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Efficacy and safety of a novel hydrogel (HYADD4-G) in degenerative disc disease patients: a multicentric open label study

E. MAZZA[1], S. MARCIA[2], F. MONDAINI[3], E. PIRAS[2], N. GIORDAN[4], T. TORRI[5], G. BARBANTI-BRÒDANO[6], F. PARODI[7]

[1]Interventional Radiology, Villa Donatello, Florence, Italy
[2]Radiology Santissima Trinità Hospital, ASSL Cagliari, ATS Sardegna, Italy
[3]Department of Extravascular Interventional Radiology, AOU-Careggi, Florence, Italy
[4]Clinical Research Department, Fidia Farmaceutici S.p.A., Abano Terme, Padua, Italy
[5]Diagnostic and Interventional Radiology, Azienda Unità Sanitaria Locale 1 Massa-Carrara, Pisa, Italy
[6]Department of Oncological and Degenerative Spine Surgery, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[7]Terapia Invasiva del Dolore SSIPM, Poliambulatorio Specialistico Lugano, Switzerland

Abstract. – OBJECTIVE: In this premarket clinical study, we evaluated the efficacy and safety of a novel Hydrogel (HYADD4-G) for reducing low back pain (LBP) in patients with degenerative disc disease (DDD).

PATIENTS AND METHODS: Twenty-three patients with chronic LBP were enrolled. All patients presented with up to three lumbar black discs (Pfirrmann grade III or IV), LBP of at least 40 mm on the Visual Analogue Scale (VAS), and a Roland–Morris Disability Questionnaire (RMDQ) score of at least 9. Patients received a single 1.5 ml intradiscal injection of HYADD4-G (8 mg/ml), guided by X-ray. Our primary endpoint was the change in VAS score from baseline (day 0) to 4, 12, and 24 weeks. Our secondary endpoints were black disc hydration by Magnetic Resonance Imaging (MRI); the patient's therapeutic response according to the RMDQ; the quality of life, as determined by the EuroQol-5 Dimension (EQ-5D) Index; and a global assessment of patient health status, safety, and local tolerability.

RESULTS: Compared with baseline values, VAS score showed a significant reduction at each time point, and across the overall 24-week follow-up period ($p < 0.0001$). MRI scanning observed a significant reduction in Pfirrmann grade from baseline, by at least one grade, at both week 4 ($p = 0.0039$) and week 24 ($p = 0.0010$). Furthermore, compared with baseline values, there was a significant reduction in RMDQ score at each timepoint, and across the entire study period ($p < 0.0001$). The EQ-5D index increased significantly from baseline to week 24 ($p = 0.0001$). Finally, mean VAS scores for Patient Global Assessment (PTGA), and Clinical Observer Global Assessment (COGA), decreased significantly at each time point ($p < 0.0001$), except for week 4.

CONCLUSIONS: HYADD4-G proved to be an efficient reliever of low back pain due to DDD.

Key Words:
Degenerative disc disease, Hydrogel, Back pain, Efficacy, Safety.

Abbreviations

DBP: Diastolic blood pressure; DDD: Degenerative disc disease; LBP: Low back pain; VAS: Visual analogue scale; RMDQ: Roland-Morris Disability Questionnaire; PTGA: Patient Global Assessment; COGA: Clinical Observer Global Assessment; SAS: Safety Analysis Set; FAS: Full Analysis Set; AE: Adverse event; CI: confidence interval; SD: Standard deviation; HA: Hyaluronic acid; EQ-5D: EuroQol-5 Dimension; MRI: Magnetic Resonance Imaging; IVD: Intervertebral Disc; NP: Nucleus Pulposus; AF: Annulus Fibrosus; SBP: Systolic Blood Pressure; SOC: System Organ Class; PT: Preferred Term.

Introduction

Degenerative Disc Disease (DDD) is a complex pathological process that involves narrowing of the disc space due to a reduction in the amount of water contained within the intervertebral discs. Intervertebral Disc (IVD) degeneration is a commonly diagnosed disorder that can lead to nerve compression and chronic back pain and has a

Corresponding Author: Ernesto Mazza, MD; e-mail: ernestomazza@outlook.it

negative impact on the quality of life[1]. IVD is a complex process involving three features: (i) the nucleus pulposus (NP) centrally, (ii) the annulus fibrosus (AF) peripherally, and (iii) the cartilaginous endplates cranially and caudally at the junction to the vertebral bodies. Within the NP, an abundance of proteoglycans normally allows for the absorption of water. This property is essential as it allows the IVD to handle axial loads. In a healthy disc, type II collagen is the most common type of collagen within the NP. The AF surrounds the NP and consists primarily of type I collagen[2]. With increasing age, the water content of the IVD decreases, and fissures in the NP can occur and potentially extend into the AF. The initiation of this process, referred to as osteochondrosis intervertebralis, can mark the beginning of degenerative destruction for the IVD, the endplates, and the vertebral bodies[3].

One recognized treatment option for DDD is spinal fusion surgery; however, the efficacy and success of this form of surgery remain controversial. Spinal fusion surgery can be achieved by a variety of approaches and techniques, including posterolateral fusion, anterior lumbar interbody fusion, and posterior lumbar interbody fusion[4]. Although fusion procedures provide us with a way of eliminating motion between spinal segments, and alleviating discogenic pain associated with degenerative changes, these techniques address only a symptom and not the cause of DDD[5-7]. Consequently, motion-preserving procedures have been introduced to assist in preventing changes in the adjacent segments. Disc arthroplasty has the potential advantage of removing the degenerated intervertebral disc and replacing it with a prosthesis that will allow motion between the segments. However, the purpoted advantages of preventing adjacent segment disease remain unclear and require additional long-term research[8]. Hence, a variety of invasive, surgical options have been developed for the treatment of lumbar DDD. Over recent years, emphasis has been directed towards the reversal of disc degeneration, or replacement of the affected disc. Furthermore, a variety of different therapies have been investigated for DDD, including biological growth factors, stem cells, and gene transplants. While these novel therapeutic modalities have shown some early promising results with regards to reversing the degenerative cascade, the clinical effects of such treatments, especially over the long-term, have not been elucidated[9]. The transduction of genes with the potential to interfere with disc degeneration, or to induce disc regeneration, is a concept that has been recently applied to DDD by researchers. This strategy requires the identification of relevant genes that play a key role in the disc degeneration cascade, as well as ways of delivering such therapeutic genes into disc cells. Delivery can be achieved by gene vector systems, which include a variety of viral and, more recently, non-viral vectors[10]. However, safety issues remain a challenge when using such vectors and the absence of adverse effects is imperative to any such vector system.

Tissue engineering approaches that combine growth factors with scaffolds are now leading the way for the treatment of DDD[11-14]. One of the substances under investigation is hyaluronic acid (HA), both as a gel that can be injected into the degenerating NP to improve hydration and consequently, its mechanical properties[15], as well as a form of scaffold for the implantation of combined cell-scaffold grafts[16]. Rheological HA properties vary significantly according to the degree of inter-molecular or intra-molecular cross-linking, and the presence of additional chemical groups on the polymer chain[17,18]. HYADD4-G is a new amidic derivative of hyaluronan. In nature, this biopolymer plays several functional roles, generally involving rheological and osmotic regulatory mechanisms in mammalian extracellular matrices and in synovial fluids[19]. The high viscosity and elasticity of HYADD4-G, together with its prolonged residence time, enables this compound to both relieve pain and to improve joint function. Recently, a controlled pilot study investigated the effects of Hymovis in 11 patients with lumbar black disc in comparison to ozone in the treatment of DDD[20]. As demonstrated by Boraso et al[20], HYADD4-G could provide similar results in the treatment of disc disease. Based on this information, the aim of this study was to investigate the efficacy of HYADD4-G to reduce low back pain arising from disc degeneration. Such pain is considered to represent an early sign of OA; the application of HYADD4-G takes advantage of the rheological properties of HA.

Patients and Methods

Study Design and Patient Recruitment

This was a multicenter, open-label pre-market clinical study with a follow-up period of 6 months. The study was conducted at four investigational sites in Italy; the enrollment period was between

June 2014 and July 2017. Twenty-three patients (14 males and 9 females) were enrolled in the study across the four investigational sites. Local Ethical Committees approved the study (approval number of coordinator EC: SPE14022 AOUC) and written informed consent was obtained from each patient prior to inclusion.

Inclusion Criteria

Our inclusion criteria were as follows: male or female patients aged >18 years; provision of written informed consent; willing and able to comply with the protocol for the duration of the study; chronic low back pain for at least 3 months from the screening; patients with one, two, or three lumbar (L1-S1) black discs (Pfirrmann grade III or IV) seen on MRI (Magnetic Resonance Imaging) examination (MRI performed within 3 months from baseline); low back pain of at least 40 mm on a 100 mm VAS (Visual Analogue Scale) at screening; or an RMDQ (Roland-Morris Disability Questionnaire) score of at least 9 on the 24-point questionnaire at screening.

Exclusion Criteria

Patients with any of the following criteria were excluded from the study: cauda equina syndrome; active malignancy or tumours as the source of symptoms; patients with more than 3 mm bulging discs seen on MRI examination; current infection or prior history of spinal infection (e.g., discitis, septic arthritis, epidural abscess) or an active systemic infection; previous lumbar spine surgery; spondylolisthesis (> grade 1) with or without spondylolysis at the symptomatic level(s); radiological sacroiliac joint/facet joint involvement; sacroiliac synchondrosis agenesis seen on MRI; or significant systemic disease, including unstable angina, autoimmune disease, rheumatoid arthritis, and muscular dystrophy.

Concomitant Therapy

During the study, patients were allowed to take drugs for diseases that were unrelated to DDD. In such cases, drug's name, indication, and date of administration had to be documented on the Case Report Form (CRF). Patients were also permitted to use non-steroidal paracetamol or anti-inflammatory drugs (NSAIDs) with a low half-life ($\leq$ 5 hours) for pain relief, according to the investigator's prescription. However, patients were not allowed to take any pain medication within the 24 hours prior to a clinical visit; otherwise the visit had to be postponed by one day.

Treatment

HYADD4-G was supplied at a concentration of 8 mg/ml in a prefilled syringe with a graduated label. In this study, the single intradiscal injection of HYADD4-G was performed in a single session under X-ray guidance using a 22-gauge needle. Other CE certificated and sterilized needles were accepted as long as they were designed for analgesic treatments or diagnostic use. The investigator used one syringe for each of the discs involved, up to a maximum of three discs. The maximum volume injected was 1.5 ml for each of the discs involved. The investigator recorded the injected volume in the accountability log. The volume (1.5 ml) and dose (8 mg/ml) of HYADD4-G were analogous to those used in a previous pilot study[19] that compared the effects of HYADD4-G and ozone in the treatment of DDD. The injection was administered at visit 1 (baseline, day 0). Each patient was then followed-up for a total of 24 weeks (6 months). During this period, each patient visited the investigation site five times: a screening visit (visit 0), a baseline/treatment visit (visit 1) that was scheduled within 7 days of the initial screening visit and during which HYADD4-G was administered; and then three follow-up visits that were performed after 4 weeks (visit 3), 12 weeks (visit 4), and 24 weeks (visit 5, final visit) of HYADD4-G treatment.

Outcome Assessment Methods

Primary efficacy Endpoint Assessment

Low back pain was measured using a VAS scale at baseline, and then, again at 4, 12, and 24 weeks. Patients were asked to report on VAS scale their pain intensity 24 hours prior to each visit. The VAS scale is a horizontal line that is 100 mm in length, anchored by word descriptors at each end. Patients marked a point on this line that they felt best represented their perception of their current state of pain. The VAS score was determined by measuring from the left-hand end of the line to the point marked by the patient (in millimetres)[21].

Secondary Efficacy Endpoint Assessment

Black disc hydration was defined as a reduction of at least one grade on the Pfirrman scale[22]. This was evaluated by MRI at baseline, at week 4, and at week 24 post-treatment. The Pfirrmann grading system is a non-invasive, simple, and convenient MRI imaging method that can provide a morphological and semi-quantitative evaluation

of intervertebral disc degeneration *in vivo* and provides a standardized and reliable assessment of MRI disc morphology for research and clinical purposes.

The RMDQ is a self-administered disability questionnaire consisting of 24 items that is administered to patients at screening, and at each subsequent visit, in order to evaluate clinical improvement. This study used a validated Italian version of the RMDG[23]. The 24 items on the RMDQ are specifically related to physical functions that are likely to be affected by low back pain. Patients completing the RMDQ were asked to place a check mark alongside a statement when it specifically applied to them. The RMDQ is scored by adding up the number of items checked by the patient and the final score was determined by simply counting the total number of scores; the final RMDQ score fell within a range of 0 and 24. Greater levels of disability are reflected by higher numbers[24].

Patient Global Assessment (PTGA) and Clinical Observer Global assessment (COGA) on how the DDD affected the patient's status were performed at the baseline visit and at visits 2, 3, and 4. Both patient and investigator made their global assessment on a 0-100 mm VAS where 0 corresponded to "not at all" and 100 to "extremely ill"[25].

The Italian version of the EQ-5D (Quality of Life Measure) questionnaire was administered at baseline and at visit 4[26]. The EQ-5D self-reporting questionnaire consists of two pages comprising the EQ-5D descriptive system and the EQ VAS. The patient was asked to indicate his/her state of health by ticking the box for the most appropriate statement in each of the 5 dimensions. This decision resulted in a one-digit number expressing the level selected for that dimension. The digits for five dimensions could then be combined in a five-digit number describing the respondent's overall state of health. With the EQ-5S, it should be noted that the numerals 1-3 have no arithmetic properties and are not to be used as a cardinal score[27].

The assessment of Safety Variables

The safety variables of the study included the occurrence of adverse events, and changes in vital signs (heart rate and blood pressure) when compared between baseline and week 24.

We recorded all adverse events (AEs) following injection, whether revealed by the subject, discovered by investigator questioning, detected through physical examination, or identified by other means. As far as possible, each adverse event was described by duration (start and end dates), intensity (mild, moderate, severe), causality (relationship) to the study product, actions taken, and outcome. The intensity of AEs was determined by the clinical investigator based on his/her direct observations, or by the patient's own reporting. Vital signs (heart rate, systolic/diastolic blood pressure) were measured at each visit. Any clinically significant change that was observed at the final evaluation, in comparison with baseline, was evaluated carefully and a relationship with a possible cause was assigned (test medication, other treatment received, or concomitant pathology).

Statistical Analysis

All data summaries and listings were generated using the Statistical Analysis Software System version 9.4 under Windows 10 PRO. Continuous variables were summarized by descriptive statistics (number of cases, mean, standard deviation [SD], median, minimum, maximum, first quartile [Q1], and third quartile [Q3]). Categorical variables were summarized using counts of patients and percentages. Dynamic changes in the VAS for low back pain, RMDQ score, PTGA VAS, and COGA VAS, were investigated by analysis of variance (ANOVA) for repeated measures. Data from the repeated ANOVA model were used to determine least square means (LSMs) with 95% confidence intervals (CIs) at each time-point, and overall. Adjusted mean differences (post-baseline visit *vs.* baseline) with 95% CIs were also determined. Changes from baseline to week 4 and week 24 on the Pfirrmann grade, and in the EQ-5D index, were analysed using the Wilcoxon Signed-Rank Test, using Bonferroni correction for the *p*-value. All statistical testing was conducted using a two-sided α of 0.05 and 95% CIs, unless otherwise specified. For all efficacy endpoints (primary and secondary), the Last Observation Carried Forward (LOCF) technique was used to correct missing data. The number of events and the number of patients with AEs, treatment-related serious adverse events, ADEs, and AEs that led to study discontinuation were presented. All AEs were first coded in accordance with the MedDRA thesaurus and the primary system organ class (SOC). Then, the preferred term (PT) was used to analyse the frequency distribution. The changes in vital signs (heart rate, systolic blood pressure, and diastolic blood pressure) from baseline to week 24 were analysed using descriptive statistics.

Determination of Sample Size

Statistical power calculations showed that the effect size on a single sample of 40 patients, with

Table I. Demographic characteristics and vital signs at screening SAS.

	(N=22)
Gender:	n=22
Males (N, %)	14 (63.6%)
Females (N, %)	8 (36.4%)
Age, years	n=22
Mean ± SD	42.86 ± 6.84
Median (range)	43.00 (33 to 58)
Ethnicity:	n=22
Caucasian (N, %)	21 (95.5%)
Hispanic (N, %)	1 (4.5%)
Weight, kg	n=21
Mean ± SD	70.76 ± 13.40
Median (range)	70.00 (52.0 to 98.0)
Height, cm	n=21
Mean ± SD	172.71 ± 7.01
Median (range)	174.00 (160 to 185)
BMI, kg/m^2	n=21
Mean ± SD	23.56 ± 3.44
Median (range)	23.10 (17.4 to 33.1)
Heart rate, bpm	n=21
Mean ± SD	74.95 ± 11.95
Median (range)	72.00 (56 to 102)
SBP, mmHg	n=21
Mean ± SD	125.33 ± 13.22
Median (range)	120.00 (107 to158)
DBP, mmHg	n=21
Mean ± SD	76.81 ± 5.89
Median (range)	78.00 (65to 87)

N = number of patients in the SAS; n = number of observations.

4 repeated measurements, an alpha of 0.05, and a potency of 80%, was 0.19. This was below the value of 0.25 that is conventionally considered to represent a small effect size[28]. When considering an effect size of 0.25, we determined that the sample size should be 24 patients. Therefore, we considered that a sample size of 40 patients was sufficient for this pilot study in order to assess small differences in the primary outcome parameter (pain VAS) over time.

Results

Due to difficulties during the recruitment phase, 23 patients were enrolled in the study. Of these, only 22 were treated with the product under investigation. Due to problems associated with needle management, one of the patients could not be treated. Figure 1 illustrates the patient enrolment and study set up.

Table I provides a summary of demographic characteristics and vital signs at screening (SAS). The SAS included a larger number of males (14 patients, 63.6%) than females (8 patients, 36.4%). All patients were Caucasians, except for one patient of Hispanic ethnicity. The mean age (± standard deviation, SD) was 42.86 ± 6.84 years (median: 43 years; range: 33-58 years).

Primary Efficacy Endpoint Results

Table II shows how the VAS for pain changed between baseline and weeks 4, 12, and 24 post-treatment in the full analysis set (FAS). There was a marked reduction in both mean and median VAS for pain when compared between baseline and

Table II. Results of change from baseline in pain measured by VAS (mm) at week 4, 12, and 24 (FAS).

	Value	Change from baseline
Baseline	n = 22	
Mean ± SD	67.1 ± 15.87	
Median (range)	68.5 (42 to 93)	
Week 4	n = 22	n = 22
Mean ± SD	33.5 ± 21.49	-33.5 ± 24.20
Median (range)	29.0 (1 to 72)	-31.0 (-92 to 5)
Week 12	n = 22	n = 22
Mean ± SD	33.8 ± 22.88	-33.3 ± 25.50
Median (range)	25.0 (2 to 72)	-31.5 (-91 to 19)
Week 24	n = 22	n = 22
Mean ± SD	29.1 ± 23.39	-38.0 ± 24.23
Median (range)	21.0 (0 to 79)	-32.0 (-93 to 3)

N = number of patients in the FAS; n = number of observations.

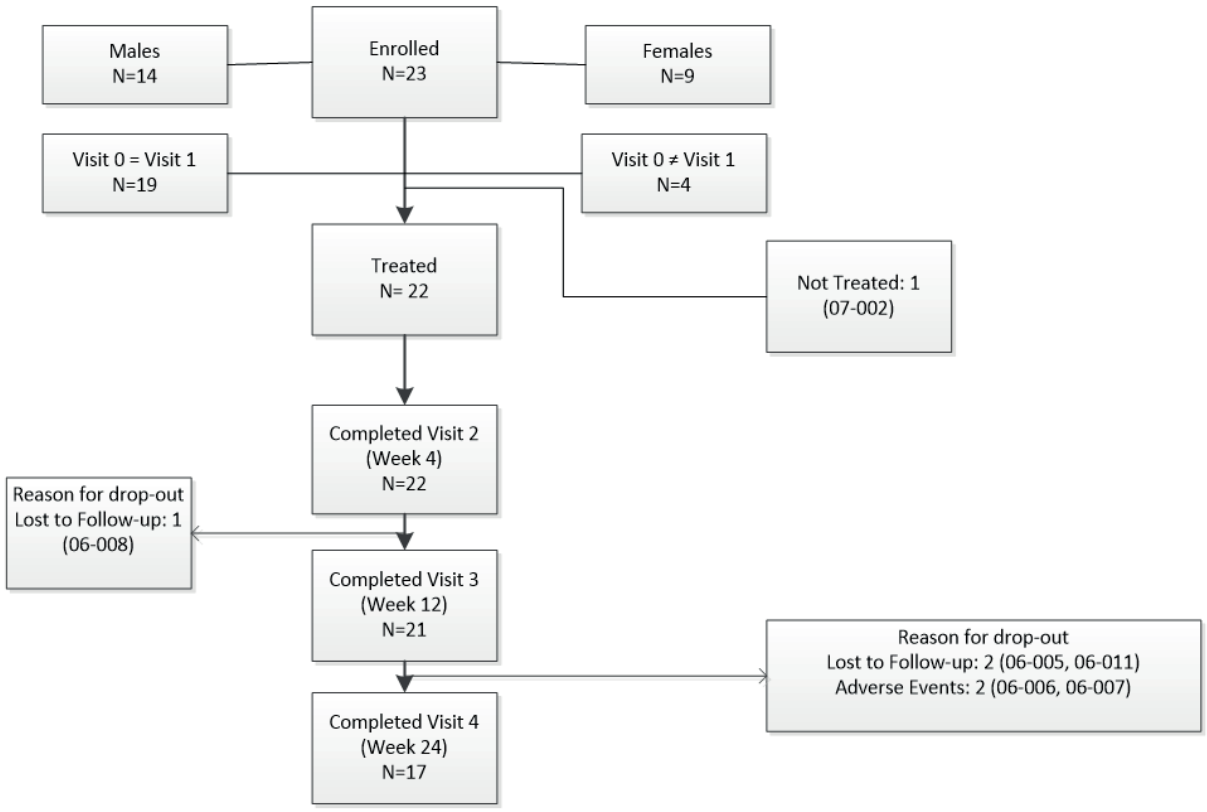

Figure 1. Study set-up.

any post-baseline time point. This reduction from baseline was statistically significant for any time point, and when considered across the entire study period ($p < 0.0001$ for each time point). Within the FAS, the mean and median VAS for pain decreased markedly from baseline to any of the post-baseline time points. These reductions from baseline were statistically significant for any time point, and when considered across the entire study period ($p < 0.0001$ for each time point).

Table III. Pfirrmann Grade during the study.

	(N = 22)
Baseline	
II	0 (0%)
III	17 (56.7%)
IV	13 (43.3%)
V	0 (0%)
Week 4	
II	3 (10%)
III	20 (66.7%)
IV	7 (23.3%)
V	0 (0%)
Week 24	
II	4 (13.3%)
III	20 (66.7%)
IV	6 (20%)
V	0 (0%)

N = number of patients in the FAS.

Secondary Efficacy Endpoint Results

Shifts in the Pfirrmann grade, as evidenced by MRI, are shown in Table III. Data showed clear improvements when tested in week 4 and 24 compared with baseline; post-treatment MRI showed clear augmentation in the hydration of the NP (Figure 2B, D) when compared with the pre-treatment condition (Figure 2 A, C) in two representative cases. Patients with Pfirrmann grade II were 10% and 13.3% at week 4 and 24 respectively. Compared with baseline (43%), there was a significant reduction in the proportion of patients diagnosed as Pfirrmann grade IV in weeks 4 (23.3%, $p = 0.0039$) and 24 (20.0%, $p = 0.0010$). None of our patients was diagnosed with Pfirrmann grade V at any time point. At the end of the study, there was no apparent change in the disc profile of patients; this was because their final disc bulge was equal to their baseline status. Table IV shows the dynamic

changes in RMDQ score from baseline at weeks 4, 12, and 24, in the FAS.

The mean and median RMDQ score decreased significantly from baseline to all of the post-baseline time points and when considered over the entire study period ($p < 0.0001$ for each time point).

Table V shows changes in the EQ-5D index between baseline and week 24 in the FAS. The mean and median EQ-5D index increased significantly from baseline to week 24 ($p = 0.0001$); the mean (± SD) change from baseline to week 24 was 0.2 ± 0.2.

Table VI shows changes in the PGTA VAS (mm) between baseline and weeks 4, 12, and 24, in the FAS. The mean and median VAS for PGTA decreased significantly from baseline to all of the post-baseline time points, and across the entire study period ($p < 0.0001$ for all time points, except for $p = 0.0017$ for week 4).

Table VII shows changes in the COGA VAS (mm) between baseline and weeks 4, 12, and 24, in the FAS. The mean and median VAS for COGA decreased significantly from baseline to all of the post-baseline time points, and across the entire study period (($p < 0.0001$ for all time points, except for $p = 0.0002$ at week 4).

Concomitant Therapy

In total, 11 patients (50.0%) used concomitant medications during the study. Of these, 5 patients (22.7%) used non-steroidal anti-inflammatory and anti-rheumatic products.

Safety Variables

A total of 14 AEs was reported in 9 patients (40.9%). Three of the AEs reported in 2 patients (9.1%) were considered to be related to the treat-

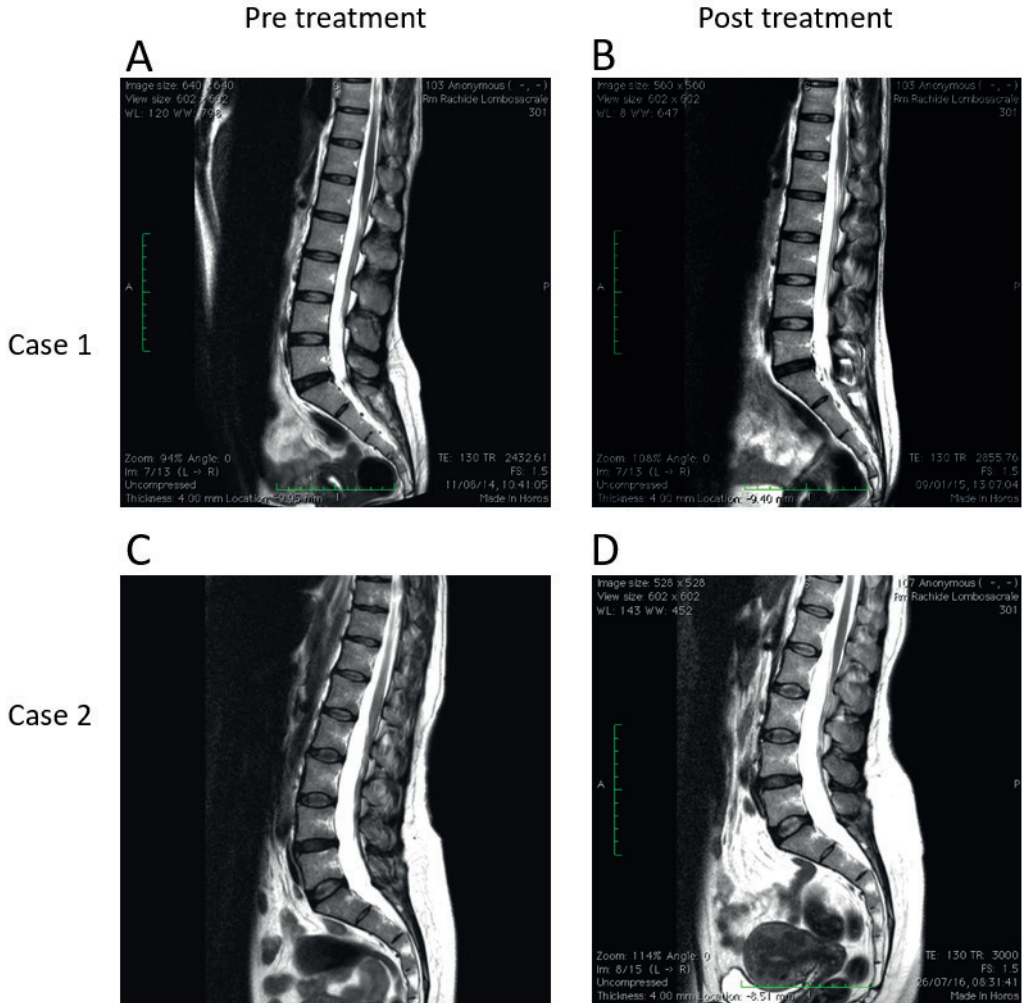

Figure 2. T2-weighted Sagittal MRI of the lumbar spine. **A-C,** Dehydration of L5-S1 discs, and L4-L5 discs, respectively. **B, D,** Augmented hydration of the nucleus pulposus with HYADD4-G.

Table IV. Results of change from baseline in RMDQ score at week 4, 12, and 24 (FAS).

	(N=22)	
	Value	**Change from baseline**
Baseline	n = 22	
Mean ± SD	12.1 ± 3.35	
Median (range)	10.5 (9 to 21)	
Week 4	n = 22	n = 22
Mean ± SD	7.6 ± 5.37	-4.5 ± 5.53
Median (range)	7.5 (0 to 18)	-4.0 (-15 to 7)
Week 12	n = 22	n = 22
Mean ± SD	5.7 ± 4.90	-6.4 ± 3.74
Median (range)	4.0 (0 to 17)	-7.0 (-14 to 0)
Week 24	n = 22	n = 22
Mean ± SD	5.0 ± 4.86	-7.1 ± 3.44
Median (range)	3.0 (0 to 15)	-7.0 (-14 to -1)

N = number of patients in the FAS; n = number of observations.

Table V. Results of change from baseline in the EQ-5D Index at week 24 (FAS).

	(N=22)	
	Value	**Change from baseline**
Baseline	n = 22	
Mean ± SD	0.329 ± 0.302	
Median (range)	0.172 (-0.016 to 0.760)	
Week 24	n = 22	n = 22
Mean ± SD	0.574 ± 0.339	0.245 ± 0.265
Median (range)	0.638 (-0.003 to 1.000)	0.157 (0.000 to 0.828)

N = number of patients in the FAS; n = number of observations.

Table VI. Results of change from baseline in PGTA VAS (mm) at week 4, 12 and 24 (FAS).

	(N=22)	
	Value	**Change from baseline**
Baseline	n = 22	
Mean ± SD	66.4 ± 18.23	
Median (range)	64.5 (28 to 94)	
Week 4	n = 22	n = 22
Mean ± SD	41.5 ± 26.75	-24.9 ± 27.16
Median (range)	40.0 (1 to 100)	-25.5 (-93 to 11)
Week 12	n = 22	n = 22
Mean ± SD	35.5 ± 28.14	-31.0 ± 26.15
Median (range)	24.5 (2 to 99)	-33.0 (-92 to 10)
Week 24	n = 22	n = 22
Mean ± SD	32.5 ± 29.13	-33.9 ± 27.75
Median (range)	18.0 (0 to 97)	-38.0 (-94 to 10)

N = number of patients in the FAS; n = number of observations.

Table VII. Results of change from baseline in COGA VAS (mm) at week 4, 12, and 24 (FAS).

	(N=22)	
	Value	**Change from baseline**
Baseline	n = 22	
Mean ± SD	51.0 ± 19.34	
Median (range)	56.5 (6 to 75)	
Week 4	n = 22	n = 22
Mean ± SD	27.4 ± 20.31	-23.7 ± 24.92
Median (range)	18.0 (0 to 73)	--23.0 (-75 to 25)
Week 12	n = 22	n = 22
Mean ± SD	23.2 ± 20.26	-27.8 ± 26.23
Median (range)	18.5 (0 to 74)	-33.0 (-75 to 23)
Week 24	n = 22	n = 22
Mean ± SD	18.0 ± 20.433	-33.0 ± 23.75
Median (range)	11.5 (0 to 78)	-39.5 (-75 to 16)

N = number of patients in the FAS; n = number of observations.

ment. Treatment-related AEs involved back pain of moderate intensity in one patient, and mild pain and moderate procedural pain in another patient. No serious adverse events, or adverse device effects, were reported in any patient. Two patients experienced to AEs that led to premature discontinuation of the study; these AEs involved intervertebral disc protrusion in one patient, and facet joint syndrome in another patient, both unrelated to treatment. The most commonly involved SOCs were musculoskeletal and connective tissue disorders (5 events in 5 patients, 22.7%), general disorders, and administration site conditions (5 events in 3 patients, 13.6%). The most commonly reported AEs were back pain (3 events in 3 patients, 13.6%), pain (4 events in 2 patients, 9.1%), and sciatica (2 events in 2 patients, 9.1%). None of the other AEs by PT was reported by more than one patient (4.5%). Treatment-related AEs included back pain of moderate intensity in one patient, and mild pain and moderate procedural pain in another patient. Three patients reported AEs that occurred in the first few minutes after injection (13.6%). In comparison with baseline values, there were no significant changes in heart rate, Systolic Blood Pressure (SBP), or Diastolic Blood Pressure (DBP), when analysed in week 24.

Discussion

There are several current surgical approaches for DDD, including discectomy and spinal fusion. However, while such methods can relieve pain, they also alter the biomechanics of the spine, eventually promoting further degeneration of the disc that was affected initially[29,30]. At present, the most widely studied materials for nucleus replacement are hydrogels; this is because hydrogels exhibit low levels of hydraulic permeability, thus allowing maintenance of hydrostatic pressure on the anulus under sustained loading. This has led to the consideration of a number of natural and synthetic chemically or physically crosslinked hydrogel-based materials[31,32]. However, synthetic polymer-based hydrogels exhibit low levels of bioactivity, thus leading to the focus shifting to natural polymer-based hydrogels. Of these natural polymers, HA has attracted considerable research attention; this is because HA has been identified as one of the glycosaminoglycans present in the NP[33]. By virtue of its physical, chemical, and biological properties, HA is already used in several biomedical applications, including ophthalmology, surgery, and orthopaedics. However, HA can degrade rapidly and therefore exhibits a short residence time. Furthermore, HA has poor mechanical characteristics, thus limiting our ability to broaden its range of biomedical applications, including NP substitution[34]. HYADD4-G is an innovative chemical derivate of HA and is associated with improved viscoelasticity and a higher residence time, keeping the safety of the natural polymer naturally present in the human body. Intradiscal injections of HA can reduce, or even help avoid, the potential complications associated with spinal surgery, including allergic reactions to the implant material; the bending, breakage, loosening, or moving of implants; the bending or

breakage of instruments; wounds; local, and/or systemic infections; nerve or spinal cord injury; loss of motion, or fusion, at the treated level; and the development or progression of disease at other disc levels. Thus, it is believed that intradiscal application of HA is advantageous, in terms of both safety and simplicity, as well as in terms of cost effectiveness in the clinical environment.

In this study, we investigated the efficacy and safety of an X-ray guided intradiscal injection of HYADD4-G for the treatment of low back pain in patients with degenerative disc disease. Data relating to our primary endpoint showed that HYADD4-G treatment was associated with a marked, and statistically significant, reduction of pain intensity when compared between the baseline status and any post-baseline time point (weeks 4, 12, or 24). This reduction in the intensity of pain from baseline was sustained and remained statistically significant up to 24 weeks after the injection. MRI analysis of black disc hydration revealed statistically significant improvements from baseline to both week 4 and week 24; this occurred because of a shift in Pfirrmann grade from grade III at baseline to grade II at the follow-up visits, and from grade IV at baseline to grade III at the follow-up visits in a significant proportion (approximately one third of the patient cohort). The patient's perception of physical function, as assessed by the RMDQ, improved significantly from baseline up to week 24. Quality of life, as measured by the EQ-5D index, also improved significantly from baseline to week 24. Consistent with the other efficacy endpoints, the VAS data for both the PTGA and COGA showed statistically significant reductions (i.e., improvements) from baseline up to week 24. The intradiscal injections of HYADD4-G were safe and well tolerated as no serious adverse events, or adverse device effects, were reported by any patient. Heart rate, SBP, and DBP, showed no significant changes from baseline to week 24 in any of the patients. With regards to concomitant therapy, our data showed that 17 patients did not use any form of anti-inflammatory or anti-rheumatic drug to relieve pain during the study. According to the protocol study design, the 5 patients requiring painkillers stopped taking their drugs at least 24 hours prior to the hospital visit. Therefore, considering that patients were not permitted to take any form of pain medication within the 24 hours prior to a clinical visit, we assumed that the results obtained during the hospital visits in weeks 4, 12, and 24, were related only to HYADD4-G treatment.

This study was limited by the lack of a control group. Prospective and randomized controlled trials are now required in a large cohort of patients as these will shed light on the long-term efficacy of HYADD4-G treatment for patients with degenerative disc disease.

Conclusions

The efficacy and safety results obtained in this study confirm the fact that intradiscal injections of HYADD4-G can be considered as a line of treatment for patients suffering from degenerative disc disease and alleviate low back pain.

Conflict of Interests

None of the authors had any conflicts of interest to declare. Nicola Giordan is an employee of Fidia Farmaceutici SpA. (Abano Terme, PD, Italy). However, Fidia Farmaceutici SpA did not participate in the decision to submit this manuscript for publication.

Ethics Approval and Consent to Participate

This study was approved by the Local Ethical Committee (approval number of coordinator EC: SPE-14022 AOUC) and written informed consent was obtained from each patient prior to inclusion.

Availability of Data and Material

All data generated or analysed during this study are included in this published article.

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Author Contributions

All authors contributed to the conception and design of the original study and approved the final submitted manuscript. EM, FM, EP, TT, and GBB were responsible for the acquisition of data. EM, FM, EP, TT, and GBB performed clinical evaluations, joint infiltrations, and clinical follow-up. The first draft of this manuscript was prepared by FP, and was critically reviewed by NG, EM, FM, EP, TT, and GBB. EM and FP take full responsibility for the integrity of the work.

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