Safety and Efficacy of Intra-Articular Injection of Umbilical Cord-Derived Extracellular Vesicles in Patients With Knee Osteoarthritis: A Retrospective Study

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abstract

Background: Extracellular vesicles derived from the umbilical cord (UC-EVs) have emerged as a potential biological therapy for knee osteoarthritis (KOA). However, their clinical efficacy remains unknown. The purpose of this study was to evaluate the safety and efficacy of intra-articular injections of umbilical cord stem cell-derived UC-EVs in patients with KOA. **Materials and Methods:** A total of 29 patients (33 knees) with KOA received 6 intra-articular injections of UC-EVs (1×10^{11} particles per dose) every 2 weeks. Clinical outcomes were assessed at 3, 6, and 12 months using the Knee Injury and Osteoarthritis Outcome Score (KOOS), visual analog scale (VAS), and Outcome Measures in Rheumatology and Osteoarthritis Research Society International (OMERACT-OARSI) responder criteria. **Results:** Significant improvements in all KOOS subscale scores and VAS for pain were observed at all follow-up time points compared with baseline ($P < .01$), with all scores exceeding predefined minimal clinically important difference thresholds. VAS for pain decreased by a mean of 38.8 ± 25.5 mm, and the greatest KOOS improvement was seen in quality of life (32.8 ± 21.9) and sport and recreation function (28.6 ± 25.2). The OMERACT-OARSI responder rate was 66.7% overall (22/33 knees), with rates of 83.3%, 66.7%, and 44.4% for Kellgren-Lawrence grades 2, 3, and 4, respectively. No serious adverse events were observed. **Conclusion:** Intra-articular injection of UC-EVs demonstrated meaningful clinical benefits in patients with KOA, including reductions in VAS pain scores and improvements across all KOOS domains. Patients with Kellgren-Lawrence grade 2 or 3 showed greater treatment responses compared with grade 4. These findings suggest that intra-articular UC-EVs therapy may represent a promising next-generation treatment option for KOA.

Knee osteoarthritis (KOA) is among the most prevalent joint disorders worldwide, affecting more than 500 million patients.[1] It causes chronic pain, stiffness, deformity, and functional limitation, resulting in significant restrictions in activities of daily living (ADL) and deterioration in quality of life (QOL).[2-5] Although surgical interventions, such as total knee arthroplasty, are effective for end-stage KOA,[6] they are invasive and often accompanied by complications and residual symptoms.[7,8] Therefore, conservative approaches, including physical therapy and intra-articular injections, are typically adopted as first-line

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Disclosure: The authors have disclosed no potential conflicts of interest, financial or otherwise.

Acknowledgment: The authors thank Youngji Kim, Momoko Abe, Takaaki Ishii (Frontier Co, Ltd), Tomoaki Inoue (Frontier Co, Ltd), Yousuke Sasaki (Satista Co, Ltd), Aoi Otsuka (x-ray), and Misaki Takehana for their invaluable support and contributions to this study.

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Submitted: March 15, 2026. Accepted: July 17, 2026. Published online: August 14, 2026.

doi: 10.3928/01477447-20260721-01

treatments. However, the clinical efficacy of corticosteroid and hyaluronic acid injections remains limited.[9]

Biological therapies have recently gained increasing attention.[10] Autologous approaches, such as platelet-rich plasma, adipose-derived stem cells (ASC), and autologous protein solution, have shown potential benefits in the management of KOA and other orthopedic disorders.[11-15] However, the therapeutic efficacy of ASC therapy may be influenced by the degree of joint degeneration and patient age, resulting in reduced effectiveness among older individuals and those with advanced KOA.[12-16]

By contrast, extracellular vesicles (EVs) enriched in the conditioned medium of young donor cells, including umbilical cord-derived mesenchymal stromal cells (MSCs), are nanosized particles containing bioactive molecules, such as proteins, lipids, and nucleic acids, that participate in intercellular communication.[17] These UC-EVs exhibit anti-inflammatory properties and promote tissue regeneration.[18-25]

EVs share key biological functions with MSCs, although they remain more stable under various pathophysiological conditions and present a lower risk of immune rejection.[26] EVs-based therapy will address several limitations of autologous ASC therapy and is viewed as a potential next-generation option; however, clinical evidence supporting its efficacy remains limited.[26-28]

This pilot study retrospectively evaluated the safety and efficacy of intra-articular injections of umbilical cord-derived (UC) EVs administered to patients with KOA at our institution. We hypothesized that intra-articular injection of allogeneic cell-free EVs would be effective, and that any adverse events would be mild and transient in the short term.

Materials and Methods

Ethical Approval

This pilot study was conducted as a single-center retrospective cohort study. Because the intervention was experimental and not covered by national health insurance, patients received written information explaining that treatment would be provided on a self-funded basis and that participation would constitute involvement in a clinical research protocol. Patients who had sufficient time to review the documents and provided written informed consent were enrolled in the study. The study protocol was approved by the institutional review board (IRB) of our hospital (IRB number 2500143; approval number TKOC-EV-2025-01) and was performed in accordance with the Declaration of Helsinki. The cost of UC-EVs products and treatment was borne entirely by the patients themselves; no financial support was provided by the institution or the manufacturer (Frontier Co, Ltd).

Patient Selection

Patients who received intra-articular injections of UC-EVs between May 2024 and December 2024 were included. The inclusion criteria were as follows: Patients who (1) had KOA of Kellgren-Lawrence (K-L) grade 2, 3, or 4[29] and (2) experienced medial or lateral knee pain. The exclusion criteria were as follows: Patients who (1) had a history of malignant tumor treatment, active infectious diseases (eg, human immunodeficiency virus), pregnancy, drug dependence, or ongoing dialysis, or were unable to provide written informed consent or communicate adequately; (2) had received intra-articular injections or undergone surgical procedures within the preceding 3 months; or (3) had severe systemic comorbidities.

Study Design

Patients received a total of 6 intra-articular injections of UC-EVs, administered at approximately 2-week intervals over a 3-month period (weeks 0, 2, 4, 6, 8, and 10). The treatment protocol consisted of intra-articular injections of UC-EVs (2 mL) mixed with 3 mL of normal saline, yielding a total volume of 5 mL. The UC-EVs used in this study were stem cell secretome victory premium (Frontier Co, Ltd),[29] manufactured in accordance with the minimal information for studies of extracellular vesicles (MISEV) 2018 and MISEV2023 guidelines recommended by the International Society for Extracellular Vesicles, and guided in clinical application of EVs by The Japan Society for Regenerative Medicine (version 1).[21,22,25] The donor was a Japanese woman, and the product was cryopreserved after passing 10 safety tests in accordance with Japanese guidelines, including assessments of appearance, endotoxin, mycoplasma negativity, sterility, and screening for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, human T-lymphotropic virus 1, cytomegalovirus, and parvovirus B19. Injections were administered approximately every 2 weeks, with an intended dose of 1×10^{11} UC-EVs particles per session. Patients received a total of 6 injections using a 23-gauge needle over a 3-month period, corresponding to an approximate cumulative dose of 6×10^{11} particles.

UC-EVs preparations were stored in a frozen state and were thawed for approximately 15 minutes before administration. After thawing, 3 mL of normal saline was added and mixed with the UC-EVs solution. The prepared mixture was then injected into the medial or lateral compartment of the knee joint, with the target compartment selected based on the site of greatest joint-space narrowing on standing plain radiographs. Although UC-EVs are expected to diffuse throughout the joint cavity, injections were directed toward the compartment with the greatest radiographic narrowing to maximize local particle concentration at the site of greatest cartilage degeneration and symptomatic burden.

Clinical Outcomes

The primary outcome was change in each Knee Injury and Osteoarthritis Outcome Score (KOOS) subscale (pain,

symptoms, ADL function, sport and recreation function, and QOL) and visual analog scale (VAS) for pain from baseline to 12 months. The minimal clinically important difference (MCID) thresholds were set at 10 points for KOOS and 14 mm for VAS.[30,31] Responder rates based on the Outcome Measures in Rheumatology and Osteoarthritis Research Society International (OMERACT-OARSI) criteria were also calculated.[32] Secondary outcomes included the acceptability and safety of the intervention. Adverse events and serious adverse events (SAEs), defined as death, hospitalization, disability, or malignancy, were continuously monitored following each injection and throughout the entire follow-up period. These assessments were conducted at baseline, at each injection visit, and at 1, 3, and 6 months after the completion of treatment, with a final follow-up at 12 months.

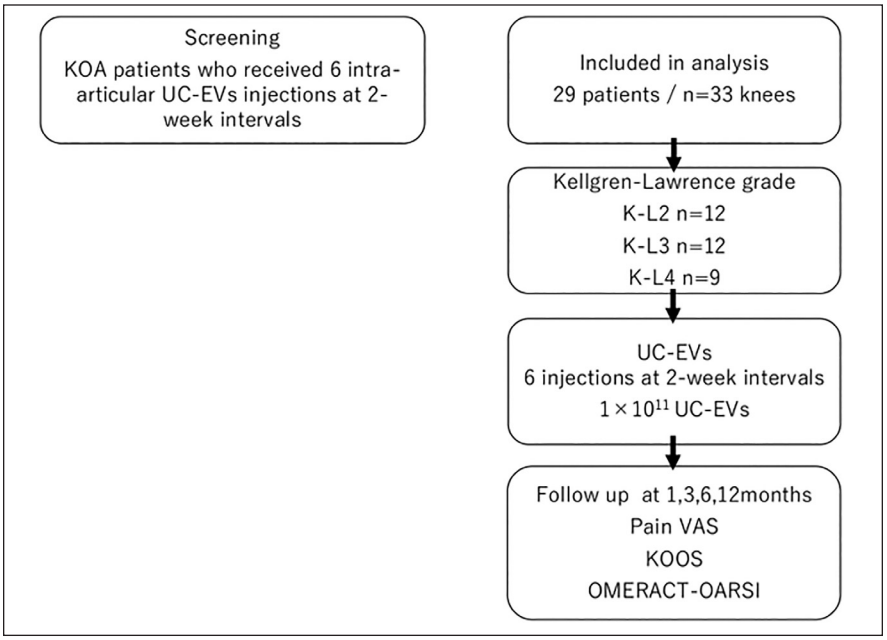

Figure 1. Flowchart of patient selection. KOA, knee osteoarthritis; K-L grade, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OMERACT-OARSI, Outcome Measures in Rheumatology and Osteoarthritis Research Society International; UC-EVs, umbilical cord-derived extracellular vesicles; VAS, visual analog scale.

Statistical Analysis

Data were analyzed using SPSS Statistics version 29.0.2.0 (IBM Japan, Ltd.) and GraphPad Prism (GraphPad Software, Inc.). Descriptive statistics are presented as mean ± standard deviation. Student's paired *t* test was used to compare clinical outcomes between baseline and final follow-up. One-way analysis of variance with Tukey's post hoc test was used to compare changes in clinical outcomes across K-L grades. Statistical significance was set at $P < .05$.

Results

Patient Characteristics

A total of 29 patients (33 knees) with K-L grades 2 to 4 KOA were included in the analysis, with a follow-up rate of 100% (**Figure 1**). The mean age was 62.3 ± 11.4 years, and 10 men and 19 women comprised the cohort. The mean body mass index was 23.3 ± 3.4 kg/m^2. The distribution of K-L grades was as follows: 12 knees with grade 2, 12 knees with grade 3, and 9 knees with grade 4. Three patients had a history of arthroscopic knee surgery.

Table 1

Patient Characteristics (N = 33 knees/29 patients)

Characteristic	Value
Age (years), mean ± SD	62.3 ± 11.4
Sex, n	
Male	10
Female	19
BMI (kg/m^2), mean ± SD	23.3 ± 3.4
K-L grade, n (%)	
2	12 (36.4)
3	12 (36.4)
4	9 (27.3)
Median duration since onset (years), median (IQR)	2.5 (1.4-4.3)
Previous knee arthroscopy, n	3

Abbreviations: BMI, body mass index; IQR, interquartile range; K-L, Kellgren-Lawrence.

Baseline clinical characteristics are summarized in **Table 1**.

Extraction and Identification of UC-EVs

Five manufacturing lots of the UC-EVs product were used in this study. Component analysis of the conditioned medium supernatant revealed vascular endothelial growth factor, epidermal growth factor, and hepatocyte growth factor concentrations of 13,230 ± 3,165 pg/mL, 4,968 ± 2,101 pg/mL, and 7,065 ± 1,630 pg/mL, respectively. The EVs particle count was (9.45 ± 0.6) × 10^{10} particles/mL, and the

Table 2

Cytokine Concentrations and Exosome Characteristics of the Conditioned Medium Supernatant

Variable	VEGF (pg/mL)	EGF (pg/mL)	HGF (pg/mL)	Exosome particle count (×10^{10} particles/mL)	Exosome protein concentration (pg/mL)
Supernatant, mean ± SD	13,230 ± 3,165	4,968 ± 2,100	7,065 ± 1,630	9.45 ± 0.6	110.0 ± 5.3

Abbreviations: EGF, epidermal growth factor; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor.

Table 3

Clinical Outcomes and MCID Achievement at Baseline and Final Follow-up After UC-EVs Injection

Variable	Baseline	Final follow-up	*P*	MCID achieved
VAS, mm	57.0 ± 23.4	18.2 ± 20.1	<.01*	81.8% (27/33)
KOOS				
Symptoms	69.7 ± 17.4	84.3 ± 15.9	<.01*	54.5% (18/33)
Pain	63.2 ± 15.0	84.1 ± 14.8	<.01*	66.7% (22/33)
ADL function	75.9 ± 16.3	91.0 ± 10.2	<.01*	69.7% (23/33)
Sport and recreation function	42.1 ± 26.6	70.8 ± 27.0	<.01*	75.8% (25/33)
QOL	33.2 ± 19.1	66.0 ± 21.7	<.01*	84.8% (28/33)

Abbreviations: ADL, activities of daily living; KOOS, Knee Injury and Osteoarthritis Outcome Score; MCID, minimal clinically important difference; QOL, quality of life; UC-EVs, umbilical cord-derived extracellular vesicles; VAS, visual analog scale.
** P < .05.*

EVs protein concentration was 110.0 ± 5.3 pg/mL. The intended dose was 1 × 10^{11} UC-EVs per injection, administered 6 times for a total planned dose of 6 × 10^{11} UC-EVs per knee. The actual measured total dose per knee was approximately 5.7 × 10^{11} particles. These data are summarized in **Table 2**.

Clinical Outcomes at Baseline and Final Follow-up

Changes in clinical outcomes between baseline and final follow-up demonstrated significant improvements in all KOOS subscales: pain increased from 63.2 ± 15.0 to 84.1 ± 14.8, symptoms from 69.7 ± 17.4 to 84.3 ± 15.9, ADL function from 75.9 ± 16.3 to 91.0 ± 10.2, sport and recreation function from 42.1 ± 26.6 to 70.8 ± 27.0, and QOL from 33.2 ± 19.1 to 66.0 ± 21.7 (all $P < .01$). VAS pain scores significantly decreased from 57.0 ± 23.4 to 18.2 ± 20.1 mm ($P < .01$). All outcome measures exceeded the predefined MCID thresholds (10 points for KOOS, 14 mm for VAS), with the highest achievement rates observed for the KOOS QOL subscale (84.8%) and VAS for pain (81.8%). These data are summarized in **Table 3**.

Changes in Clinical Outcomes According to K-L Grade

When stratified by K-L grade, grade 2 showed significant improvements in VAS and all KOOS subscales except for QOL at all follow-up time points (1, 3, 6, and 12 months; all $P < .01$). K-L grade 3 demonstrated significant improvements in VAS and all five KOOS subscales throughout the entire follow-up period (all $P < .05$). For K-L grade 4, significant improvements in VAS were observed at all time points (1 month: $P < .001$, 3 months: $P < 0.001$, 6 months: $P < .001$, 12 months: $P < .01$). Among KOOS subscales in K-L grade 4, significant improvements were noted in the symptoms subscale at 3 and 6 months ($P < .05$ and $P < .01$, respectively), in the pain subscale at 6 months ($P < .01$), in the sport and recreation function subscale at 6 months ($P < .05$), and in the QOL subscale at 3, 6, and 12 months ($P < .05$, $P < .01$, and $P < .05$, respectively). No significant improvements were observed in the ADL function subscale for K-L grade 4 at any time point. The OMERACT–OARSI responder rate was 66.7% overall (22/33 knees), with rates of 83.3% for K-L grade 2, 66.7% for K-L grade 3, and 44.4% for K-L grade 4. These data are summarized in **Table 4**, **Figure 2**, and **Table A**.

Safety

SAEs were not observed in any patient. Mild adverse events occurred in a total of 8 cases (8 knees) in the UC-EVs group, with an overall incidence of 3.3% per injection (8/240 injections). Following the first injection, no cases of infection, pain, or swelling were observed; minor adverse reactions included low-grade fever in 1 case, drowsiness in 1 case, and increased

Table 4

Changes in Clinical Outcomes According to K-L Grade Following UC-EVs Injection

Variable	All (N = 33)	K-L grade 2 (n = 12)	K-L grade 3 (n = 12)	K-L grade 4 (n = 9)	*P*
ΔVAS, mm	−38.8 ± 25.5	−54.2 ± 26.4	−33.3 ± 22.7	−25.6 ± 18.1	.02*
ΔKOOS					
Symptoms	14.6 ± 14.5	17.0 ± 14.6	12.8 ± 11.7	13.9 ± 18.7	.36
Pain	20.8 ± 18.3	28.7 ± 15.9	19.7 ± 15.8	11.9 ± 21.8	.11
ADL function	15.1 ± 13.1	19.7 ± 9.3	16.6 ± 12.2	7.1 ± 15.9	.08
Sport and recreation function	28.6 ± 25.2	42.5 ± 24.0	20.0 ± 16.8	21.7 ± 30.2	.06
QOL	32.8 ± 21.9	43.8 ± 18.4	26.6 ± 19.6	26.4 ± 24.8	.09
OMERACT-OARSI responder rate	66.7% (22/33)	77.8% (10/12)	66.7% (8/12)	44.4% (4/9)	

Abbreviations: ADL, activities of daily living; K-L, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OMERACT-OARSI, Outcome Measures in Rheumatology and Osteoarthritis Research Society International; QOL, quality of life; UC-EVs, umbilical cord-derived extracellular vesicles; VAS, visual analog scale.
** P < .05.*

hair growth at the injection site in 1 case, occurring across 3 knees (9.1%). Following subsequent injections, mild pain and transient swelling were reported in 5 knees (15.2%). No patient experienced adverse reactions on more than one occasion, and no new adverse events were observed on the fourth through sixth injections. The incidence of pain or swelling associated with subsequent injections was 15.2% (5/33 knees), with an incidence per injection of 2.5% (5/200 injections) (**Table B**). None of these adverse events required additional treatment such as analgesic medication or joint aspiration, and all resolved spontaneously.

Discussion

The most important finding of the current study was that intra-articular injection of UC-EVs in patients with KOA led to significant improvements in both KOOS and VAS for pain over a 12-month follow-up period, with no SAEs observed in any patient.

In our previous studies, intra-articular injection of ASC or stromal vascular fraction (SVF) was proven to be safe and effective for treating KOA.[13-15] Those studies primarily demonstrated symptomatic improvement in patients with K-L grades 2 and 3, but not in those with grade 4 KOA. In contrast to ASC therapy, in which K-L grade 4 knees demonstrated significantly inferior outcomes compared with K-L grades 2 and 3,[15] UC-EVs therapy in the current study showed significant improvements in VAS at all follow-up time points even in K-L grade 4 knees (ΔVAS: –25.6 ± 18.1 mm), along with significant improvements in several KOOS subscales. Unlike ASC therapy, SVF therapy has been reported to demonstrate improvements regardless of K-L grade, a pattern similarly observed with UC-EVs in the current study. However, UC-EVs therapy offers the additional advantage of being allogeneic and cell free, eliminating donor-site morbidity and the age-related decline in cell quality inherent to autologous approaches.

We have observed that (1) younger age is associated with better clinical outcomes, (2) milder deformity yields more favorable results, and (3) lower body mass index generally correlates with a longer duration of therapeutic effect.[13-15] Owing to age-related cellular deterioration, the clinical benefits of cell-based therapies are particularly limited in patients aged ≥65 years and in those with advanced-stage KOA, representing a major limitation of autologous biotherapy. Therefore, UC-EVs therapy may help bridge the limitations of existing treatments, including corticosteroid injections, hyaluronic acid injections, and age-related declines in the effectiveness of autologous ASC therapy. This approach could offer a meaningful treatment option for middle-aged and older patients who wish to avoid surgery but seek improvement beyond what conservative care can provide. In particular, UC-EVs may represent a promising next-generation therapeutic option for this population.

Regarding safety, no SAEs were observed in the current study. Minor and transient reactions, such as mild pain or temporary swelling at the injection site, were the only events reported, with no cases of intra-articular infection or systemic reactions, suggesting a high level of clinical acceptability. In our previous studies using ASCs, the first injection was generally well tolerated with minimal adverse effects.[13-15] However, after the second or subsequent administrations, the incidence of post-injection pain and swelling increased more than tenfold. These reactions typically appeared several hours after injection and subsided following the administration of anti-allergic medication. Because repeated

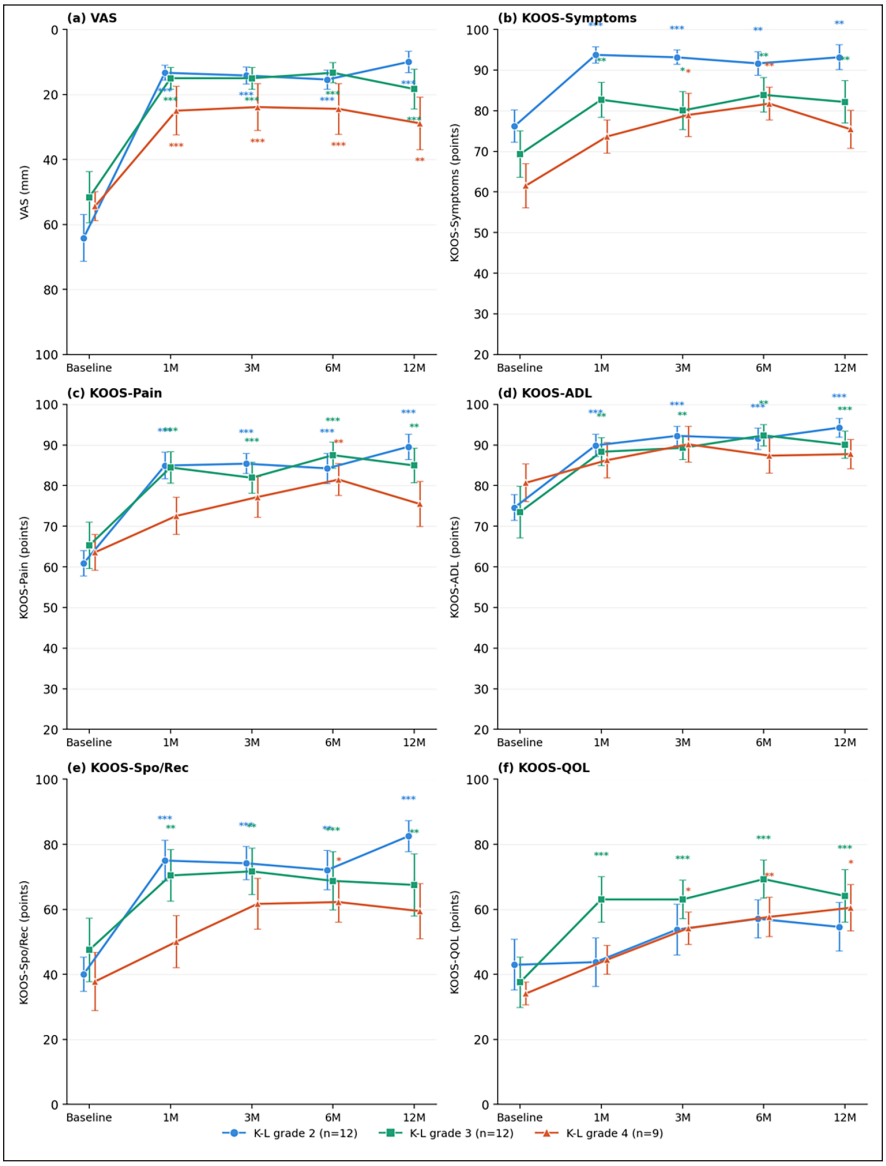

Figure 2. Changes in clinical outcomes following UC-EVs injection by Kellgren-Lawrence (K-L) grade at baseline, 1 month (1M), 3 months (3M), 6 months (6M), and 12 months (12M). Panels show scores for (a) visual analog scale (VAS) for pain, (b) KOOS symptoms subscale, (c) KOOS pain subscale, (d) KOOS ADL function subscale, (e) KOOS sport and recreation (spo/rec) function subscale, and (f) KOOS QOL subscale. Error bars represent standard error (SE). Significant differences from baseline are indicated as * $P < .05$, ** $P < .01$, *** $P < .001$. ADL, activities of daily living; KOOS, Knee Injury and Osteoarthritis Outcome Score; QOL, quality of life; UC-EVs, umbilical cord-derived extracellular vesicles.

ASC therapy was not associated with further therapeutic benefits, it was not considered effective for repeated use.

Intra-articular administration of MSCs is safe and effective for KOA treatment.[33,34] Several recent studies have reported the important role of EVs derived from MSCs in their therapeutic mechanisms.[20,35] These findings emphasize the critical role of the paracrine effects of MSCs, which involve the regulation of inflammatory mediators, maintenance of immune homeostasis, and stimulation of cell proliferation and differentiation through the secretion of soluble cytokines. EVs are considered key mediators of these paracrine mechanisms in a cell-free manner. Before the MISEV guidelines standardized the terminology, EVs were commonly referred to as exosomes.[20-22] These vesicles contain a range of bioactive molecules, including transforming growth factor-beta, hepatocyte growth factor, various growth factors, and multiple microRNAs,[35-37] all of which contribute to immune regulation and the maintenance of cartilage matrix. The clinical improvements observed in this study are likely driven by paracrine mechanisms mediated by UC-EVs.

EVs therapy also provides several advantages: Their small size supports efficient migration into target tissues; repeated dosing is more feasible than with cell-based treatments; large-scale production allows for a nearly unlimited supply; and the active therapeutic components within each vesicle can be quantified with relative ease. However, EVs therapy also carries certain limitations. Compared with cell-based treatments, significantly more vesicles are required, and the regulatory framework for non-cellular therapeutics remains underdeveloped, leaving uncertainties regarding how best to ensure safety and efficacy.[25,38] Nevertheless, in countries where cultured stem cell therapies remain restricted by regulation, EVs-based approaches may represent the only feasible treatment option.

This study has several limitations. First, the sample size was small. Second, the study was designed as a real-world clinical data analysis, and no randomized controlled trial was conducted. Third, no comparative analysis based on magnetic resonance imaging was performed, as the study was conducted in a self-pay clinical setting where these data were not obtained for all participants. Fourth, the follow-up period was limited to 12 months. Future well-designed randomized controlled trials with appropriate control groups are therefore warranted to more rigorously evaluate the safety and efficacy of allogeneic UC-EVs therapy.

Despite these limitations, to the best of our knowledge, this study is among the first to evaluate UC-EVs therapy for knee joint disease in humans using a structured dosing protocol and assessing clinical outcomes including KOOS and VAS over a 12-month follow-up period. EVs therapy may offer a less invasive and potentially promising treatment option for patients who wish to avoid surgery. Furthermore, this study did not include biological or imaging-based analyses (eg, synovial fluid biomarkers, cartilage magnetic resonance imaging) to directly elucidate the mechanism underlying the observed clinical improvements; the proposed paracrine mechanisms remain inferential and warrant confirmation in future mechanistic studies.

Conclusion

Intra-articular injection of UC-EVs demonstrated meaningful clinical benefits in patients with KOA, including reductions in pain and improvements in function as assessed by KOOS and VAS. Patients with K-L grade 2 or 3 showed greater treatment responses compared to those with K-L grade 4. No serious adverse events were observed; only mild, transient local reactions occurred, supporting a favorable safety profile. The adverse reactions following additional injections were mild and transient, consistent with our initial hypothesis. Overall, intra-articular UC-EVs therapy may represent a promising next-generation treatment option that could complement and potentially overcome the limitations of hyaluronic acid injections and aging-dependent declines associated with autologous biotherapies.

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Table A. Changes in clinical outcome in K-L grade following UC-EVs injection

(a) VAS for pain

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
1M		—	0.339	0.499	0.339
3M			—	0.674	0.210
6M				—	0.035 *
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
1M		—	1.000	0.166	0.529
3M			—	0.166	0.540
6M				—	0.324
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	0.003 **
1M		—	0.347	0.760	0.367
3M			—	0.681	0.256
6M				—	0.237
12M					—

(b) KOOS-Symptoms

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	0.001 **	0.002 **
1M		—	0.504	0.374	0.795
3M			—	0.477	0.744
6M				—	0.537
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	0.002 **	0.020 *	0.002 **	0.003 **

1M		—	0.359	0.583	0.859
3M			—	0.209	0.482
6M				—	0.360
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	0.052	0.016 *	0.004 **	0.056
1M		—	0.040 *	0.002 **	0.574
3M			—	0.130	0.233
6M				—	0.015 *
12M					—

(c) KOOS-Pain

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
1M		—	0.846	0.845	0.146
3M			—	0.708	0.021 *
6M				—	0.070
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	0.001 **
1M		—	0.434	0.118	0.844
3M			—	0.077	0.436
6M				—	0.199
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	0.182	0.092	0.007 **	0.140
1M		—	0.017 *	0.023 *	0.437
3M			—	0.252	0.543
6M				—	0.169
12M					—

(d) KOOS-ADL

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
1M		—	0.202	0.456	0.061
3M			—	0.655	0.145
6M				—	0.073
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	0.002 **	0.004 **	0.002 **	<0.001 ***
1M		—	0.297	0.034 *	0.268
3M			—	0.069	0.546
6M				—	0.300
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	0.373	0.178	0.293	0.220
1M		—	0.008 **	0.471	0.548
3M			—	0.237	0.433
6M				—	0.773
12M					—

(e) KOOS-Spo/Rec

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	0.001 **	<0.001 ***
1M		—	0.748	0.630	0.101
3M			—	0.681	0.044 *
6M				—	0.026 *
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	0.001 **	0.001 **	<0.001 ***	0.002 **
1M		—	0.633	0.666	0.570
3M			—	0.409	0.367

6M				—	0.463
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	0.212	0.062	0.026 *	0.064
1M		—	0.034 *	0.004 **	0.082
3M			—	0.886	0.731
6M				—	0.499
12M					—

(f) KOOS-QOL

K-L grade 2 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	0.934	0.353	0.153	0.264
1M		—	0.016 *	<0.001 ***	0.012 *
3M			—	0.331	0.869
6M				—	0.420
12M					—

K-L grade 3 (n=12)

	Baseline	1M	3M	6M	12M
Baseline	—	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
1M		—	0.413	0.068	0.654
3M			—	0.026 *	0.792
6M				—	0.250
12M					—

K-L grade 4 (n=9)

	Baseline	1M	3M	6M	12M
Baseline	—	0.183	0.020 *	0.009 **	0.013 *
1M		—	0.008 **	0.042 *	0.018 *
3M			—	0.466	0.170
6M				—	0.402
12M					—

** P<0.05 ** P<0.01 *** P<0.001*

Abbreviations: K-L, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; ADL, activities of daily living; spo/rec, sport and recreation function subscale; QOL, quality of life; UC-EVs, umbilical cord-derived extracellular vesicles; VAS, visual analog scale.

Table B. Adverse event incidence K-L grade following UC-EVs injection

	All (N=33)	K-L grade 2 (n=12)	K-L grade 3 (n=12)	K-L grade 4 (n=9)
First injection				
Serious adverse events[a]	0	0	0	0
Infection	0	0	0	0
Pain/swelling	0	0	0	0
Other minor reaction[b]	3 (9.1%)	3 (25.0%)	0	0
Additional intra-articular injections				
Serious adverse events[a]	0	0	0	0
Infection	0	0	0	0
Pain/swelling	5 (15.2%)	1 (8.3%)	2 (16.7%)	2 (22.2%)
Other minor reaction[b]	0	0	0	0

Abbreviations: K-L, Kellgren-Lawrence; UC-EVs, umbilical cord-derived extracellular vesicles

[a]: Serious adverse events included death, hospitalization, disability, and malignancy.

[b]: Other minor reactions include low-grade fever, drowsiness, and increased hair growth at the injection site.