79)	0.0	0.40	<0.0005
KOOS activities	−3.67 (−8.54 to 1.20)	−3.45 (−8.33 to 1.43)	0.0	0.52	<0.0005
KOOS pain	−1.41 (−5.79 to 2.97)	−1.59 (−5.98 to 2.80)	0.0	0.63	<0.0005
KOOS sports	−1.31 (−7.13 to 4.50)	−4.19 (−10.0 to 1.64)	0.0	0.84	<0.0005
KOOS quality of life	−2.72 (−7.31 to 1.87)	−2.06 (−6.66 to 2.55)	0.0	0.72	<0.0005
Extension gap (deg)	0.844 (−0.59 to 2.28)	1.88 (0.44–3.31)	0.0	0.038	0.40
Flexion (deg)	−1.27 (−4.33 to 1.79)	−1.57 (−4.65 to 1.50)	0.0	0.10	0.91

The value shown for outcome is the group mean difference from the reference group (physiological saline 2 mL) and the corresponding 95% confidence interval. The p-values are based on the results of an analysis only including the main effects of intervention group and time.

Table 21. Outcome, mixed model analysis.

Secondary outcomes	Hyaluronate 2 mL	Physiological saline 20 mL	Physiological saline 2 mL	p-value
				Rank differences between groups
Global assessment by patient				
Improvement	57 (67.9)	52 (62.7)	42 (51.9)	0.070
Deterioration	19 (22.6)	17 (20.5)	29 (35.8)	
Global assessment by physician				
Improvement	68 (81.0)	56 (67.5)	47 (58.0)	0.010
Deterioration	13 (15.5)	17 (20.5)	29 (35.8)	
Consumption of analgesics				
Decreased	30 (35.7)	23 (27.7)	17 (21.0)	0.29
Increased	22 (26.2)	18 (21.7)	23 (28.4)	
				Group effect by logistic regression
Assessment by OARSI criteria				
Class-1 responders*	50 (61.0)	42 (51.9)	33 (41.8)	0.053
Odds ratio (95% CI)	2.18 (1.15–4.14)	1.51 (0.79–2.83)	reference	
Class-2 responders**	30 (36.6)	27 (33.3)	27 (34.2)	0.90
Odds ratio (95% CI)	1.12 (0.58–2.16)	0.96 (0.49–1.88)	reference	
				Main effect of groups
Quadriceps circumference (cm)	47.3 (46.2–48.5)	47.0 (45.9–48.2)	46.5 (45.4–47.6)	0.36
Body mass index (kg/m^2)	29.4 (28.5–30.4)	29.0 (27.9–30.1)	29.3 (28.3–30.3)	0.69

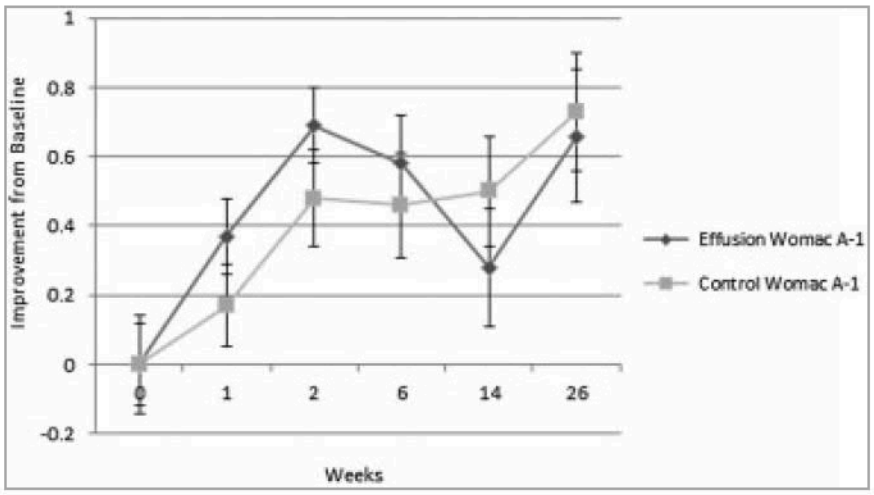
Values are given as n (%) or, for continuous quantities, mean (95% confidence interval). *Class-1 responders are patients who responded according to the OARSIS criteria. If this response could not be determined because of insufficient information on global assessment, the patient was classified as a responder. **Class-2 responders are patients who responded according to the OARSIS criteria. If this response could not be determined because of insufficient information on global assessment, the patient was classified as a non-responder.

	Table 22. Secondary outcomes. For all KOOS and VAS outcome measures, there were no significant differences between the three intervention groups. All three groups showed initial improvement, which later declined somewhat. The investigators' global assessment differed significantly between the three groups: the highest proportion with improvement was found in the hyaluronate group. In the 20 mL saline group the percentage improvement was intermediate. The smallest proportion was found in the placebo group. The outcome as assessed using the OARSI criteria did not differ significantly between the three intervention groups either when the 41 patients without data were classified as responders (p=0.053) or when they were classified as non-responders (p=0.90).
Study Results **Safety**	No serious or non-serious adverse events were reported, thus no local reactions at the injection site with pain, tenderness, and erythema were seen. No post-injection 'flares' were reported.
Limits of the study	A break in the blinding, creating a wish bias, might have occurred. The break in the blinding might originate from the physicochemical differences between the interventions, causing different local sensations in the knee.
Discussion	The analysis of the primary outcome measure of VAS-movement in this trial demonstrated no significant differences between the three intervention groups. In all of the intervention groups a significant improvement was demonstrated during the intervention compared with baseline. The investigators observed no local adverse events after injection, possibly because of the injection technique. The investigators' global assessment of the response to treatment differed significantly between the groups, with the hyaluronate group receiving a better assessment than the distension group, which in turn received a better assessment than the placebo group. Similar results, but of borderline significance, were observed for the patients' global assessment in the hyaluronate group. These findings may suggest either a subtle effect not measurable by the other outcome measures or a break in the blinding, creating a wish bias.
Conclusions of the authors	Intra-articular hyaluronate or distention with physiological saline did not significantly reduce pain compared with physiological saline placebo in patients with OA of the knee.

Citation 21

Title	Waddell BS, Waddell WH, Waddell DD. **Comparison of Efficacy and Tolerability of Hylan G-F 20 in Patients with and**

	without Effusions at the Time of Initial Injection. *J Knee Surg.* 2015 Jun;28(3):213-22.
Aim of the study	This study compares efficacy of hylan G-F 20 in patients with and without an effusion.
Relevance of the study	The efficacy and tolerability of hylan G-F 20 for the treatment of pain associated with knee OA have been demonstrated in numerous clinical trials. Fundamental to viscosupplementation is aspiration of any excess abnormal synovial fluid at the onset of therapy. All viscosupplements direct the user to aspirate any existing synovial fluid before the injection of the viscosupplement. The principle behind this directive is to avoid diluting the hyaluronan with excess synovial fluid. As previously reported, this dilution effect can be as great as a 67% reduction in hyaluronan concentration below the maximum achievable concentration. Many physicians believe that the presence of an effusion at the onset of therapy could adversely affect good clinical outcome and increase AE rates. So, this study determined if an aspirated joint effusion at the onset of viscosupplementation would adversely affect the outcomes of therapy and the AE rate in patients with diagnosis of osteoarthritis (OA).
Equivalent Device	Hylan G-F 20 (**Sinvisc**) - hyaluronic acid for viscosupplementation.
Study Design	Retrospective review of prospectively collected data. Study design called for one control patient (no effusion) for every effusion patient.
Study period	Not available.
Sample size	A total of 50 patients with an effusion requiring aspiration were compared with 50 matched patients without an effusion.
Inclusion Criteria	Patients were generally healthy, ambulatory men or women at least 47 years of age with an OA diagnosis based on American College of Rheumatology criteria (knee pain, radiographic confirmation of osteophytes, and any one of the following: age > 50, crepitus, or morning stiffness <= 30 minute in duration). Patients with mechanical symptoms or deformities due to OA, including flexion contracture > 20 degrees, valgus malalignment > 15 degrees, or varus malalignment > 10 degrees are not considered for hylan G-F 20 therapy. Patients with effusions were selected on the basis of having a palpable joint effusion of the knee upon arrival at the office for their first viscosupplementation injection.
Exclusion Criteria	Not available.
Intervention	Study treatment was administered as three separate, intra-articular injections (2 mL) of hylan G-F 20 at baseline (week 0) and weeks 1 and 2. In the effusion group, sterile technique with ethyl chloride spray and/or local anesthetic and aspiration of the joint effusion using an 18-guage 3" spinal needle was performed before injection of the hylanG-F 20 through the same needle before removing it from the joint. In the control group, hylan G-F 20 was injected using a 21-guage 2" needle

	with aseptic technique, along with either an anesthetic skin spray or subcutaneous local anesthetic (1% plain Xylocaine [AstraZeneca, Wilmington, DE]), under fluoroscopic control to confirm proper needle placement.
Outcomes	A WOMAC questionnairewas completed by patients in both the effusion and control groups at screening, baseline, and all follow-up visits. Along with the full WOMAC, WOMAC Index A-1 (primary endpoint), and WOMAC Domain C were used to determine efficacy of the therapy. The investigator also evaluated patients with the visual analog scale (VAS) for pain at each visit (100 mm).
Study Results **Performance**	Both effusion and control group VAS was significantly lowered at all time points. WOMAC scores improved ($p < 0.025$) at all visits in the effusion group except for WOMAC A-1 week 14. Control WOMAC scores also significantly improved at all visits ($p < 0.027$), except for full WOMAC and WOMAC A-1 at week 1.  **Figure 16.** Western Ontario and McMaster's Universities Osteoarthritis (WOMAC) A-1 (pain while walking on a flat surface) comparison between the two groups. This graph shows the improvement from baseline of the effusion and the control group for the WOMAC A-1. At all time points, there is no statistical difference between the groups. At all times points, the control group is statistically improved from baseline. In the effusion group, only week 14 shows a lack of statistical difference from baseline. This returns to significantly improved at week 26.

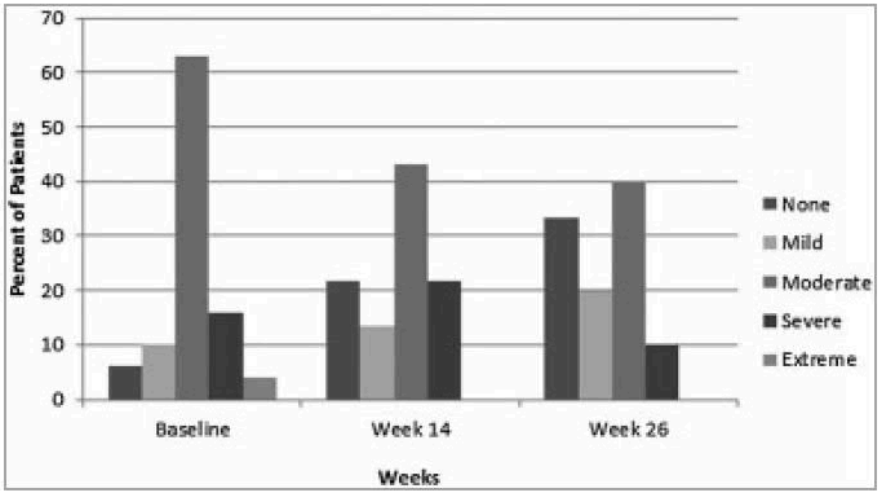


Figure 17. Distribution of Western Ontario and McMaster's Universities Osteoarthritis (WOMAC) A-1 scores in the effusion group. Moderate-to-severe pain scores diminished as none to mild increased throughout study. This bar graph shows the effusion group's WOMAC A-1distribution. At baseline, very few patients rated their pain as none to mild. Most patients rate their pain as moderate and 4% had extreme pain. By week 26, there are no patients who rate their pain as extreme and most rate their pain as none to mild.

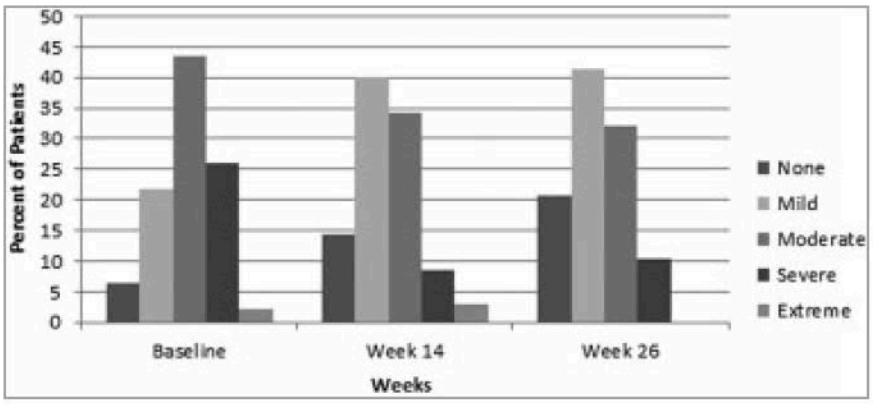


Figure 18. Distribution of Western Ontario and McMaster's Universities Osteoarthritis (WOMAC) A-1 scores in the control group. Moderate-to-severe pain scores diminished as none to mild increased throughout study. This bar graph shows the control group's WOMAC A-1 distribution. At baseline, most patients rate their pain as moderate to severe. About 2% of patients rate their pain as extreme. By the end of the study, most patients rate their pain as none to mild. No patients have

	extreme pain at week 26.
Study Results **Safety**	Neither group experienced an adverse event.
Limits of the study	Follow-up, though similar in each group, was low at the 26-week mark and could have skewed our results. Lack of randomization and its retrospective nature for the control group could lead to bias in selection. Finally, some patients did not fully fill out the survey forms.
Discussion	Both the effusion group and the control group demonstrated improvement with the treatment protocol as described. In addition, no local AEs were noted in either group. No patient had pain, swelling, or any postinjection synovial flare reaction. The only patient that went on to a total knee replacement during the study was a patient in the control group. Although there were patients in both the groups that did not complete the study, follow-up numbers and results were similar in both groups.
Conclusions of the authors	The results of this study demonstrate that there is no negative effect of beginning a course of hylan G-F 20 in patients with a knee effusion that has been aspirated on the day of treatment initiation. Patients with an effusion at the beginning of viscosupplementation with hylan G-F 20 responded as well or better than the control group without an effusion, when the effusion was aspirated before the initiation of treatment.

Citation 22

Title	Karalezli N, Ogun TC, Kartal S, Saracgil SN, Yel M, Tuncay I. **The pain associated with intraarticular hyaluronic acid injections for trapeziometacarpal osteoarthritis.** *Clin Rheumatol.* 2007 Apr;26(4):569-71
Aim of the study	The purpose of this study was to evaluate the tolerability of viscosupplementation in patients with trapeziometacarpal osteoarthritis and to compare the pain of injections given with and without fluoroscopy control.
Relevance of the study	Trapeziometacarpal osteoarthritis predominantly affects middle-aged women. Most cases with rhizarthrosis can be managed successfully by conservative means. The purpose of this prospective study was to evaluate pain and tolerability of viscosupplementation therapy with hyaluronic acid (HA) for trapeziometacarpal osteoarthritis.
Equivalent Device	Sodium hyaluronate (**Ostenil**, TRB Chemedica, Munich, Germany).
Study Design	Prospective study.
Study period	Not available.
Sample size	Sixteen patients agreed to participate in the study.
Inclusion Criteria	Patient with radiographic osteoarthritis and pain in the first CMC joints agreed to participate in the study.
Exclusion Criteria	Exclusion criteria were as follows: psychotic disorders, mental retardation, infection or any skin conditions at the injection site, non-osteoarthritic joint disease (rheumatoid arthritis and infection), malignant disease, use of anticoagulants, and patients with known allergy to administered agents.
Intervention	Groups A and B consisted of eight patients each with Eaton stage 3 or 4 osteoarthritic changes. The patients in both groups A and B underwent one cycle of three injections (one per week) of 0.3 cm^3 sodium hyaluronate (Ostenil) with an insulin syringe needle (Medset, Anhui Tiankang Medical Products, China) inserted in the original syringe by one investigator (NK). Injections for Group A was administered under fluoroscopy control, but fluoroscopy was not used for group B. The mean ages of the patients in groups A and B were 52 (48–58) and 57 (48–63), respectively.
Outcomes	Pain during the injection was assessed using 10 mm visual analogue scale (VAS), with 0 representing no pain and 10 representing the worst imaginable pain. The patients of both groups were also asked to evaluate the tolerability of the treatment (0, poor; 1, slight; 2, moderate; 3, good; 4, excellent).

	The follow-up time was 1 month from the first injection.
Study Results **Performance**	All the patients in groups A and B complained of pain and discomfort during the injections. The mean VAS scores of the pain in groups A and B were 4.1 (range 3–6) and 5.6 (range 3–7), respectively. The difference of the VAS scores between the three groups was statistically significant ($p<0.005$). The mean score of the tolerability of the injection in groups A and B was 2.5 (moderate–good) (range 1–3).
Study Results **Safety**	There were no complications with the sodium hyaluronate intra-articular injections.
Limits of the study	Not available.
Discussion	Intra-articular HA and steroid injection for the treatment of osteoarthritis of first CMC joint is an effective procedure. The authors of this study, however, observed that the intra-articular injections into the CMC joint is a painful procedure especially if it is done without fluoroscopy control. The reason may be para-articular injection or periosteal irritation.
Conclusions of the authors	Viscosupplementation for the treatment of trapeziometacarpal osteoarthritis is a viable treatment option for stages 3 and 4 patients when they do not want to be operated on. It is a tolerable but not a painless procedure especially when it is done without fluoroscopy control.

Citation 23

Title	Di Sante L, Villani C, Santilli V, Valeo M, Bologna E, Imparato L, Paoloni M, Iagnocco A. **Intra-articular hyaluronic acid vs platelet-rich plasma in the treatment of hip osteoarthritis.** *Med Ultrason*. 2016 Dec 5;18(4):463-468
Aim of the study	The aim of the present study was to test the efficacy of PRP intra-articular (IA) therapy as compared to HA IA treatment in terms of pain relief and functional recovery in a population of hip OA patients.
Relevance of the study	Platelet-rich plasma (PRP) therapy is a feasible, minimally invasive and relatively inexpensive treatment that allows a natural concentrate of autologous growth factors to be obtained from the blood. This therapy is widely experimented in different fields of medicine to test its potential role to enhance tissue re-generation. More recently, ultrasound-guided injection of platelet-rich plasma and HA were used separately and in combination in hip OA patients in a randomized controlled study. Moreover, the efficacy of ultrasound-guided intra-articular injections of PRP versus HA was assessed in another study focused on hip OA. Based on the previously reported researches, this study tested the efficacy of PRP IA therapy as compared to HA IA treatment in patients suffering from hip OA. It might be useful to sustain both the efficacy and safety of hyaluronic acid intended for viscosupplementation.
Equivalent Device	Hyaluronic acid (molecular weight 1,000 to 2,900 kDa) vs platelet rich plasma (PRP).
Study Design	Prospective randomized comparative study.
Study period	Not available
Sample size	Assuming a mean difference from baseline of 3-points of the VAS score at T2 with an α error of 0.05, a β error of 0.2 the minimum sample size was 17 for each group. Assuming a dropout of 15%, 20 patients per group were required. The statistical analysis was performed using the MedCalc version 10.2.0.0 for Windows. All primary and secondary outcome analyses were performed according to the principle of intention-to-treat. The chi-square or 2-sample t-tests were ap applied to compare the differences of the baseline data. A 2-way ANOVA with group (experimental versus control) as the between-subjects factor, and time (T0, T1 and T2) as the within-subjects factor was used to detect any significant differences between the experimental and control groups and within each group. A Tukey post-hoc comparison was used to detect any significant differences between the mean values when a significant main effect and interaction were found. The level of significance was set at $p<0.05$ for all analyses.

	A total of 43 patients were randomized into two groups: HA group (n=22) or PRP group (n=21).
Inclusion Criteria	Consecutive patients of both genders with a diagnosis of hip OA, according to American College of Rheumatology criteria were enrolled in the study.
Exclusion Criteria	Exclusion criteria were the following: I and IV Kellgren and Lawrence scores; clinical evidence of hip joint instability; previous open or arthroscopic hip surgery; history of systemic or local infectious, neoplastic and/or other rheumatic diseases; haematological diseases (coagulopathy); severe cardiovascular diseases; infections; immunodepression; patients in therapy with antiplatelet drugs; and patients with Hb values <11g/dl and platelet values <150,000/mmc.
Intervention	Patients were randomized to either receiving Na-HA (30 mg/2 ml of HA with molecular weight 1,000 to 2,900 kDa) or PRP (3 ml) injections (3 injections in total – 1/week). The injection was performed under sterile conditions by means of a 3.5 MHz convex probe. After a few minutes of rest, the patients were allowed to walk and leave the clinic. They were also advised to rest until the next morning and during the follow-up period (16 weeks); the use of any anti-inflammatory or analgesic medication was not allowed.
Outcomes	Self-rated pain intensity at the moment of the evaluation was measured on a 10-cm horizontal visual-analogue scale (VAS), with 0 cm labeled as “no pain” and 10 cm labeled as “worst pain I have ever had”. The Italian version of the Western Ontario and McMaster Universities (WOMAC) OA index, a self-assessment multidimensional instrument that evaluates 17 functional activities, 5 pain-related activities, and 2 joint stiffness categories in 3 different subscales, was used to measure dysfunction and pain.
Study Results **Performance**	VAS scores were significantly lower than T0 values at T1, but not at T2 in the PRP group, thereby indicating an immediate effect on pain of PRP which was afterward lost (at T2 VAS value was further reduced but this reduction was not statistically significant). In contrast, in the HA group the significance between VAS values was reached only between T0 and T2 values. At T2, patients in the HA group had lower VAS values than those in the PRP group, the difference being significant at the 2-sample t-test (p=0.0004). Two-way ANOVA showed a significant group (F=32.070; p<0.0001) and time (F=6.036; p=0.003) effect for WOMAC A, while no significant group xtime interaction effect was found (F=2.488; p=0.09). Post hoc analysis revealed that WOMAC A scores were significantly lower than T0 values at T2 but not at T1 in the HA group. No differences between T0, T1 and T2 values were discernible in the PRP group. As regards to secondary outcome measures, a significant time (F=4.436; p=0.01) effect was found for WOMAC B, while no significant group (F=0.471; p=0.49) or group xtime interaction (F=1.653; p=0.20) effects were found. Significant differences at post-hoc analysis were found only in the HA group between T0 and T2 values. A

	significant group (F=14.177; p<0.0001) and time (F=3.680; p=0.03) effect was found for WOMAC C, while no group xtime interaction effect was found (F=0.789; p=0.457). Again, post-hoc analysis revealed a significant difference between T0 and T2 values in the HA group.

	Hyaluronic acid group			Platelet rich plasma group		
	Mean ± SD (95% CI)	p-value[a]		Mean ± SD (95% CI)	p-value[a]	
VAS						
T0	6.32±1.7 (5.527-7.113)	*T0 vs T1*	NS	7.08±2.0 (6.067-8.100)	*T0 vs T1*	<0.01
T1	5.27±1.6 (4.538-6.002)	*T0 vs T2*	<0.01	4.73±3.4 (3.016-6.437)	*T0 vs T2*	NS
T2	3.63±2.1 (2.624-4.636)	*T1 vs T2*	NS	6.36±2.1 (5.307-7.415)	*T1 vs T2*	NS
WOMAC A						
T0	42.36±20.5 (32.757-51.963)	*T0 vs T1*	NS	58.89±22.0 (47.952-69.837)	*T0 vs T1*	NS
T1	29.6±13.4 (23.333-35.867)	*T0 vs T2*	<0.01	44.27±28.8 (29.964-58.574)	*T0 vs T2*	NS
T2	19.9±11.4 (14.553-25.247)	*T1 vs T2*	NS	53.47±22.3 (42.391-64.554)	*T1 vs T2*	NS
WOMAC B						
T0	57.65±26.2 (45.365-69.935	*T0 vs T1*	NS	53.72±22.7 (42.414-65.030)	*T0 vs T1*	NS
T1	47.69±21.2 (37.787-57.593)	*T0 vs T2*	<0.01	46.42±27.5 (32.738-60.096)	*T0 vs T2*	NS
T2	32.91±20.6 (23.249-42.567)	*T1 vs T2*	NS	47.22±22.7 (35.936-58.505)	*T1 vs T2*	NS
WOMAC C						
T0	45.83±21.7 (35.663-55.991)	*T0 vs T1*	NS	59.87±22.5 (48.683-71.057)	*T0 vs T1*	NS
T1	39.13±17.2 (31.096-47.158)	*T0 vs T2*	<0.01	49.13±29.1 (34.675-63.583)	*T0 vs T2*	NS
T2	28.39±17.2 (20.360-36.420)	*T1 vs T2*	NS	50.80±22.8 (39.480-62.122)	*T1 vs T2*	NS

[a]Bonferroni corrected, NS: not significant, VAS: visual-analogue scale, WOMAC: Western Ontario and McMaster Universities index, SD: standard deviation.

Table 23. VAS and WOMAC scores at baseline (T0), 4-week (T1) and 16-weeks (T2) evaluations in the analysed group.

Study Results **Safety**	No complications related to the IA injections were registered during the treatment and follow-up period and all patients completed the treatment and performed the post-treatment assessment.
Limits of the study	The main limitation of the present study is its lack of a control group without therapy. However, the authors aimed to compare PRP injections directly with HA injections by means of a non-inferiority study, considering the HA injections as a “gold standard” IA therapy.
Discussion	The efficacy of PRP IA therapy as compared to HA on pain relief and functional recovery in patients with hip OA was tested in the present study which demonstrated an early effect (4 weeks) of PRP treatment on hip joint pain that however, was not maintained at follow-up. On the contrary, HA produced a long term (16-weeks) pain relief and not a short term response. Interestingly, for all the other outcome measures, no significant effect could be demonstrated.
Conclusions of the authors	HA, in elderly patients with OA of the hip, has been proven to be safe and without risks. However, the functional WOMAC and VAS score in the HA has shown to be more effective in the long-term (T2) than the PRP group which presents only significant improvement in VAS scores 4 weeks after treatment (T1). Further studies are needed to confirm these results with longer follow-ups especially if the mechanism of regeneration is proposed and in order to understand the mechanism of action, particular with a view to finding different platelet concentrations and injection timing.

Citation 24

Title	Trueba Davalillo CÁ, Trueba Vasavilbaso C, Navarrete Álvarez JM, Coronel Granado P, García Jiménez OA, Gimeno Del Sol M, Gil Orbezo F. **Clinical efficacy of intra-articular injections in knee osteoarthritis: a prospective randomized study comparing hyaluronic acid and betamethasone.** *Open Access Rheumatol.* 2015 Jan 9;7:9-18.
Aim of the study	This clinical trial was conducted in order to compare HA with a corticosteroid (CS), betamethasone (BM), evaluating both treatments in terms of clinical efficacy and enlarging the follow-up period up to 12 months.
Relevance of the study	Osteoarthritis (OA) is the most common joint disease and leading cause of disability. Intra-articular (IA) administration of hyaluronic acid (HA) or corticosteroids (CS) have been previously studied, though using insufficient number of patients or short follow-up periods. Previous studies did not find significant differences in clinical effects between CS and HA at 3 months or even at 6 months' follow-up, leaving open the discussion on the relative efficacy of the two products.
Equivalent Device	HA with a mean molecular weight of 900,000 Da, obtained by a fermentation process (**Suprahyal®**) or IA injections of BM: BM dipropionate 5.0 mg + BM sodium phosphate 2.0 mg in 1 mL (**Diprospan Hypack®**).
Study Design	Prospective, randomized, open study with parallel groups.
Study period	Patients were recruited between April 2008 and February 2011.
Sample size	A total of 320 patients were screened, of whom 120 (40%) were screening failures. Five patients did not provide any efficacy data after randomization and were, therefore, not included in the analysis of efficacy, leaving a total of 195 patients in the mITT population. Finally, 89 and 91 patients receiving HA or BM, respectively, completed the study according to protocol (PP population).
Inclusion Criteria	Eligible patients were men and women from 40 years to 85 years of age suffering from knee OA, with radiographic OA grade II–III according to Kellgren and Lawrence (KL) with a body mass index (BMI) >35 kg/m^2, who had signed the informed consent form for participation.
Exclusion Criteria	Main exclusion criteria were a history of trauma or surgery on the target knee, inflammatory arthritis, microcrystalline arthropathies, previous unspecific knee synovitis, knee infection, angular deformity >10°, and neoplasia, as well as other conditions where the administration of CS would be specifically contraindicated such as diabetes mellitus, and metabolic syndrome.
Intervention	Patients suffering from knee OA were randomized to receive IA injections of 2.5 mL of 1% HA with a mean molecular weight of 900,000 Da, obtained by a fermentation

	process from Streptococcus zoopidemicus strains (Suprahyal®) or IA injections of BM: BM dipropionate 5.0 mg + BM sodium phosphate 2.0 mg in 1 mL (Diprospan Hypack®). More precisely, the treatment consisted of five IA injections of HA (day 0 and weekly injections afterward) or two injections of BM (day 0 and in the fourth week), and the follow-up visits were scheduled at 3 months, 6 months, 9 months, and 12 months.
Outcomes	The primary efficacy outcomes were reduction in global pain and function improvement using Western Ontario McMaster University Osteoarthritis (WOMAC) subscale at the end of follow-up (12 months), in comparison to baseline and the difference between both treatment groups. A 0–10 cm Visual Analog Scale (VAS) was used for global pain measurement, and a five-point Likert scale (0–4) for WOMAC. For both VAS and Likert scales, the higher the score, the worse is the patient's condition. Efficacy along the different visits scheduled in the protocol and consumption of acetaminophen as rescue medication for OA were assessed as secondary outcomes. Additionally, the number of patients achieving the Minimal Clinically Important Improvement (MCII) on each treatment group was also calculated. The safety and tolerability of the interventions were evaluated based on the incidence and type of adverse events that could have arisen throughout the study.
Study Results **Performance**	In the mITT population, the raw values for pain showed a significant reduction in both groups from early follow-up. Percentages of reduction in pain at 3 months were notably higher in the BM group (66.3%, 95% CI: 63.3–69.3) compared to the HA group (48.5%, 95% CI: 45.8–51.3) ($p<0.0001$). These results showed a reversion in the following visits, with the reduction in pain being significantly higher in the HA group. At 12 months, the mean reduction in pain in the HA group was 33.6% (95% CI: 31.1–36.1) compared to 8.2% (95% CI: 5.2–11.1) in patients treated with BM ($P<0.0001$). The PP population showed similar results, with the mean reduction in pain at 12 months of 34.4% (95% CI: 31.7–36.1) in the HA group and 7.7% (95% CI: 4.4–9.7) for the BM patients ($P<0.0001$). WOMAC's total score and the subscales of pain, function, and stiffness also showed significant improvement in both treatment group. When the WOMAC function scores in HA and BM at different time points were analyzed, the comparison was distinctly favorable to HA at all visits. Tje percentage of patients achieving the MCII for both pain and function was nearly 100% in both groups up to 6 months' follow-up. From this visit onward, the values decreased dramatically in the BM group in such a way that at 9 months the MCII for a change of at least 15 of 100 for absolute change established in the literature was 81.4% in the HA group and only 9.2% in those treated with BM ($P<0.0001$).

	In the PP population, the MCII values when the 15 of 100 cutoff for absolute improvement was used were 82.0% for HA and 5.5% for BM at 9 months, and 77.5% and 2.2% at 12 months for HA and BM, respectively (P<0.0001). When the cutoff was 20% for relative improvement, the values were 88.8% for HA and 6.6% for BM at 9 months and 85.4% and 1.1% at 12 months, for HA and BM, respectively (P<0.0001). Overall, 67.4% of patients in the mITT population and 70.6% in PP took acetaminophen as rescue medication during the follow-up period, with no differences between groups.
Study Results **Safety**	Adverse reactions were all related to the administration procedure, and experienced by 3.5% of the patients: 6 cases of pain (four in the group treated with HA and two in BM) and 1 erythema in the HA group. Effusion was detected in 3.5% of the patients (five patients in the HA group) when attending the second (three patients), third (one patient), and fifth (one patient) injection, and two in the BM group when attending for the second injection.
Limits of the study	Not available.
Discussion	This clinical trial comparing HA and BM showed remarkable long-term improvement in knee OA symptoms after treatment in both groups, with statistical and clinical differences favoring HA (P<0.0001). Adverse reactions were rare and related to the administration procedure, concluding that both treatments were safe and well tolerated, in accordance with other publications.
Conclusions of the authors	The results of our study showed that both treatments are effective in controlling OA symptoms, but the pattern varies over time. The two treatments showed equal efficacy at initial follow-ups, but HA demonstrated a clearly superior long-term effectiveness than BM, with sustained clinical efficacy levels in a significant number of patients 1 year after administration.

Citation 25

Title	de Campos GC, Rezende MU, Pailo AF, Frucchi R, Camargo OP. **Adding triamcinolone improves viscosupplementation: a randomized clinical trial.** *Clin Orthop Relat Res*. 2013 Feb;471(2):613-20
Aim of the study	The objectives of this study was answering the following questions: Does the addition of triamcinolone to viscosupplementation (1) improve first-week pain and function compared with viscosupplementation alone, (2) diminish adverse effects of viscosupplementation alone, and (3) alter 6-month pain and function of viscosupplementation alone?
Relevance of the study	Intra-articular injections, mainly using long- lasting corticosteroid suspensions, have long been used to treat knee osteoarthritis. Viscosupplementation is a relatively new approach with injection of a variety of agents. When comparing viscosupplementation with intra-articular injections of corticosteroids from baseline to the fourth week, steroids have been more effective for pain relief. By the fourth week they provide similar relief, but beyond that viscosupplementation appears to provide greater pain reduction. The delayed onset of symptomatic improvement combined with reports of reactive synovitis may discourage physicians and patients.
Equivalent Device	**Hylan** GF-20 (Group viscosupplementation [Group VS]) and triamcinolone **hexacetonide** (Group VS + T).
Study Design	Prospective, double-blind parallel, group-controlled trial.
Study period	Patients were recruited between January 2011 and March 2011. All patients were evaluated clinically and received intra-articular injections between March 2011 and April 2011. The trial ended by October 2011, Week 24 of the follow-up.
Sample size	Of the approximately 250 patients, 147 met the eligibility criteria for the study, and 104 were randomly selected with a computer-generated program. All invited patients agreed to participate. Patients were randomly divided into two groups of 52 patients.
Inclusion Criteria	To meet the eligibility requirements, a patient had to have: (1) met the American College of Rheumatology criteria for knee OA; (2) no previous fractures of the index knee; (3) no previous surgeries on the index knee; (4) no allergies to any of the substances used; (5) no rheumatoid arthritis; (6) no intra-articular injection in the index knee in the past 6 months; (7) been receiving usual care for OA for at least 6 months; and (8) been able to understand and agree with the informed consent.
Exclusion Criteria	The exclusion criteria were: (1) undergoing surgery during the study; (2) receiving an intraarticular injection during the study; (3) having a severe reaction to the procedure; and (4) having an articular infection of the index joint develop during the study.

Intervention	All procedures were performed in an outpatient setting with the patients receiving local anesthesia. The joint punctures were performed by three orthopaedic surgeons (GCC, AFP, RF) who had experience in viscosupplementation. If present, knee effusion was extracted before the injection. Patients received single intraarticular injection (6 mL) of hylan GF-20 (Group viscosupplementation [Group VS]), or a single intraarticular injection of hylan GF-20 (6 mL) and 1 mL (20 mg) of triamcinolone hexacetonide (Group VS + T).
Outcomes	The VAS, WOMAC, and Lequesne questionnaires were given again at the scheduled visits at Weeks 1, 4, 12 and 24. The primary outcomes were improvements in knee pain and function, as expressed by the results of the questionnaires. Secondary outcomes were the presence of adverse effects (knee pain, effusion, or erythema at Week 1), and any correlation between the anthropometric data and the clinical outcomes.
Study Results **Performance**	Baseline scores were similar (p = 0.062 to p = 0.969) between the groups. At Week 1, Group VS + T showed improvement in all the scores, with a difference from baseline. Group VS showed mild improvement at Weel 1, with a difference from baseline (p=0.009) only in VAS. Comparing the two groups, Group VS+T showed lower levels in WOMAC (p=0.038) and VAS (p=0.014) at Week 1. Seventeen percent of all patients reported knee pain or discomfort and 4.8% had joint effusions after the injections. There were no differences between the groups. During the follow-up, the difference between the groups decreased and at Week 4, 12, and 24 there were no differences between the groups in any score. At 6 months follow-up, both groups showed similar values in WOMAC (p > 0.999), VAS (p>0.999) and Lequesne index (p=0.942).
Study Results **Safety**	One patient in Group VS presented with severe effusion and pain at Week 1 and was treated with arthrocentesis and an intraarticular corticosteroid injection. This patient was excluded from the study. All other cases of adverse events were mild, and the symptoms were relieved with ice, rest, and analgesics.
Limits of the study	The authors did not limit the use of analgesics or any other non-pharmacologic treatment. Moreover, clinical scores such as the WOMAC and Lequesne, cannot distinguish one knee from another when the patient has bilateral OA. No saline injection placebo group was identified.
Discussion	The addition of triamcinolone hexacetonide improved first-week symptom and functional scores of viscosupplementation, but not beyond. It did not seem to increase the likelihood of adverse effects.
Conclusions of the authors	The addition of triamcinolone hexacetonide improved first-week symptom and functional scores of viscosupplementation, but not beyond. It did not seem to increase the likelihood of adverse effects.

Citation 26

Title	Vanelli R, Costa P, Rossi SM, Benazzo F. **Efficacy of intra-articular polynucleotides in the treatment of knee osteoarthritis: a randomized, double-blind clinical trial.** *Knee Surg Sports Traumatol Arthrosc*. 2010 Jul;18(7):901-7
Aim of the study	The aim of the study was to assess the efficacy and safety profile of intra-articular polynucleotides gel injections in the treatment of knee osteoarthritis associated with persistent knee pain.
Relevance of the study	This was the first clinical trial to assess the efficacy of an intra-articular preparation based on polynucleotides in the treatment of osteoarthritis (OA) associated with persistent pain by comparing its effects with standard HA viscosupplementation.
Equivalent Device	2006 - 2007
Study Design	Randomized, double-blind clinical trial.
Study period	Not available.
Sample size	Sixty patients were enrolled and randomized.
Inclusion Criteria	The main inclusion criteria were subjects between 18 and 80 years, having developed persistent pain for at least 2 months and affected by knee osteoarthritis (diagnosis based on the ACR-American College of Rheumatology classification.
Exclusion Criteria	Exclusion criteria included alcohol or drug abuse, pregnancy or breastfeeding, hypersensibility to study products, hyaluronic acid or steroid infiltration therapy ongoing or suspended for less than 3 months, systemic treatment with anticoagulants and steroids ongoing or suspended for less than 1 month, previous bone fractures or severe traumas of the interested knee, presence of rheumatoid arthritis and of relevant haematological pathologies.
Intervention	A total of 60 patients were enrolled and randomized to receive intra-articular polynucleotides (n = 30) or hyaluronan (n = 30); patients received five weekly intra-articular knee injections and the follow-up period was 3 months after the end of treatment. There were no restrictions on the use of NSAIDs during the study period.
Outcomes	Primary endpoint was to determine polynucleotides (PN) efficacy in reducing knee pain at the end of the study, over baseline value and over standard hyaluronan viscosupplementation (HA). Pain levels were measured using a 0–10 cm Visual Analogue Scale (VAS). Secondary endpoints included Knee Osteoarthritis Outcome Score (KOOS), NSAIDs consumption, crackling during movement and articular mobility limitation.

Study Results

Performance

The mean global VAS pain decreased from 5.7 ± 1.9 cm (T0) to 1.9 ± 1.5 cm (T16) in polynucleotide group and from 4.9 ± 2.0 cm (T0) to 2.1 ± 1.4 cm (T16) in hyaluronan group. The reduction in pain was statistically significant for both groups. KOOS increases from baseline values were statistically significant in both groups.

Pain at rest	Group A (PN) (cm)	Group B (HA) (cm)
T0	4.5 ± 3.0	2.9 ± 2.4
T1	3.4 ± 2.8	2.7 ± 2.1
T2	3.0 ± 2.4	2.2 ± 1.7
T3	2.7 ± 2.5	1.9 ± 1.6
T4	2.2 ± 2.5	1.7 ± 1.5
T8	1.7 ± 1.9	1.7 ± 1.9
T16	1.3 ± 1.5	1.2 ± 1.3

Data are presented as mean ± SD

Table 24. VAS scores (pain at rest) for Group A and Group B.

Pain at weight-bearing	Group A (PN) (cm)	Group B (HA) (cm)
T0	5.5 ± 2.3	5.0 ± 2.8
T1	5.1 ± 2.4	4.3 ± 2.4
T2	4.2 ± 2.4	3.7 ± 2.0
T3	3.9 ± 2.4	3.8 ± 1.8
T4	3.8 ± 2.2	3.2 ± 1.8
T8	2.8 ± 1.8	2.5 ± 1.7
T16	2.1 ± 1.6	2.1 ± 1.4

Data are presented as mean ± SD

Table 25. VAS scores (pain at weight-bearing) for Group A and Group B.

Table 4 VAS scores (pain during physical activity) for Group A and Group B

Pain during physical activity	Group A (PN) (cm)	Group B (HA) (cm)
T0	6.9 ± 1.9	7.0 ± 2.2
T1	5.7 ± 1.9	6.2 ± 2.0
T2	4.9 ± 1.9	5.4 ± 2.0
T3	4.7 ± 2.2	4.7 ± 2.0
T4	3.8 ± 2.4	4.1 ± 2.1
T8	3.2 ± 1.8	3.6 ± 2.1
T16	2.4 ± 1.8	3.1 ± 2.2

Data are presented as mean ± SD

	Table 26. VAS scores (pain during physical activity) for Group A and Group B.
Study Results **Safety**	No significant adverse events were reported.
Limits of the study	The principal limitation of this study is represented by the short follow-up period (3 months): another clinical study with an extended follow-up might confirm these preliminary results and should also investigate whether the efficacy of polynucleotides can be maintained changing the posologic scheme (e.g., three injections only).
Discussion	In this trial, the authors verified that intra-articular polynucleotides are able to decrease substantially the pain associated with osteoarthritis and to enhance the global quality of life of patients (as demonstrated by KOOS questionnaire). Polynucleotides are physiological molecules endowed with remarkable viscoelastic and trophic properties that represent an innovation in the field of intra-articular viscosupplementation. Overall efficacy of polynucleotides, both in terms of pain reduction and KOOS results, was comparable to hyaluronic acid. The mean global VAS pain decreased significantly from T0 to T16 in both groups. In the same way, KOOS scores showed significant improvements between baseline and T16 values in both groups. Other secondary parameters (i.e., crackling, LMA and NSAIDs consumption) seem to show, at some timepoints, a greater efficacy of PN over HA, although a statistical comparison between the two groups was not performed.
Conclusions of the authors	The results of this study suggested that intra-articular polynucleotides can be a valid alternative to traditional hyaluronan supplementation for the treatment of knee osteoarthritis.

Citation 27

Title	Raman R, Dutta A, Day N, Sharma HK, Shaw CJ, Johnson GV. **Efficacy of Hylan G-F 20 and Sodium Hyaluronate in the treatment of osteoarthritis of the knee -- a prospective randomized clinical trial.** *Knee*. 2008 Aug;15(4):318-24.
Aim of the study	The aim of this study is to compare the clinical effectiveness, functional outcome and patient satisfaction following intra articular injection with Hylan G-F 20 and Sodium Hyaluronate in patients with symptomatic primary OA of the knee.
Relevance of the study	In recent years, viscosupplementation with Hylan G-F 20 and Sodium Hyaluronate has been successfully used for short-term relief of arthritic symptoms in the knee. Whilst there is a general acceptance of the beneficial effects of hyaluronan, there still exists an ongoing debate about the degree of efficacy of these individual products. Additionally there have been controversies in the therapeutic efficacy of cross-linked/non cross-linked and high/low molecular weight products. This has prompted the need for well-designed prospective randomized trials to resolve the continued uncertainty about the magnitude of therapeutic effects of various products. This study might be useful to sustain the safety and efficacy of hyaluronan in viscosupplementation of the knee.
Equivalent Device	**Synvisc®** (Genzyme Biosurgery, Oxford, UK), composed of cross-linked derivatives of hyaluronan (Hylan G-F 20) with an average molecular weight of 6 million Daltons for its fluid component. **Hyalgan®** (Fidia Farmaceutici S.p.A, Italy), viscous solution consisting of a fraction of purified natural sodium hyaluronate with a molecular weight of 0.50–0.73 million Daltons.
Study Design	Independent single centre prospective randomized study.
Study period	Not available.
Sample size	The sample size was calculated from a two arm pilot study, which was performed with 10 patients in each group. The pilot study was performed over 6 months. Using a power of 80% and α=0.05, the required sample was 156 per group for a total of 312 patients. The final sample required was 344 patients to accommodate a 10% expected dropout. An end point analysis of the intent-to-treat patients was undertaken using the last recorded observation carried forward. All scale variables were tested for normality with the Kolmogorov–Smirnov test. Student's t-test was used for parametric and Mann–Whitney U test for non-parametric data. Fisher's exact test was used for all nominal comparisons. A total of 392 patients who met our criteria and participated in the study. Following randomization; 199 patients received Hylan G-F 20 and 193 received Sodium

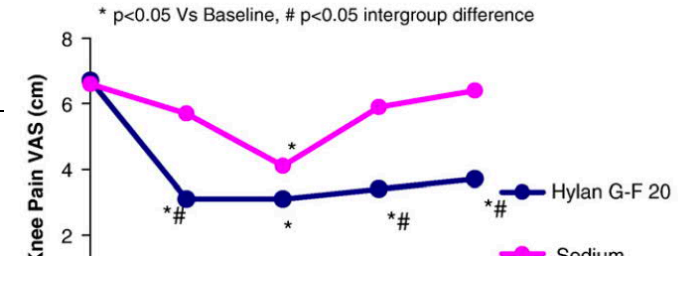
	Hyaluronate.
Inclusion Criteria	The inclusion criterion was a minimum pain score of 6 on a visual analogue scale (VAS) (0–10, 10 as worst pain) in the affected knee.
Exclusion Criteria	The exclusion criteria were surgery to the knee, previous intra articular treatment with corticosteroids, local anaesthetic agents or viscosupplementation agents to the target knee. Patients who had bilateral disease warranting treatment on both knees were excluded from the trial as they received the same treatment agent in both knees.
Intervention	Hylan G-F 20 was administered as a series of 3 weekly injections and Sodium Hyaluronate as a series of 5 weekly injections as per the manufacturer's recommendations. All injections were performed using the default blind technique by the same surgeon (AD), who did not participate in the evaluation of the patients. Any synovial fluid that was present in the knee was aspirated before the injection.
Outcomes	All patients were prospectively reviewed by independent assessors who were blinded for the treatment at pre injection, 6 weeks, 3, 6 and 12 months. Weight bearing radiographs were reviewed at baseline to grade the degree of OA using the Kellgren–Lawrence (KL) system. The follow up was 12 months. Knee pain on a VAS (0–10, 10 as worst pain) was recorded at each visit by the patient. The primary outcome variable was the inter-group difference in the knee pain as measured by VAS at 6 months. Measures of secondary effectiveness were WOMAC 3.1 (Likert) and Oxford knee scores. Patient satisfaction was quantified on VAS. Health related quality of life was measured using EuroQol-5D index[*1]. Safety was assessed at each visit. AE were classified into those occurring within 48 h of injection and those occurring at any other time.
Study Results **Performance**	There was a reduction in knee pain as measured by VAS in both groups at 6 months. However, there was a statistically significant improvement from the baseline score at 6 months only in the Hylan G-F 20 group. Knee pain as measured by VAS improved from 6.7 to 3.1 (median = 2.9) by 6 weeks (p = 0.01) and was sustained until 12 months (3.7, median = 3.5, p = 0.04) with Hylan G-F 20. In the Sodium Hyaluronate group, pain improved from 6.6 to 5.7 (median = 5.8) at 6 weeks (p N 0.05) and to 4.1 (median = 4.0) at 3 months (p = 0.04) but was sustained only until 6 months (5.9, median= 6.0, p >0.05) (Figure 19). When comparing the knee pain improvement from baseline between the two groups, the Hylan G-F 20 group was statistically superior (2.5 mm, p = 0.02) at 6 months. This difference was as early as 6 weeks (p = 0.001) and was observed until 12 months (p = 0.01). However, there was no difference in the magnitude of pain relief at 3 months between the groups. 

Figure 19. Knee pain (VAS) in both groups.

There was improvement in the WOMAC pain subscales in both groups compared to the baseline measurements. The pain subscale scores were significantly better than the pre-treatment scores at all assessment periods in the Hylan G-F 20 group. In the Sodium Hyaluronate group, it was significant only at 3 months, mimicking the results of the primary outcome variable. Pain subscale improvements between the two groups were significantly better in the Hylan G-F 20 group at 3 months (p=0.02), 6 months (p=0.01) and 12 months (p=0.007). Similarly there was an improvement in WOMAC physical activity subscale in both groups. However, the physical activity subscale improvement was significantly better in the Hylan G-F 20 group at 6 months (p=0.02) and 12 months (p=0.004) when compared to the Sodium Hyaluronate group. There was improvement in the WOMAC stiffness subscale in both groups at 3, 6 and 12 months, but no statistical difference was observed between the two groups at these timescales. WOMAC improvement from baseline in both groups at 6 months is illustrated in Figure 20.

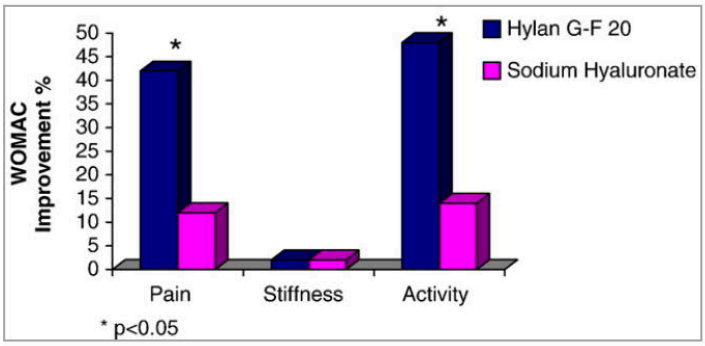


Figure 20. WOMAC improvement at 6 months.

With regard to the Oxford scores, a significant improvement from baseline values was observed in the Hylan G-F 20 group at 6 weeks, 6 months and 12 months. In the Sodium Hyaluronate group, the improvement from the pretreatment value was significant only at 3 months. Analysis of the magnitude of improvement between the two groups suggested a significantly better outcome at 6 (p = 0.009) and 12

	months (p = 0.02) in the Hylan G-F 20 group. General patient satisfaction of the treatment and health related quality of life as measured by EQ-5D assessment tool at baseline, 6 weeks, 3, 6, and 12 months is provided in the following table (Table 27). Patient satisfaction was highest at 3 months in both groups. At 6 months, patient satisfaction was significantly better in the Hylan G-F 20 group. Overall, patients were generally more satisfied with their treatment in the Hylan G-F 20 group. In the Hylan G-F 20 group, EQ-5D description and valuation subscales improved from baseline at 6 weeks and was sustained until 12 months. In the Sodium Hyaluronate group, significant improvement was observed only in the description subscale at 3 months. There was a significant decrease in the requirement of Paracetamol in the Hylan G-F group at 6 months (p = 0.01) and 12 months (p = 0.03) as compared to the Sodium Hyaluronate group.
Study Results **Safety**	Treatment related adverse events (AE) were reported in 39 patients in the Hylan G-F 20 group and in 30 patients in the Sodium Hyaluronate group (p > 0.05). In the Hylan G-F 20 group all AE were minor except one major AE. The major AE occurred in a patient aged 62 years with Grade III OA of the knee. Patient developed severe pain, moderate effusion, erythema, and swelling in the treated knee after 5 days following the third injection. The patient was admitted to the hospital and clinical examination revealed a picture akin to 'pseudo-sepsis' in the knee. The knee aspirate was sterile and the symptoms settled completely by 4 weeks with oral NSAID. This patient was reviewed according to the trial protocol and the outcome was included in the final analysis. 32 of the minor AE in the Hylan G-F 20 group occurred within 48 h and the rest after. All minor AE were related to the treated knee. All AE in the Sodium Hyaluronate group were minor such as injection site pain and occurred within 48 h and relating to the treated knee. No systemic AE were recorded in either of the groups. There were no other withdrawals from the study owing to AE.
Limits of the study	The patients were not blinded to the treatment.
Discussion	Although both treatments offered significant pain reduction, it was earlier and sustained for a longer period in patients with Hylan G-F 20 as seen in other studies. Both treatments were well tolerated, however, a local reaction of pseudo-sepsis was observed with Hylan G-F 20 in one patient. One patient with a serious adverse event in the Hylan G-F 20 group was also included in the final outcome analysis. Few

		Pre injection	6 weeks	3 months	6 months	12 months
EQ-5D description	Hylan G-F 20	0.65	0.72 (p>0.05)	0.76 (p=0.03)	0.74 (p=0.03)	0.76 (p=0.03)
	Sodium Hyaluronate	0.61	0.68 (p>0.05)	0.69 (p=0.04)	0.65 (p>0.05)	0.67 (p>0.05)
EQ-5D valuation	Hylan G-F 20	68	81 (p=0.03)	82 (p=0.03)	80 (p>0.05)	78 (p>0.05)
	Sodium Hyaluronate	69	72 (p>0.05)	72 (p>0.05)	68 (p>0.05)	70 (p>0.05)
Treatment satisfaction (VAS)	Hylan G-F 20	N/A	7.8	8.1	7.9	6.2
	Sodium Hyaluronate	N/A	4.9	5.1	5.0	4.9

p values against pre injection scores.

Table 27. EuroQol-5D Index and treatment satisfaction scores.

	recent publications have reported similar reactions particularly in patients with repeat injections. The shorter treatment regime of Hylan G-F 20 reduces the overall operational cost, both for the patient and the hospital.
Conclusions of the authors	From this study, it appeared that the clinical effectiveness and general patient satisfaction were better amongst patients who received Hylan G-F 20.

[*1] **EuroQol-5D index**

Applicable to a wide range of health conditions and treatments, the EQ-5D provides both a compact descriptive profile and a single index value that can be used in the clinical and economic evaluation of the health care. The EQ-5D has been found to be acceptable, valid, and reliable in population studies and with other patient groups. It consists of five dimensions — mobility, self-care, usual activity, -anxiety/depression, and pain/discomfort. Each dimension has 3 levels of statement representing degrees of perceived problem. In addition to the five dimensions, the EQ-5D also incorporates a visual analogue scale (VAS) on which patients are requested to rate their health on a scale of 0 (worst imaginable health) to 100 (best imaginable health).

Citation 28

Title	Iannitti T, Rottigni V, Palmieri B. **A pilot study to compare two different hyaluronic acid compounds for treatment of knee osteoarthritis.** *Int J Immunopathol Pharmacol.* 2012 Oct-Dec;25(4):1093-8.
Aim of the study	The aim of this pilot clinical study was to investigate the clinical effectiveness of viscosupplementation with the new highly cross-linked HA, Variofill®, in patients affected by bilateral knee OA, in comparison with the widely used Synvisc®.
Relevance of the study	High molecular weight cross-linked HA has been widely used in clinical practice due to its high viscosity allowing a better lubrication and a stronger shock-absorbing affect. Hylan G-F 20 (Synvisc®) is a formaldehyde and divinyl sulfone cross-linked molecule composed of two hylan polymers within a buffered physiological NaCl solution with different rheological properties characterized by a viscosity and elasticity comparable with synovial fluid. The investigators had a previous experience with Variofill®, a highly cross-linked HA formula characterized by a very high density. So, they investigated the clinical effectiveness of viscosupplementation with a new highly cross-linked HA, namely Variofill®, in patients with bilateral knee OA, in comparison with Synvisc®.
Equivalent Device	**Synvisc®** (Hylan G-F 20), viscoelastic fluid containing hylans. Hylans are derivatives of HA sodium salt of avian origin. Synvisc® contains 80% Hylan A fluid and 20% Hylan B gel in buffered physiological sodium chloride solution (pH = 7.2). **Variofill®**, gel of sodium hyaluronate purified from a streptococcus species of bacteria. It is chemically cross-linked and suspended in physiologic buffer at pH = 7.0 to a concentration of 33 mg/ml.
Study Design	Pilot randomized triple-blind clinical study.
Study period	Not available.
Sample size	Twenty patients met the inclusion criteria and were randomized to receive Synvisc® on their left knee and Variofill ® on their right knee.
Inclusion Criteria	The inclusion criteria were bilateral knee OA (Kellgren-Lawrence grade II and III (18)), as diagnosed by Magnetic Resonance Imaging (MRI) and a minimum pain score 2: 30 on both knees as assessed by Visual Analogue Scale (VAS; 0-100 mm, 0 = no pain, 100 = very severe pain). All patients signed the informed consent.
Exclusion Criteria	Exclusion criteria were: patients with unilateral knee OA or unilateral/bilateral knee OA concerning predominantly the patellofemoral region; meniscal- or ligamentous-related instability, as assessed by physical examination; any prior viscosupplementation or intra-articular injection of corticosteroids or any other drug in the knee within 5 months prior to the beginning of the study; concomitance of other pathologies affecting the knee; anticoagulant therapy.

Intervention	With the patients lying in bed, 2 injections (2 ml each) were performed spaced 15 days apart. Variofill® injection was performed on their right knee while Synvisc® was injected into their left knee by the same surgeon who was blinded for the duration of the study and did not participate in the data evaluation. For ethical reasons, Synvisc® was used as control, given that it is a widely used HA product for viscosupplementation for knee OA. Data were evaluated by a blinded allied health professional. Following viscosupplementation, all patients were advised to avoid NSAIDs for 6 months, while paracetamol, at a maximum dose of 2000 mg/day, was allowed for pain management. All patients were advised to stop analgesics for 24 h before each assessment (3, 6 months).
Outcomes	VAS and Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) scores were used to evaluate the efficacy of HA injections before and 6 months after treatment. Patients were advised not to use any analgesic drug 24 hours before baseline assessment.
Study Results **Performance**	Variofill® and Synvisc® administration showed a significant reduction in VAS pain, WOMAC pain, physical activity and stiffness at 3 and 6 months vs baseline ($P < 0.001$) in knee OA patients. A decrease in VAS from a baseline value of 73.3 ± 1.7 to 52.7 ± 1.6 at 3 months and 39.3±2.2 at 6 months was observed in the Synvisc® group ($P < 0.001$ at all time points). A decrease in VAS from a baseline value of 74.7±1.5 to 53.4±1.4 at 3 months and 31.8±0.9 at 6 months was observed in the Variofill® group ($P < 0.001$). The same result was observed when pain was assessed using WOMAC. A decrease in WOMAC pain from a baseline value of 15.05 ± 0.65 to 11.5 ± 0.5 at 3 months and 7 .05±0.3 at 6 months was observed in the Synvisc® group ($P < 0.001$). A decrease in Womac pain from a baseline value of 14.9±0.5 to 10.8 ± 0.4 at 3 months and 5.9 ± 0.3 at 6 months was observed in the Variofill® group ($P < 0.001$). A significant decrease in Womac stiffness from a baseline value of 5.7±0.2 to 3.9±0.2 at 3 months and 2.4±0.1 at 6 months was observed in the Synvisc® group ($P < 0.001$). A significant decrease in Womac stiffness from a baseline value of 6.2±0.2 to 4.1±0.2 at 3 months and 2.5±0.2 at 6 months was observed in the Variofill® group ($P < 0.001$). A decrease in WOMAC physical activity from a baseline value of 53.1±2.4 to 33.5±1.6 at 3 months and 19.6 ± 1.06 at 6 months was observed in the Synvisc® group ($P < 0.001$). A decrease in WOMAC physical activity from a baseline value of 57.2±1.4 to 33.9±1.4 at 3 months and 15.8±1.05 at 6 months was observed in the Variofill® group ($P < 0.001$). Inter-group analysis showed no significant difference between the two treatments at 3 months for VAS pain, WOMAC pain, stiffness and physical activity. At 6 months, Variofill® induced a significant percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity if compared to Synvisc® ($p < 0.05$ vs Synvisc® group; Figs. 2, 3A, 3C). No difference in percentage improvement in Womac stiffness between groups was observed. The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Variofill® group at 6 months was

	56.94±1.18%, 59.54±2.55% and 72.84±3.32% respectively ($p < 0.05$ vs Synvisc® group). The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Synvisc® group at 6 months was 46.2±3.1 %, 52.02±1.9% and 62.003±2.4%, respectively.
Study Results **Safety**	No serious adverse events were observed during treatment at all time points.
Limits of the study	Not available.
Discussion	The results of the present study can be explained by the greatest density ofVariofill® due to its high cross-linking density and concentration, a more sustained coating and antifriction effect across the areas where the cartilage is fractured or damaged, achieving, during its degradation, a progressive lubricant and protecting effect on synoviocyte recovery. The authors suggested that the relented Variofill® turnover, especially in an acute or subacute inflammatory environment, accounts for a quicker functional knee reactivation with reduced pain
Conclusions of the authors	The authors concluded that the results of our study can support Variofill® potential clinical use in patients affected not only by knee OA, but also in other different joints where the persistence of cross-linked HA is required notwithstanding the high pressure of the body weight over the cartilage, either at rest or while performing daily activities.

Citation 29

Title	Rat AC, Baumann C, Guillemin F. **National, multicentre, prospective study of quality of life in patients with osteoarthritis of the knee treated with hylane G-F 20.** *Clin Rheumatol*. 2011 Oct;30(10):1285-93
Aim of the study	The aim of this study was to describe the changes in QoL in patients receiving hylane G-F 20 in routine practice for the treatment of knee osteoarthritis and to determine the factors associated with changes in QoL.
Relevance of the study	The efficacy of viscosupplementation for the relief of pain and disability caused by knee osteoarthritis has been demonstrated, but its effects on Quality of Life (QoL) are less well known. These were investigated in the present study.
Equivalent Device	Hylane G-F 20 (**Synvisc**)
Study Design	Observational, prospective, multicentre study.
Study period	Study conducted between March 2005 and July 2006.
Sample size	To be able to evidence a five-point difference in the SF36 scores between the scores recorded at the third and sixth months and the baseline scores (inclusion) on a scale of 0 to 100 (this difference is considered to be clinically significant for the SF36), with a risk α of 0.05, a power of 80%and a standard deviation of 18 for each dimension, a total of 104 patients was necessary for the primary objective. However, the authors continued to include patients for the secondary objective that was more demanding in the number of necessary subjects. Three hundred patients were included in the study; 226 continued to be followed-up at 6 months, and complete data at 3 and 6 months were available for the 221.
Inclusion Criteria	To be eligible for inclusion, patients had to be aged over 18 years old and present with symptomatic (pain and/or loss of function), Kellgren and Lawrence (KL) radiological stage II or III osteoarthritis of the knee
Exclusion Criteria	Patients receiving treatment for secondary osteoarthritis or who had undergone intra-articular injection of corticosteroids or intra-articular lavage of the target knee in the month preceding inclusion were not included in the study. Patients presenting with bilateral osteoarthritis of the knee and/or symptomatic homolateral or contralateral osteoarthritis of the hip were also not included in the study.
Intervention	The intra-articular injection [three injections (2 ml, average molecular weight of approximately 6,000,000 Da) hylane G-F 20 (Synvisc®) given at weekly intervals] method and care dispensed to patients were those generally employed by the physicians who agreed to take part in the study.

Outcomes	The primary objective was to describe changes in the QoL of patients treated with hylane G-F 20 for knee osteoarthritis in routine practice. The secondary objectives were to describe changes in the other clinical indices of these patients (pain and Lequesne index) and to determine the factors associated with changes in QoL. Two QoL scales were used before and after treatment: these were the generic SF-36 questionnaire (Medical Outcome Study 36-Item Short Form-36) and the specific OAKHQOL questionnaire (OsteoArthritis Knee and Hip Quality Of Life)[*1]. Patients were seen each time they received their hylane G-F 20 injection as per the standard protocol, so once a week for 3 weeks, and then after 3 and 6 months.
Study Results **Performance**	Three and 6 months after treatment, a statistically signifi-cant improvement in the SF36 dimensions was observed with the exception of the general health dimension. With regard to the OAKHQOL questionnaire, a significant improvement ($p<0.0001$) was observed in three of the five dimensions measured, i.e. the physical activity, pain and mental health dimensions, after 3 and 6 months. Conversely, no improvement was measured in the social activity dimension. As expected, the social support dimension did not change. No significant difference was found between the results of the different QoL scores at 3 and 6 months. The mean value for pain on the 0 to 100 VAS scale decreased significantly from 52.3 (20.2) at inclusion to 27.3 (22.6) at 3 months ($p<0.0001$) and 25.6 (21.9) after 6 months ($p<0.0001$). Similarly, the Lequesne index decreased after treatment with hylane G-F 20, dropping from a mean of 10.9 (4.3) at inclusion to 7.9 (4.7) after 3 months ($p<0.0001$) and 7.0 (4.9) at 6 months ($p<0.0001$). The change in the index between 3 and 6 months was not significant. The use of concomitant treatments for knee osteoarthritis (pain relief, NSAIDs and steroids injections) dropped from 87% to 44% at 6 months in the patients monitored for the entire 6 months.
Study Results **Safety**	During this study, overall tolerability was considered to be good. Twenty-seven adverse events were reported in 25 patients. These were expected events, such as local post-injection reactions, most of which were mild to moderate in intensity and resolved spontaneously and rapidly. One serious adverse event was reported: a patient presented with septic *Staphylococcus aureus* osteoarthritis, probably of iatrogenic origin, occurring after the third injection and responding well to antibiotics. A case of severe knee effusion was also reported; this was observed after the third injection and resolved spontaneously. No unexpected adverse events were reported.
Limits of the study	Not all the factors related to changes in QoL are taken into account. It was not

	possible to look for some of the factors that may be linked with changes in QoL. This was particularly the case for the radiographic factors, since this was an observational study conducted on the basis of physicians' findings in which interpretation of radiographic images was not centralised. The number of patients enrolled was low for the analysis of factors associated with response to treatment. Moreover, the number of lost to follow-up was quite high.
Discussion	This study and the different data reported in the literature show that HA has a beneficial effect on the pain and physical dimensions and, to a lesser extent on, the mental health and social functioning dimensions. More precisely, in this study, low QoL scores (high level of limitation or pain) are associated with a larger improvement of the corresponding scores but no other factors emerged as being clearly associated with a change in QoL scores. Prior viscosupplementation, which is probably a reflection of disease severity, is associated with a less important improvement for two dimensions. Effusion was associated with a more modest improvement of the SF36 physical functioning score.
Conclusions of the authors	Not available.

[*1] **OAKHQOL**

The questionnaire consists of 43 items: 40 items divided into five dimensions (physical activity, pain, mental health, social activities and social support) and three independent items. Each of the items in this self-questionnaire is scored on a 1 to 10 visual analog scale and the scores are standardized from 0 (worst QoL) to 100 (best QoL). The OAKHQOL provides a certain amount of information on the patient's private or social life that is not covered by the SF36, including social support, sleep, side effects of treatments, future perspectives, use of public transport, stiffness when changing positions and sexuality.

Citation 30

Title	Di Martino A, Tentoni F, Di Matteo B, Cavicchioli A, Lo Presti M, Filardo G, Zaffagnini S, Marcacci M, Kon E. **Early Viscosupplementation After Anterior Cruciate Ligament Reconstruction: A Randomized Controlled Trial.** *Am J Sports Med*. 2016 Oct;44(10):2572-2578. Epub 2016 Jul 27.
Aim of the study	The aim of the present trial was to evaluate pain control and functional recovery provided by a single injection of HA performed the day after anterior cruciate ligament (ACL) reconstruction.
Relevance of the study	Hyaluronic acid (HA) has been widely used to treat osteoarthritis given its biological and mechanical properties. Because HA is an intra-articular treatment approach that affects the joints, it could be used in the management of acute conditions, such as during the early postsurgical phase, to improve articular function.
Equivalent Device	Hyaluronic acid alkyl derivative (HYADD)–4, 24 mg per 3 mL (**Hymovis**; Fidia Farmaceutici SpA), or **saline** solution.
Study Design	Double-blinded, placebo-controlled, randomized study.
Study period	The study was conducted over a 3-year time span (2012-2015).
Sample size	Sixty patients were included.
Inclusion Criteria	The inclusion critera were: (1) Chronic and symptomatic anterior cruciate ligament (ACL) tear requiring surgical reconstruction, (2) age between 18 and 50 years, (3) no concurrent articular lesion requiring surgical treatment (only isolated partial meniscectomy was permitted), (4) no axial malalignment in the index limb, (4) healthy contralateral knee (ie, absence of functional limitation or pain), (5) no con-current rheumatic or metabolic disease, and (6) no alterations of the index limb in the other joints (e.g. hip or ankle disease).
Exclusion Criteria	Patients with an International Cartilage Repair Society (ICRS) grade 3 to 4 focal chondral-osteochondral lesion were excluded.
Intervention	All patients were operated on by experienced surgeons of the same team, and the same surgical technique was adopted to reconstruct the torn ACL, using both hamstring tendons sutured together as a double-stranded graft. The day after the procedure, patients were randomized to receive a single injection of hyaluronic acid alkyl derivative (HYADD)–4, 24 mg per 3 mL (Hymovis; Fidia Farmaceutici SpA), or 3 mL of saline solution. To maintain patient blinding to the treatment, a surgical drape was placed to prevent patients from observing the injection. After the injection, a sterile dressing was placed and patients were instructed to keep the limb elevated and to use topical cryotherapy in the following hours. All patients were discharged on the

	second day postoperatively and were assigned to the same rehabilitation protocol. To manage pain and swelling after discharge, the patients of both treatment groups were advised to use local cryotherapy 3 times a day and oral ketoprofen 80 mg twice a day for 5 days in total.
Outcomes	All patients were evaluated at baseline and at 15, 30, 60, and 180 days and 12 months after surgery by a physician not involved in the injective treatment to maintain the double-blind design of the study. The following evaluation tools were used: Short Form–36 Health Survey (SF-36), International Knee Documentation Committee (IKDC) subjective score, visual analogue scale (VAS) for pain, VAS for general health status, and Tegner score. Furthermore, during the baseline evaluation and at each follow-up visit up to 6 months, active and pas-sive range of motion (ROM) of both the operated and contralateral knee was documented; the transpatellar circumference of both knees was also recorded to assess knee swelling over time. The healthy contralateral knee was used as control for the operated knee. Both ROM and transpatellar circumference of the index knee were compared over time with the contralateral knee to further assess whether a single HA injection could restore the operated side to the same ROM and circumference of the contralateral healthy knee. Adverse events and patient satisfaction rate at 12-month follow-up were documented.
Study Results **Performance**	With regard to the clinical outcome, a significant improve-ment was documented in both treatment groups without any statistically relevant intergroup difference in any of the scores used. In particular, the IKDC subjective score increased from 65.8 6 16.2 to 90.8 6 9.1 (12-month follow-up) and from 60.0 6 17.3 to 91.5 6 8.8 (12-month follow-up) in the HA and pla-cebo groups, respectively. The Tegner score and all the sub-scales of the SF-36 questionnaire showed a similar trend of improvement in both treatment groups. Similarly, the VAS for pain and for general health sta-tus revealed a significant improvement from baseline to the final 12-month evaluation, without reaching statistical intergroup difference at any follow-up evaluations. With regard to the objective measurements, a significant difference between groups was observed in the transpatellar circumference of the operated knee. In the HA group, a lower difference in transpatellar circumference between the contra-lateral nonoperated knee and the ACL-reconstructed knee was documented at 60 days postoperatively, meaning that at this time point, in the HA group, the circumference of the operated knee was more similar to the circumference of the healthy contralateral knee used as a control (P = 0.022). Another significant difference was observed in the active ROM at 30 days postoperatively. The difference between the active ROM of the contralateral healthy knee versus that of the ACL-reconstructed knee was considered: The patients who received HA had less difference in active ROM of the treated knee versus the contralateral healthy knee at 30 days postoperatively, indicating that

	viscosupplementation helped bridge the gap between the operated knee and the contralateral nonoperated knee (P = 0.027).
Study Results **Safety**	No severe adverse events were reported.
Limits of the study	Not available.
Discussion	The present randomized, double-blinded, placebo-con-trolled trial investigated the effects of HA administration after one of the most common procedures in sport orthopaedic practice—ACL reconstruction. The main finding of the present randomized trial is that the early administration of HA after ACL reconstruction does not provide substantial beneficial effects in terms of pain control and functional recovery in the short term.
Conclusions of the authors	The study documented no adverse events and had some positive findings in terms of active ROM recovery and transpatellar circumference reduction. However, the early postoperative application of viscosupplementation did not lead to significant improvement in clinical scores after ACL reconstruction.

Citation 31

Title	Panuccio E, Memeo A, Richetta S. **[Evaluation of the combined treatment of oral viscosupplementation with hyaluronic acid intra-articular injection on symptomatic knee osteoarthritis].** *Clin Ter*. 2015; 166(5):e321-6
Aim and relevance of the study	The goal was to evaluate whether combined treatment with intra-articular injection of HA and AI is more effective than treatment with HA only for the symptomatic treatment of knee OA.
Equivalent Device	The formulation aim of the present study (IA) combines a hydrolyzed low molecular weight collagen matrix providing high content of depolymerised HA and CS, with methylsulfonylmethane (MSM), Manganese and a milk glycoprotein.
Study Design	Randomized, double-blind, placebo-controlled clinical trial.
Study period	Not available.
Sample size	A hundred patients were randomized to receive IA or placebo.
Inclusion Criteria	Not available.
Exclusion Criteria	Not available.
Intervention	Patients, after undergoing an intra-articular injection with HA, were randomized to receive IA or placebo for 3 months.
Outcomes	The efficacy of the treatment was assessed by measuring at baseline, 1 and 3 months, the values of the VAS pain scale, the Knee injury and Osteoarthritis Outcome Score, the Tegner Lysholm Knee Scoring Scale, Lequesne algofunctional index and the consumption of NSAIDs and analgesics.
Study Results Performance	The treatment group HA + IA showed a positive trend compared to the group treated with HA only for all the efficacy variables observed, in particular regarding the VAS and the analgesic consumption.
Study Results Safety	Not available.
Limits of the study	Not available.
Discussion	Not available.
Conclusions of the authors	The evidences obtained in this study point out that the oral viscosupplementation with the formulation aim of the present study (IA) represents a valuable, manageable, effective and well tolerated aid, useful to maintain and extend the benefits obtained with intra - articular injection of HA, helping to significantly reduce the use of painkillers by patients.

Citation 32

Title	Van Den Bekerom MPJ, Rys B, Mulier M. **Viscosupplementation in the hip: evaluation of hyaluronic acid formulations.** *Arch Orthop Trauma Surg* 2008; 128(3): 275-80.
Aim of the study	This was a clinical trial comparing three formulations of HA.
Relevance of the study	Viscosupplementation (VS) is the administration of hyaluronan and/or hyaluronic acid preparations to joint synovial fluid for the treatment of OA in order to restore the biologic properties of normal hyaluronic acid (HA). The use of VS with HA was first described to provide pain relief and to increase mobility of the knee joint. The VS is an effective treatment for OA of the knee with beneficial effects on pain, function and patient global assessment. HA products have more prolonged effects than intraarticular corticosteroids. Since 1984, this technique is also used for the management of OA of the hip joint.
Equivalent Device	**Test devices:** Adant (viscosupplementation containing biofermentative low molecular weight Hyaluronic acid); Synocrom (viscosupplementation containing biofermentative high molecular weight Hyaluronic acid); Synvisc (viscosupplementation containing high molecular weight Hyaluronic acid of animal origin - chicken combs) .
Study Design	This was a prospective clinical study.
Study period	Treatment was performed Between March 2001 and February 2005. Assessment was carried out in April 2005.
Sample size	120 patients (126 hips), 49 males and 71 females, with an age betwwen 30 and 70 years, received the treatment.
Inclusion Criteria	• Age between 30 and 70 years and suV ering idiopathic radiologically conW rmed hip OA. • Visual Analogue Scale (VAS) score for pain greater then 30 (on a 100-point scale; 0 no pain and 100 “the worst pain imaginable”) • Have persistent pain for longer than 1 month despite use of analgesics or NSAID’s. • Be candidate for surgical treatment with a THA, according to the following criteria: o continuous hip pain, also during the night, requiring daily intake of NSAID’s or pain medication o disabled gait pattern and need of walking aid • Be able to understand the information relative to viscosupplementation and to give informed consent.
Exclusion Criteria	• Pregnancy • Contraindications to intra-articular hyaluronic-acid preparations • Major hip dysplasia or congenital abnormality of the hip • Patients with systemic corticosteroids or intra-articular corticosteroid injections in the last 6 months • Contra-lateral THA or hip arthroscopy in the last 6 months • Oral or parenteral anticoagulant therapy

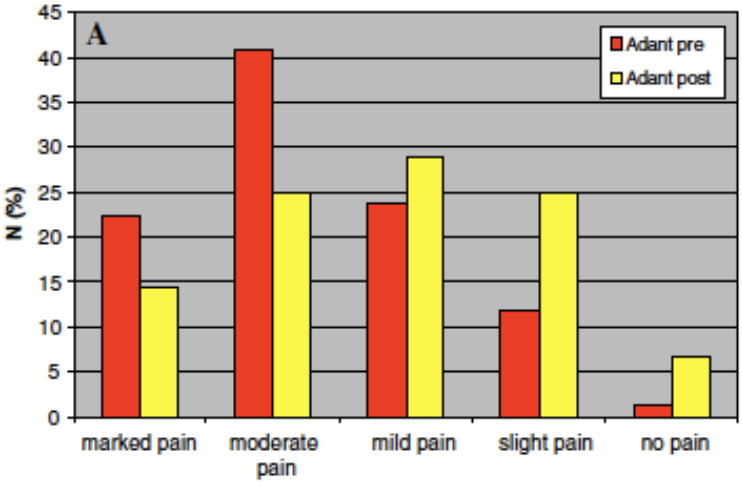
	• Previous hyaluronic acid hip inW ltrations • Skin diseases or infections • Signs of haemarthrosis • History of allergy or hypersensitivity to iodated contrast
Intervention	Name and type of intervention Intra-articular (hip joint) administration of HA-based viscosupplementations Aim of the intervention Comparison of three different hyaluronate formulations and evaluates functionality, time of satisfactory pain relief and also the delay in performing a total hip arthroplasty. Duration 3-year follow-up. Description of the intervention Patients received an intra articular infiltration with one of the three products. The manufacturer's treatment recommendations were followed. Patients having initially experienced a satisfactory pain relief are offered a second and third infiltration or THA when the condition deteriorates. Injection of the viscosupplementation was performed under sterile conditions by the same experienced orthopaedic surgeon (MM) in all patients. After skin cleaning a lumbar puncture needle was inserted in a lateral approach. Layer by layer local anaesthesia was performed using lidocaine 1%. Iodinated contrast agent was injected. The needle positioning into the joint cavity was fluoroscopically controlled. Arthrocentesis was carefully performed prior to each injection to remove any effusion. After resting for 2 h, the patient was allowed to walk and to return home. The patient was advised to rest at home until the next morning. Oral symptomatic slow acting drugs for osteoarthritis were authorized if they were taken at a stable dose for more than 3 months prior to inclusion in the study. These analgesics were continued at a stable dose during the VS treatment.
Outcomes	Primary outcomes Pain and functionality were evaluated using the VAS pain during walking score (100-point scale) and the Harris Hip Score (HHS). The latter is a clinical scoring system on a total of 100 points whereby the following subscales are rated: function (47 points), pain (44 points), range of motion of the hip (5 points) and absence of muscle contractures and length discrepancy (4 points). All side effects and complications of viscosupplementation were noted. In April 2005, all patients were contacted for follow-up assessment over the phone VAS and HHS. Secondary outcomes

	Not reported. Measures and timepoints All patients were assessed at baseline and 6 weeks after each infiltration.
Study Results **Performance**	The mean pre-infiltration HHS was comparable for the three groups and varied from 64.8 points in the Adant group to 66.8 points in the Synocrom-group. The post-infltration HHS increased with 6.3 points in the Adant group ($P < 0.001$), with 10.6 points in the Synocrom group ($P < 0.05$) and with 6.1 points in the Synvisc-group ($P > 0.05$; Figure 28). There was no statistical significant difference in the evolution of the HHS between the three groups (Figure 28).

	HHS pre	HHS post	Difference	*P*
Adant	64.8 ± 13.8	71.1 ± 15.7	+ 6.3	< 0.001
Synocrom	66.8 ± 13.8	77.4 ± 14.7	+ 10.6	< 0.05
Synvisc	66.3 ± 13.5	72.4 ± 14.5	+ 6.1	> 0.05

Figure 28. Evolution in average HHS score.

Viscosupplementation provided a highly significant pain reduction in the Adant-group ($P < 0.0001$), a significant pain reduction in the Synocrom-group ($P < 0.05$) and a pain reduction that did not reach significance in the Synvisc group ($P > 0.05$). There was no significant difference in pain relief between the three treatment products (Figure 29 and 30).



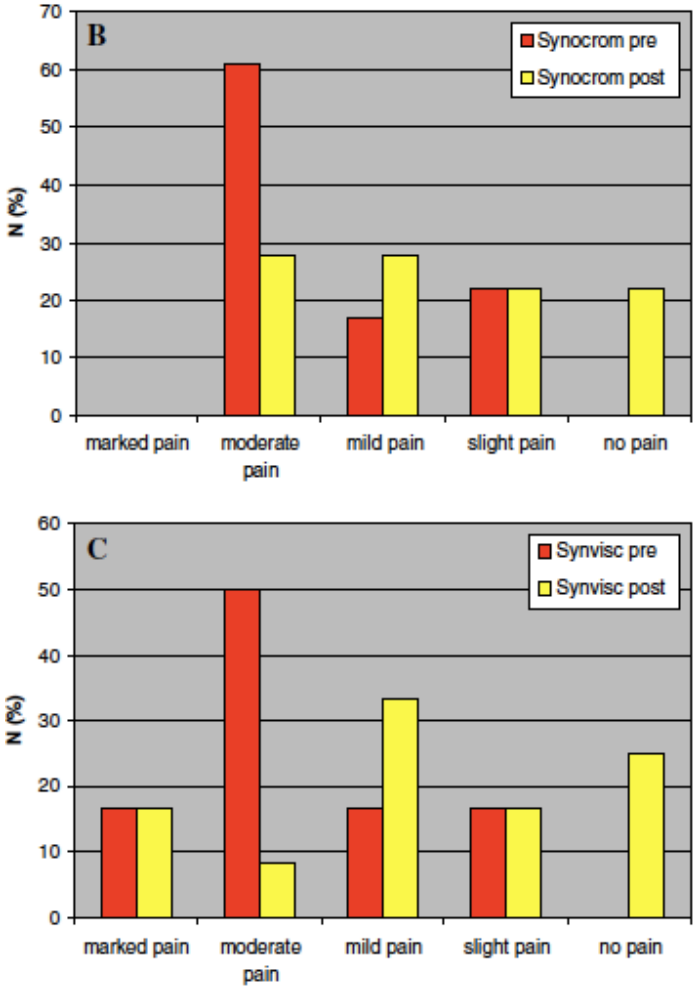


Figure 29. Pain relief in the three treatment groups a Adant, b Synocrom and c

Synvisc.

	VAS pre	VAS post	Difference	*P*
Adant	51 ± 23	39 ± 27	− 12	< 0.0001
Synocrom	43 ± 22	29 ± 23	− 14	< 0.02
Synvisc	47 ± 26	30 ± 29	− 17	> 0.05

Pre: at first infiltration
Post: at follow-up

Figure 30. Duration of treatment effect after first infiltration.

The duration of the effect of the first infiltration in the three groups is shown in a Kaplan-Meier curve (Figure 31).

The first infiltration was the starting point. Endpoints were the second infiltration or operation of the afflicted hip, or when these were not applicable the latest patient contact, which can be considered as ongoing effect.

There is no significant difference between the three groups (P = 0.61).

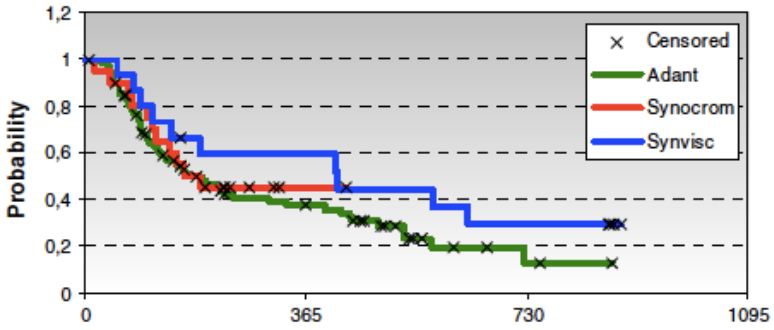
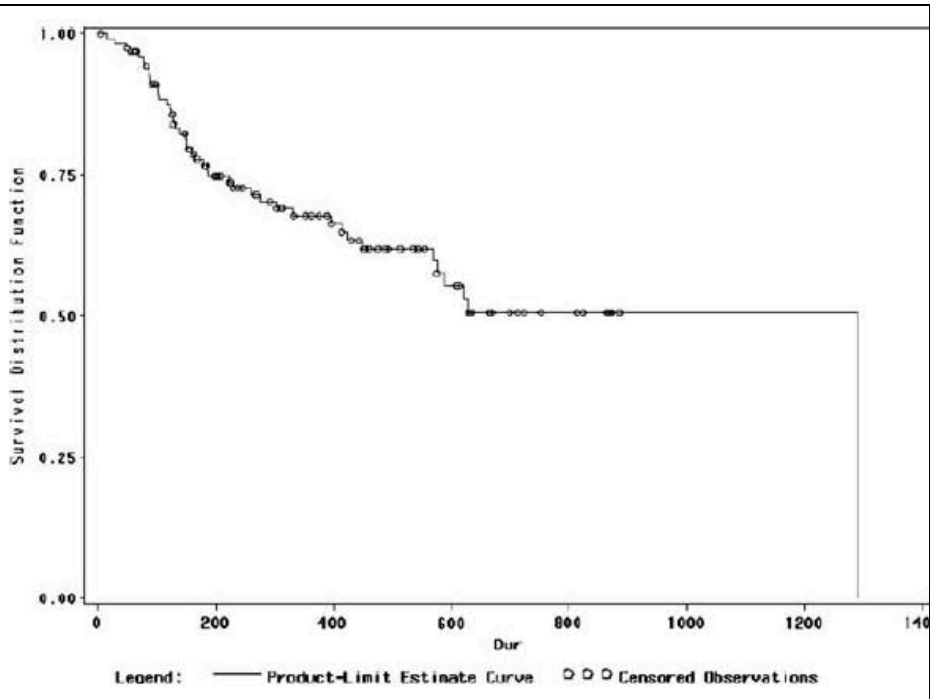


Figure 31. Kaplan-Meier survival curve of the duration of effect of the first infiltration in days for the three different treatment groups.

The positive effect was still ongoing in 46 hips, while in 80 hips patients had either received a second inW ltration or THA at, the end of the study.

The delay in performing a hip operation is analysed using a Kaplan-Meier survival curve (Figure 32). After 3 years, 51% of the patients have not undergone surgery.

	 **Figure 32.** Kaplan-Meier survival curve for the delay to surgery in days for the three groups confounded.
Study Results **Safety**	Not reported.
Limit/s of the study	The results of this study should be considered in the light of the limitations of the design of this study. It is a nonplacebo controlled nonrandomised prospective study. It is known from experience with knee OA that the placebo effect of VS tends to be substantial. The dimension of the groups treated with the three different products differs from 15 to 91 patients.
Discussion	The VS method is widely used for OA of the knee joint, but there are only a few studies about its use in OA of the hip. Most authors agree that there should be a role for viscosupplementation in the treatment of hip OA. The findings of this study confirm the effect of VS in patients suffering OA of the hip. This is the largest series of patients with hip osteoarthritis treated with viscosupplementation. The three preparations provided a significant pain relief and improvement of the HHS. The isolated Synvisc group never reached statistical significance in HHS score evolution and VAS during walk test after VS treatment; possibly due to the small number of patients (N = 15) in this group. We saw no infectious adverse events and no serious systemic reactions, but all the interventions are performed in the operating theatre under strict aseptic conditions. The adverse events rates ranged from 10 to 30% that is slightly higher than the rates reported in VS treatment of knee OA. Repeated injections did not increase the risk

	of adverse events.
Conclusions of the authors	Not reported.

7. ANALYSIS OF THE CLINICAL DATA

7.1. INTENDED PURPOSE

"*HiLow - Visco-Suppletive Joint device*" is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, "*HiLow - Visco-Suppletive Joint device*" reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action.

7.2. CRITICAL ANALYSIS AND COMPARISON WITH THE "STANDARD OF CARE"

Osteoarthritis is a very common disease, and its prevalence increases with age. According to the American College of Rheumatology, nearly 70 % of people over age 70 have X–ray evidence of osteoarthritis, although only half ever develop symptoms. Notwithstanding, due to the huge amount of persons affected, osteoarthritis is a frequent cause of disability.

The management of OA has been described in evidence-based guidelines from important musculoskeletal organizations. There is a general consensus on recommended therapy across these guidelines, although discordance exists on particular therapies [19].

Treatment choices fall into four main categories: nonpharmacologic, pharmacologic, complementary and alternative, and surgical. Surgical management should be reserved for those who do not improve with behavioral and pharmacologic therapy, and who have intractable pain and loss of function [97].

A multidisciplinary, patient-centred combination of education, self-management, exercise, weight loss with realistic goals, encouragement and regular reassess-ment is recommended for individuals with OA.

Topical, oral and injectable pharmacological treatments are available for individuals with OA. Age, concurrent medications, comorbid conditions (in particular, cardiovascular and gastrointestinal problems) and predicted adherence should be considered for each individual before prescribing pharmacological interventions. First-line therapies include topical NSAIDs and oral paracetamol. Topical NSAIDs have better safety pro-files than oral NSAIDs as systemic drug

levels are much lower. However, they are limited by joint penetration and multiple daily applications [19].

Systemic treatment with nutraceuticals — including glucosamine and chondroitin sulfate products, which are natural compounds that consist of GAG unit components and GAGs, respectively — is not recommended by the UK National Institute for Health and Care Excellence (NICE) or guidelines owing to the lack of certainty of clinically important analgesic benefit. Conversely, Cochrane reviews and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) guidelines conclude that these therapies may have analgesic effects beyond the placebo effect [98,99,100].

Intra-articular corticosteroids might be recommended in patients in whom pain is preventing appropriate muscle strengthening exercise or, more uncommonly, in which large effusions are painful or limit joint movement. They provide short-term analgesic benefits typically for 3–4 weeks in individuals with moderate-to-severe OA pain presumably due to their anti-inflammatory actions [101,102]. Side effects of corticosteroids injections in OA patients may be the thinning of the cartilage, the weakening of the ligaments of the joint, the increase of the inflammation caused by a corticosteroid that has crystzallized, the infecting of the joint or irritation of the nerves by the needle or by the medication itself [103].

Arthroscopic lavage and debridement (flushing debris out of the joint space or resecting cartilage and/or meniscus) are not recommended for the treatment of knee OA without a clear history of true mechanical locking, as the clinical outcomes are not improved [104]. If medical interventions fail to sufficiently improve per-sistent debilitating symptoms of OA, joint replacement surgery should be considered. Joint replacement surgery has been highly effective for the hip and increasingly so for the knee joint; the evidence for other joints lags behind. The patient should be adequately informed about the relative benefits of surgery, the risks of continued medical treatment and surgical options along with a realistic understanding of the postsurgical rehabilitation. As a general rule, joint replacement surgery in younger patients (<60 years of age) might be delayed because joint prostheses have a finite life expectancy and revision surgery offers less-favourable outcomes [105,106].

Among the available pharmacologic solutions, despite contradictory findings and controversies regarding its effective usefulness, intra-articular hyaluronic acid (HA) is widely applied in clinical practice, with good results reported in many studies.
HA is the molecule responsible for synovial rheological properties, enabling it to act as a lubricant or shock absorber depending upon the forces exerted upon it. The HA concentration is 2-3 mg/ml in normal joints and is reduced to 0.8-2 mg/ml in joints of arthritic patients. In its altered state, HA contributes to inflammation and no longer has lubricating and hydrophilic properties [67,107]. The purpose of viscosupplementation with HA is to restore the rheologic properties, namely viscosity and elasticity, of synovial fluid by normalizing the concentration and molecular weight of hyaluronan [108].
Viscosupplementation based on HA injections is recommended by the current Osteoarthritis Research Society International (OARSI) guidelines and previous practice guidelines [109].

Hyaluronic acid (HA) is a glycosaminoglycan constituent of synovial fluid and cartilage matrix in normal joints. In osteoarthritis, HA molecular weight (MW) and concentration are decreased. Exogenous HA is available as a vis-cosupplementation device or a drug for intra-articular use in the treatment of knee osteoarthritis symptoms. Different HA formulations are currently available worldwide: from the reference low MW preparation (range 500.000–730.000 Daltons) to more recent intermediate MW (range 800.000–2.000.000) and even cross-linked, high MW formulations (average 6 000 000 Daltons) including hylans, non-animal-derived HA and others.
HA was found to have longer-lasting pain control compared with intra-articular corticosteroids, and the majority of trials has been performed with the low MW HA product. Low MW HA thus often remains the preferred option when using HA in knee osteoarthritis. However, there is a paucity of appropriately sized, high-quality trials comparing the effects of different MW preparations, with particular regard to potential differences between low and intermediate MW products, given the worse safety profile of high MW formulations [110].

There are different commercially available products acting as viscosupplements for the relief from pain due to osteoarthritis. Most of them have Hyaluronic acid as the key ingredient, thanks to its high tolerability and efficacy.

Native HA of synovial fluid has a high molecular weight (4-10 $*10^6$ Da) and a concentration of about 0.35 g/100 ml. At present, preparations with different molecular weight are available (Low and High Molecular Weight). Some clinical study were carried out to compare clinical outcomes of low and high molecular weight; in some of them, high molecular weight hyaluronic acid (HMWHA) is considered more effective in relieving pain, compared to low molecular weight HA. This is due to the fact that HMWHA molecules are bigger than LMWHA: this property allows the compound to not penetrate the extracellular matrix and to concentrate in the joint cavity, leading to a higher lubrication and protection of the joint [103]. However, no conclusive theories have been confirmed regarding the correlation between molecular weight and efficacy [108-110].

Moreover, HA of *"HiLow - Visco-Suppletive Joint Device"* has a biofermentative origin, i.e. it is obtained from microbial fermentation using bacterial strains. Bacterial hyaluronan is not immunogenic and therefore is an excellent source for medical grade hyaluronan. Extracting hyaluronan from microbial fermentation broth is a relatively simple process with high yields. An additional and important advantage of microbial hyaluronan production on avain Hyaluronan is that microbial cells can be physiologically and/or metabolically adapted to produce more hyaluronan of high molecular weight. Therefore, microbial hyaluronan production using either pathogenic streptococci or safe recombinant hosts, containing the necessary hyaluronan synthase, is nowadays more and more preferred [111]. In addition, in relation to hyaluronic acid synthesis, these substances can be classified into two types: hyaluronans, composed of long-chain molecules of avian or biofermentation origin, with a molecular weight of between 0.5 and 1.8 x 10^6 Da; hylan, i.e. hyaluronan molecule chemically modified by means of cross-links, with a liquid phase of higher molecular weight (around 6x10^6 Da), through crosslinking connections

between long chains of hyaluronan, and a solid portion (of infinite molecular weight) formed by even greater presence of links [112].

Furthermore, efficacy and safety of Hyaluronic acid-based viscosupplementations are supported by some clinical trials [113-116].

7.3 *SUMMARY OF CONFORMITY ASSESSMENT WITH REQUIREMENT ON SAFETY (MDD ER1)*

According to Directive 93/42/EEC Essential Requirements (Annex I), 1:

"The devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons, provided that any risks which may be associated with their intended use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety.

This shall include:

- *Reduce as far as possible the risk of use error due to the ergonomic features of the device and the environment in which the device is intended to be used (design for patient safety), and*
- *Consideration of the technical knowledge, experience, education and training and where applicable the medical and physical conditions of intended users (design for lay, professional, disabled or other users)".*

"*HiLow - Visco-Suppletive Joint Device*" is an intra-articular visco-supplementation product that allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis.

7.3.1 Safety features related to "HiLow - Visco-Suppletive Joint Device" ingredients

No special formulation features of the device have been identified that could pose particular safety concerns. In particular, "*HiLow - Visco-Suppletive Joint Device*" mechanism of action is based only on Hyaluronic acid, the key ingredient, while the other compounds, sodium chloride and sodium phosphate, are excipients. Excipients are more or less inert substances added to a compound to give suitable consistency or form to the compound; they are also named vehicles.

As excipients of intra-articular viscosupplementations, Sodium chloride and Sodium phosphate irrigate the joint and are both proven to be nontoxic and nonirritant. Sodium phosphate is often in the form of Sodium phosphate dibasic anhydrous & sodium phosphate monobasic monohydrate.

Hyaluronic acid is a viscous ingredient widely use in cosmetic products and medical devices, thanks to its safety and tolerability. Regarding interactions with other drugs or products, no one is known between HA viscosupplementations and products commonly used for osteoarthritis, such as oral drugs, supplements or corticosteroid/PRP injections.

Furthermore, HA-based intra-articular viscosupplementations are safe and highly tolerable, as proved by clinical data commented below and in Section 7.2.1 and 7.2.2 of this Clinical Evaluation report.

"HiLow - Visco-Suppletive Joint Device" is intended for the treatment for osteoarthritis, and is particularly indicated for pain or reduced mobility due to degenerative diseases (arthrosis), post-traumatic diseases and tendinopathy associated with joint disabilities.

The sodium salt of hyaluronic acid is formed of repeated chains of disaccharide units of N-acetylglucosamine and sodium glucuronate, and is a fundamentally important component of the synovial fluid to which it gives its visco-elastic properties.

In 2008, the Osteoarthritis Research Society International (OARSI) cited intra-articular hyaluronic acid as a useful therapeutic modality that has delayed onset, but prolonged duration of symptomatic benefit, in treating patients with osteoarthritis of the knee or hip [119]. In addition, although marketed as analgesics, viscosupplements have been postulated to have potential chondroprotective effects as well [117].

Hyaluronic acid, also known as hyaluronan or hyaluronate, is a high-molecular-weight glycosaminoglycan made up of repeating disaccharide units of N-acetylglucosamine and glucoronic acid [118]. The average molecular weight of synovial fluid HA is 5 to 7 × 10^6 Da. It is widely present in mammalian tissues and has the highest concentration in synovial fluid. Type B synoviocytes and fibroblasts synthesize HA and secrete it into the joint space. HA molecules occupy a large spheroidal space while in their fully hydrated state. Therefore, the viscoelasticity and flow characteristics of synovial fluid are intimately tied to its HA content [120,121].

HA provides important viscoelasticity and lubricating properties to synovial fluid, thereby reducing articular cartilage wear and acting as a lubricant during slow movements and as a shock absorber during rapid movements [118,120-122]. Furthermore, HA molecules restrict large plasma protein from entering into the synovial fluid while facilitating the passage of small molecules into the joint for maintenance of nutrition.

The normal adult knee contains approximately 2 mL of synovial fluid, with a HA concentration of 2.5 to 4.0 mg/mL. In the arthritic joint, the concentration and molecular weight of HA are decreased by 33% to 50% because the synthesis of HA in OA is disrupted by increased levels of pro-inflammatory cytokines, free radicals and proteinases [121,123]. These alterations lead to dramatically poorer viscous and elastic properties and, thus, distorted joint mechanics. Decreased lubrication leads to increased stress on the already diseased cartilage, which further disrupts the collagen network and the integrity of the chondral surface. The loss of barrier integrity also adversely affects cartilage nutrition and waste removal.

The goal of IA HA injections is to replenish the pathologically altered SF and to restore its normal viscoelastic properties, creating a sort of pad into the synovial cavity thanks to the viscosity of Hyaluronic acid [118]. HA temporarily restores the lubricating and shock-absorbing effects of SF. Moreover, several studies suggest that viscosupplements also have effects, such as protection against cartilage erosion [124,125], and promotion of intra-articular HA production.

The safety profile of HA viscosupplementation has been well established over its 20 years of clinical use. In fact, no viscosupplement product has been withdrawn because of safety concerns. Intra-articular HA is generally well tolerated with low incidence of local adverse events [126]. The overall incidence of adverse events has been reported to be approximately 1% to 4% per injection [127,128]. The most common adverse event is local reaction at the injection site, consisting of mild pain, swelling, or effusion, and warmth or redness, or both. Such injection site reactions are usually mild and self-limited, resolving with 1 to 3 days and generally respond to NSAIDs and local modalities. Other mild adverse effects that have been reported include post-injection itching, headaches, and calf pain [129]. Furthermore, the incidence of adverse events with viscosupplementation is similar to that observed with other intra-articular procedures. The incidence of adverse events has been proved to being significantly related to the injection technique used: a medial approach to a partially bent knee was associated with 5.2% adverse events by injection, compared with 1.5% with straight lateral injections. Interestingly, injection laterally has also been shown to have a higher incidence of intra-articular injection accuracy when compared with injection into the flexed knee using conventional arthroscopic portal approaches [130]. Brockmeir and Schaffer [131] postulated that adverse reactions are related more closely to the accuracy of intra-articular injection than to the substance itself.

Although the cause of local adverse events associated with HA injection is not clear, these events are typically mild-to-moderate in nature, resolve spontaneously or after treatment of symptoms, and do not result in any longterm sequelae. Therefore, it is often difficult to clinically distinguish the symptoms of a reaction from the symptoms of osteoarthritis. Additionally, the types of usual local adverse events observed after viscosupplementation are not as potentially serious as the systemic adverse effects that may occur with NSAIDs or COX-2 inhibitors [132].

An important property of hyaluronic acid influencing its efficacy and safety is the molecular weight (MW). HA contained in the synovial fluid has a MW of 5 to 7 × 10^6 Da, classified as high molecular weight (HMW). High molecular weight hyaluronic acid (HMWHA) is considered more effective in relieving pain, compared to low molecular weight HA. This is due to the fact that HMWHA molecules are bigger than LMWHA: this property allows the compound to not penetrate the extracellular matrix and to concentrate in the joint cavity, leading to a higher lubrication and protection of the joint

[133]. However, no conclusive theories have been confirmed regarding the correlation between molecular weight and efficacy [134-136].

In order to join peculiarities of both low and high molecular weight hyaluronic acid viscosupplementations, recently combined forms have been introduced. In this context applies "*HiLow - Visco-Suppletive Joint device*". This medical device consists of a buffered saline solution of hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA), thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.

It is now known that hybrid cooperative HA complexes, produced through a patented technology, represent a new and valuable alternative, permitting to deliver the double of the HA amount in the same volume with a contained and even reduced dynamic viscosity. In particular, D'Agostino *et al*. in 2015 reported the efficiency of hybrid complexes (H-HA; MW 1200 ± 200 kDa and L-HA: Mw = 100 ± 5 kDa) molecular weight HA on a scratch *in vitro* model. It was found that H-HA/L-HA hybrid complexes improved the reparation processes compared to control and even H-HA alone. These hybrid cooperative hyaluronan complexes - due to their outstanding biochemical and biophysical features, and to the remarkable biological action - could represent a valuable alternative to cross-linked HA for different biomedical device applications [96].

Furthermore, Petrella *et al.* [96]. evaluated clinical outcomes of a low and high molecular weight HA combined viscosupplementation. In this study, there were no serious adverse events up to 104 weeks. Non-serious adverse events were associated with the injection procedure and included pain and local swelling at the injection site (21%), erythema at the injection site (12%) and stiffness in the index knee (7%). By 104 weeks, only 2 patients opted for surgical intervention and only 5 opted for an alternate therapy. There was no difference between groups for any of these reported events. None of the adverse events resulted in delay in study procedures. Moreover, global satisfaction was significantly higher for the DMW group compared to the other groups at 16, 52 and 104 weeks. Further, fewer concomitant treatments (ie PT, acupuncture) were utilized by those who received DMW compared to the other treatments at all follow-up periods.

Generally, both LMW and HMW HA are very well tolerated treatments. The most common adverse effect is mild, short-lived pain and inflammation at the injection site. Two meta-analyses assessed the frequency of adverse events vs. placebo and noted only a slight increase in the risk of mild adverse events (RR 1.19, 95% CI 1.01-1.41 and RR 1.08, 95% CI 1.01-1.15).

Another safety concern regarding exogenous Hyaluronic acid is its derivation. *"HiLow - Visco-Suppletive Joint device"* contains biofermentative Hyaluronic acid, i.e. obtained from bacterial chains. The alternative is HA derived from chicken combs.

The former process is still an important technology for commercial products, but is hampered by several technical limitations. One drawback in the extraction process is the inevitable degradation of hyaluronan, caused by (a) the endogenous hyaluronidase activity in animal tissues, breaking down the polymer chain through enzymatic hydrolysis, and (b) the harsh conditions of extraction. Extraction protocols have been improved over the years, but still suffer from low yields, due to the intrinsic low concentration of hyaluronan in the tissue, and from high polydispersity of polymer products due to both the natural polydispersity of hyaluronan and to the uncontrolled degradation during extraction. As in any process for the production of therapeutic compounds from animal sources, there is a potential risk of contamination with proteins and viruses, but this can be minimized by using tissues from healthy animals and extensive purification. Nevertheless, concerns on viral (particularly avian) and protein (particularly bovine) contamination increased the interest in the biotechnological production of hyaluronan [111].

Since the hyaluronan polymer produced in animals and bacteria is identical, bacterial hyaluronan is not immunogenic and therefore is an excellent source for medical grade hyaluronan. Extracting hyaluronan from microbial fermentation broth is a relatively simple process with high yields. An additional and important advantage of microbial hyaluronan production is that microbial cells can be physiologically and/or metabolically adapted to produce more hyaluronan of high molecular weight. Therefore, microbial hyaluronan production using either pathogenic streptococci or safe recombinant hosts, containing the necessary hyaluronan synthase, is nowadays more and more preferred [111].

7.3.2 Safety results of clinical supportive data analysed

Some of the clinical studies commented in this Clinical Evaluation report have evaluated safety outcomes of Hyaluronic acid-based viscosupplementations, finding favourable results.

Filardo *et al.* (Citation 1) compared platelet-rich plasma (PRP) and Hyaluronic acid injections to treat knee joint degeneration. Two patients reported severe pain and swelling after HA injections, while no major adverse events were noted in the PRP group. However, PRP presented overall significantly more post injection swelling and pain.

Jüni *et al.* (Citation 4) carried out a clinical study aimed to compare the efficacy and safety of intraarticular hylan and 2 hyaluronic acids (HAs) in osteoarthritis (OA) of the knee. Serious adverse events during the first cycle occurred in 15 of 222 patients

allocated to receive hylan and in 25 of 438 patients allocated to receive Has. There was little evidence for a difference between groups. Two serious adverse events were judged to be probably related to the evaluated intervention. These included 1 episode of septic arthritis, which occurred after injection of the avian HA, and 1 episode of anaphylactic shock, which occurred after injection of the hylan.

Three hundred thirty patients were randomly allocated to receive a second cycle of treatment with the originally assigned preparations, 110 in the hylan group and 220 in the HA groups. Local adverse events occurred more frequently in the hylan group than in the HA groups (difference 6.4% [95% CI 0.6, 12.2]). This difference was most pronounced for flares (difference 6.4% [95% CI 1.8, 10.9]), but was apparent for all outcome measures.

Petrella *et al.* (Citation 5) evaluated the long-term efficacy and safety of a combined HA of low and high molecular weight and different concentrations (DMW) in comparison to low molecular weight (LMW 500-730 KDa) or high molecular weight (HMW 6000 KDa) HA products in reducing pain at rest and pain at walking associated with knee osteoarthritis, as compared to placebo. Safety evaluation reported no serious adverse events. DMW and LMW had no reported side effects. HMW had two local reactions at 52 weeks and 1 at 104 weeks. DMW and LMW had no reported adverse events; HMW had 2 local reactions at 52 weeks and 1 at 104 weeks. Non-serious adverse events included pain and local swelling at the injection site (21%), erythema at the injection site (12%) and stiffness in the index knee (7%).

Roux *et al.* (Citation 6) compared the efficacy on pain relief and function of one, two or three injections of intra-articular hyaluronic acid in symptomatic osteoarthritis (OA) of the carpometacarpal joint of the thumb (CMCJ). Injections were well tolerated. Pain and/or swelling and/or heat and/or redness, always moderate happened equally in the 3 groups in about 30% of cases. When occurring they lasted less than 3 h in most cases, and always less than 2 days in few cases. No septic arthritis was observed.

Berenbaum *et al.* (Citation 7) carried outa a clinical study aimed to compare the effects of an intermediate molecular weight (MW) intra-articular hyaluronic acid (HA) with a low MW product on knee osteoarthritis (OA) symptoms. Results showed that GO-ON and Hyalgan were equally well tolerated at the injection site. The proportion of patients reporting any AE in the safety population was similar: 74 out of 223 (33.2%) and 75 out of 213 (35.2%) with GO-ON and Hyalgan, respectively, most AE being unrelated to treatment.

Lucas *et al.* (Citation 9) and Kon *et al.* (Citation 10) reported no adverse events from the evaluation of HA vsicosupplementations and the comparison between them and PRP injections, respectively. Similar results were obtained by Diracoglu *et al.* (Citation 11), who investigated the short-term effects of intra-articular injection of hyaluronan (Hylan G-F 20) on proprioception, isokinetic muscle force, self reported pain and functional condition in patients with knee OA. No pseudoseptic reaction or adverse event was determined. Local adverse events were not reported in any patient.

The clinical study carried out by Carpenter *et al.* (Citation 12) showed that none of the patients suffered with any type of postoperative complication, and none of those receiving hylan G-F 20 injections displayed any type of local or systemic adverse reaction to the agent.

Conrozier *et al.* (Citation 13) assessed different dosing regimens of hylan G-F 20 in the treatment of pain due to knee OA. The treatment was well tolerated. There were no serious or severe, device-related AE's in any of the studied dosing regimens, nor were any new safety concerns identified following initial or repeat treatment. Group 4 (3 x 4mL) had the highest percentage of patients reporting device-related local AE's (30%) while Group 1 (1 x 6 mL) and Group 5 (3 x 2 mL) had only 10%. These device-related local AE's consisted mostly of mild or moderate post-injection pain (n = 12 patients) with local inflammation (described as synovitis by some investigators, n =3) or effusion (n = 1). Twenty-four patients (24%) were re-treated in the extension study; no safety concerns were raised by re-treatment with the same injection schedules. Four patients that were re-treated experienced five target knee AE's. No patients experienced AE's in Group 1, while one patient reported an AE in each of Groups 2–5. None of the target knee, treatment-emergent AE's was serious. One patient in Group 4 (3 x 4 mL) discontinued from the study due to synovitis with eVusion at the target knee. One case of synovitis in Group 5 (3 x 2 mL) was severe.

Results obtained by Borràs-Verdera *et al.* (Citation 14) in their clinical study evaluating safety and efficacy of a single intra-articular injection of 2.0% hyaluronic acid (HA)+mannitol in symptomatic knee OA showed that safety was positively evaluated by investigators and patients. No serious adverse events were observed. Mild side effects were reported in 4 patients (local pain and swelling in the infiltration area).

Also Palmieri *et al.* (Citation 15) reported favourable safety results in their clinical trial investigating, for the first time, the effects of a highly cross-linked hyaluronic acid, alone or in combination with diclofenac sodium or sodium clodronate, for the management of bilateral knee OA-related pain. No serious adverse events were observed in any group. Some bruising at 4 hours after injection containing sodium clodronate was reported by four patients, but resolved without any further treatment. No pain was observed at the injection site.

Strand *et al.* (Citation 16) compared safety and efficacy of a single intra-articular (IA) injection of a new cross-linked hyaluronic acid product, with PBS in patients with symptomatic knee OA. The incidence of AEs was similar in both treatment groups; 182 treatment-related AEs were reported in 100 patients: 67 (26.9%) in Gel-200 and 33 patients (25.8%) in PBS groups, respectively. Most common treatment-related AEs included joint swelling, effusions and arthralgia, without significant differences between treatment groups. Serious adverse events (SAEs) were reported in eight patients, including five cases of cancer. None were judged by investigators to be related to study treatment, although all SAEs occurred in the Gel-200 group, including one death. No clinically notable changes in laboratory results were identified.

Navarro-Sarabia *et al.* (Citation 17) clinical study aimed to compare against placebo the efficacy and safety of repeated injections of hyaluronic acid and its effect on disease progression over 40 months. The number of patients who experienced at least one adverse event was the same in both treatment groups. Twenty-two patients (11 in each group) experienced a total of 29 related adverse events. Most of them were related to the study intervention, such as local bleeding, pain of mild intensity or allergic reaction, none of them was serious.

Munteanu *et al.* (Citation 18) evaluated the effectiveness of a single intra-articular injection of hylan G-F 20 (Synvisc) for symptomatic first metatarsophalangeal joint (MTPJ) OA. The proportion of local adverse events at 1 month was significantly less in the hylan G-F 20 group (RR=0.602, 95% CI 0.378 to 0.960). There were no other statistically significant differences in the proportion of participants reporting adverse events or in the frequency of local adverse events for any time period. One participant (hylan G-F20 group) developed cellulitis at the injection site 2 days after injection that was defi nitely related to the study treatment and this resolved after treatment with antibiotics for 12 days. There were no serious adverse events.

Chevalier *et al.* (Citation 19) primary objective was to compare a single, 6 ml, intra-articular injection of hylan G-F 20 with placebo in patients with symptomatic knee OA. Moreover, the safety of a repeat injection of hylan G-F 20 was also assessed. There were no target knee serious AE and no serious AE that were related to the study treatment or the study procedure. The overall frequency of AE was comparable between the two treatment groups (hylan G-F 20, n = 70, 56.9%; placebo, n = 79, 60.8%). The most commonly reported AE were pain in the target knee (coded as ‘‘arthralgia’’), joint stiffness, joint effusion and joint swelling. The incidence of AE was slightly higher in the hylan G-F 20 group (n = 7, 5.7%) than in the placebo group (n = 4, 3.1%) but this was not statistically significant (p=0.366). In addition, there were no statistically significant differences between the groups in treatment-related (p=0.203) or procedure-related (p=0.531) target knee AE, all of which were of mild or moderate severity. A total of 160 patients was treated in the open, repeat treatment phase, of which 77 received a second injection of hylan G-F 20 and 83 received a first injection of hylan G-F 20, having received placebo during the initial treatment phase. There were no target knee serious AE. In the group receiving a second injection of hylan G-F 20 one patient (1.3%) experienced target knee AE related to the study treatment and four patients (5.2%) experienced target knee AE related to the study procedure. Patients who developed target knee AE during the initial phase of the study, and who subsequently received repeat treatment, did not experience target knee AE on repeat exposure to hylan G-F 20. All treatment-related and procedure-related target knee AE were of mild or moderate severity.

Lundsgaard *et al.* (Citation 20) evaluated intra-articular viscosupplementation in patients with painful knee OA. Patients were randomized to receive four weekly intra-articular injections of sodium hyaluronate 2 mL (Hyalgan 10.3 mg/mL) versus physiological saline

20 mL (distention) versus physiological saline 2 mL (placebo); they were followed up for 26 weeks. Autjors reported no serious or non-serious adverse events were reported, thus no local reactions at the injection site with pain, tenderness, and erythema were seen. No post-injection 'flares' were reported.

Waddell *et al.* (Citation 21) compared efficacy of hylan G-F 20 in patients with and without an effusion. No adverse events were reported.

Karalezli *et al.* (Citation 22) evaluated the tolerability of viscosupplementation in patients with trapeziometacarpal osteoarthritis and to compare the pain of injections given with and without fluoroscopy control. According to safety results obtained, there were no complications with the sodium hyaluronate intra-articular injections.

Di Sante *et al.* (Citation 23) tested the efficacy of PRP intra-articular (IA) therapy as compared to HA IA treatment in terms of pain relief and functional recovery in a population of hip OA patients. No complications related to the IA injections were registered during the treatment and followup period and all patients completed the treatment and performed the post-treatment assessment.

Trueba *et al.* (Citation 24) conducted a clinical study in order to compare HA with a corticosteroid (CS), betamethasone (BM), evaluating both treatments in terms of clinical efficacy and enlarging the follow-up period up to 12 months. Adverse reactions were all related to the administration procedure, and experienced by 3.5% of the patients: 6 cases of pain (four in the group treated with HA and two in BM) and 1 erythema in the HA group. Effusion was detected in 3.5% of the patients (five patients in the HA group) when attending the second (three patients), third (one patient), and fifth (one patient) injection, and two in the BM group when attending for the second injection.

De Campos *et al.* (Citation 25) investigated the effect of the addition of triamcinolone to viscosupplementation on viscosupplementation's outcomes. In this study one patient in Group VS presented with severe effusion and pain at Week 1 and was treated with arthrocentesis and an intraarticular corticosteroid injection. This patient was excluded from the study. All other cases of adverse events were mild, and the symptoms were relieved with ice, rest, and analgesics.

Vanelli *et al.* (Citation 26) assessed the efficacy and safety profile of intra-articular polynucleotides gel injections in the treatment of knee osteoarthritis associated with persistent knee pain. No significant adverse events were reported.

The clinical trial carried out by Raman *et al.* (Citation 27) aimed to compare the clinical effectiveness, functional outcome and patient satisfaction following intra articular injection with Hylan G-F 20 and Sodium Hyaluronate in patients with symptomatic primary OA of the knee. Treatment related adverse events (AE) were reported in 39 patients in the Hylan G-F 20 group and in 30 patients in the Sodium Hyaluronate group ($p > 0.05$). In the Hylan G-F 20 group all AE were minor except one major AE. The major AE occurred in a patient aged 62 years with Grade III OA of the knee. Patient developed severe pain, moderate effusion, erythema, and swelling in the treated knee after 5 days following the third injection. The patient was admitted to the hospital and clinical

examination revealed a picture akin to 'pseudo-sepsis' in the knee. The knee aspirate was sterile and the symptoms settled completely by 4 weeks with oral NSAID. This patient was reviewed according to the trial protocol and the outcome was included in the final analysis. 32 of the minor AE in the Hylan G-F 20 group occurred within 48 h and the rest after. All minor AE were related to the treated knee. All AE in the Sodium Hyaluronate group were minor such as injection site pain and occurred within 48 h and relating to the treated knee. No systemic AE were recorded in either of the groups. There were no other withdrawals from the study owing to AE.

Iannitti *et al.* (Citation 28) investigated the clinical effectiveness of viscosupplementations with the new highly cross-linked HA, Variofill®, in patients affected by bilateral knee OA, in comparison with the widely used Synvisc®. No serious adverse events were observed during treatment at all time points.

Rat et al. (Citation 29) described the changes in QoL in patients receiving hylane G-F 20 in routine practice for the treatment of knee osteoarthritis and to determine the factors associated with changes in QoL. During this study, overall tolerability was considered to be good. Twenty-seven adverse events were reported in 25 patients. These were expected events, such as local post-injection reactions, most of which were mild to moderate in intensity and resolved spontaneously and rapidly. One serious adverse event was reported: a patient presented with septic Staphylococcus aureus osteoarthritis, probably of iatrogenic origin, occurring after the third injection and responding well to antibiotics. A case of severe knee effusion was also reported; this was observed after the third injection and resolved spontaneously. No unexpected adverse events were reported.

Finally, Di Martino *et al.* (Citation 30) evaluate pain control and functional recovery provided by a single injection of HA performed the day after anterior cruciate ligament (ACL) reconstruction. No severe adverse events were reported.

7.3.3 Summary of "HiLow - Visco-Suppletive Joint device" preclinical studies' results

Pre-clinical studies carried out on *"HiLow - Visco-Suppletive Joint device"* aimed to prove the product cytotoxicity, systemic toxicity, hypersensitivity, intracutaneous reactivity, subcutaneous implantation toxicity, genotoxicity and delayed hypersensitivity. Biocompatibility tests concluded that the medical device is noncytotoxic, non sensitizing, non irritant, non genotoxic, without systemic toxicity and with no adverse effects due to the intra-articular injection ("implantation") of HA.

Actually, tests of sub-acute and sub-chronic toxicity were excluded, because not required by ISO 10993 and because data in the literature (SAX'S DANGEROUS PROPERTIES OF INDUSTRIAL MATERIALS) show that the LD50 value, under the least favorable conditions, is 1500 mg/kg and this value is far higher than the dosage at which the substance in question is administered. Moreover, the positive results obtained in the tests performed led to the consideration that it was not necessary to conduct further tests. Therefore, the

results obtained allow state that "HiLow - Visco-suppletive Joint device" is non-cytotoxic, non-irritant, non-sensitizing and non-mutagenous.

For further details, please refer to paragraph 4.4 ("Preclinical studies carried out on *"HiLow - Visco-Suppletive Joint device"*) and paragraph 8.7 ("Adequacy of preclinical testing").

7.3.4 Medical device interactions with other substances/treatments

Another critical point is the use of *"HiLow - Visco-Suppletive Joint device"* with other concomitant therapies.

Hyaluronic acid is a compound commonly combined with other ingredients, thanks to its water binding and moisturizing properties; moreover, it is used as a vehicle for the local delivery of compounds, allowing to avoid systemic therapies. No particular side effects deriving from the combined use of HA with active ingredients have been identified in literature during this Clinical Evaluation, due to the established safety and biocompatibilty of Hyaluronic acid. This compound is a key ingredient of ophthalmic, nasal, pulmonary, parenteral and topical products, and no safety concern has commonly been highlighted.

However, the Manufacturer has identified a harmful interaction between Hyaluronic acid and quaternary ammonium salts or chlorhexidine, commonly contained into disinfectants used for skin preparation before injections. The product leaflet states *"Do not mix the device with disinfectants containing quaternary ammonium salts or chlorhexidine, as hyaluronic acid can precipitate in their presence."*

The only interaction between HA and other drugs specified on the leaflet is *"On the basis of the results of in vitro studies available to date, there isn't any biological and chemical-physical interaction between "HiLow - Visco-Suppletive Joint device" and Platelet-rich Plasma (PRP), used for the treatment of the intra-articular infiltrative osteoarthritis."*

No sufficient data have been found in literature with regard to the use of HA-based viscosupplementations during pregnancy or breastfeeding. Commonly, HA is not recommended to pregnant women, lactating women, and children under 18, because the safety and effectiveness have not been established.

However, no contraindications regarding pregnant or breastfeeding women have been specified on the product leaflet. This is due to the fact that *"HiLow - Visco-Suppletive Joint device"* is indicated to be sold by medical prescription only and to be administered only by a healthcare professional, who may exclude these classes of patients from the target population of the device.

7.3.5 Medical device posology justification

For the medical device *"HiLow - Visco-Suppletive Joint device",* the following posology has been proposed and reported on the leaflet:

"The treatment can be carried out up to three injections depending on the severity of joint degeneration. It is the doctor's responsibility to evaluate the appropriateness of repeating the treatment and its frequency for each patient, taking into consideration the risk/benefit ratio of the treatment in each case".

The product packaging contains:

- 1ml pre-filled syringe (16 mg (H-HA) + 16 mg (L-HA) of hyaluronic acid sodium salt in 1 ml of sodium chloride buffered physiological solution);
- 2 ml pre-filled syringe (32 mg (H-HA) + 32 mg (L-HA) of hyaluronic acid sodium salt in 2 ml of sodium chloride buffered physiological solution).

The medical devices described in the Clinical Evaluation Plan, fully and partially equivalent to *"HiLow - Visco-Suppletive Joint device"*, are all HA-based viscosupplementations and are indicated to be administered up to 5 times, commonly at week intervals. However, the frequency of treatments is established by the doctor and depends on the severity of the joint disease.

No one of the products identified contain a combination of High and Low Molecular weight Hyaluronic acid. The product's action and consequently its dosage are influenced by this aspect, since a more concentrate and weighed Hyaluronic acid has a lubricating and shock-absorbing effect more durable than the low-molecular weight compound, thanks to its higher viscosity. Therefore, fewer administrations are needed to reach the expected efficacy. Moreover, single-injection or few injections schedules, which often involve reticulated HA derivatives reduce the number of injections, thus reducing the risk of infection and, in patients under anticoagulants, the risk of hemorrhage.

Moreover, also clinical trials described in the present Clinical Evaluation Report evaluating safety and efficacy of intraarticular combined molecular weight hyaluronic acid follow the same dosage protocol, i.e. weekly injections up to 3 weeks (Citation 5, 8). Results obtained show that treatment was highly tolerable and with no significant adverse events.

Based on these data, and considered the posology and the frequency of application of *"HiLow - Visco-Suppletive Joint device"*, no safety concerns are expected after product's administration according to directions for use and posology as reported on product's leaflet.

7.3.6 Relevant safety features

Absence of preservative substances

"HiLow - Visco-Suppletive Joint device" does not contain preservatives, i.e. compounds intended to prevent decomposition by microbial growth or by undesirable chemical changes. These substances may cause sensitizing or irritation reactions to some people.

The absence of preservatives in *"HiLow - Visco-Suppletive Joint device"* is justified since the device is sterilized and contained in a sterile syringe.

However, the damaged packaging, the reuse of the product or the use after the expiry date may lead to a risk of contamination of the device. The leaflet states: "

- ! *Do not use the device if the packaging is open or damaged.*
- ! *Do not reuse to avoid any risk of contamination.*
- ! *Do not use the device after the expiry date shown on the pack.***"**

Absence of perfumes

"HiLow - Visco-Suppletive Joint device" does not contain fragrances, i.e. compounds that, such as preservatives, may cause sensitizing or irritation in some people.

HA molecular weight

Hyaluronic Acid contained in the synovial fluid has a MW of 5 to 7 × 10^6 Da, classified as high molecular weight (HMW). High molecular weight hyaluronic acid (HMWHA) is considered more effective in relieving pain, compared to low molecular weight HA. This is due to the fact that HMWHA molecules are bigger than LMWHA: this property allows the compound to not penetrate the extracellular matrix and to concentrate in the joint cavity, leading to a higher lubrication and protection of the joint [103]. However, no conclusive theories have been confirmed regarding the correlation between molecular weight and efficacy [134-136].

"HiLow - Visco-Suppletive Joint device" contains a combination of low and high molecular weight Hyaluronic acid [800-1400 kDa]. The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA), thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.

It is now known that hybrid cooperative HA complexes, produced through a patented technology, represent a new and valuable alternative, permitting to deliver the double of the HA amount in the same volume with a contained and even reduced dynamic viscosity. In particular, D'Agostino *et al*. in 2015 reported the efficiency of hybrid complexes (H-HA; MW 1200 ± 200 kDa and L-HA: Mw = 100 ± 5 kDa) molecular weight HA on a scratch *in vitro* model. It was found that H-HA/L-HA hybrid complexes improved

the reparation processes compared to control and even H-HA alone. These hybrid cooperative hyaluronan complexes - due to their outstanding biochemical and biophysical features, and to the remarkable biological action - could represent a valuable alternative to cross-linked HA for different biomedical device applications [96].

Exogenous HA derivation

Another safety concern regarding Hyaluronic acid is its derivation. *"HiLow - Visco-Suppletive Joint device"* contains biofermentative Hyaluronic acid, i.e. obtained from bacterial chains. The alternative is HA derived from animal tissues, especially chicken combs. In the former case, Hyaluronan is exctracted from animal waste. It is still an important technology for commercial products, but is hampered by several technical limitations. However, one drawback in the extraction process is the inevitable degradation of hyaluronan, caused by (a) the endogenous hyaluronidase activity in animal tissues, breaking down the polymer chain through enzymatic hydrolysis, and (b) the harsh conditions of extraction. [111]. Bacterial hyaluronan is not immunogenic and therefore is considered an excellent source for medical grade hyaluronan. Extracting hyaluronan from microbial fermentation broth is a relatively simple process with high yields. An additional and important advantage of microbial hyaluronan production is that microbial cells can be physiologically and/or metabolically adapted to produce more hyaluronan of high molecular weight. Therefore, microbial hyaluronan production using either pathogenic streptococci or safe recombinant hosts is nowadays more preferred [111].

7.3.7 Risk analysis outcomes

The results of the risk analysis of *"HiLow - Visco-Suppletive Joint device"* lead to consider the residual risk acceptable for each hazard identified. The overall residual risk has been judged as acceptable by the Risk Management Team, as reported in the risk management report for “Hyaluronic acid sodium salt, viscosuppletive joint device”. More precisely, no unacceptable risks related to the use of the medical device were detected.

7.3.8 Post-market information about similar/predicate devices

No recall of fully equivalent or partially equivalent medical devices has been retrieved on MoH, FDA and MHRA medical devices recall databases.
Information regarding Post market Surveillance of *"HiLow - Visco-Suppletive Joint device"* and similar/equivalent products are enclosed in **Appendix 9.**

7.4 *SUMMARY OF CONFORMITY ASSESSMENT WITH REQUIREMENT ON PERFORMANCE (MDD ER3)*

According to Directive 93/42/EEC Essential requirements (Annex I), 3:

The devices must achieve the performances intended by the manufacturer and be designed, manufactured and packaged in such a way that they are suitable for one or more of the functions referred to in Article 1 (2) (a), as specified by the manufacturer.

7.4.1 Medical device overview and general features

It is expected that a device achieves its intended performance during normal conditions of use, and that the intended performances are supported by sufficient clinical evidence.

"HiLow - Visco-Suppletive Joint device" is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, *"HiLow - Visco-Suppletive Joint device"* reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action.

"HiLow - Visco-Suppletive Joint Device" consists of a buffered saline solution of hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The other components of the product are: sodium chloride, sodium phosphate and water for injections.

The sodium salt of hyaluronic acid is formed of repeated chains of disaccharide units of N-acetylglucosamine and sodium glucuronate, and is a fundamentally important component of the synovial fluid to which it gives its visco-elastic properties.

The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA) contained in “this medical device, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.

High and Low Molecular Weight Hyaluronic Acid contained in this device is produced through the biosynthesis of a natural substrate, without further chemical transformations, thus having excellent biocompatibility and allowing the natural re-establishment of the viscoelastic properties of the synovial fluid when injected in the joints.

Moreover, the results of the studies carried out on cultured human mesenchymal stem cells (MSC) differentiated in chondrocytes demonstrate that the Platelet-Rich Plasma (PRP) therapy, used for the treatment of the intra-articular infiltrative osteoarthritis, doesn’t modify the rheological structure of sodium hyaluronate, which therefore retains its viscosuppletive function.

The key ingredient of *"HiLow - Visco-Suppletive Joint Device"* is hyaluronic acid.

Other ingredients contained in *"HiLow - Visco-Suppletive Joint Device"* are represented by sodium chloride, sodium phosphate and water for injections. In the contest of *"HiLow - Visco-Suppletive Joint Device"* formulation, Sodium chloride and Sodium phosphate are used as excipients, which irrigate the joint cavity. Also water acts as a vehicle.

7.4.2 Specific physico-chemical requirements for HA-based viscosupplementations

Intra-articular viscosupplementations are a non-pharmacological approach for the management of osteoarthritis symptoms. They consist of injection of exogenous hyaluronic acid into diarthrodial joints, with the aim of restoring the rheological properties of the synovial fluid, thereby producing mechanical and chondroprotective effects.

Hyaluronic acid is a high-viscosity polysaccharide that is produced naturally by the B-cells of the synovial membrane. From a biochemical point of view, it is classified in the glycosaminoglycan (GAG) group. It is a component of the synovial fluid, enhancing viscosity and elastic nature of SF. SF with normal HA concentration acts as a viscous lubricant during slow joint movements and as an elastic shock absorber during rapid joint movements [137]. Moreover, several studies suggest that viscosupplements also have effects, such as protection against cartilage erosion [98,99], and promotion of intra-articular HA production [123,125,122,138].

The mechanism of action of Hyaluronic acid contained in *"HiLow - Visco-Suppletive Joint Device"* is mechanical: it promotes better force distribution, diminishes the pressure due to weight and recovers the rheological properties of the synovial fluid. In particular, it creates a sort of pad improving the shock-absorbing and lubricating properties of the synovial fluid, which are decreased due to osteoarthritis, and enhancing joint and limb mobility.

Physico-chemical properties of Hyaluronic acid are mainly determined by its molecular weight.

Native HA of synovial fluid has a high molecular weight ($4*10^6$ Da) and a concentration of about 0.35 g/100 ml. At present, preparations with different molecular weight are available (Low and High Molecular Weight). Some clinical study were carried out to compare clinical outcomes of low and high molecular weight; in some of them, high molecular weight hyaluronic acid (HMWHA) is considered more effective in relieving pain, compared to low molecular weight HA, since it is composed of bigger mulecules. Therefore, it does not penetrate the extracellular matrix, but it concentrate in the joint cavity, leading to a higher lubrication and protection of the joint [133]. However, no

conclusive theories have been confirmed regarding the correlation between molecular weight and efficacy [134-136]. Some studies shows that low weight HAs are more effective than high molecular weight HAs in restoring the rheological properties of synovial fluid [139]. Also, several preclinical studies evaluating joint-structure modification in animal models of OA have reported advantages of using HAs of molecular weight in the low- to mid-range, as they can access diseased tissue more easily, suggesting potential for disease modification [140].

It is now known that hybrid cooperative HA complexes, produced through a patented technology, represent a new and valuable alternative, permitting to deliver the double of the HA amount in the same volume with a contained and even reduced dynamic viscosity.

Molecular weight of Hyaluronic acid is related also to its derivation. Exogenous hyaluronic acid is produced from two sources: avian origin, i.e. from poultry material (cock crest). This presents allergenic potential due to avian antigens; non-avian origin, i.e. bio-fermentation - obtained from fermentation of bacteria (*Streptococcus zooepidermicus*). These have lower allergenic potential [112].

7.4.3 Performance data from pivotal studies

Filardo *et al.* (Citation 1) carried out a clinical study aimed to evaluate the benefit provided by platelet-rich plasma (PRP) injections to treat knee joint degeneration in comparison with hyaluronic acid (HA), the most common injective treatment currently adopted for this condition. In the PRP group, a statistically significant improvement in all clinical scores was documented. In particular, the IKDC subjective score increased from 52.4 ± 14.1 to 63.2 6 16.6 at 2 months ($p<0.0005$) and remained stable for up to 12 months (66.2 ± 16.7; p=nonsignificant vs 2 months). Similarly, an increase was recorded in all KOOS subscales. The evaluation of sport activity level through the Tegner score showed a significant improvement from pretreatment (2.9 ± 1.3) to 2 months (3.6 6 1.4; $p < 0.0005$) and then values were stable up to the final follow-up (3.7 ± 1.3; p = nonsignificant), although it was not pos-sible to regain the same preinjury level (5.2 ± 1.9). The EQ-VAS score for general health revealed a significant increase from baseline to the 12-month follow-up (73.2 ± 12.0 vs 77.6 ± 11.1; p = 0.006). A significant reduction in transpatellar circumference was also observed from the baseline evaluation to 12-month follow-up (410 ± 34 vs 402 ± 33 mm; p = 0.001), whereas no significant changes occurred in knee ROM at any follow-up. In the HA group, two patients reported severe pain and swelling after the first HA injection, which led them to withdraw from the injective treatment. A statistically significant improvement in all clinical scores was found. In particular, the IKDC subjective score increased from 49.6 ± 13.0 to 63.6 ± 15.2 at 2 months ($P < 0.0005$) and remained stable for up to 12 months (64.2 ± 18.0; p = nonsignificant vs 2 months). Similarly, an increase was recorded in all KOOS subscales.

The Tegner score showed a significant improvement from pretreatment level (2.8 ± 1.3) to 2 months (3.3 ± 1.5; p<0.0005) and then remained stable up to the final follow-up (3.4 ± 1.5; p = nonsignificant) but without reaching the preinjury value (4.9 ± 1.7). No significant variation was reported in the EQ-VAS score. A statistically significant reduction in transpatellar circumference was observed from the baseline evaluation to the final follow-up (415 ± 35 vs 406 ± 34 mm; p = 0.002), whereas no significant changes occurred in the knee ROM at any follow-up.

Furthermore, both treatments proved to be effective in improving knee functional status and reducing symptoms, but the comparative analysis showed no significant inter-group difference at any follow-up in any of the clinical scores adopted.

The objective evaluation of the transpatellar circumference and knee ROM with respect to the contralateral joint and in terms of changes over time did not show any difference when the measurements of the two treatment groups were compared. The satisfaction rate was 88.3% in the PRP group and 89.9% in the HA group.

Giarratana *et al.* (Citation 2) assessed the equivalence of intra-articular polynucleotides compared to standard hyaluronic acid (HA) viscosupplementation in the treatment of knee osteoarthritis (OA). In the first analysis, the KOOS parameters in Group C and Group H were considered separately, observing the trend of their values at different time-points with respect to their baselines. The most remarkable re-sult was achieved for the parameter “Symptoms”: in fact the outcome obtained with the treatment with Condrotide was statistically significant already after 2 weeks since the beginning of the treatment (at T2 p = 0.003), while the results achieved with Hyalubrix became significant only after 18 weeks (at T18 p = 0.010). Another important result concerns the parameters “pain” and “Function in sports and recreation”: Condrotide showed statistically significant results after 6 weeks (for KOOS “pain”: at T6 p = 0.03; for KOOS “Function in sports and recrea-tion”: at T6 p = 0.012) since the beginning of the treatment, while Hyalubrix outcome became significant only after 18 weeks (for KOOS “pain”: at T18 p = 0.0001; for KOOS “Function in sports and recreation”: at T18 p = 0.003). Finally, considering the parameters “Function in daily living” and “Quality of life”, the results of both treatments be-came statistically significant after 6 weeks.

Concerning the comparison between Condrotide and Hyalubrix at different time-points a statistically significant difference in favour of Condrotide was observed at T10 for parameters “Pain”, “Function in daily living”, and “Function in sports and recreation”. In all the other cases the efficacy of both treatments can be considered equal.

As regards parameters “Symptoms” and “Pain”, the linear fit of group C is clearly steeper, showing that Condrotide has a faster effect on their reduction if compared to Hyalubrix. Concerning the other parameters, the slopes of groups C and H are similar, illustrating that both treatments almost perform in the same way. As regards VAS “at rest” since T2 both groups C (at T2 p = 0.043) and H (at T2 p=0.043) showed a statistically significant difference, that was also later maintained. Analyzing VAS values

"standing" and "walking", Condrotide showed a statistically significant difference earlier than Hyalubrix (T1 for group C vs T2 for group H).

The evaluation of COMP showed a statistically significant reduction of its serum levels since the beginning to the end of the treatment (T26-T0) in group H ($p = 0.001$), while the treatment with Condrotide caused a mild increase of COMP levels at T6 with a new successive reduction. Besides, the comparison between the two treatments did not show any statistical significance.

Zoboli *et al.* (Citation 3) compared two different dosages of an intermediate molecular weight sodium hyaluronate (HA) assessing whether a single 6 ml application of this HA has the same effectiveness as the classical three-weekly 2 ml dose. An increase (improvement) in IKDC after one month in both groups and a small reduction after three months. Only the group that received the weekly application (W Group) presented an improvement in the WOMAC questionnaires and their pain subscale (WOMAC pain) over the course of treatment, particularly during the first month.

Jüni *et al.* (Citation 4) carried out a clinical study aimed to compare the efficacy and safety of intraarticular hylan and 2 hyaluronic acids (HAs) in osteoarthritis (OA) of the knee. It could not be detected a difference in the WOMAC pain score between the hylan group and the HA groups at 3 and 6 months. No differences were observed in the number of patients receiving intraarticular steroid injections in the 4 weeks before the 6-month assessment. Results of the stratified analyses of the primary outcome are reported in the figure below.

7.4.4 Performance data from indirect supportive articles

Petrella *et al.* (Citation 5) evaluated the long term efficacy and safety of a combined HA of low and high molecular weight and different concentrations (DMW) in comparison to low molecular weight (LMW 500-730 KDa) or high molecular weight (HMW 6000 KDa) HA products in reducing pain at rest and pain at walking associated with knee OA, as compared to placebo. At 16, 52 and 104 weeks respectively, walking VAS pain was significantly improved in all treatment groups vs. placebo: DMW (89.3%, $p< 0.001$; 87.4%, $p < 0.001$; 88.1%, $p < 0.001$); LMW (81.3%, $p < 0.001$; 78.2%, $p < 0.001$; 77%, $p < 0.001$); HMW (78.1%, $p < 0.001$; 81.1%, $p < 0.001$; 79.4%, $p < 0.001$). At 52 weeks, 8 patients in DMW group has resting VAS < 45 mm. No patient in the LMW or HMW groups has VAS at rest. Similar differences were observed for walking VAS (77 mm vs 89 mm vs 91 mm, respectively). 39, 41 and 43 (DMW, LMW, HMW) received repeat injections. At 104 weeks, these differences were similar.

Roux *et al.* (Citation 6) carried out a clinical study to investigate a difference of efficacy in pain relief of one, two or three injections of hyaluronic acid in OA of the CMC1 (carpometacarpal joint of the thumb). The authors studied the effect of injections all the study long and looked for a difference in efficacy at three months on pain and functionality. In group 1, the mean VAS was 58.4 (16.2) at baseline, 46.2 (21.9) at month

1, and 43.1 (22.8) at month 3. Dreiser test results were 12.1 (5.2), 9.0 (5.1), and 9.7 (4.9), respectively. The reduction in pain (VAS) between baseline and different evaluations times did not reach statistical significance (Baseline-3 months: p = 0.18; baseline-1 month: p = 0.09). In group 2, the mean VAS was 54.6 (18.9) at baseline, 48.1 (27.9) at month 1, and 39.5 (28.6) at month 3. Dreiser values were 13.4 (5.9); 10.7 (9.7), and 10.1 (7.9), respectively. Pain reduction (VAS) between baseline and different evaluation times was statistically significant (Baseline-3 months: p =0.01; baseline-1 month: p = 0.01). In group 3, the mean VAS value was 60.1 (17.0) at baseline, 28.4 (20.8) at month 1, and 29.8 (21.9) at month 3. Dreiser test values were 11.9 (6.6), 5.9 (3.7), and 7.1 (4.6), respectively. Pain reduction (VAS) between baseline and 3 months was statistically significant (p = 0.002) as between baseline and 1 month (p = 0.001). No significant difference was found between 1 month and 3 month VAS (p = 0.5).

Berenbaum *et al.* (Citation 7) compared the effects of an intermediate molecular weight (MW) intra-articular hyaluronic acid (HA) with a low MW product on knee osteoarthritis (OA) symptoms. Patients in both groups improved markedly during the first month after treatment and the effect was maintained for the duration of the study, with GO-ON exhibiting an overall better trend, that was particularly consistent between 12 and 24 weeks after the end of treatment. After 6 months from the end of treatment (week 26), patients who had received GO-ON had decreased their WOMAC pain score by 22.9±1.4 mm (mean±SE), compared with 18.4±1.5 mm with Hyalgan in the ITT population. It was concluded a statistical superiority of GO-ON versus Hyalgan (p=0.021). Global knee pain VAS decreased by over 50% with GO-ON at week 26, but less with Hyalgan (effect size 0.26). A similar degree of effi cacy was detected for all WOMAC scales and the Lequesne index underwent an over 4-point decrease with GO-ON versus 3 points with Hyalgan (effect size 0.34). The degree of improvement was similar for the ICOAP index, but the difference between groups was barely significant only for constant pain, while the two preparations behaved similarly on intermittent pain. Patients had also improved their global assessment VAS by almost 20 mm with GO-ON, but the better trend versus Hyalgan was not significant in ITT (p=0.068), but only in the PP analysis (p=0.044). There were 73% OARSI/OMERACT responders 6 months after the end of treatment with GO-ON, versus 58% with Hyalgan (difference 14.9%, p=0.001). The proportion of patients achieving MCII and PASS for global knee pain, function and PGA was also high with both treatments but significantly higher with GO-ON than with Hyalgan except for global pain PASS and MCII for PGA. All superiority trends were similar at the 12-week endpoint (data not shown), with a signifi cant difference in the proportion of OARSI/OMERACT responders, 69.6% with GO-ON versus 60.3% with Hyalgan (p=0.044). Patients used the rescue medications in a similar proportion: 166 out of 217 on GO-ON (77%) and 154 out of 209 (74%) with Hyalgan (p=0.50), with a low paracetamol daily mean consumption (218 and 223 mg/day, respectively, p=0.60).

Promising results were also obtained by Lucas *et al.* (Citation 8), who evaluated the efficacy of a three-injection HA viscosupplementation protocol. The average follow-up was 45.5 months (range 22.5-71.8), with no patients being lost to follow-up. With regard

to subjective effect of the injections, nineteen of the 26 injection series were evaluated was being satisfactory. With regard to the AOFAS score, the average score went from 61.8 ± 15.0 before the viscosupplementation to 73.7 ± 16.6 at 12 months after, with variations seen depending on the initial AOFAS grouping.

Kon *et al.* (Citation 10) compared the efficacy of platelet-rich plasma (PRP) and viscosupplementation (hyaluronic acid [HA]) intra-articular injections for the treatment of knee cartilage degenerative lesions and osteoarthritis (OA). A statistically significant improvement in all clinical scores from basal evaluation to the 2- and 6-month follow-up visits was observed in all treatment groups. In the PRP group a higher IKDC improvement at 6 months was observed in patients affected by cartilage degeneration compared with patients affected by early OA (P = 0.004) or advanced OA (P < 0.0005). In the LW HA group patients affected by advanced OA showed worse IKDC results at 2 months compared with patients affected by cartilage degeneration (P = 0.001) or early OA (P = 0.002). In the HW HA group higher EQ VAS results were found at 2 months in patients affected by cartilage degeneration compared with patients af-fected by early OA (P = 0.003) or advanced OA (P = 0.05). Comparison of the satisfaction level obtained in the 3 groups showed a significant difference, with a higher number of satisfied patients in the PRP group (82% [41 of 50] v 64% [32 of 50] in the LW HA group and 66% [33 of 50] in the HW HA group; P = 0.04). At the 2-month evaluation, the same results were found in the PRP and LW HA groups, whereas lower IKDC (P = 0.009) and EQ VAS (P = 0.001) scores were observed in the patients treated with HW HA. The analysis at the 6-month follow-up, the primary outcome of our study, showed better IKDC results in the PRP group compared with the LW HA group (P = 0.003), as well as compared with patients treated with HW HA (P = 0.005), and the same results were found with the EQ VAS (PRP v LW HA, P = 0.001; PRP v HW HA, P = 0.002). After the 2-month follow-up (at which the same results were obtained from the PRP and LW HA groups), a significant difference was documented over time (P = 0.001), with a further improvement in the PRP group and a worsening of the results obtained in the patients treated with LW HA injections.

Diracoglu *et al.* (Citation 11) investigated the short-term effects of intra-articular injection of hyaluronan (Hylan G-F 20) on proprioception, isokinetic muscle force, self reported pain, and functional condition in patients with knee osteoarthritis (OA). Here, the investigators tested this hypothesis: “One of the mode of actions of intra-articular hyaluronan in knee OA is the increase of proprioception.” The AAAE values of the treatment group were detected to be significantly lower at the measurements performed following the 3rd injection and one week after. 120 knees of 60 patients were evaluated at the isokinetic measurements. With respect to 60◦/sec angular speed, the post-injection differences were determined to be significantly higher in the treatment group com-pared to placebo group ($p<0.05$). However there was no significant difference between the differences obtained in 180 and 240◦/sec angular speed ($p>0.05$). Before the injections, there was no significant difference between the treatment and placebo groups regard ing the VAS and WOMAC parameters ($p>0.05$). After the injections, activity and resting VAS-pain values, all WOMAC parameters (except

the WOMAC stiffness) were detected to be significantly lower in the treatment group ($p<0.05$). There was no significant difference between the groups in WOMAC-stiffness values.

The clinical study carried out by Carpenter et al. (Citation 12), aimed to compare pain reduction following ankle arthroscopy versus that following ankle arthroscopy combined with weekly intra-articular instillation of hylan G-F 20 during the first 3 postoperative weeks, showed promising results. Overall, the median and interquartile range for the pre-intervention and post-intervention pain scores was 8.5 (8, 9) and 2 (1, 3), respectively, and this difference was statistically significant ($P <0.0001$). Overall, the median and interquartile range for the reduction in pain (the difference between the pre- and post-intervention pain scores) was 6 (5, 8). For the AAA group, the median and interquartile range for the pre-intervention pain score was 8 (7.5, 9.5), whereas that for the post-intervention score was 3 (2, 3.5), and this difference was statistically significant ($P <0.002$). For the AA+H group, the median and interquartile range for the pre-intervention pain score was 9 (8, 9), and that for the post-intervention pain score was 1 (0, 2), and this difference was highly statistically significant ($P <0.0009$). The median and interquartile range for the pre-intervention pain score for the AAA group was 8 (7.5, 9.5); whereas that for the AA+H group was 9 (8, 9), and this difference was not statistically significant ($P <0 .6525$). The median and interquartile range for the post-intervention pain score for the AAA group was 3 (2, 3.5); whereas that for the AA+H group was 1 (0, 2), and this difference was statistically significant ($P <0.0002$). The median and interquartile range for the reduction in pain for the AAA group was 5.5 (5, 6); whereas that for the AA+H group was 7.5 (6, 9), and this difference was statistically significant ($P <0.0014$).

Conrozier *et al.* (Citation 13) assessed different dosing regimens of hylan G-F 20, a high molecular-weight cross-linked derivative of HA, in the treatment of pain due to knee OA.

Treatment with hylan G-F 20 resulted in a statistically significant improvement from baseline to week 24 in all end-points for all treatment regimens. The largest changes were observed in Group 5 (3 x 2 mL) with a mean change [SD] from baseline at week 24 in the patient-completed knee OA pain VAS score of -36.7 mm [26.9]. Groups 1 (1 x 6 mL) and 4 (3 x 4 mL) consistently showed similar mean improvement (respectively -34.9 mm [16.4] and -32.6 mm [25.3]). Smaller changes (-24.0 mm [22.9] and -24.3 mm [28.3]) were found in Group 3 (2 x 4mL) and Group 2 (1 x 4mL). The group with the highest number of re-treated patients (n = 7) was Group 3 (2 x 4mL). Group 1 (1 x 6 mL) had the lowest number of patients qualifying for repeat treatment.

Borràs-Verdere *et al.* (Citation 14) clinical study aimed to evaluate the safety and efficacy of a single intra-articular injection of 2% hyaluronic acid (HA) + mannitol in symptomatic knee osteoarthritis (KOA). A significant reduction in joint pain, stiffness and functional disability compared with baseline was observed at every follow-up visit ($P<0.001$). Joint function improved by 38.7% on Day 30, reaching 47.5% on Day 180.

Rescue medication use decreased from 58.2% at baseline to 2.5% on Day 90, increasing in the last visits. Efficacy was positively evaluated by investigators and patients.

Palmieri *et al.* (Citation 15) investigate, for the first time, the effect of a highly cross-linked hyaluronic acid, Variofill®, alone or in combination with diclofenac sodium or sodium clodronate, for the management of bilateral knee OA-related pain. Group 1 showed a decrease in VAS pain score from a mean baseline value of 67.5 ± 2.04 mm to 46.8 ± 2.09 mm at 3 months and to 31.3 ± 2.4 mm at 6 months. Group 2 showed a decrease in VAS pain score from a mean baseline value of 71.9 ± 1.1 mm to 48.86 ± 0.9 mm at 3 months and to 32.1 ± 1.1 mm at 6 months. Group 3 showed a decrease in VAS pain score from a mean baseline value of 76.9 ± 1.9 mm to 47.5 ± 1.05 mm at 3 months and to 26.8 ± 1.2 mm at 6 months. When comparing the percentage change in mean VAS pain score from baseline in the three treatment groups, the therapy including sodium clodronate was the most beneficial in terms of percentage improvement in VAS pain score. A significant decrease in ESR and CRP versus baseline was observed at 6 months after the procedure in each treatment group. In group 1, ESR decreased from 76.4 ± 2.6 mm/hr to 23.7 ± 1.5 mm/hr (P <= 0.001) and CRP decreased from 7.4 ± 0.3 mg/L to 1.5 ± 0.09 mg/L (P <= 0.001). In group 2, ESR decreased from 77.1 ± 2.5 mm/hr to 23.2 ± 1.1 mm/hr (P <= 0.001) and CRP decreased from 7.1 ± 0.3 mg/L to 1.8 ± 0.1 mg/L (P <= 0.001). In group 3, ESR decreased from 76.7 ± 2.5 mm/hr to 22.8 ± 1.2 mm/hr (P <= 0.001) and CRP decreased from 6.8 ± 0.3 mg/L to 1.5 ± 0.08 mg/L (P <= 0.001). No significant difference was observed when the percentage change from baseline related to these parameters was compared among the groups.

Strand *et al.* (Citation 16) compare the safety and efficacy of a single intra-articular (IA) injection of a new cross-linked hyaluronic acid product, Gel-200, with phosphate buffered saline (PBS, control) in a multi-center randomized controlled trial in patients with symptomatic osteoarthritis (OA) of the knee. Mean changes from baseline in WOMAC pain subscores demonstrated a statistically significant advantage of 6.39 mm for Gel-200 treatment over PBS at week 13 (P = 0.037). Treatment differences at weeks 3 and 6 exceeded 8 mm (P = 0.001 and P = 0.003, respectively), and the overall difference over weeks 3 through 13 was 7.10 mm (P = 0.005). Mean improvements from baseline in WOMAC pain subscores consis-tently favored Gel-200 at each visit, with improvements of 40.6% at week 3 and 44.1% at week 6. Effectiveness in the Gel-200 treated group was sustained over weeks 3-13 by WOMAC total score, physical function, and physician global evaluations with statistical significance (P < 0.05) in addition to WOMAC pain. In the ITT population, the odds ratio (OR) for “strict” OMERACTeOARSI responders was statistically significant for Gel-200 vs PBS from weeks 6 to 13 [OR = 1.59; P = 0.022] There were no statistically significant differences in SF-36 between weeks 0 and 13, although benefit was demonstrated in both treatment groups. In terms of clinically meaningful responses over weeks 3e13, 64.5-72.8% of patients reported improvements >=MCID in Gel-200; compared with 57.1-69.5% in PBS, moderate improvements >=30%in a maximum of 62.1% vs 54.0% at week 6 and substantial improvements >=50% in a maximum of 49.4% vs 37.9% at week 6.

Navarro-Sarabia *et al.* (Citation 17) carried out the AMELIA OsteoArthritis Modifying Effects of Long-term Intra-articular Adant) study clinical study to compare against placebo the efficacy and safety of repeated injections of hyaluronic acid (HA) and its effect on disease progression over 40 months. At the end of follow-up (40 months) significantly more patients receiving HA responded to treatment in comparison with placebo according to OARSI 2004 criteria (p=0.004), the number of responders being 22% higher in HA group after the four treatment cycles (RR 1.22, 95% CI 1.07 to 1.41). The number of responders to HA injections progressively increased after each treatment cycle (from 71.1% to 80.5%), whereas responses to placebo remained fairly stable (from 67.8% to 65.8%). This progression gave results with strong statistical significance and differences between the two groups from the second until the last evaluation at 40 months. Among those non-responders after the first cycle, up to 54% of HA and 38% of placebo patients evolved positively over the study. At the 40-month visit the number of responders in this subgroup was 54% with HA versus 31% in the placebo group (p=0.026). All of the OARSI components (pain, function and patient global assessment) were analysed at the end of the study, showing that the degree of improvement in the HA group was signifi cantly higher compared with placebo (p values = 0.025, 0.023 and 0.002, respectively). A total of 26.8% of patients receiving HA did not complete the study compared with 38.2% in the placebo group. It is noteworthy that the number of losses due to lack of efficacy were significantly higher in the placebo group (p=0.027). The demographic and baseline characteristics of completers and dropouts were analysed, and no differences were found with the exception of age in the placebo group, with the completers being younger than the dropouts (p=0.047). Aspiration in the target knee was performed in 22.82% of patients in the HA group and 21.05% of the placebo group (p=0.712), with a median of two aspirations per patient in both groups during the overall study period. Overall, rescue medication (paracetamol/NSAID) was consumed during the study by 71.1% and 71.7% of the HA and placebo patients, respectively. Paracetamol was consumed by 48% of the patients and the mean daily dose during the study experienced a 27% reduction in the HA group compared with baseline versus only a 4% reduction in the placebo group. A logistic regression analysis was performed with no differences between the HA and placebo (p=0.9129) groups, concluding that rescue medication did not interfere with the clinical assessment of patients.

Munteanu *et al.* (Citation 18) evaluated the effectiveness of a single intra-articular injection of hylan G-F 20 (Synvisc) for symptomatic first metatarsophalangeal joint (MTPJ) osteoarthritis (OA). Both groups experienced improvements in foot pain compared with baseline, but there were no statistically significant differences between the hylan G-F 20 or placebo groups at any time point.

The study carried out by Chevalier et al. (Ciattion 19) aimed to to compare a single, 6 ml, intra-articular injection of hylan G-F 20 with placebo in patients with symptomatic knee osteoarthritis. The treatment effect with hylan G-F 20 was statistically significantly superior to placebo for the primary endpoint, change in WOMAC A (pain) over 26 weeks.

The estimated treatment difference between the hylan G-F 20 group and placebo group over the 26-week study was statistically significant (p=0.047). Some, but not all, of the secondary endpoints, including WOMAC A1 (walking pain), PGA and COGA, showed statistically significant differences between the two groups favouring hylan G-F 20 treatment. Seventy-one per cent (88/124) of the patients were WOMAC A1 (walking pain) responders at week 18 in the hylan G-F 20 group compared with 53% (69/129) in the placebo group (p=0.003). At week 26, 64% (79/124) of patients in the hylan G-F 20 group were WOMAC A1 responders compared with 50% (64/129) in the placebo group (p=0.028). The change in WOMAC C (function) scores did not reach statistical significance. Further exploratory analyses of predefined covariates were carried out to understand better the lack of effect of hylan G-F 20 on the WOMAC C endpoint. In patients without any other lower limb osteoarthritis (defined as hip or contralateral knee involvement), those treated with Synvisc experienced a greater change in WOMAC C than those treated with placebo (20.71 and 20.55, respectively). The OMERACT–OARSI responder analysis over 26 weeks approached statistical significance (p=0.059). At week 26, 73 patients (59%) in the hylan G-F 20 group and 66 patients (51%) in the placebo group were responders. Overall, patients consumed a mean daily dose of 0.26 g (SD 0.654 g) of paracetamol in the hylan G-F 20 group, and 0.28 g (SD 0.570 g) in the placebo group. Throughout the study there was no statistically significant difference in paracetamol consumption between the two groups (p=0.370).

Lundsgaard *et al.* (Citation 20) compared hyaluronate 2 mL (HyalganH 10.3 mg/mL) versus physiological saline 20 mL (distention) versus physiological saline 2 mL (placebo) in elderly patients with osteoarthritic knee pain resistant to analgesics. No significant interaction between time and group was observed (the range of p-values was 0.13–0.91). Thus, the time curves of the three intervention groups were parallel except for random variation. The model was therefore simplified to include only main effects of time and of group, that is only differences between mean levels. The mean levels of the primary and secondary outcome measures did not differ significantly between the three intervention groups except for extension gap, where a difference in borderline significance was noted. Pairwise comparisons revealed that only the difference between the 20 mL vs. the 2 mL physiological saline groups was significant (p=0.033).

Waddell *et al.* (Citation 21) carried out a clinical study to compare efficacy of hylan G-F 20 in patients with and without an effusion. Both effusion and control group VAS was significantly lowered at all time points. WOMAC scores improved ($p < 0.025$) at all visits in the effusion group except for WOMAC A-1 week 14. Control WOMAC scores also significantly improved at all visits ($p < 0.027$), except for full WOMAC and WOMAC A-1 at week 1.

The purpose of the study carried out by Karalezli *et al.* (Citation 22) was to evaluate the tolerability of viscosupplementation in patients with trapeziometacarpal osteoarthritis and to compare the pain of injections given with and without fluoroscopy control. All the patients in groups A and B complained of pain and discomfort during the injections. The

mean VAS scores of the pain in groups A and B were 4.1 (range 3–6) and 5.6 (range 3–7), respectively. The difference of the VAS scores between the three groups was statistically significant (p<0.005). The mean score of the tolerability of the injection in groups A and B was 2.5 (moderate–good) (range 1–3).

Di Sante *et al.* (Citation 23) test the efficacy of PRP intra-articular (IA) therapy as compared to HA IA treatment in terms of pain relief and functional recovery in a population of hip OA patients. VAS scores were significantly lower than T0 values at T1, but not at T2 in the PRP group, thereby indicating an immediate effect on pain of PRP which was afterward lost (at T2 VAS value was further reduced but this reduction was not statistically significant). In contrast, in the HA group the significance between VAS values was reached only between T0 and T2 values. At T2, patients in the HA group had lower VAS values than those in the PRP group, the difference being significant at the 2-sample t-test (p=0.0004). Two-way ANOVA showed a significant group (F=32.070; p<0.0001) and time (F=6.036; p=0.003) effect for WOMAC A, while no significant group xtime interaction effect was found (F=2.488; p=0.09). Post hoc analysis revealed that WOMAC A scores were significantly lower than T0 values at T2 but not at T1 in the HA group. No differences between T0, T1 and T2 values were discernible in the PRP group.

As regards to secondary outcome measures, a significant time (F=4.436; p=0.01) effect was found for WOMAC B, while no significant group (F=0.471; p=0.49) or group xtime interaction (F=1.653; p=0.20) effects were found. Significant differences at post-hoc analysis were found only in the HA group between T0 and T2 values. A significant group (F=14.177; p<0.0001) and time (F=3.680; p=0.03) effect was found for WOMAC C, while no group xtime interaction effect was found (F=0.789; p=0.457). Again, post-hoc analysis revealed a significant difference between T0 and T2 values in the HA group.

Trueba *et al.* (Citation 24) compared HA with a corticosteroid (CS), betamethasone (BM), evaluating both treatments in terms of clinical efficacy and enlarging the follow-up period up to 12 months. In the mITT population, the raw values for pain showed a significant reduction in both groups from early follow-up. Percentages of reduction in pain at 3 months were notably higher in the BM group (66.3%, 95% CI: 63.3–69.3) compared to the HA group (48.5%, 95% CI: 45.8–51.3) (p<0.0001). These results showed a reversion in the following visits, with the reduction in pain being significantly higher in the HA group. At 12 months, the mean reduction in pain in the HA group was 33.6% (95% CI: 31.1–36.1) compared to 8.2% (95% CI: 5.2–11.1) in patients treated with BM (P<0.0001). The PP population showed similar results, with the mean reduction in pain at 12 months of 34.4% (95% CI: 31.7–36.1) in the HA group and 7.7% (95% CI: 4.4–9.7) for the BM patients (P<0.0001). WOMAC's total score and the subscales of pain, function, and stiffness also showed significant improvement in both treatment group. When the WOMAC function scores in HA and BM at different time points were analyzed, the comparison was distinctly favorable to HA at all visits. The percentage of patients achieving the MCII for both pain and function was nearly 100% in both groups up to 6 months' follow-up. From this visit onward, the values decreased dramatically in the BM

group in such a way that at 9 months the MCII for a change of at least 15 of 100 for absolute change established in the literature was 81.4% in the HA group and only 9.2% in those treated with BM (P<0.0001). In the PP population, the MCII values when the 15 of 100 cutoff for absolute improvement was used were 82.0% for HA and 5.5% for BM at 9 months, and 77.5% and 2.2% at 12 months for HA and BM, respectively (P<0.0001). When the cutoff was 20% for relative improvement, the values were 88.8% for HA and 6.6% for BM at 9 months and 85.4% and 1.1% at 12 months, for HA and BM, respectively (P<0.0001). Overall, 67.4% of patients in the mITT population and 70.6% in PP took acetaminophen as rescue medication during the follow-up period, with no differences between groups.

De Campos *et al.* (Citation 25) carried out a clinical study to investigate the effect of the addition of triamcinolone on viscosupplementations. Baseline scores were similar (p = 0.062 to p = 0.969) between the groups. At Week 1, Group VS + T showed improvement in all the scores, with a difference from baseline. Group VS showed mild improvement at Weel 1, with a difference from baseline (p=0.009) only in VAS. Comparing the two groups, Group VS+T showed lower levels in WOMAC (p=0.038) and VAS (p=0.014) at Week 1. Seventeen percent of all patients reported knee pain or discomfort and 4.8% had joint effusions after the injections. There were no differences between the groups. During the follow-up, the difference between the groups decreased and at Week 4, 12, and 24 there were no differences between the groups in any score. At 6 months follow-up, both groups showed similar values in WOMAC (p > 0.999), VAS (p>0.999) and Lequesne index (p=0.942).

Vanelli *et al.* (Citation 26) assessed the efficacy and safety profile of intra-articular polynucleotides gel injections in the treatment of knee osteoarthritis associated with persistent knee pain. The mean global VAS pain decreased from 5.7 ± 1.9 cm (T0) to 1.9 ± 1.5 cm (T16) in polynucleotide group and from 4.9 ± 2.0 cm (T0) to 2.1 ± 1.4 cm (T16) in hyaluronan group. The reduction in pain was statistically significant for both groups. KOOS increases from baseline values were statistically significant in both groups.

The clinical study performed by Raman *et al.* (Citation 27), comparing the clinical effectiveness, functional outcome and patient satisfaction following intra articular injection with Hylan G-F 20 and Sodium Hyaluronate in patients with symptomatic primary OA of the knee, showed that there was a reduction in knee pain as measured by VAS in both groups at 6 months. However, there was a statistically significant improvement from the baseline score at 6 months only in the Hylan G-F 20 group. Knee pain as measured by VAS improved from 6.7 to 3.1 (median = 2.9) by 6 weeks (p = 0.01) and was sustained until 12 months (3.7, median = 3.5, p = 0.04) with Hylan G-F 20. In the Sodium Hyaluronate group, pain improved from 6.6 to 5.7 (median = 5.8) at 6 weeks (p N 0.05) and to 4.1 (median = 4.0) at 3 months (p = 0.04) but was sustained only until 6 months (5.9, median= 6.0, p >0.05). When comparing the knee pain improvement from baseline between the two groups, the Hylan G-F 20 group was statistically superior (2.5 mm, p = 0.02) at 6 months. This difference was as early as 6 weeks (p = 0.001) and was

observed until 12 months (p = 0.01). However, there was no difference in the magnitude of pain relief at 3 months between the groups. There was improvement in the WOMAC pain subscales in both groups compared to the baseline measurements. The pain subscale scores were significantly better than the pre-treatment scores at all assessment periods in the Hylan G-F 20 group. In the Sodium Hyaluronate group, it was significant only at 3 months, mimicking the results of the primary outcome variable. Pain subscale improvements between the two groups were significantly better in the Hylan G-F 20 group at 3 months (p=0.02), 6 months (p=0.01) and 12 months (p=0.007). Similarly there was an improvement in WOMAC physical activity subscale in both groups. However, the physical activity subscale improvement was significantly better in the Hylan G-F 20 group at 6 months (p=0.02) and 12 months (p=0.004) when compared to the Sodium Hyaluronate group. There was improvement in the WOMAC stiffness subscale in both groups at 3, 6 and 12 months, but no statistical difference was observed between the two groups at these timescales. With regard to the Oxford scores, a significant improvement from baseline values was observed in the Hylan G-F 20 group at 6 weeks, 6 months and 12 months. In the Sodium Hyaluronate group, the improvement from the pretreatment value was significant only at 3 months. Analysis of the magnitude of improvement between the two groups suggested a significantly better outcome at 6 (p = 0.009) and 12 months (p = 0.02) in the Hylan G-F 20 group. General patient satisfaction of the treatment and health related quality of life as measured by EQ-5D assessment tool at baseline, 6 weeks, 3, 6, and 12 months is provided in the following table (Table 25). Patient satisfaction was highest at 3 months in both groups. At 6 months, patient satisfaction was significantly better in the Hylan G-F 20 group. Overall, patients were generally more satisfied with their treatment in the Hylan G-F 20 group. In the Hylan G-F 20 group, EQ-5D description and valuation subscales improved from baseline at 6 weeks and was sustained until 12 months. In the Sodium Hyaluronate group, significant improvement was observed only in the description subscale at 3 months. There was a significant decrease in the requirement of Paracetamol in the Hylan G-F group at 6 months (p = 0.01) and 12 months (p = 0.03) as compared to the Sodium Hyaluronate group.

Iannitti *et al.* (Citation 28) investigated the clinical effectiveness of viscosupplementation with the new highly cross-linked HA, Variofill®, in patients affected by bilateral knee OA, in comparison with the widely used Synvisc®. Variofill® and Synvisc® administration showed a significant reduction in VAS pain, WOMAC pain, physical activity and stiffness at 3 and 6 months vs baseline (P < 0.001) in knee OA patients. A decrease in VAS from a baseline value of 73.3 ± 1.7 to 52.7 ± 1.6 at 3 months and 39.3±2.2 at 6 months was observed in the Synvisc® group (P < 0.001 at all time points). A decrease in VAS from a baseline value of 74.7±1.5 to 53.4±1.4 at 3 months and 31.8±0.9 at 6 months was observed in the Variofill® group (P < 0.001). The same result was observed when pain was assessed using WOMAC. A decrease in WOMAC pain from a baseline value of 15.05 ± 0.65 to 11.5 ± 0.5 at 3 months and 7 .05±0.3 at 6 months was observed in the Synvisc® group (P < 0.001). A decrease in Womac pain from a baseline value of 14.9±0.5 to 10.8 ±

0.4 at 3 months and 5.9 ± 0.3 at 6 months was observed in the Variofill® group (P < 0.001). A significant decrease in Womac stiffness from a baseline value of 5.7±0.2 to 3.9±0.2 at 3 months and 2.4±0.1 at 6 months was observed in the Synvisc® group (P < 0.001). A significant decrease in Womac stiffness from a baseline value of 6.2±0.2 to 4.1±0.2 at 3 months and 2.5±0.2 at 6 months was observed in the Variofill® group (P < 0.001). A decrease in WOMAC physical activity from a baseline value of 53.1±2.4 to 33.5±1.6 at 3 months and 19.6 ± 1.06 at 6 months was observed in the Synvisc® group (P < 0.001). A decrease in WOMAC physical activity from a baseline value of 57.2±1.4 to 33.9±1.4 at 3 months and 15.8±1.05 at 6 months was observed in the Variofill® group (P < 0.001). Inter-group analysis showed no significant difference between the two treatments at 3 months for VAS pain, WOMAC pain, stiffness and physical activity. At 6 months, Variofill® induced a significant percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity if compared to Synvisc® (p < 0.05 vs Synvisc® group; Figs. 2, 3A, 3C). No difference in percentage improvement in Womac stiffness between groups was observed. The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Variofill® group at 6 months was 56.94±1.18%, 59.54±2.55% and 72.84±3.32% respectively (p < 0.05 vs Synvisc® group). The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Synvisc® group at 6 months was 46.2±3.1 %, 52.02±1.9% and 62.003±2.4%, respectively.

Rat *et al*. (Citation 29) described the changes in QoL in patients receiving hylane G-F 20 in routine practice for the treatment of knee osteoarthritis and to determine the factors associated with changes in QoL. Three and 6 months after treatment, a statistically significant improvement in the SF36 dimensions was observed with the exception of the general health dimension. With regard to the OAKHQOL questionnaire, a significant improvement (p<0.0001) was observed in three of the five dimensions measured, i.e. the physical activity, pain and mental health dimensions, after 3 and 6 months. Conversely, no improvement was measured in the social activity dimension. As expected, the social support dimension did not change. No significant difference was found between the results of the different QoL scores at 3 and 6 months. The mean value for pain on the 0 to 100 VAS scale decreased significantly from 52.3 (20.2) at inclusion to 27.3 (22.6) at 3 months (p<0.0001) and 25.6 (21.9) after 6 months (p<0.0001). Similarly, the Lequesne index decreased after treatment with hylane G-F 20, dropping from a mean of 10.9 (4.3) at inclusion to 7.9 (4.7) after 3 months (p<0.0001) and 7.0 (4.9) at 6 months (p<0.0001). The change in the index between 3 and 6 months was not significant. The use of concomitant treatments for knee osteoarthritis (pain relief, NSAIDs and steroids injections) dropped from 87% to 44% at 6 months in the patients monitored for the entire 6 months.

Di Martino *et al.* (Citation 30) evaluate pain control and functional recovery provided by a single injection of HA performed the day after anterior cruciate ligament (ACL) reconstruction. With regard to the clinical outcome, a significant improve-ment was documented in both treatment groups without any statistically relevant intergroup difference in any of the scores used. In particular, the IKDC subjective score increased

from 65.8 6 16.2 to 90.8 6 9.1 (12-month follow-up) and from 60.0 6 17.3 to 91.5 6 8.8 (12-month follow-up) in the HA and pla-cebo groups, respectively. The Tegner score and all the sub-scales of the SF-36 questionnaire showed a similar trend of improvement in both treatment groups. Similarly, the VAS for pain and for general health sta-tus revealed a significant improvement from baseline to the final 12-month evaluation, without reaching statistical intergroup difference at any follow-up evaluations. With regard to the objective measurements, a significant difference between groups was observed in the transpatellar circumference of the operated knee. In the HA group, a lower difference in transpatellar circumference between the contra-lateral nonoperated knee and the ACL-reconstructed knee was documented at 60 days postoperatively, meaning that at this time point, in the HA group, the circumference of the operated knee was more similar to the circumference of the healthy contralateral knee used as a control (P = 0.022). Another significant difference was observed in the active ROM at 30 days postoperatively. The difference between the active ROM of the contralateral healthy knee versus that of the ACL-reconstructed knee was considered: The patients who received HA had less difference in active ROM of the treated knee versus the contralateral healthy knee at 30 days postoperatively, indicating that viscosupplementation helped bridge the gap between the operated knee and the contralateral nonoperated knee (P = 0.027).

Panuccio *et al.* (Citation 31) carried out a clinical study to evaluate whether combined treatment with intra-articular injection of HA and AI is more effective than treatment with HA only for the symptomatic treatment of knee OA. The treatment group HA + IA showed a positive trend compared to the group treated with HA only for all the efficacy variables observed, in particular regarding the VAS and the analgesic consumption.

Van Den Bekerom *et al.* (Citation 32) carried out a prospective clinical study comparing three different hyaluronate formulations and evaluating functionality, time of satisfactory pain relief and also the delay in performing a total hip arthroplasty. One hundred and twenty patients (126 hips) received viscosupplementation with one of the three hyaluronate formulations. All patients were candidate for surgical treatment with a total hip arthroplasty. Results reported that there was no significant difference in duration of the effect of the first infiltration between the three groups. The positive effect was still ongoing at the end point of the study in 46 hips: 51% of the patients did not undergo total hip arthroplasty, 3 years after viscosupplementation.

7.5 *SUMMARY OF CONFORMITY ASSESSMENT WITH REQUIREMENT ON ACCEPTABILITY OF UNDESIRABLE SIDE-EFFECTS (MDD ER6)*

According to Directive 93/42/EEC Essential requirements (Annex I), 6:

Any undesirable side effect must constitute an acceptable risk when weighed against the performances intended.

There are no undesirable side effects expected deriving from the instillation of *"HiLow - Visco-Suppletive Joint device"*, according to its indications, target population and mode of use as described on product's leaflet. Hazards leading to harm to the user/patients due to various causes will be covered by the risk analysis, for example use in case of known or suspected hypersensitivity, use of the product after the expiry date, etc.

There are no particular concerns about the use of the device, since *"HiLow - Visco-Suppletive Joint device"* is intended to be administered by a doctor and to be sold by medical prescription only. In addition, no training is necessary due to the easiness of use of the device. Therefore, risks related to the improper use of *"Hyaluronic acid sodium salt, viscosuppletive joint device"* may be reasonably considered negligible.

However, the product leaflet specifies: *"*

- *The injection site must be on healthy skin.*
- *Do not inject intravenously. Do not inject outside the joint cavity, into the synovial tissue or into the articular capsule.*
- *Do not administer the device in the presence of heavy intra-articular effusion."*

Some adverse events (no SAEs) occurred during the studies described. In the majority of the cases, these events were not related to the test product. No clinical data from literature describe particular side effects or severe adverse events that may derive from a Hyaluronic acid-based intra-articular viscosupplementation for the relief from pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendons alterations.

7.6 *SUMMARY OF CONFORMITY ASSESSMENT WITH REQUIREMENT ON ACCEPTABLE BENEFIT/RISK PROFILE (MDD ER1)*

The information material supplied by the manufacturer has been reviewed. The description provided by the manufacturer correctly and precisely identifies the medical conditions for which *"HiLow - Visco-Suppletive Joint device"* is intended to be used.

To date no clinical investigation has been performed with *"HiLow - Visco-Suppletive Joint device"*, according with EN ISO 14155:2011 (Clinical investigation of medical devices for human subjects – Good clinical practice). So, the clinical evaluation of *"Hyaluronic acid sodium salt, viscosuppletive joint device"* is based exclusively on literature route.

All risks addressed in the risk analysis are within an acceptable range or as far as possible. It is believed that the benefits deriving from the use of *"HiLow - Visco-Suppletive Joint device"* outweigh the risks.

Altogether, the clinical evaluation of *"Hyaluronic acid sodium salt, viscosuppletive joint device"* results in a positive risk/benefit ratio for the application of the product after assessment of the

risks and benefits with regard to its specific intended use, as discussed in the previous paragraphs (Section 8.3, 8.4, 8.5).

7.7 *ADEQUACY OF PRECLINICAL TESTING*

According to the classification criteria set out by Italian Legislative Decree no. 46/97, amended by Italian Legislative Decree no. 37/2010, Annex IX at paragraph 2.4, Rule 8 the product is defined as long-term surgically invasive device intended to be absorbed. Therefore the "*HiLow - Visco-suppletive joint device"* falls into risk class III.

"*HiLow - Visco-suppletive joint device"* is an invasive device, since it is intended to be injected into the joint cavity through the joint skin. For permanent contact (> 24 h to 30 days) devices, ISO 10993-1 suggests the following tests: cytotoxicity, sensitization, irritation, systemic toxicity and implantation.

The following biocompatibility studies have been conducted on "*HiLow - Visco-suppletive joint device":*

1. Cytotoxicity by direct contact (ISO 10993-5);
2. Intracutaneous reactivity (ISO 10993-10);
3. Subcutaneous implant (ISO 10993-6);
4. Systemic toxicity (ISO 10993-11);
5. Salmonella typhimurium – reverse mutation assay (Ames test) (ISO 10993-3);
6. Delayed hypersensitivity test (ISO 10993-10).

Therefore, the results obtained allow state that "HiLow - Visco-suppletive Joint device" is non-cytotoxic, non-irritant, non-sensitizing and non-mutagenous.

7.8 *USABILITY*

The Risk Management Team did not prepare a specific Usability Report for "*HiLow - Visco-suppletive joint device"*, due to the fact that no critical usability-related risks were identified. Possible risks and related hazards resulting from non-correct use (improper or wrong use) of the medical device shall be taken into account in the Risk Management for "*HiLow - Visco-suppletive joint device"*, although the device is intended to be administered only by a doctor and the intended use of the product does not require training to end users, according also to the safety characteristics of the medical device.

7.9 *CLAIMS' CONFIRMATION*

A resuming table for claims confirmation is reported here below. Once the device leaflet and labeling will be finalized, the table below would be updated according to the claims highlighted by the Manufacturer.

Table 28. Confirmation of "HiLow - Visco-suppletive joint device" claims.

Claim	Evidence	Reference
"HiLow - Visco-Suppletive Joint device" is indicated for pains or reduced joints mobility due to degenerative diseases, post-traumatic diseases or joint and tendon alterations. It substitutes the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, *"HiLow - Visco-Suppletive Joint device"* reduces the pain quickly and re-establishes joint and tendon mobility acting only at the level of the joint into which it is injected, without exercising any systemic action.	Clinical data	Clinical Evaluation: *CEP_IAHiLow, rev.05 (03/03/2017)* *CER_IAHiLow, rev.05 (03/03/2017)*
HiLow - Visco-Suppletive Joint Device" consists of a buffered saline solution of hyaluronic acid with visco-elastic properties. It contains 3.2% of highly purified sodium hyaluronate with high and low molecular weight. The other components of the product are: sodium chloride, sodium phosphate and water for injections.	Clinical data	Clinical Evaluation: *CEP_IAHiLow, rev.05 (03/03/2017)* *CER_IAHiLow, rev.05 (03/03/2017)*
The High Molecular Weight Hyaluronic Acid chains (H-HA) and Low Molecular Weight Hyaluronic Acid chains (L-HA) contained in “this medical device, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device thus allowing the	Clinical data	Clinical Evaluation: *CEP_IAHiLow, rev.05 (03/03/2017)* *CER_IAHiLow, rev.05 (03/03/2017)*

administration of higher concentrations of hyaluronic acid at the equal level of viscosity. High and Low Molecular Weight Hyaluronic Acid contained in this device is produced through the biosynthesis of a natural substrate, without further chemical transformations, thus having excellent biocompatibility and allowing the natural re-establishment of the viscoelastic properties of the synovial fluid when injected in the joints. Moreover, the results of the studies carried out on cultured human mesenchymal stem cells (MSC) differentiated in chondrocytes demonstrate that the Platelet-rich Plasma (PRP) therapy, used for the treatment of the intra-articular infiltrative osteoarthritis, doesn't modify the rheological structure of sodium hyaluronate, which therefore retains its viscosuppletive function.		
"*HiLow - Visco-Suppletive Joint Device*" is a medical device that integrates the synovial fluid and allows the re-establishment of the physiological and rheological properties of joints affected by arthrosis. By naturally re-establishing the viscoelastic properties of the synovial fluid, the device reduces the pain quickly and re-establishes joint and tendon mobility. "*HiLow - Visco-Suppletive Joint Device*" acts only at the level of the joint into which it is injected, without exercising any systemic action.	Clinical data	Clinical Evaluation: *CEP_IAHiLow, rev.05 (03/03/2017)* *CER_IAHiLow, rev.05 (03/03/2017)*

The therapeutic action of the medical device is carried out by the particular characteristics of the Hyaluronic Acid used. The hyaluronic acid contained in this product is a combination of high- and low- molecular weight hyaluronic acid and it is produced by fermentation and without any chemical modification, so it can reach an excellent tolerability. The chains of HA with different molecular weight contained, thanks to a specific and patented treatment of the solution, interact each other providing unique rheological characteristics to the device, thus allowing the administration of higher concentrations of hyaluronic acid at the equal level of viscosity.	Clinical data	Clinical Evaluation: *CEP_IAHiLow, rev.05 (03/03/2017)* *CER_IAHiLow, rev.05 (03/03/2017)*

8 CONCLUSIONS IN COMPLIANCE WITH THE ESSENTIAL REQUIREMENTS OF EC-DIRECTIVE

The information presented in this clinical evaluation indicates that "*HiLow - Visco-suppletive joint device*" is similar to some products available on the market, as described in the Clinical Evaluation Plan.

The Clinical Evaluation of "*HiLow - Visco-suppletive joint device*" resulted in a positive benefit/risk ratio for the application of the product after assessment of the risks/benefit specifically with regard to the intended use. A critical assessment of data collected from literature supports "*HiLow - Visco-suppletive joint device*" safety and performance in compliance with Essential Requirements and Annex X of EC-Directive 93/42/EEC as amended by 2007/47/EC and with the European guideline MEDDEV 2.7.1 of June 2016.

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10 DECLARATIONS OF INTERESTS

Declarations of interests of all the authors of the Clinical Evaluation are enclosed in **Appendix 7**.

11 DOCUMENT APPROVAL / 1MED CERTIFICATIONS

The present Clinical Evaluation has been draw up and internally approved by 1MED sa. 1MED is a consulting Company based in Switzerland, ISO 9001 and ISO 13485 certified by TÜV Rheinland Notified Body. The scope of the certifications (**Appendix 8**) covers the activities conducted (bibliographic clinical evaluation for the medical device "*HiLow - Visco-suppletive joint device"*):

"Design and provision of consultancy services and management of clinical trials in the field of medical devices and in vitro diagnostics medical devices"

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Enrico Perfler
Chief Executive Officer 1MED sa

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12 APPENDICES

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