Advancing Osteoarthritis Treatment: Translational Insights from HYADD®4-Based Combination Therapies in a Postmenopausal Rat Model

Anna Scanu[1], Roberto Luisetto[2], Mauro Pavan[3], Cristian Guarise[3], Chiara Giraudo[4], Francesca Galuppini[5], Vanni Lazzarin[5], Gianmaria Pennelli[5], Maddalena Zangari[1], Devis Galesso[3], Stefano Masiero[1]

[1]Physical Medicine and Rehabilitation Unit, Dept. of Neuroscience-DNS, University of Padova, Padova, Italy; [2]Dept. of Surgery, Oncology and Gastroenterology-DISCOG, University of Padova, Padova, Italy; [3]Fidia Farmaceutici S.p.A., Abano Terme, Italy; [4]Dept. of Cardiac, Thoracic, Vascular Sciences and Public Health-DSCTV, University of Padova, Padova, Italy; [5]Pathology Unit, Department of Medicine-DIMED, University of Padova, Padova, Italy.

Email of Presenting Author: mpavan@fidiapharma.it

Disclosures: Anna Scanu (N), Roberto Luisetto (N), Mauro Pavan (Fidia Farmaceutici), Cristian Guarise (Fidia Farmaceutici), Chiara Giraudo (N), Francesca Galuppini (N), Vanni Lazzarin (N), Gianmaria Pennelli (N), Maddalena Zangari (N), Devis Galesso (Fidia Farmaceutici), Stefano Masiero (N)

INTRODUCTION: HYADD®4 is a hexadecylamide-modified derivative of hyaluronic acid (HA) that forms hydrogels with superior lubricating and viscoelastic properties; HYADD®4 formulated in PBS at 0.8% is widely utilized as a viscosupplement in the clinical management of knee osteoarthritis (OA). In the present study the therapeutic effects of two novel intra-articular formulations were evaluated in an OA model induced by destabilization of the medial meniscus (DMM) in ovariectomized (OVX) female rats, representing the clinical population of post-menopausal OA patients. The formulations included FID-337, a ready-to-use combination of HYADD®4 and alendronate (ALN), and FID-338, a composite treatment comprising HYADD®4 and rapamycin (RAP).

METHODS: This study was performed in accordance with relevant guidelines and regulations and adhere to the ARRIVE guidelines; experimental procedures were approved by the Padova University Animal Ethic Committee and Italian Ministry of Health (Rome, Italy) registered under #189/2024-PR and #190/2024-PR. Eight week old female Sprague Dawley rats underwent bilateral ovariectomy, and after a four-week recovery period, destabilization of the medial meniscus (DMM) surgery was performed on the right knee. Four weeks post-DMM, animals were randomly allocated into four experimental groups (n = 9 per group) based on the intra-articular (i.a.) treatment administered once every two weeks, for a total of three injections: FID-337, FID-338, HYADD®4, or saline. A separate cohort of sham-operated animals (SHAM) served as controls. Mechanical allodynia was evaluated weekly using the Von Frey filament test. Four weeks following the final i.a. injection, animals were euthanized. Micro-computed tomography (micro-CT) was employed to quantify bone volume, trabecular thickness and separation, and bone mineral density. Histological assessment of cartilage integrity was conducted using hematoxylin–eosin and safranin O/fast green staining. Cartilage degradation was scored according to the Osteoarthritis Research Society International (OARSI) grading system, and proteoglycan content was also evaluated. Serum CTX-I, CTX-II and CRTAC-1 levels were evaluated longitudinally using dedicated ELISA kits, while immunohistochemical (IHC) evaluation of MMP-13, CASP-3, CRTAC-1, LC-3B, COLL10A1, TRPV1, TRPV4 was determined at sacrifice.
An a priori sample size calculation (one-way ANOVA: effect size = 0.6; $1-\beta = 0.80$; $\alpha = 0.05$) was performed in order to determine the number of animals needed for the study, and found that 9 rats per group was adequate. Data are expressed as the mean ± SD. Statistical differences between experimental groups were assessed by nonparametric one-way analysis of variance (ANOVA). Multiple comparisons were performed by Dunnett's test.

RESULTS SECTION: In the Von-Frey filament test the withdrawal thresholds remained consistently low throughout the study period in saline-treated animals, confirming the symptomatic onset of the disease. In contrast, all treatment groups exhibited an increase in withdrawal thresholds following the first intra-articular injection. Notably, thresholds in the FID-338 group reached levels comparable to the SHAM group by week 10, while in the FID-337 group, thresholds improved more gradually until the study endpoint. Structural alterations in subchondral bone architecture were evident in the saline group and were only partially ameliorated by treatment with FID-337, FID-338, or HYADD®4. Preservation of the synovial membrane was also improved in these treatment groups relative to saline controls. Histological evaluation revealed significantly lower OARSI scores and proteoglycan (PG) loss in HYADD®4-treated joints, with even greater reductions observed following treatment with FID-337 or FID-338 (Fig. 1). Serum CTX-I was significantly lower in FID-337, FID-338 and HYADD®4 groups at sacrifice, while no significant reduction of CRTAC-1 or CTX-II was observed. IHC revealed in the same groups a reduction in MMP-13, CASP-3, CRTAC-1, COLL10A1 and TRPV4 expression, and an increase in LC-3B compared to saline-treated rats.

DISCUSSION: Intra-articular administration of HYADD®4 alone was effective in attenuating OA progression by reducing nociceptive responses and preserving cartilage integrity in the OVX+DMM rat model, which recapitulates a specific OA endotype. Moreover, administration of HYADD®4 formulations with either ALN or RAP resulted in a significantly greater therapeutic effect compared to HYADD®4 treatment. Accordingly, synovial membrane was preserved and most of the cartilage and serum biomarkers were reverted towards the sham condition. This enhanced efficacy suggests a synergistic interaction between the HA-based viscosupplement and the repurposed pharmacological agents with hypothesized efficacy on OA. The findings underscore the potential of targeted, localized drug delivery to enhance efficacy while minimizing systemic side effects, paving the way for innovative, combination-based viscosupplement therapies in OA management.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a compelling advancement in the treatment of osteoarthritis (OA), particularly in post-menopausal populations, by evaluating two novel intra-articular formulations—FID-337 and FID-338—based on HYADD®4, a modified hyaluronic acid with enhanced viscoelastic and lubricating properties. Using a clinically relevant animal model that mimics OA progression in estrogen-deficient conditions, the research demonstrates that combining HYADD®4 with either alendronate or rapamycin significantly improves therapeutic outcomes compared to HYADD®4 alone.

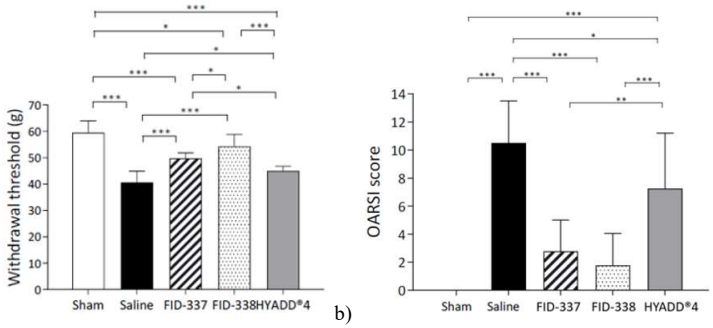

Figure 1. a) Withdrawal threshold and b) OARSI score at sacrifice. Results are presented as mean ± SD of 9 rats per group. *p < 0.05, **p < 0.005, ***p < 0.001.