

RESEARCH ARTICLE

Open Access



# Hyaluronan derivative HYMOVIS® increases cartilage volume and type ii collagen turnover in osteoarthritis knee: data from MOKHA study

Yves Henrotin<sup>1,2\*</sup> , Raveendhara Bannuru<sup>3</sup>, Michel Malaise<sup>4</sup>, Hang-korng Ea<sup>5</sup>, Cyrille Confavreux<sup>6</sup>, Jacques Bentin<sup>7</sup>, Didier Urbin-Choffray<sup>8</sup>, Thierry Conrozier<sup>9</sup>, Jean-Pierre Brasseur<sup>10</sup>, Philippe Thomas<sup>11</sup>, Anne-Christine Hick<sup>2</sup>, Alessandro Marinello<sup>12</sup>, Nicola Giordan<sup>12</sup> and Pascal Richette<sup>5</sup>

## Abstract

**Background:** The objective of this pilot study was to identify biological, clinical or structural biomarkers of an intra-articular hyaluronic acid injection efficacy (HYMOVIS®) for the design of a larger placebo-controlled clinical trial studying the disease-modifying activity of this treatment.

**Methods:** Forty six patients with symptomatic knee Osteoarthritis (OA) were enrolled in this open-label, prospective, multicenter, pilot study. Patients received two treatment cycles of intra-articular injections (3 mL) of HYMOVIS® (8 mg/mL of hyaluronic acid hexadecylamide) at 6 months interval. Each treatment cycle involved two intra-articular injections 1 week apart. All patients had Magnetic Resonance Imaging (MRI) of the target knee at baseline and 1 year, and blood samples to assess joint biomarkers. The primary outcome was the change in type II collagen-specific biomarkers (Coll2-1, Coll2-1NO2 and CTX-II) after HYMOVIS® treatment versus baseline. Secondary endpoints included levels changes in aggrecan chondroitin sulfate 846 epitope (CS-846), Cartilage Oligomeric Matrix Protein (COMP), procollagen type II N-terminal propeptide (PIIANP), Matrix Metalloprotease (MMP)-3, Myeloperoxidase (MPO) and Interleukin (IL)-6 serum biomarkers, the ratio Coll2-1/PIIANP, CTX-II/PIIANP, variation of MRI cartilage volume, and Knee injury and Osteoarthritis Outcome Score (KOOS) index.

**Results:** Coll2-1 serum levels significantly increased overtime while Coll2-1NO2 levels were only increased at D360. Serum PIIANP levels also progressively and significantly enhanced with time. In contrast, other serum biomarker levels including CTX-II, CS-846, COMP, MMP-3, MPO or IL-6 did not change significantly overtime. Interestingly, the ratios Coll2-1/PIIANP and CTX-II/PIIANP decreased, indicating a decrease of cartilage catabolism. Compared to baseline value, MRI cartilage volume and thickness increased in lateral femoral and lateral trochlea compartments and not in medial compartment. These results, in addition to an improvement of T2 mapping score suggest a positive structural effect of the product. Interestingly, WORMS effusion score, an indicator of synovitis, significantly decreased. Finally, global KOOS score and subscales significantly increased overtime while pain at rest, walking pain and patients or investigators global assessment of disease activity decreased. The safety profile was favorable with a low incidence of injection-site pain.

(Continued on next page)

\* Correspondence: [yhenrotin@uliege.be](mailto:yhenrotin@uliege.be)

<sup>1</sup>Bone and Cartilage Research Unit, Arthropole Liège, Université de Liège, Institute of Pathology, CHU Sart-Tilman, 4000 Liège, Belgium

<sup>2</sup>Artialis SA, GIGA Tower, CHU Sart-Tilman, Liège, Belgium

Full list of author information is available at the end of the article



© The Author(s). 2019 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

(Continued from previous page)

**Conclusion:** HYMOVIS®, a well-tolerated intra-articular treatment, significantly enhanced type II collagen turnover as suggested by the increase in Coll2-1 and PIIANP levels and cartilage volume observed by MRI in lateral knee compartment. Importantly, this study provides critical information for the design of a larger phase III clinical trial investigating Disease Modifying effect of HYMOVIS®.

**Trial registration:** <http://www.isrctn.com/ISRCTN12227846> 11/02/2015.

**Keywords:** Hyaluronic acid, Osteoarthritis, Cartilage, Biomarkers

## Background

Osteoarthritis (OA) is a disorder involving movable joints characterized by cartilage degradation initiated by micro- and macro-injury, synovial membrane inflammation and abnormal subchondral bone remodeling. The disease manifests first as a molecular derangement followed by anatomic, and/or physiologic derangements that can culminate in illness [1]. The knee is the most affected joint by OA. In United States, it has a high prevalence of 40% for men and 47% for women [2].

Up to date, there is no curative treatment for knee OA despite availability of a large number of therapeutic options, including nonpharmacological, pharmacological and surgical therapies. The aim of the pharmacological treatment remains symptomatic to relieve pain and restore function [3–8]. The first-line pharmacological therapy is the use of analgesics such as paracetamol (acetaminophen) up to 4 g per day. However, paracetamol has a short-term analgesic effect and some meta-analysis indicated the occurrence of adverse events i.e. liver injury [9, 10]. The second line therapy is the use of non-steroidal anti-inflammatory drugs (NSAIDs). However, chronic use of NSAIDs can result in serious complications e.g. gastrointestinal bleeding, renal failure, coronary heart disease even at normal dosage [11–13].

Viscosupplementation is recommended in the management of symptomatic knee OA, for appropriate patients, by many scholarly societies of rheumatology and orthopaedics [4, 14–16] sport medicine [17] and geriatrics [18], on the basis of recent systematic reviews and metaanalyses [19–25]. Even though the clinical efficacy is now proved, at least in selected patients, the structure-modifying effect remains to be demonstrated. Two recent studies have suggested that repeat intra-articular (IA) injections of hyaluronic acid (HA) may delay the time to prosthetic replacement [26, 27]. Total knee replacement (TKR) is a valuable surrogate marker of severe OA and a possible endpoint for clinical trials, but unfortunately, is neither a reliable marker of the lack of treatment efficacy nor of the anatomical progression of the disease. Indeed, TKR is highly dependent on intrinsic problems such as access barriers due to geographical and financial considerations, the availability of the resources for TKR and in the willingness of patients to be operated [28]. Therefore, additional studies are required to demonstrate

the structure-modifying effect HA viscosupplementation in knee OA.

HYMOVIS® is a sterile, non-pyrogenic, viscoelastic hydrogel for intra-articular injection. The principal component is HYADD®, a novel linear (i.e., not cross-linked) HA chemical derivative containing between 1250 to 1800 disaccharides with a molecular weight comprised between 500 to 730 kDa, displaying unique rheological properties. Indeed, the modification with hexadecylamine creates a network stabilized by reversible hydrophilic and hydrophobic interactions (not by rigid covalent cross-links), conferring high viscoelasticity to this HA derivative. Unlike rigid chemically cross-linked HAs, the reversible interactions stabilizing the mobile reticulum allow for a complete recovery of the 3D structure of the gel (and, therefore, of its elasticity) after mechanical shocks [29]. Thus, hexadecylamine structure improves shock absorbing function of synovial fluid, and potentially protect cartilage and soft tissues against mechanical injuries. These properties together with the prolonged residence time (between 2 to 5 weeks in rodents) in the articular joints enable HYMOVIS® to relieve pain and to improve joint function with a short treatment regimen. In a recent preclinical study [30], HYMOVIS® has exhibited beneficial effects on both chondrocyte and synovial fibroblast expression of catabolic enzymes and inflammatory cytokines/mediator. Recently, a retrospective study and one open-label study have reported that two intra-articular injections of HYMOVIS® 1 week apart reduced pain and improved function for at least 1 year after the first injection in knee OA patients [31, 32] and one single-center single-blind prospective randomized clinical trial evidenced that two injections 7 days apart of HYMOVIS® provided better short-term (at 26 weeks but not 52 weeks) effects on pain and function than two injections of methylprednisolone acetate in patients with mild to moderate knee osteoarthritis [33].

This pilot study aimed to explore the potential structure-modifying effect of HYMOVIS® in patients suffering of knee OA using a combination of scientifically sound, objective measurements of clinical, biological and MRI-based imaging markers [34].

**Fig. 1** Study design. KOOS = Knee Injury and Osteoarthritis Outcome Score; VAS = Visual Analog Scale; MRI = Magnetic Resonance Imaging

**Table 1** Exclusion criteria

Related to the OA pathology
<ul style="list-style-type: none"> <li>• Bilateral (except asymptomatic and grade I) OA of the knee</li> <li>• Radiological K&amp;L grade I or IV</li> <li>• Chondromatosis or villonodular synovitis of the knee</li> <li>• Recent trauma (&lt; 1 month) of the knee responsible of the symptomatic knee</li> <li>• Acute inflammatory osteoarthritis (Kofus <math>\geq 7</math>)</li> <li>• Articular disease resulting from articular dysplasia, aseptic osteonecrosis, acromegaly, Paget's disease, hemophilia, hemochromatosis....</li> <li>• Inflammatory disease i.e. rheumatoid arthritis, gout and infectious arthritis, acute calcium pyrophosphate arthritis</li> <li>• Pathologies interfering with the evaluation of OA (radiculargia in the lower limbs, arteritis....)</li> <li>• Presence of another joint (other than the target knee) affected by OA (confirmed in radiographs and symptomatic)</li> </ul>
Related to treatments
<ul style="list-style-type: none"> <li>• Corticosteroids injection in the target knee in the last month before first injection</li> <li>• Hyaluronan injection in the target knee in the last 6 months before first injection</li> <li>• Arthroscopy and surgery in the target knee in the last 6 months before first injection</li> <li>• Oral corticotherapy <math>\geq 5</math> mg/day (in Prednisone equivalent) in the last 3 months before first injection</li> <li>• OA treatments based on curcuma extract (e.g. FLEXOFYTOL) in the last 3 months before first injection</li> <li>• Change in the dosage of symptomatic slow-acting drugs (SYSAD) i.e. chondroitin, glucosamine, diacerein or avocado-soya unsaponifiables in the last 3 months before first injection</li> <li>• Paracetamol and oral NSAIDs before inclusion and follow-up visits (washout period depending on the half-life of the drug).</li> <li>• Osteoporosis-related treatments based on strontium ranelate, selective estrogen-receptor modulator (SERM) and parathormone (PTH) in the last 12 months before first injection</li> <li>• An anticipated need for any forbidden OA treatments during the trial</li> <li>• Contraindications to HYMOVIS®: hypersensitivity to the product components and infections or skin diseases in the area of the injection site</li> <li>• Non-pharmacologic therapy (including physical therapy) for the lower extremities initiated in the month before first injection</li> <li>• Anticoagulant (coumarinic compound) and heparin</li> </ul>
Related to associated diseases
<ul style="list-style-type: none"> <li>• Severe diseases (liver or renal failure, lung/heart disease, tumor, HIV....)</li> <li>• Allergy or contra-indication to hyaluronan and constituents</li> <li>• Severe alteration of mobility unabling functional evaluation</li> <li>• High risk of hemorrhage and risk of infection at the site of injection</li> <li>• Anticipated need for any surgical or other invasive procedure during the trial including prosthesis in the target knee</li> </ul>
Related to patients
<ul style="list-style-type: none"> <li>• Participation to a therapeutic clinical trial in the last 3 months before first injection</li> <li>• Under guardianship or judicial protection</li> <li>• Pregnancy, breastfeeding, planned conception, premenopausal women without contraception, tubal ligation or hysterectomy</li> </ul>
Related to MRI counter-indication
<ul style="list-style-type: none"> <li>• Patient with a pacemaker, an implantable defibrillator, neurosurgical clips, a neurostimulator, cochlear implant, a stent from less than 3 weeks, an insulin pump</li> <li>• Patient with a ferromagnetic splinter in the body, or having wire sutures</li> <li>• Serious mobility problem (Parkinson, tremors),</li> <li>• Claustrophobia</li> </ul>

compound) and heparin and osteoporosis-related treatments based on strontium ranelate, selective estrogen-receptor modulator (SERM) and parathormone (PTH).

### Outcomes measures

Demographic data, medical history, eligibility criteria, concomitant treatments were recorded at screening visit scheduled 30 days (D30) before the first injection regimen. MRI acquisition was performed in the month preceeding the first injection, but also 6 months (D180) and 12 months (D360) after this injection. Images were transferred to the centralized imaging platform (ARTIALIS SA, Liège, Belgium) for quality check and reading. The semi-quantitative Whole-Organ Magnetic Resonance Imaging Score (WORMS) and its 14 features (articular cartilage integrity, subarticular bone marrow abnormality, subarticular cysts, subarticular bone attrition, marginal osteophytes, medial and lateral meniscal integrity, anterior and posterior cruciate ligament integrity, medial and lateral collateral ligament integrity, synovitis/effusion, intraarticular loose bodies, and peri-articular cysts/bursitis) were evaluated in 14 subregions (patella/femur/tibia) of the knee [35]. T2 relaxation time were evaluated in patella, femur and tibia subregions and in the following cartilage sub-regions medial tibia, medial weight bearing femur, medial trochlea, lateral tibia, lateral weight bearing femur and lateral trochlea. Cartilage volume ( $\text{mm}^3$ ), thickness (mm), and bone curvature were reported for femur, tibia, patella, and for the following cartilage subregions: medial tibia, medial weight bearing femur, medial trochlea, lateral tibia, lateral weight bearing femur and lateral trochlea.

The Knee Injury and Osteoarthritis Outcome Score (KOOS) and its subscale scores using a self-administered questionnaire, the mean knee pain over the last 24 h at rest and while walking using a VAS, the global assessment of disease activity (by patient and by investigator) using a VAS, responder rate to treatment (following OARSI OMERACT criteria), patient satisfaction by a five-category scale (i.e. better, little better, same, little lower or far lower), concomitant treatments, adverse events and drop-off were recorded at each visit. Adverse events were recorded immediately after the injections and during the follow-up visits.

Serum (s) and urine (u) were collected at D0, D30, D90, D180, D210 and D360 for each subject by the clinical laboratory of each investigation site and shipped to the centralized biomarker platform (Artialis SA, Liège, Belgium) for biomarker testing at appropriate frequency. Samples have been assayed in pooled test series using validated immunoassay assays and according to written procedures provided by the manufacturer. sColl2-1 (Artialis SA, Liège Belgium), sColl2-1NO2 (Artialis SA, Liège Belgium), uCTXII (Immunodiagnostic Systems Limited (IDS), Boldon,

Tyne and Wear, UK), sCS-846 (IBEX, Montréal, Canada), sCOMP (BioVendor, Brno, Czech Republic), sPIIANP (Merck, Darmstadt, Germany), and sMMP-3, sMPO, sIL-6 (Bio-Techne, Abingdon, UK). CTX-II was normalized on creatinine (Quidel Corporation, San Diego, USA).

### Primary outcomes

This is a post-marketing study designed to explore the effect of HYMOVIS on cartilage metabolism and to explore the potential of Coll2-1, Coll2-1NO2 and CTX-II as companion biomarkers for the follow-up of patients treated with HYMOVIS. This was the reason for which we have selected Coll2-1, Coll2-1NO2 and CTX-II as primary end-points. The primary outcome measures were the levels of sColl2-1, sColl2-1NO2 and uCTX-II biomarkers at baseline (D0) and at the different time points.

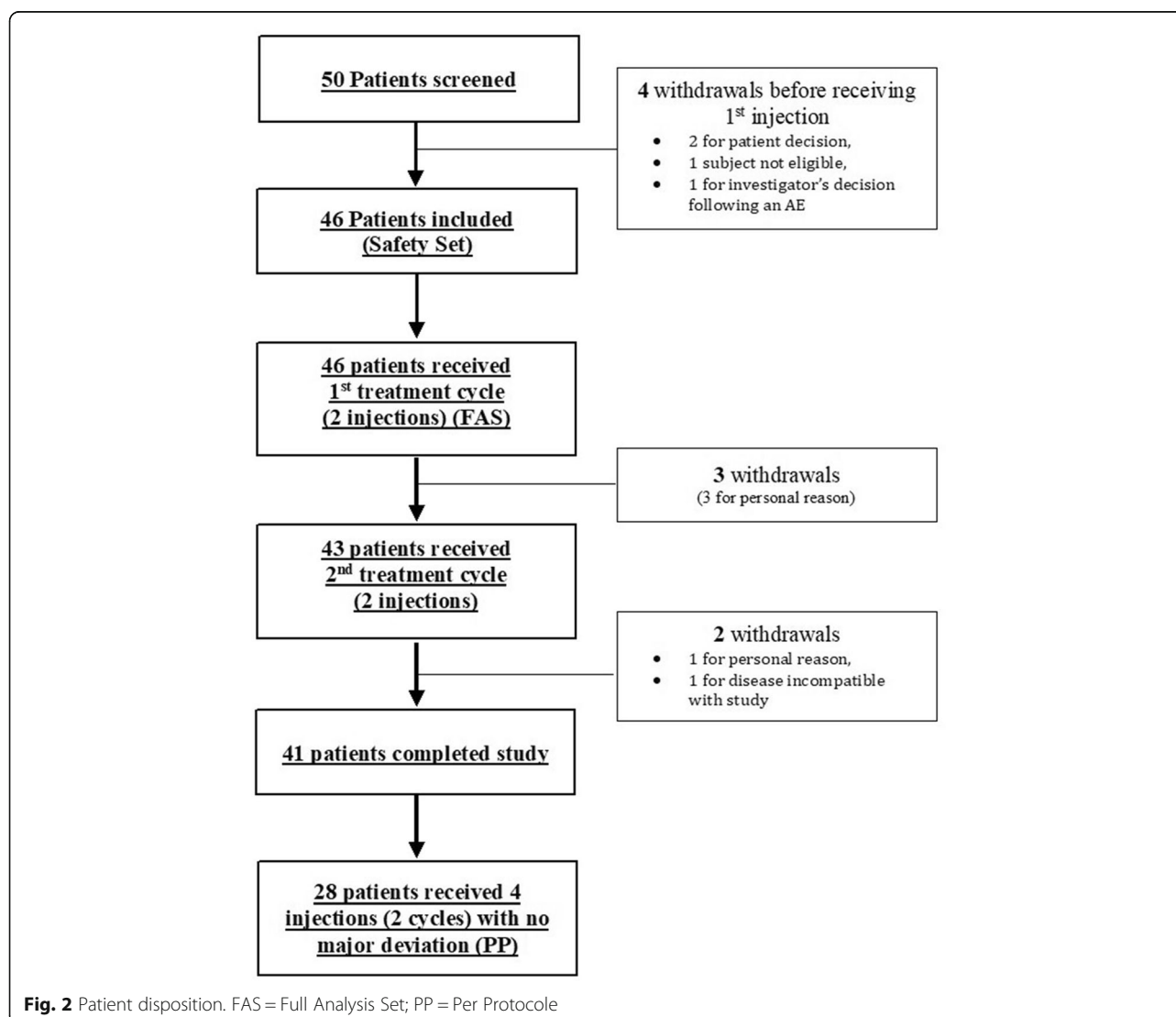
### Secondary outcomes

The secondary outcome measures were the levels of sCS-846, sCOMP, sPIIANP, sMMP-3, sMPO and sIL-6 biomarkers; the measure of MRI WORMS score, T2 relaxation time (whole, top, middle and bottom layers), cartilage volume, thickness and curvature; function evaluated with KOOS index, VAS for the mean knee pain over the last 24 h at rest and while walking, VAS for the global assessment of disease activity (by patient and by investigator), responder rate to treatment (OARSI-OMERACT criteria), adverse events, drop-off and patient satisfaction scale.

### Statistical analysis

#### Sample size

The sample size was calculated following recommendations and guidance on statistical principles for clinical trials [36, 37], considering a minimal biomarker difference between time points at least equal to the variability of the





assay (primary endpoint,  $s = \theta = 10\%$ ). It was calculated to estimate a sample size for a future trial studying the disease-modifying effect of the product. Sample size was calculated according to the paired t-test formula assuming a type I error rate of 0.05 and an 80% power (type II error). The sample size varied according to the assumed correlation (Corr) between the pre and post visits. The sample was sized on the worst case (10% correlation). With 17 cases, the power was 90.5, 97.2 and 99.9% in case of higher correlations, respectively 0.3, 0.5 and 0.7. Moreover, the sample size was inflated according to the theoretical responder rate (RR) determined from the literature [38] (RR = 56.8%) and potential study drop-off (DO = 40%). Therefore, a total number of 50 patients was planned to be enrolled.

### Analysis of the primary outcome

Analyses were performed using the Statistical Analysis Software SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). First, baseline (D0) sColl2-1, sColl2-1 NO<sub>2</sub> and uCTX-II concentrations were compared with those obtained at D30, D90, D180, D210 and D360 days by using a paired t-test (or a Wilcoxon signed-rank test if the data were deemed non-normal). Moreover, a repeated measures mixed model was applied using the change from baseline in biomarker levels as the outcome variable, visit as qualitative independent variable and baseline x visit interaction as covariate. *P*-values of the fixed effects and covariates have been presented and estimates at each time point have been given with 95% confidence intervals. Analyses were performed both in the Full Analysis Set (FAS) and Per-Protocol (PP) populations. Full Analysis Set (FAS) corresponded to all subjects who received at least one treatment cycle consisting of two injections at 1 week interval. Per-Protocol Set (PP) were all subjects who received all the injections and had no major protocol deviations.

**Table 2** Demographic data and OA history of the FAS population (*N* = 46)

Age (years; mean (SD))	61.4 (9.6)
Sex (%)	
Women	67.4%
Men	32.6%
BMI (Kg/m <sup>2</sup> ; mean (SD))	30.6 (5)
Disease duration (years; mean (SD))	4.54 (5.9)
Evaluated knee (%)	
Left	32.6%
Right	67.4%
Radiological score (Kellgren & Lawrence)	
Score II	63%
Score III	37%

### Analysis of the secondary outcomes

The secondary biomarkers endpoints were analyzed using the same statistical methods that described for the primary endpoints. The MRI endpoints (WORMS, T2 relaxation time, cartilage volume, thickness and curvature) have been analyzed with the Paired t-test or Wilcoxon signed-rank tests comparing the results observed at D180 and D360 versus the baseline visit (D0). The WORMS values have been analyzed by compartments and using the total score. The T2 relaxation time have been analyzed by layers and using the total score as well. The KOOS (sub-score and total score) and VAS scales have been analyzed using the same statistical methods that biomarkers endpoints. The responder rate according to the OARSI-OMERACT ratio was calculated at each time point and all the analyses of biomarkers has been repeated in subgroups of responders and non-responders. Moreover, the level of biomarkers at each assessment has been compared between responders and non-responders with the T-test (or the Wilcoxon rank-sum test if the data are deemed non-normal). Correlations between variables (biological and/or MRI-based imaging markers) has been obtained using the Spearman rank's correlation test. Patient's satisfaction has been analyzed using shift tables and the McNemar test. The analyses have been performed in the FAS population. No formal hypothesis testing of safety data (Adverse Effect (AE) and drop-off) has been undertaken, but descriptive statistics have been performed. The Safety Analysis Set (SAS) has been used when summarizing safety data. Safety Set included all subjects who received at least one treatment cycle consisting of two injections at a 1 week interval.

## Results

### Study population

Fifty patients [39] were screened from which 46 entered the Multicenter Osteoarthritis Knee Hyaluronic Acid (MOKHA) Study. Forty-one (41; 89%) completed the study and 5 (11%) discontinued: 4 for personal reasons not related to the study and 1 for disease incompatible with the pursuing of the study decided by the patient. Eighteen (39.1%) patients out of 46 presented at least one major deviation. Major deviations were intake of prohibited medication (*n* = 10; 21.7%), no respect of the treatment regimen (*n* = 3; 6.5%), the presence of at least one exclusion criteria (*n* = 2; 4.3%) or the non-respect of delays between visits (*n* = 7; 15.2%). Forty-six patients received at least one treatment injection (SAS), 46 patients received at least one treatment cycle consisting of two injections at one week intervals (FAS) and 28 patients received a total dose of 4 injections without any major protocol violations (PP) (Fig. 2).

**Table 3** levels (median (IQR)) of soluble biomarkers in the Full Analysis Set Population

	D0	D30	D90	D180	D210	D360
sColl2-1(nM)	<i>n</i> = 46; 498.66 (297.61;647.44)	<i>n</i> = 46; 578.30 (399.97;724.51)	<i>n</i> = 43; 634.46 (439.25;787.76)	<i>n</i> = 43; 635.01 (484.51;824.38)	<i>n</i> = 39; 622.47 (476.03;858.14)	<i>n</i> = 42;722.09 (469.99;883.08)
<i>Change from baseline</i>		<i>n</i> = 46; 20.84 (-42.75;132.08)	<i>n</i> = 43; 50.25 (-42.03;242.73)	<i>n</i> = 43; 149.62 (28.17;297.19)	<i>n</i> = 39; 138.98 (-52.42;241.88)	<i>n</i> = 42;135.36 (-6.52;319.60)
<i>P-value vs D0</i>		0.098	0.021	< 0.001	0.010	< 0.001
sColl2-NO2(pg/ml)	<i>n</i> = 30; 340.21 (266.24;425.99)	<i>n</i> = 36; 268.61 (206.13;370.75)	<i>n</i> = 34; 293.75 (233.64;464.92)	<i>n</i> = 34; 336.82 (265.68;450.43)	<i>n</i> = 29; 396.35 (284.85;598.79)	<i>n</i> = 31; 384.53 (303.84;663.99)
<i>Change from baseline</i>		<i>n</i> = 30; -28.54 (-71.03;53.08)	<i>n</i> = 27; -17.33 (-64.21;67.82)	<i>n</i> = 26; 18.57 (-49.93;120.00)	<i>n</i> = 22; 29.31 (- 67.38;198.32)	<i>n</i> = 25; 69.75 (- 13.51;294.24)
<i>P-value vs D0</i>		0.243	0.907	0.225	0.272	0.027
uCTX-II (ug/mmol creatinine)	<i>n</i> = 37; 0.012 (0.009;0.015)	<i>n</i> = 37; 0.012 (0.009;0.015)	<i>n</i> = 34; 0.010 (0.008;0.016)	<i>n</i> = 37; 0.012 (0.009;0.015)	<i>n</i> = 34; 0.011 (0.008;0.015)	<i>n</i> = 36; 0.012 (0.008;0.016)
<i>Change from baseline</i>		<i>n</i> = 33; 0.001 (-0.002;0.003)	<i>n</i> = 31; 0.000 (-0.003;0.002)	<i>n</i> = 33; 0.000 (- 0.002;0.002)	<i>n</i> = 31; 0.000 (- 0.002;0.003)	<i>n</i> = 33; 0.000 (- 0.003;0.003)
<i>P-value vs D0</i>		0.733	0.924	0.882	0.924	0.910
sPIINP (ng/ml)	<i>n</i> = 45; 599.50 (502.45; 812.42)	<i>n</i> = 42; 667.45 (519.13; 827.70)	<i>n</i> = 40; 708.90 (548.63; 873.46)	<i>n</i> = 41; 780.20 (572.88; 983.53)	<i>n</i> = 37; 858.79 (738.85;1061.52)	<i>n</i> = 37; 1057.41 (726.91;1268.47)
<i>Change from baseline</i>		<i>n</i> = 41; 31.2 (-87.64;117.27)	<i>n</i> = 40; 74.82 (-83.03;193.95)	<i>n</i> = 41; 96.97 (5.27;298.16)	<i>n</i> = 37; 190.11 (138.91;319.81)	<i>n</i> = 37; 433.90 (136.71;573.78)
<i>P-value vs D0</i>		0.954	0.038	< 0.001	< 0.001	< 0.001
sColl2-1(nM)/sPIINP (ng/ml)	<i>n</i> = 45; 0.72 (0.53; 0.96)	<i>n</i> = 42; 0.80 (0.56; 1.03)	<i>n</i> = 40; 0.69 (0.56; 1.05)	<i>n</i> = 41; 0.81 (0.62; 1.00)	<i>n</i> = 37; 0.69 (0.54;0.93)	<i>n</i> = 37; 0.56 (0.48;0.81)
<i>Change from baseline</i>		<i>n</i> = 41; 0.04 (-0.11; 0.26)	<i>n</i> = 40; - 0.02 (- 0.18; 0.30)	<i>n</i> = 41; 0.04 (- 0.14; 0.36)	<i>n</i> = 37; - 0.04 (- 0.26; 0.22)	<i>n</i> = 37; - 0.11 (- 0.28; 0.00)
<i>P-value vs D0</i>		0.291	0.677	0.257	0.678	0.005
uCTX-II (ng/mmol creatinine)/sPIINP (ng/ml)	<i>n</i> = 36; 0.019 (0.012; 0.027)	<i>n</i> = 33; 0.018 (0.012; 0.026)	<i>n</i> = 33; 0.015 (0.011; 0.022)	<i>n</i> = 36; 0.016 (0.011; 0.024)	<i>n</i> = 33; 0.014 (0.011; 0.020)	<i>n</i> = 31; 0.012 (0.008; 0.019)
<i>Change from baseline</i>		<i>n</i> = 28; -0.001 (- 0.004; 0.003)	<i>n</i> = 30; - 0.004 (- 0.008; 0.002)	<i>n</i> = 32; - 0.003 (- 0.008; 0.001)	<i>n</i> = 30; - 0.004 (- 0.010; - 0.001)	<i>n</i> = 28; - 0.006 (- 0.013; - 0.001)
<i>P-value vs D0</i>		0.219	0.041	0.010	0.004	0.001
sCS-846 (ng/ml)	<i>n</i> = 38; 153.03 (124.60;186.18)	<i>n</i> = 38; 141.94 (125.27;185.74)	<i>n</i> = 36; 150.27 (114.88;180.88)	<i>n</i> = 37; 163.23 (134.32;185.58)	<i>n</i> = 36; 153.50 (121.16;178.61)	<i>n</i> = 39; 157.41 (130.60;186.27)
<i>Change from baseline</i>		<i>n</i> = 34; -6.35 (-19.86;12.92)	<i>n</i> = 32; -5.52 (-24.24;19.69)	<i>n</i> = 31; -1.54 (- 18.28;22.55)	<i>n</i> = 30; - 1.97 (- 19.82;20.00)	<i>n</i> = 33; 2.67 (- 17.60;19.17)
<i>P-value vs D0</i>		0.086	0.419	0.863	0.756	0.787
sCOMP (ng/ml)	<i>n</i> = 46; 796.15 (615.97;1044.40)	<i>n</i> = 45; 823.23 (617.95;1032.53)	<i>n</i> = 43; 737.14 (602.20;982.47)	<i>n</i> = 43; 740.58 (626.94;968.86)	<i>n</i> = 39; 763.79 (573.95;991.30)	<i>n</i> = 42; 846.46 (613.54;1060.12)
<i>Change from baseline</i>		<i>n</i> = 45; -14.69 (-94.76;118.464)	<i>n</i> = 43; -4.86 (- 164.98;89.46)	<i>n</i> = 43; -26.32 (- 116.87;79.57)	<i>n</i> = 39; -24.32 (- 98.96;108.31)	<i>n</i> = 42; 15.30 (- 123.31;109.46)
<i>P-value vs D0</i>		0.934	0.310	0.404	0.701	0.936
sMMP-3 (ng/ml)	<i>n</i> = 46; 14.86 (11.99;20.90)	<i>n</i> = 46; 15.75 (12.48;23.64)	<i>n</i> = 43; 16.36 (13.71;22.17)	<i>n</i> = 42; 15.87 (12.05;21.91)	<i>n</i> = 38; 15.95 (12.54;22.27)	<i>n</i> = 42; 14.87 (11.03;19.16)
<i>Change from baseline</i>		<i>n</i> = 46; 1.19 (-1.11;3.17)	<i>n</i> = 43; 1.61 (- 1.37;3.53)	<i>n</i> = 42; 0.62 (-2.64;3.33)	<i>n</i> = 38; 0.85 (- 1.81;2.68)	<i>n</i> = 42; -0.03 (-3.21;2.57)
<i>P-value vs D0</i>		0.138	0.041	0.726	0.420	0.792
sIL-6 (pg/ml)	<i>n</i> = 46; 1.62 (1.23;2.16)	<i>n</i> = 45; 1.70 (1.35;2.63)	<i>n</i> = 41; 1.67 (1.29;2.61)	<i>n</i> = 42; 1.54 (1.20;2.33)	<i>n</i> = 37; 1.71 (1.21;2.73)	<i>n</i> = 42; 1.95 (1.35;2.86)
<i>Change from baseline</i>		<i>n</i> = 45; 0.19 (-0.23;0.51)	<i>n</i> = 41; 0.04 (- 0.30;-0.61)	<i>n</i> = 42; 0.014 (- 0.23;0.49)	<i>n</i> = 37; 0.175 (- 0.28;0.44)	<i>n</i> = 42; 0.26 (- 0.31;1.04)
<i>P-value vs D0</i>		0.119	0.448	0.534	0.410	0.081
sMPO (ng/ml)	<i>n</i> = 44; 176.08 (128.28;251.10)	<i>n</i> = 44; 189.13 (104.79;260.37)	<i>n</i> = 41; 182.85 (132.41;288.12)	<i>n</i> = 41; 184.19 (105.37;264.12)	<i>n</i> = 35; 158.36 (87.61;250.25)	<i>n</i> = 38; 168.92 (11.21;239.39)

**Table 3** levels (median (IQR)) of soluble biomarkers in the Full Analysis Set Population (*Continued*)

	D0	D30	D90	D180	D210	D360
Change from baseline		<i>n</i> = 43; -3.77 (-52.92;69.34)	<i>n</i> = 40; 1.32 (-45.19;75.32)	<i>n</i> = 39; 0.07 (-71.70;78.37)	<i>n</i> = 34; -24.03 (-64.88;39.60)	<i>n</i> = 36; -11.28 (-39.45;12.45)
P-value vs D0		0.767	0.412	0.816	0.183	0.202

### Patient characteristics

Patients were mainly women (67.4%) with a mean age of 61.4 (9.6) years and a BMI of 30.60 [5] kg/m<sup>2</sup>. Patients were suffering from knee OA for a mean of 4.54 (5.9) years. The most painful knee was the right knee for 67.4% of the patients. Sixty-three (63) percents had a KL grading of II and 37% a KL of III (Table 2).

### Primary efficacy outcomes

sColl2-1 showed a significant increase at D90 and over in both FAS and PP population (Tables 3 and 4). sColl2-1 NO2 showed a significant increase only at D360 in the FAS (Table 3), but not in the PP population (Table 4). At D360, the effect size calculated on the FAS population was 0.47 ( $p = 0.022$ ) for Coll2-1 and 0.32 for Coll2-1NO2 ( $p = 0.048$ ). uCTX-II Biomarker did not show any significant change over time in both FAS and PP population (Tables 3 and 4).

### Secondary efficacy outcomes

#### Biological parameters

Among all biomarkers tested as secondary outcomes, only sPIIANP levels significantly changed during the study (Tables 3 and 4). A significant increase of sPIIANP was observed from D90 in the FAS population and from D180 in the PP set. The other biomarkers did not vary overtime.

Further, the increase of PIIANP between D0 and D90 was significantly lower in OARSI-OMERAC responders than in non-responders (responders 39.3157.43 (208–84.65; 172.50) ng/ml vs non-responders 239.41258.27 (46.09182.03; 273.51) ng/ml;  $p = 0.008$ ). No difference was observed if we consider the variation of PIIANP between D0 and D360.

No significant differences at baseline or changes over time were observed in sColl2-1, Coll2-1 NO2 or CTX-II levels between clinical responders and non-responders. The ratios Coll2-1/PIIANP at D360 ( $p = 0.005$ ) and CTX-II/PIIANP from D90 to D360 ( $0.05 < p < 0.001$ ) decreased, indicating a decrease of cartilage catabolism. At D360, the effect size calculated on the FAS population was 0.46 ( $p = 0.005$ ) and 0.720 ( $p = 0.001$ ) for the ratio Coll2-1/PIIANP and CTX-II/PIIANP respectively.

#### MRI features

WORMS total score, that represents a summation of grades for all of the knee features, showed a significant

( $p = 0.037$ ) increase at D360. WORMS total cartilage and total cysts features were also significantly increased (cartilage: mean change (SD): 0.45 (1.21);  $p = 0.025$ ); cysts: mean change (SD): 0.23 (0.63),  $p = 0.047$ ) at D360. Interestingly, WORMS effusion score was significantly decreased at D180 that was not maintained at D360. Other WORMS total features or WORMS compartments were not significantly modified over time (Table 5).

Mean T2 relaxation time (ms) was significantly decreased overtime in the femur compartment for 8 distinct features (Table 6): femur whole layers (median change: -0.537 ms,  $p = 0.050$  at D180 and -1.595 ms,  $p = 0.014$  at D360), femur middle layer (median change: -0.596 ms,  $p = 0.03$  at D180 and -1.249 ms,  $p = 0.008$  at D360), femur bottom layer (median change: -2.051 ms,  $p = 0.009$  at D360), lateral weight bearing femur whole (median change: -3.129 ms,  $p = 0.011$  at D180 and -1.748 ms,  $p = 0.032$  at D360), lateral weight bearing femur middle (median change: -1.075 ms,  $p = 0.005$  at D180 and -1.264 ms,  $p = 0.045$  at D360), lateral weight bearing femur bottom (median change: -1.599 ms,  $p = 0.015$  at D180). In contrast, mean T2 relaxation time was not modified in the medial compartment. Standard deviation of T2 relaxation was significantly reduced in the femoral cartilage for the 5 following distinct features: femur bottom (median change: -0.944 ms,  $p = 0.027$  at D180), lateral weight bearing femur bottom (median change: -1.053 ms,  $p = 0.005$  at D180), medial weight bearing femur middle (median change: -1.355 ms,  $p = 0.037$  at D180), medial weight bearing femur bottom (median change: -1.506 ms,  $p = 0.010$  at D180 and median change: -2.298 ms,  $p = 0.012$  at D360). Similarly, at patella level, mean and SD of T2 relaxation time showed a significant decrease, the majority being observed starting from D180, for 7 distinct features: patella medial trochlea whole, patella medial trochlea top, patella medial trochlea middle, patella medial trochlea bottom, patella lateral trochlea whole, patella lateral trochlea middle, patella lateral trochlea bottom. No significant changes were observed at the tibia level (Table 6).

Cartilage volume significantly increased at D360 in the lateral weight bearing femur (median change = 66 mm<sup>3</sup>,  $p = 0.03$ ) and the patella lateral trochlea (median change = 77 mm<sup>3</sup>,  $p = 0.028$ ). No significant volume change was observed in the other compartments.

Cartilage thickness significantly increased at D360 in the lateral weight bearing femur (median change: 0.023



**Table 4** levels (median (IQR)) of soluble biomarkers in the Per Protocol Population

	D0	D30	D90	D180	D210	D360
sColl2-1 (nM)	<i>n</i> = 28; 462.04 (297.03;604.49)	<i>n</i> = 28; 556.76 (399.84;692.02)	<i>n</i> = 28; 653.36 (429.74;757.79)	<i>n</i> = 28; 599.67 (456.70;725.51)	<i>n</i> = 24; 557.73 (460.48;818.09)	<i>n</i> = 27; 598.44 (408.51;858.57)
<i>Change from baseline</i>		<i>n</i> = 28; 37.03 (-45.30;140.52)	<i>n</i> = 28; 61.29 (-31.90;250.43)	<i>n</i> = 28; 106.88 (31.31;265.17)	<i>n</i> = 24; 145.72 (-40.33;201.72)	<i>n</i> = 27; 123.74 (-6.52;273.51)
<i>P-value vs D0</i>		0.162	0.018	0.001	0.066	0.005
sColl2-NO2 (pg/ml)	<i>n</i> = 20; 335.13 (268.79;467.17)	<i>n</i> = 23; 271.58 (222.80;393.76)	<i>n</i> = 23; 283.65 (212.03;599.42)	<i>n</i> = 22; 317.89 (264.34;450.43)	<i>n</i> = 18; 491.21 (271.25;624.31)	<i>n</i> = 20; 485.38 (307.04;740.40)
<i>Change from baseline</i>		<i>n</i> = 20; -34.88 (-65.31;62.77)	<i>n</i> = 18; -36.62 (-73.09;87.12)	<i>n</i> = 18; -9.71 (-65.55;104.32)	<i>n</i> = 14; 44.37 (-67.38;202.55)	<i>n</i> = 16; 76.89 (-55.92;334.31)
<i>P-value vs D0</i>		0.498	0.832	0.899	0.426	0.175
uCTX-II (ug/mmol creatinine)	<i>n</i> = 24; 0.012 (0.008;0.014)	<i>n</i> = 25; 0.012 (0.009;0.015)	<i>n</i> = 23; 0.010 (0.008;0.016)	<i>n</i> = 26; 0.012 (0.009;0.014)	<i>n</i> = 23; 0.011 (0.007;0.015)	<i>n</i> = 23; 0.010 (0.008;0.014)
<i>Change from baseline</i>		<i>n</i> = 23; 0.001 (-0.002;0.003)	<i>n</i> = 21; 0.001 (-0.003;0.001)	<i>n</i> = 23; 0.000 (-0.003;0.003)	<i>n</i> = 21; 0.000 (-0.002;0.003)	<i>n</i> = 22; -0.001 (-0.003;0.002)
<i>P-value vs D0</i>		0.357	0.626	0.791	0.987	0.420
sPIINP (ng/ml)	<i>n</i> = 27; 590.14 (500.89; 740.87)	<i>n</i> = 24; 651.57 (529.91; 875.12)	<i>n</i> = 26; 697.9 (544.21; 858.85)	<i>n</i> = 27; 780.2 (572.88; 999.99)	<i>n</i> = 22; 865.69 (752.23;1075.72)	<i>n</i> = 24; 1002.69 (664.39;1263.72)
<i>Change from baseline</i>		<i>n</i> = 23; 5.83 (-87.64;87.38)	<i>n</i> = 26; 57.68 (-96.03;172.51)	<i>n</i> = 27; 90.35 (-23.12;298.16)	<i>n</i> = 22; 198.77 (144.54;368.52)	<i>n</i> = 24; 417.05 (115.26;529.05)
<i>P-value vs D0</i>		0.791	0.434	0.004	< 0.001	< 0.001
sColl2-1 (nM)/sPIINP (ng/ml)	<i>n</i> = 27; 0.66 (0.49; 0.96)	<i>n</i> = 24; 0.69 (0.45; 0.93)	<i>n</i> = 26; 0.78 (0.60; 1.00)	<i>n</i> = 27; 0.76 (0.60; 0.94)	<i>n</i> = 22; 0.61 (0.54;0.82)	<i>n</i> = 24; 0.52 (0.44;0.62)
<i>Change from baseline</i>		<i>n</i> = 23; 0.07 (-0.09; 0.24)	<i>n</i> = 26; 0.00 (-0.08; 0.34)	<i>n</i> = 27; 0.04 (-0.21; 0.20)	<i>n</i> = 22; -0.04 (-0.40; 0.12)	<i>n</i> = 24; -0.12 (-0.30; -0.01)
<i>P-value vs D0</i>		0.465	0.258	0.665	0.306	0.022
uCTX-II (ng/mmol creatinine)/ sPIINP (ng/ml)	<i>n</i> = 23; 0.018 (0.010; 0.028)	<i>n</i> = 21; 0.025 (0.011; 0.029)	<i>n</i> = 22; 0.015 (0.010; 0.018)	<i>n</i> = 25; 0.016 (0.011; 0.024)	<i>n</i> = 22; 0.012 (0.008; 0.016)	<i>n</i> = 20; 0.011 (0.007; 0.019)
<i>Change from baseline</i>		<i>n</i> = 18; -0.001 (-0.004; 0.003)	<i>n</i> = 20; -0.002 (-0.007; 0.002)	<i>n</i> = 22; -0.004 (-0.008; 0.002)	<i>n</i> = 20; -0.003 (-0.011; -0.001)	<i>n</i> = 19; -0.007 (-0.013; -0.002)
<i>P-value vs D0</i>		0.777	0.218	0.046	< 0.001	< 0.001
sCS-846 (ng/ml)	<i>n</i> = 23; 140.14 (117.75;186.18)	<i>n</i> = 23; 127.73 (123.57;188.92)	<i>n</i> = 24; 131.47 (113.90;174.13)	<i>n</i> = 24; 156.04 (127.58;183.30)	<i>n</i> = 23; 161.33 (120.75;174.33)	<i>n</i> = 25; 164.94 (135.04;204.99)
<i>Change from baseline</i>		<i>n</i> = 21; -1.82 (-17.50;5.25)	<i>n</i> = 21; -7.30 (-23.49;7.85)	<i>n</i> = 20; 13.64 (-5.99;20.73)	<i>n</i> = 19; 1.534 (-15.11;29.84)	<i>n</i> = 21; 11.30 (-4.93;24.80)
<i>P-value vs D0</i>		0.352	0.212	0.231	0.651	0.143
sCOMP (ng/ml)	<i>n</i> = 28; 802.85 (613.44;1050.03)	<i>n</i> = 28; 855.56 (607.44;1097.99)	<i>n</i> = 28; 746.93 (611.46;979.58)	<i>n</i> = 28; 751.8 (617.94;977.22)	<i>n</i> = 24; 770.23 (584.69;1014.59)	<i>n</i> = 27; 800.09 (613.54;1060.12)
<i>Change from baseline</i>		<i>n</i> = 28; 26.33 (-43.24;152.94)	<i>n</i> = 28; 5.75 (-107.05;107.18)	<i>n</i> = 28; -10.06 (-109.63;121.00)	<i>n</i> = 24; -33.70 (-113.39;103.32)	<i>n</i> = 27; 13.56 (-133.86;109.46)
<i>P-value vs D0</i>		0.176	0.929	0.947	0.678	0.797
sMMP-3 (ng/ml)	<i>n</i> = 28; 14.67 (11.89;21.58)	<i>n</i> = 28; 16.43 (12.14;23.93)	<i>n</i> = 28; 15.98 (13.92;22.67)	<i>n</i> = 28; 16.00 (12.23;22.15)	<i>n</i> = 24; 16.53 (12.9;23.0)	<i>n</i> = 27; 15.63 (11.56;24.06)
<i>Change from baseline</i>		<i>n</i> = 28; 1.19 (-0.91;2.97)	<i>n</i> = 28; 1.94 (-0.19;4.29)	<i>n</i> = 28; 0.91 (-2.52;3.48)	<i>n</i> = 24; 1.39 (-0.92;2.7)	<i>n</i> = 27; 0.46 (-1.99;3.58)
<i>P-value vs D0</i>		0.282	0.006	0.348	0.071	0.511
sIL-6 (pg/ml)	<i>n</i> = 28; 1.47 (1.10;2.00)	<i>n</i> = 27; 1.60 (1.22;2.35)	<i>n</i> = 27; 1.67 (1.28;2.42)	<i>n</i> = 28; 1.49 (1.11;2.47)	<i>n</i> = 23; 1.48 (1.13;2.23)	<i>n</i> = 27; 1.83 (0.97;2.86)
<i>Change from baseline</i>		<i>n</i> = 27; 0.21 (-0.12;0.48)	<i>n</i> = 27; 0.03 (-0.30;-0.61)	<i>n</i> = 28; -0.004 (-0.11;0.49)	<i>n</i> = 23; 0.21 (-0.09;0.46)	<i>n</i> = 27; 0.33 (-0.13;1.13)
<i>P-value vs D0</i>		0.030	0.481	0.348	0.108	0.017
sMPO (ng/ml)	<i>n</i> = 27; 199.49 (140.46;264.05)	<i>n</i> = 28; 240.45 (134.93;308.59)	<i>n</i> = 28; 187.17 (142.02;336.18)	<i>n</i> = 28; 208.81 (114.126;285.47)	<i>n</i> = 22; 161.96 (82.81;241.45)	<i>n</i> = 24; 174.51 (93.23;216.50)

**Table 4** levels (median (IQR)) of soluble biomarkers in the Per Protocol Population (*Continued*)

	D0	D30	D90	D180	D210	D360
Change from baseline		<i>n</i> = 27; 36.24 (−41.33;96.54)	<i>n</i> = 27; 30.40 (−48.99;84.18)	<i>n</i> = 27; 22.83 (−71.70;95.12)	<i>n</i> = 21; −16.46 (−62.60;39.60)	<i>n</i> = 23; −4.74 (−43.30;24.41)
P-value vs D0		0.160	0.237	0.481	0.317	0.555

mm). No change was observed in the other compartments (Table 7).

Neutral, positive and negative curvature correspond to a flat, convex, and concave surface respectively. Cartilage curvature significantly decreased at D360 for 3 distinct convex (positive curvature) surfaces: femur ( $-0.001 \text{ mm}^{-1}$ ,  $p = 0.015$ ), medial weight bearing femur ( $-0.001 \text{ mm}^{-1}$ ,  $p = 0.027$ ), lateral tibia ( $-0.002 \text{ mm}^{-1}$ ,  $p = 0.02$ ). These results suggest a flattening of medial femur shape.

### Pain and function

In comparison to baseline, VAS at rest and while walking was significantly decreased at each time point (mean decrease from  $-20.1 \text{ mm}$  to  $-29.9 \text{ mm}$  and from  $-25.5 \text{ mm}$  to  $-31.5 \text{ mm}$  at rest and while walking respectively,  $p < 0.001$ ) (Fig. 3). Similarly, patient and investigator global assessment of the disease activity scores were significantly reduced at each time point compared to baseline value ( $p < 0.001$ ) (Fig. 3, Table 8).

KOOS global score and subscale significantly increased overtime (mean improvement from 16.173 to 21.341 and from 15.384 to 20.511 in pain sub-score and in activities of daily living score respectively;  $p < 0.001$  for all subscales) (Fig. 4, Table 9). The ratio of OARSI-OMERACT responders in the FAS population increased overtime. This ratio was 48.9% at D180 and 70.7% at D360.

**Table 5** WOMBS total score and by features (mean (SD)) in the Full Analysis Set population

	D0	D180	D360
WOMBS total score	63.95 (27.78)	64.39 (27.71)	64.08 (28.03)
Change from baseline		0.38 (1.77)	0.96 (2.75)
<i>p</i> value		0.183	0.037
Cartilage	23.83 (11.27)	23.01 (11.13)	23.03 (11.45)
Change from baseline		0.18 (0.70)	0.45 (1.21)
<i>p</i> value		0.188	0.025
Cyst	2.73 (2.65)	2.83 (2.65)	2.90 (2.62)
Change from baseline		0.10 (0.30)	0.23 (0.63)
<i>p</i> value		0.125	0.047
Effusion	0.93 (0.69)	0.76 (0.54)	0.77 (0.54)
Change from baseline		−0.17 (0.38)	−0.15 (0.54)
<i>p</i> value		0.016	0.148

### Patient's satisfaction

At D30, patient satisfaction was good, with 43.5% of patients feeling better, 37% feeling a little better, 15.2% feeling no change, 4.3% feeling little lower and none far lower. Between D30 and D360, patient's satisfaction remained stable.

### Correlations between imaging, clinical and biological parameters

At baseline, the VAS walking pain were positively and significantly correlated with cartilage volume ( $r = -0.42779$ ;  $p = 0.007$ ) and thickness ( $r = -0.38758$ ;  $p = 0.015$ ). VAS changes overtime (from baseline to D180 or from baseline to D360) were not correlated with MRI changes overtime.

At baseline, VAS walking pain was positively correlated with sColl2-1NO2 levels ( $r = -0.53617$ ;  $p = 0.002$ ), but not with the other biomarkers. No significant correlation was found between VAS walking pain and biomarkers at follow-up time points.

### Safety

Ninety-five (95) AEs were reported after the first treatment cycle in 37 patients (80% of the SAS). Eleven patients (23.9%) presented an Adverse Device Reaction (ADR). In total, 15 ADRs (16% of AE) have been reported: difficulty to extend leg, redness of the patella, knee pain after injection, knee swelling, injection site pain, pain at injection, dizziness and knee swelling. Importantly, only one AE led to treatment discontinuation and only one led to patient withdrawal but were not related to the product. Eight (15.2% of the safety population) SAEs have been recorded during the study. Importantly, no SAE was linked to the study medication.

### Discussion

This non-controlled prospective study provided a lot of precious information on the evolution of biological, structural and clinical parameters after intra-articular injection of HYMOVIS®. These results will be valuable for the design of a randomized and controlled study aiming to demonstrate the disease modifying effect of viscosupplementation. We have investigated the evolution profile of biomarkers of type II collagen metabolism. Coll2-1 and CTX-II are both biomarkers of cartilage degradation [40–43] while PIIANP is a biomarker of collagen synthesis [44]. Interestingly,

**Table 6** Mean T2 relaxation time and standard deviation of relaxation time for patella (median (IQR)) in the Full Analysis Set population

		Mean T2 relaxation time (ms)		SD of relaxation time (ms)	
		Median (IQR) <i>n</i> = 38	<i>p</i> -value	Median (IQR) <i>n</i> = 38	<i>p</i> -value
Whole Patella- <i>whole layer</i>	<i>D180 vs D0</i>	2.822 (− 3.872; 8.996)	0.403	0.627 (− 3.895; 7.427)	0.480
	<i>D360 vs D0</i>	1.168 (− 7.721; 9.410)	0.593	0.236 (− 9.654; 7.812)	0.960
Whole Patella- <i>top layer</i>	<i>D180 vs D0</i>	4.446 (− 6.977; 16.697)	0.320	6.644 (− 21.994; 20.796)	0.593
	<i>D360 vs D0</i>	4.937 (− 10.051; 21.672)	0.327	1.413 (− 16.726; 28.354)	0.603
Whole Patella- <i>middle layer</i>	<i>D180 vs D0</i>	1.236 (− 2.881; 6.889)	0.356	0.788 (− 4.667; 6.221)	0.603
	<i>D360 vs D0</i>	0.125 (− 6.598; 7.365)	0.803	− 0.333 (− 11.132; 13.976)	0.870
Whole Patella- <i>bottom layer</i>	<i>D180 vs D0</i>	0.202 (− 3.239; 5.418)	0.545	1.115 (− 2.416; 5.700)	0.320
	<i>D360 vs D0</i>	− 0.228 (− 2.205; 5.368)	0.633	0.408 (− 4.172; 6.304)	0.644
Medial trochlea - <i>whole layer</i>	<i>D180 vs D0</i>	− 2.354 (− 16.342; 4.102)	0.061	− 1.959 (− 8.472; 1.353)	0.008
	<i>D360 vs D0</i>	− 2.429 (− 12.540; 0.968)	0.019	− 0.818 (− 6.424; 1.126)	0.107
Medial trochlea - <i>top layer</i>	<i>D180 vs D0</i>	− 1.768 (− 8.476; 1.722)	0.027	− 1.774 (− 6.983; 1.804)	0.023
	<i>D360 vs D0</i>	− 1.777 (− 9.093; 2.549)	0.075	− 0.449 (− 5.186; 1.711)	0.229
Medial trochlea - <i>middle layer</i>	<i>D180 vs D0</i>	1.606 (− 14.664; 2.549)	0.032	− 2.074 (− 9.846; 2.389)	0.011
	<i>D360 vs D0</i>	− 1.45 (− 10.896; 0.991)	0.024	− 0.796 (− 7.151; 0.737)	0.033
Medial trochlea - <i>bottom layer</i>	<i>D180 vs D0</i>	− 2.303 (− 21.865; 7.574)	0.16	− 2.703 (− 13.902; 2.017)	0.006
	<i>D360 vs D0</i>	− 1.852 (− 16.764; 0.791)	0.023	− 1.574 (− 8.096; 2.390)	0.124
Lateral trochlea - <i>whole layer</i>	<i>D180 vs D0</i>	− 2.167 (− 10.398; 0.720)	0.001	− 0.804 (− 6.646; 2.799)	0.110
	<i>D360 vs D0</i>	− 2.107 (− 11.110; 0.938)	0.006	− 0.508 (− 3.996; 0.839)	0.117
Lateral trochlea - <i>top layer</i>	<i>D180 vs D0</i>	− 0.410 (− 8.520; 4.261)	0.623	1.069 (− 7.704; 10.070)	0.972
	<i>D360 vs D0</i>	0.169 (− 5.191; 7.014)	0.848	0.670 (− 3.756; 8.526)	0.387
Lateral trochlea - <i>middle layer</i>	<i>D180 vs D0</i>	− 3.663 (− 11.111; 0.482)	0.001	− 1.473 (− 9.228; 0.898)	0.009
	<i>D360 vs D0</i>	− 2.380 (− 10.532; 0.399)	0.001	− 1.547 (− 7.190; − 0.374)	0.003
Lateral trochlea - <i>bottom layer</i>	<i>D180 vs D0</i>	− 2.538 (− 26.748; 0.497)	0.001	− 1.188 (− 8.698; 0.999)	0.005
	<i>D360 vs D0</i>	− 2.677 (− 20.622; 0.621)	0.002	− 2.245 (− 9.955; 1.961)	0.012

Coll2–1 and PIIANP increased overtime while CTX-II remained stable. As the ratio Coll2–1/PIIANP and CTX-II/PIIANP decreased overtime, these biomarkers variations can be interpreted as a positive effect of the HYMOVIS® on type II collagen turnover. However, in the absence of a control group, we can not exclude that these changes are simply the consequence of changes in the cartilage matrix turnover linked to natural disease evolution. The observation that Coll2–1 increased while CTX-II remained stable is surprising. This can be explained in two ways. First, CTX-II and Coll2–1 epitopes are generated by different mechanisms. CTX-II is a neoepitope located in the C-terminal telopeptide and generated enzymatically whereas Coll2–1 is a peptide located in triple helix and released after denaturation of the collagen [45]. Secondly, there is evidence that urinary level of CTX-II is influenced by bone remodeling which is not demonstrated for Coll2–1 [46, 47]. Therefore, we can hypothesize that metabolic change induced by viscosupplementation in a single joint is not sufficient to influence the urinary CTX-II level resulting from bone and cartilage remodeling, while as demonstrated previously,

Coll2–1 is sensitive to changes occurring in one single joint [42, 48].

To demonstrate the structure-modifying effect of viscosupplementation, the EUROVISCO group recommends a combination of imaging and biological outcome measures [49]. A decrease of soluble biomarkers of cartilage degradation over time does not prove the chondroprotective effect of the treatment if this effect is not complemented by the imaging examinations. One strength of this study is that we have combined MRI and biological markers. Based on the literature review, a worsening of the MRI features was anticipated after 12 months follow-up [50]. In this study, T2 relaxation time analysis revealed an improvement of cartilage quality in total femur and in the lateral weight bearing femur as well as in the patella as soon as 6 months. WORMS effusion score, an indicator of synovitis, also revealed an improvement after 6 months. Finally, cartilage volume and thickness showed an increase in lateral weight bearing femur. The improvement in cartilage T2 values and volume in the lateral weight bearing femur can be interpreted as a beneficial effect of HYMOVIS®. These changes in

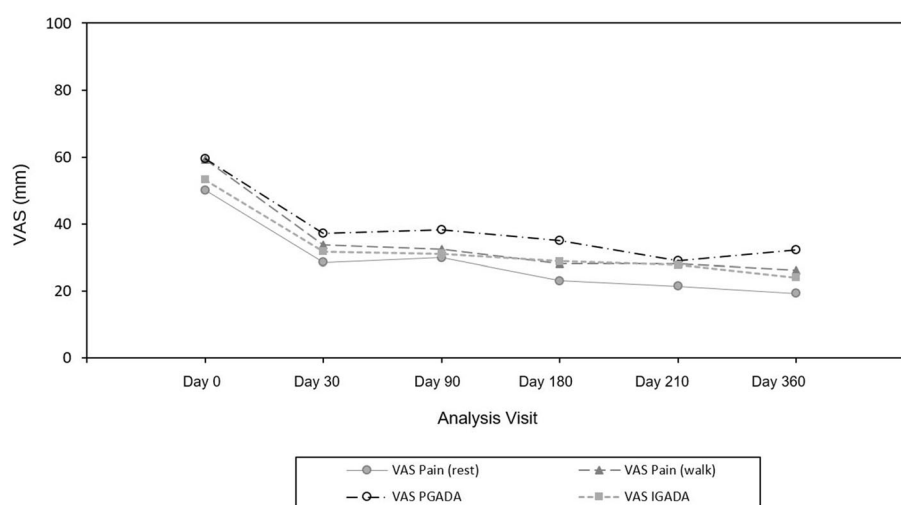
**Table 7** Mean T2 relaxation time and standard deviation of relaxation time for femur (median (IQR)) in the Full Analysis Set population

		Mean T2 relaxation time (ms)		SD of relaxation time (ms)	
		Median (IQR) <i>n</i> = 38	<i>p</i> -value	Median (IQR) <i>n</i> = 38	<i>p</i> -value
Whole Femur- <i>whole layer</i>	<i>D180</i> vs <i>D0</i>	−0.537 (−10.658; 2.368)	0.050	−1.315 (−4.361; 2.227)	0.160
	<i>D360</i> vs <i>D0</i>	−1.595 (−9.338; 1.532)	0.014	−0.588 (−4.128; 1.634)	0.279
Whole Femur- <i>top layer</i>	<i>D180</i> vs <i>D0</i>	−1.589 (−6.682; 2.216)	0.080	−0.697 (−7.715; 5.048)	0.574
	<i>D360</i> vs <i>D0</i>	−1.678 (−6.797; 3.763)	0.131	1.159 (−5.690; 6.124)	0.545
Whole Femur- <i>middle layer</i>	<i>D180</i> vs <i>D0</i>	−0.596 (10.378; 1.938)	0.030	−1.524 (−5.920; 1.637)	0.86
	<i>D360</i> vs <i>D0</i>	−1.249 (−10.187; 0.967)	0.008	−0.305 (−5.309; 1.955)	0.174
Whole Femur- <i>bottom layer</i>	<i>D180</i> vs <i>D0</i>	−0.259 (−10.454; 3.226)	0.094	−0.944 (−8.002; 1.725)	0.027
	<i>D360</i> vs <i>D0</i>	−2.051 (−10.495; 1.684)	0.009	−0.402 (−8.032; 1.057)	0.055
Medial weight bearing femur- <i>whole layer</i>	<i>D180</i> vs <i>D0</i>	0.318 (−14.381; 3.036)	0.198	−1.320 (−6.054; 2.027)	0.059
	<i>D360</i> vs <i>D0</i>	−1.680 (−13.240; 2.583)	0.147	−0.585 (−5.550; 1.085)	0.124
Medial weight bearing femur- <i>top layer</i>	<i>D180</i> vs <i>D0</i>	−1.119 (−8.54; 3.575)	0.219	0.493 (−4.423; 3.233)	0.782
	<i>D360</i> vs <i>D0</i>	−1.923 (−7.076; 4.147)	0.480	−1.741 (−3.532; 10.667)	0.717
Medial weight bearing femur- <i>middle layer</i>	<i>D180</i> vs <i>D0</i>	0.115 (−12.221; 2.005)	0.086	−1.335 (−8.136; 1.699)	0.037
	<i>D360</i> vs <i>D0</i>	−1.725 (−8.003; 0.934)	0.057	−1.718 (−6.450; 0.685)	0.050
Medial weight bearing femur- <i>bottom layer</i>	<i>D180</i> vs <i>D0</i>	−0.261 (−13.971; 5.085)	0.254	−1.506 (−11.723; 1.387)	0.010
	<i>D360</i> vs <i>D0</i>	−2.727 (−10.766; 3.163)	0.053	−2.298 (−8.391; 0.853)	0.012
Lateral weight bearing femur- <i>whole layer</i>	<i>D180</i> vs <i>D0</i>	−3.129 (−8.423; 1.453)	0.011	0.179 (−3.713; 2.078)	0.613
	<i>D360</i> vs <i>D0</i>	−1.748 (−8.187; 2.160)	0.032	−0.123 (−3.475; 2.057)	0.613
Lateral weight bearing femur- <i>top layer</i>	<i>D180</i> vs <i>D0</i>	0.580 (−7.286; 4.058)	0.664	−0.375 (−11.374; 5.426)	0.412
	<i>D360</i> vs <i>D0</i>	−0.628 (−6.597; 3.953)	0.364	0.897 (−5.299; 6.409)	0.792
Lateral weight bearing femur- <i>middle layer</i>	<i>D180</i> vs <i>D0</i>	−1.075 (−7.777; 1.477)	0.005	0.183 (−3.536; 1.522)	0.395
	<i>D360</i> vs <i>D0</i>	−1.264 (−9.870; 1.896)	0.045	−0.589 (−3.179; 1.625)	0.349
Lateral weight bearing femur- <i>bottom layer</i>	<i>D180</i> vs <i>D0</i>	−1.599 (−12.514; 2.41)	0.015	−1.053 (−5.959; 0.667)	0.005
	<i>D360</i> vs <i>D0</i>	−2.164 (−10.944; 3.286)	0.100	−0.699 (−6.740; 1.931)	0.110

cartilage structure and volume could be the result of type II collagen turnover increase as suggested by collagen-derived biomarkers. However, in this study, no significant correlation has been found between soluble biomarkers levels at baseline and the severity or

changes of MRI features. Therefore, this explanation remains hypothetical.

A significant decrease of knee pain and an improvement of joint function was observed. Similarly, a significant improvement of patient and investigator

**Fig. 3** Time evolution of pain at rest, at walking, and patients and investigators global assessment. VAS = Visual Analog scale; PGADA = Patient Global Assessment of Disease Activity; IGADA = Investigator Global Assessment of Disease Activity

**Table 8** VAS changes from baseline (mean (SD)) in the Full Analysis Set Population

VAS (mm)	D30 vs D0	D90 vs D0	D180 vs D0	D210 vs D0	D360 vsD0
Knee pain at rest	<i>n</i> = 46 −21.7 (23.7)	<i>n</i> = 43 −20.1 (24.5)	<i>n</i> = 43 −26.9 (25.1)	<i>n</i> = 39 −29.9 (24.4)	<i>n</i> = 41 −29.8 (23.8)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Knee pain while walking	<i>n</i> = 45 −25.5 (24.0)	<i>n</i> = 42 −26.9 (24.7)	<i>n</i> = 42 −30.2 (29.4)	<i>n</i> = 39 −31.3 (31.1)	<i>n</i> = 40 −31.5 (31.5)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Patient's global assessment of disease activity	<i>n</i> = 45 −22.7 (27.8)	<i>n</i> = 42 −21.0 (26.4)	<i>n</i> = 42 −23.9 (28.5)	<i>n</i> = 39 −30.5 (30.6)	<i>n</i> = 40 −25.3 (31.5)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Investigator global assessment of disease activity	<i>n</i> = 42 −21.0 (22.3)	<i>n</i> = 40 −21.4 (25.0)	<i>n</i> = 41 −23.0 (24.9)	<i>n</i> = 37 −24.4 (23.9)	<i>n</i> = 37 −25.8 (25.5)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

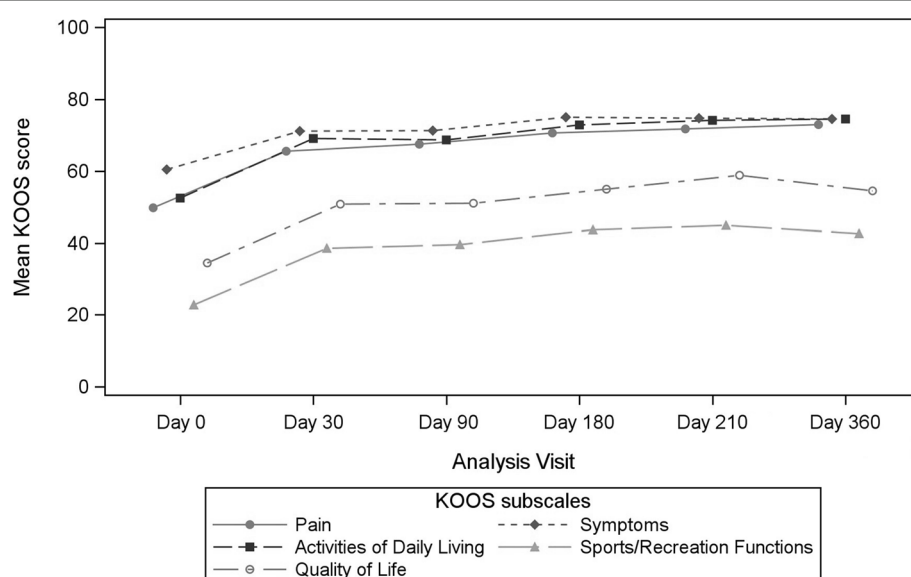
global assessment was observed. These improvements are confirmed by the number of responders to treatment which increases over time. However, in the absence of control group, the interpretation of these improvements may be less credible since placebo is known to be effective at relieving pain and at improving function and stiffness [39, 51]. None of the biomarkers were able to discriminate between OARSI-OMERACT non-responders and responders to treatments. This is probably due to the small sample size.

HYMOVIS® shows a good safety profile with no SAE related to the product. Remarkably, less than 15% of patients had definite product related reaction the majority being pain and swelling at site injection

and only one led to patient withdrawal. We can conclude that the product is well tolerated.

### Conclusion

This prospective study indicates that HYMOVIS®, a well-tolerated intra-articular treatment, significantly enhanced type II collagen turnover as suggested by the increase in Coll2-1 and PIIANP levels and cartilage volume observed by MRI in lateral knee compartment only. Importantly, this study highlighted the potential symptomatic benefit of HYMOVIS® on pain and function. Altogether, these data suggest that HYMOVIS® could have a protective effect on cartilage and provides critical information for the design of a larger phase III clinical trial.

**Fig. 4** Time evolution of Knee Injury and Osteoarthritis Outcome (KOOS) global score and subscores



**Table 9** KOOS changes from baseline (mean (SD)) in the Full Analysis Set Population

KOOS scores	D30 vs D0	D90 vs D0	D180 vs D0	D210 vs D0	D360 vs D0
Pains	<i>n</i> = 45 16.173 (18.13)	<i>n</i> = 42 16.968 (18.36)	<i>n</i> = 42 20.106 (21.62)	<i>n</i> = 39 20.664 (20.22)	<i>n</i> = 41 21.341 (18.68)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Symptoms	<i>n</i> = 45 11.164 (16.04)	<i>n</i> = 42 10.431 (17.05)	<i>n</i> = 42 13.747 (14.59)	<i>n</i> = 39 12.790 (16.27)	<i>n</i> = 41 14.184 (17.27)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Activity of daily living	<i>n</i> = 45 16.97 (16.08)	<i>n</i> = 42 15.384 (18.49)	<i>n</i> = 42 19.60 (20.80)	<i>n</i> = 39 19.92 (17.72)	<i>n</i> = 41 20.51 (16.82)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Sports/ Recreation function	<i>n</i> = 45 15.963 (19.21)	<i>n</i> = 41 17.31 (27.40)	<i>n</i> = 40 18.65 (28.17)	<i>n</i> = 38 21.930 (26.79)	<i>n</i> = 40 17.563 (24.17)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Quality of life	<i>n</i> = 45 16.898 (26.82)	<i>n</i> = 42 15.774 (25.43)	<i>n</i> = 42 19.643 (26.54)	<i>n</i> = 39 23.077 (26.45)	<i>n</i> = 41 18.902 (29.23)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
KOOS total	<i>n</i> = 45 77.171 (76.99)	<i>n</i> = 42 73.368 (85.49)	<i>n</i> = 42 90.854 (88.37)	<i>n</i> = 39 97.820 (85.82)	<i>n</i> = 41 91.95 (83.47)
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

**Acknowledgments** HY is the coordinator of the WP1 of the D-BOARD Consortium funded by European Commission Framework 7 program (EU FP7; HEALT H.2012.2.4.5–2, project number 305815, Novel Diagnostics and Biomarkers for Early Identification of Chronic Inflammatory Joint Diseases) and principal investigator of the Excellence of Science project. Joint-T-against Osteoarthritis 30,480,119 funded by the elgian FWO/FNRS.

#### Abbreviations

ADR: Adverse Device Reaction; AE: Adverse Effect; AGG: Aggrecan; BMI: Body Mass Index; COMP: Cartilage Oligomeric Matrix Protein; DO: Drop-Off; FAS: Full Analysis Set; GCLP: Good Clinical Laboratory Practice; GCP: Good Clinical Practice; HA: Hyaluronic Acid; IA: Intra-Articular; IL: InterLeukin; KL: Kelgren and Lawrence; KOOS: Knee Injury and Osteoarthritis Outcome Score; MMP: Matrix Metalloprotease; MOKHA: Multicenter Osteoarthritis Knee Hyaluronic Acid; MPO: MyeloPerOxidase; MRI: Magnetic Resonance Imaging; MW: Molecular Weight; NSAIDS: Non-Steroidal Anti-Inflammatory Drugs; PP: Per-Protocol; PTH: ParaTHormone; RR: Responder rate; SAS: Safety Analysis Set; SD: Standard Deviation; SERM: Selective Estrogen-Receptor Modulator; SYSADOA: Symptomatic Slow Acting Drugs in OA; TKR: Total Knee Replacement; VAS: Visual Analog Scale; WOMBS: Whole-Organ Magnetic Resonance Imaging Score

#### Authors' contributions

HY, MA, GN, HAC and PR conceived and designed the study; HY, BR, MM, EK, CC, BJ, UCD, CT, BJP, TP, HAC acquired the data, or analysed and interpreted the data; HY, BR, MA, NG, HAC and PR drafted and revised the manuscript; HY, MM, BR, MA, GN, PR provided final approval of the version to be published. All authors have read and approved the manuscript, and ensure that this is the case.

#### Funding

This study was funded by Fidia Farmaceutici, Italy. They fully financed the costs of the study and contributed to the design of the study, interpretation of data and in writing the manuscript.

#### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

In Belgium, the protocol has been submitted to the central Ethic Committee (EC) from the University Hospital of Liege and appropriate local ethical committee on September 9th 2014 and approved by November 12th 2014 (B707201422130–2014/247). In France, the protocol has been submitted to the Comité de Protection des Personnes Îles-de France IV and to the French competent authorities (ANSM) as Biomedical Research on February 25th 2015. Approval has been obtained on April 10th 2015 (2015/11) and May 6th 2015 (2015-A00370–49), respectively. The study was conducted in strict accordance with the declaration of Helsinki and GCP principles. Each patient received and signed an informed consent.

#### Consent for publication

Not applicable.

#### Competing interests

HY is the founder of Artialis SA a spin-off company of the University of Liège. He also received fees from Flexion, Ibsa, Expanscience, KiOmed Pharma SA (formerly Synolyne Pharma SA), Nestle, GSK, Tilman SA, Bepharbel, Labhra and Fidia Farmaceutici. BR received consulting fees from Fidia Farmaceutici. BJ received consulting fees from Kiomed Pharma. PR received fees from Artialis, IBSA, Bioiberica, Fidia, Expanscience, Genevri, Sanofi, Servier, Flexion, Pfizer, Rottapharm, Daichi-Sankyo. The authors have no conflict of interest. The other authors did not disclaim conflict of interest.

#### Author details

<sup>1</sup>Bone and Cartilage Research Unit, Arthropole Liège, Université de Liège, Institute of Pathology, CHU Sart-Tilman, 4000 Liège, Belgium. <sup>2</sup>Artialis SA, GIGA Tower, CHU Sart-Tilman, Liège, Belgium. <sup>3</sup>Center for Treatment Comparison and Integrative Analysis, Division of Rheumatology, Tufts Medical Center, Boston, MA, USA. <sup>4</sup>Service de Rhumatologie - CHU Sart-Tilman, Liège, Belgium. <sup>5</sup>Service de Rhumatologie - Centre Viggo Petersen, Hôpital Lariboisière, Paris, France. <sup>6</sup>Department of Rheumatology, Hospices Civils de Lyon, Lyon, France. <sup>7</sup>Service de Rhumatologie - CHU Brugmann, Bruxelles, Belgium. <sup>8</sup>Service de Médecine Physique - CHR Citadelle, Liège, Belgium. <sup>9</sup>Service de Rhumatologie - Hôpital Nord Franche-Comté, Trévenans, France. <sup>10</sup>Service de Rhumatologie - CHU UCL Namur - Site de Godinne, Yvoir, Belgium. <sup>11</sup>Service de Rhumatologie - CHR

Metz-Thionville - Hôpital Bel Air, Thionville, France. <sup>12</sup>Fidia Farmaceutici, S.p.A, Abano Terme, Italy.

Received: 5 May 2018 Accepted: 5 June 2019

Published online: 18 June 2019

## References

- Kraus VB, Blanco FJ, Englund M, Karsdal MA, Lohmander LS. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. *Osteoarthritis Cartil.* 2015;23(8):1233–41.
- Wallace IJ, Worthington S, Felson DT, Jurmain RD, Wren KT, Majanen H, et al. Knee osteoarthritis has doubled in prevalence since the mid-20th century. *Proc Natl Acad Sci U S A.* 2017;114:9332–6.
- Zhang W, Nuki G, Moskowitz RW, Abramson S, Altman RD, Arden NK, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P. OARS recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthritis Cartil.* 2010;18(4):476–99.
- Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, Gunther K, Hauselmann H, Herrero-Beaumont G, Kaklamani P, Lohmander S, Leeb B, Lequesne M, Mazieres B, Martin-Mola E, Pavelka K, Pendleton A, Punzi L, Serni U, Swoboda B, Verbruggen G, Zimmerman-Gorska I, Dougados M, Standing Committee for International Clinical Studies Including Therapeutic Trials ESCISIT. EULAR recommendations: an evidence-based approach to the management of knee osteoarthritis: report of a task force of the standing committee for international clinical studies including therapeutic trials (ESCISIT). *Ann Rheum Dis.* 2003;62:1145–55.
- Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P. OARS recommendations for the management of hip and knee osteoarthritis, part I: critical appraisal of existing treatment guidelines and systematic review of current research evidence. *Osteoarthritis Cartil.* 2007;15(9):981–1000.
- Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P. OARS recommendations for the management of hip and knee osteoarthritis, part II: OARS evidence-based, expert consensus guidelines. *Osteoarthritis Cartil.* 2008;16(2):137–62.
- Zhang W, Doherty M, Leeb BF, Alekseeva L, Arden NK, Bijlsma JW, Dincer F, Dziedzic K, Hauselmann HJ, Kaklamani P, Kloppenburg M, Lohmander LS, Maheu E, Martin-Mola E, Pavelka K. Punzi EULAR evidence-based recommendations for the diagnosis of hand osteoarthritis: report of a task force of ESCISIT. *Ann Rheum Dis.* 2009;68(1):8–17.
- McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, Hawker GA, Henrotin Y, Hunter DJ, Kawaguchi H, Kwoh K, Lohmander S, Rannou F, Roos EM, Underwood M. OARS guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartil.* 2014;22(3):363–88.
- Doherty M, Hawkey C, Goulder M, Gibb I, Hill N, Aspley S, Reader S. A randomised controlled trial of ibuprofen, paracetamol or a combination tablet of ibuprofen/paracetamol in community-derived people with knee pain. *Ann Rheum Dis.* 2011;70(9):1534–41.
- Machado GC, Maher CG, Ferreira PH, Pinheiro MB, Lin CW, Day RO, McLachlan AJ, Ferreira ML. Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomised placebo-controlled trials. *BMJ.* 2015;350:h1225. <https://doi.org/10.1136/bmj.h1225> Review.
- Schaffer D, Florin T, Eagle C, Marschner I, Singh G, Grobler M, Fenn C, Schou M, Curnow KM. Risk of serious NSAID-related gastrointestinal events during long-term exposure: a systematic review. *Med J Aust.* 2006;185(9):501–6.
- Ungprasert P, Cheungpasitporn W, Crowson CS, Matteson EL. Individual non-steroidal anti-inflammatory drugs and risk of acute kidney injury: a systematic review and meta-analysis of observational studies. *Eur J Intern Med.* 2015;26(4):285–91.
- Coxib and traditional NSAID Trialists' (CNT) Collaboration, Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, Bombardier C, Cannon C, Farkouh ME, Goss P, Halls H, Hawkey C, Hennekens C, Hochberg M, Holland LE, Kearney PM, Laine L, Lanus A, Lance P, Laupacis A, Oates J, Patrono C, Schnitzer TJ, Solomon S, Tugwell P, Wilson K, Wittes J, Baigent C. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet.* 2013;382(9894):769–79.
- Henrotin Y, Raman R, Richette P, Bard H, Jerosch J, Conrozier T, Chevalier X, Migliore A. Consensus statement on viscosupplementation with hyaluronic acid for the management of osteoarthritis. *Semin Arthritis Rheum.* 2016;45(2):140–9.
- Bruyère O, Cooper C, Pelletier JP, Branco J, Luisa Brandi M, Guillemin F, Reginster JY. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: a report from a task force of the European Society for Clinical and Economic Aspects of osteoporosis and osteoarthritis (ESCEO). *Semin Arthritis Rheum.* 2014;44(3):253–63.
- Rillo O, Riera H, Acosta C, Liendo V, Bolaños J, Monterola L, Nieto E, Arape R, Franco LM, Vera M, Papasidero S, Espinosa R, Esquivel JA, Souto R, Rossi C, Molina JF, Salas J, Ballesteros F, Radrigan F, Guibert M, Reyes G, Chico A, Camacho W, Urioste L, Garcia A, Iraheta I, Gutierrez CE, Aragón R, Duarte M, Gonzalez M, Castañeda O, Angulo J, Coimbra I, Munoz-Louis R, Saenz R, Vallejo C, Briceño J, Acuña RP, De León A, Reginato AM, Möller I, Caballero CV, Quintero M. PANLAR consensus recommendations for the management in osteoarthritis of hand, hip, and knee. *J Clin Rheumatol.* 2016;22:345–54.
- Trojan TH, Concoff AL, Joy SM, Hattenbuehler JR, Saulsberry WJ, Coleman CI. AMSSM scientific statement concerning viscosupplementation injections for knee osteoarthritis: importance for individual patient outcomes. *Br J Sports Med.* 2016;50(2):84–92.
- Abdulla A, Adams N, Bone M, Elliott AM, Gaffin J, Jones D, Knaggs R, Martin D, Sampson L, Schofield P, British Geriatric Society. Guidance on the management of pain in older people. *Age Ageing.* 2013;42(Suppl 1):51–7.
- Newberry SJ, Fitzgerald JD, Maglione MA, O'Hanlon CE, Booth M, Motala A, Timmer M, Shanman R, Shekelle PG. Systematic review for effectiveness of hyaluronic acid in the treatment of severe Degenerative Joint Disease (DJD) of the knee. Rockville: Agency for Healthcare Research and Quality (US); 2015.
- Strand V, McIntyre LF, Beach WR, Miller LE, Block JE. Safety and efficacy of US-approved viscosupplements for knee osteoarthritis: a systematic review and meta-analysis of randomized, saline-controlled trials. *J Pain Res.* 2015;8:217–28.
- Bannuru RR, Schmid CH, Kent DM, Vaysbrot EE, Wong JB, McAlindon TE. Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis. *Ann Intern Med.* 2015;162(1):46–54.
- Xing D, Wang B, Liu Q, Ke Y, Xu Y, Li Z, Lin J. Intra-articular hyaluronic acid in treating knee osteoarthritis: a PRISMA-compliant systematic review of overlapping meta-analysis. *Sci Rep.* 2016;6:32790.
- Campbell KA, Erickson BJ, Saltzman BM, Mascarenhas R, Bach BR Jr, Cole BJ, Verma NN. Is local viscosupplementation injection clinically superior to other therapies in the treatment of osteoarthritis of the knee: a systematic review of overlapping meta-analyses. *Arthroscopy.* 2015;31(10):2036–45.
- Bhandari M, Bannuru RR, Babins EM, Martel-Pelletier J, Khan M, Raynauld JP, Frankovich R, McLeod D, Devji T, Phillips M, Schemitsch EH, Pelletier JP. Intra-articular hyaluronic acid in the treatment of knee osteoarthritis: a Canadian evidence-based perspective. *Ther Adv Musculoskelet Dis.* 2017;9(9):231–46.
- Richette P, Chevalier X, Ea HK, Eymard F, Henrotin Y, Ornetti P, Sellam J, Cucherat M, Marty M. Hyaluronan for knee osteoarthritis: an updated meta-analysis of trials with low risk of bias. *RMD Open.* 2015;1(1):e000071.
- Altman R, Lim S, Steen RG, Dasa V. Hyaluronic acid injections are associated with delay of total knee replacement surgery in patients with knee osteoarthritis: evidence from a large U.S. Health Claims Database. *PLoS One.* 2015;10(12):e0145776.
- Altman R, Fredericson M, Bhattacharyya SK, Bisson B, Abbott T, Yadalam S, Kim M. Association between hyaluronic acid injections and time-to-total knee replacement surgery. *J Knee Surg.* 2016;29(7):564–70.
- Eymard F, Charles-Nelson A, Katsahian S, Chevalier X, Bercovy M. "Forgotten knee" after total knee replacement: a pragmatic study from a single-Centre cohort. *Joint Bone Spine.* 2015;82(3):177–81.
- Finelli I, Chiessi E, Galesso D, Renier D, Paradossi G. A new viscosupplement based on partially hydrophobic hyaluronic acid: a comparative study. *Biorheology.* 2011;48(5):263–75.
- Smith MM, Russell AK, Schiavinato A, Little CB. A hexadecylamide derivative of hyaluronan (HYMOVIS®) has superior beneficial effects on human osteoarthritic chondrocytes and synoviocytes than unmodified hyaluronan. *J Inflamm (Lond).* 2013;10:26.

31. Priano F. Early efficacy of intra-articular HYADD® 4 (Hymovis®) injections for symptomatic knee osteoarthritis. *Joints*. 2017;5(2):79–84.
32. Benazzo F, Perticarini L, Padolino A, Castelli A, Gifuni P, Lovato M, Manzini C, Giordan N. A multi-Centre, open label, long-term follow-up study to evaluate the benefits of a new viscoelastic hydrogel (Hymovis®) in the treatment of knee osteoarthritis. *Eur Rev Med Pharmacol Sci*. 2016;20(5):959–68.
33. Bisicchia S, Bernardi G, Tudisco C. HYADD 4 versus methylprednisolone acetate in symptomatic knee osteoarthritis: a single-Centre single blind prospective randomised controlled clinical study with 1-year follow-up. *Clin Exp Rheumatol*. 2016;34(5):857–63.
34. Henrotin Y, Bannuru RR, Malaise M, Ea H-K, Confavreux CB, Bontin J, Urbini-Choffray D, Conrozier T, Brasseur J-P, Thomas P, Hick AC, Marinello A, Giordan N, Richette P. (AB0983). Hyaluronan derivative HYMOVIS® increases cartilage volume and type II collagen turnover in osteoarthritic knee: data from MOKHA study. *Ann Rheum Dis*. 2018;77:1614.
35. Peterfy CG, Guermazi A, Zaim S, Tirman PF, Miaux Y, White D, Kothari M, Lu Y, Fye K, Zhao S, Genant HK. Whole-Organ Magnetic Resonance Imaging Score (WORMS) of the knee in osteoarthritis. *Osteoarthr Cartil*. 2004;12(3):177–90.
36. ICH Topic E 9, statistical principal for clinical trials step 4, consensus guideline, 05Feb1998 note for guidance on statistical principles for clinical trial. Available via <http://www.ich.org>. Accessed 1998.
37. Sakpal TV. Sample size estimation in clinical trial. *Perspect Clin Res*. 2010;1(2):67–9.
38. Henrotin Y, Conrozier T, Deberg M, Walliser-Lohse A, Richette P, Mulleman D, Maillet B, Rannou F, Piroth P, Hilliquin, E. Vignon, Chevalier X. early decrease of serum biomarkers of type II collagen degradation (Coll2-1) and joint inflammation (Coll2-1NO2) by hyaluronic acid intra-articular injections in patients with knee osteoarthritis. *J Orthop Res*. 2013;31(6):901–7.
39. Zhang W, Robertson J, Jones AC, Dieppe PA, Doherty M. The placebo effect and its determinants in osteoarthritis: meta-analysis of randomised controlled trials. *Ann Rheum Dis*. 2008;67(12):1716–23.
40. Deberg M, Labasse A, Christgau S, Cloos P, Henriksen DB, Chapelle JB, Zegels B, Reginster JY, Henrotin Y. New serum biochemical markers (Coll2-1 and Coll2-1 NO2) for studying oxidative related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. *Osteoarthr Cartil*. 2005;13:258–65.
41. Deberg M, Labasse A, Christgau S, Henriksen DB, Seidel L, Reginster JY, Henrotin Y. One-year increase of Coll2-1 level in urine is predictive of OA progression. *Osteoarthr Cartil*. 2005;13:1059–65.
42. Deberg M, Dubuc JE, Labasse A, Sanchez C, Quettier E, Bosseloir A, Henrotin Y. One-year follow-up of Coll2-1, Coll 2-1NO2 and myeloperoxidase before and after knee and hip replacement. *Ann Rheum Dis*. 2008;67:168–74.
43. Charni-Ben Tabassi N, Desmarais S, Bay-Jensen AC, Delaissé JM, Percival MD, Garnerio P. The type II collagen fragments Helix-II and CTX-II reveal different enzymatic pathways of human cartilage collagen degradation. *Osteoarthr Cartil*. 2008;16(10):1183–91.
44. Kraus VB, Collins JE, Hargrove D, Losina E, Nevitt M, Katz JN, Wang SX, Sandell LJ, Hoffmann SC, Hunter DJ; OA biomarkers consortium. Predictive validity of biochemical markers in knee osteoarthritis: data from the FNIH OA biomarkers consortium. *Ann Rheum Dis*. 2017;76(1):186–95.
45. Henrotin Y, Addison S, Kraus V, Deberg M. Type II collagen markers in osteoarthritis: what do they indicate? *Curr Opin Rheumatol*. 2007;19(5):444–50.
46. de Klerk B, Lafteber FP, van Spil WE. Associations of CTX-II with biochemical markers of bone turnover raise questions about its tissue origin: new insights from CHECK. *Ann Rheum Dis*. 2014;73(7):e39. <https://doi.org/10.1136/annrheumdis-2014-205494> Epub 2014 Apr 2.
47. van Spil WE, Drossaers-Bakker KW, Lafteber FP. Associations of CTX-II with biochemical markers of bone turnover raise questions on its tissue origin: data from CHECK, a cohort study of early osteoarthritis. *Ann Rheum Dis*. 2013;72(1):29–36.
48. Henrotin Y, Berenbaum F, Chevalier X, Marty M, Richette P, Rannou F. Reduction of the serum levels of a specific biomarker of cartilage degradation (Coll2-1) by hyaluronic acid (KARTILAGE® CROSS) compared to placebo in painful knee osteoarthritis patients: the EPIKART study, a pilot prospective comparative randomized double-blind trial. *BMC Musculoskelet Disord*. 2017;18(1):222–8.
49. Henrotin Y, Chevalier X, Raman R, Richette P, Montfort J, Jerosch J, Baron D, Bard H, Carrillon Y, Migliore A, Conrozier T. EUROVISCO guidelines for the design and conduct of clinical trials assessing the disease-modifying effect of knee viscosupplementation. *Cartilage*. 2018. <https://doi.org/10.1177/1947603518783521>.
50. Baum T, Stehling C, Joseph GB, Carballido-Gamio J, Schwaiger BJ, Müller-Höcker C, Nevitt MC, Lynch J, McCulloch CE, Link TM. Changes in knee cartilage T2 values over 24 months in subjects with and without risk factors for knee osteoarthritis and their association with focal knee lesions at baseline - data from the osteoarthritis initiative. *Magn Reson Imaging*. 2012;35(2):370–8.
51. Bannuru RR, McAlindon TE, Sullivan MC, Wong JB, Kent DM, Schmid CH. Effectiveness and implications of alternative placebo treatments: a systematic review and network meta-analysis of osteoarthritis trials. *Ann Intern Med*. 2015;163(5):365–72.

# Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

