Safety and Efficacy of an Amniotic Suspension Allograft Injection Over 12 Months in a Single-Blinded, Randomized Controlled Trial for Symptomatic Osteoarthritis of the Knee

Andreas H. Gomoll, M.D., Jack Farr, M.D., Brian J. Cole, M.D., M.B.A.,
David C. Flanigan, M.D., Christian Lattermann, M.D., Bert R. Mandelbaum, M.D.,
Sabrina M. Strickland, M.D., Kenneth R. Zaslav, M.D., Kelly A. Kimmerling, Ph.D., and
Katie C. Mowry, Ph.D.

Purpose: The purpose of this study is to determine the efficacy of amniotic suspension allograft (ASA) compared to hyaluronic acid (HA) and saline at up to 12 months of follow-up through the use of patient-reported outcomes, immunoglobulin levels, and anti-human leukocyte antigen (HLA) levels. **Methods:** Within this multicenter study, 200 patients were randomized 1:1:1 to a single intra-articular injection of saline, HA, or ASA. Patient-reported outcomes, including Knee Injury and Osteoarthritis Outcome Score (KOOS) and visual analog scale (VAS) score, were collected at multiple time points (baseline, 1 week, 6 weeks, 3 months, 6 months) out to 12 months to assess improvements in pain and function. Radiographs at baseline and 12 months were taken to determine radiographic changes, while blood was collected at baseline, 6 weeks, and 6 months to determine changes in immunoglobulins and anti-HLA levels. Statistical analyses were performed using last observation carried forward and mixed effects model for repeated measures. **Results:** Treatment with ASA resulted in significant improvements in KOOS and VAS scores that were maintained through 12 months ($P < .05$). Treatment with ASA resulted in a 63.2% responder rate at 12 months using the Outcome Measures in Arthritis Clinical Trials—Osteoarthritis Research Society International simplified definition. There were no significant differences between groups for radiographic measures in the index knee, immunoglobulins, C-reactive protein, or anti-HLA serum levels ($P > .05$). The number and type of adverse events (AEs) reported for ASA were comparable to the HA injection group, while no treatment-emergent AEs were reported for the saline group. **Conclusions:** This randomized controlled trial of ASA vs HA and saline for the treatment of symptomatic knee osteoarthritis demonstrated clinically meaningful improved outcomes with ASA over the controls out to 12 months postinjection. No concerning immunologic or adverse reactions to the ASA injection were identified with regards to severe AEs, immunoglobulin, or anti-HLA levels. **Level of Evidence:** Level I, randomized controlled multicenter trial.

See commentary on page 2257

More than 30.8 million people are estimated to be affected by osteoarthritis (OA) in the United States,[1] with a lifetime risk of 45% for its development,[2] thus constituting one of the leading causes of disability in adults.[3] While OA of the knee accounts for approximately 14 million of these

---

From the Department of Orthopaedic Surgery, Hospital for Special Surgery, New York, New York, U.S.A. (A.H.G., S.M.S.); Knee Preservation and Cartilage Restoration Center, OrthoIndy, Indianapolis, Indiana, U.S.A. (J.F.); Midwest Orthopaedics at Rush, Rush University Medical Center, Chicago, Illinois, U.S.A. (B.J.C.); Division of Sports Medicine Cartilage Repair Center, Department of Orthopaedics, The Ohio State University, Columbus, Ohio, U.S.A. (D.C.F.); Department of Orthopaedic Surgery, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A. (C.L.); Cedars-Sinai Kerlan-Jobe Institute, Santa Monica, California, U.S.A. (B.R.M.); OrthoVirginia, Richmond, Virginia (K.R.Z.); and Department of Research and Development, Organogenesis, Birmingham, Alabama, U.S.A. (K.A.K., K.C.M).

The authors report the following potential conflicts of interest or sources of funding: This study was funded by Organogenesis. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

Received November 16, 2020; accepted February 25, 2021.

Address correspondence to Andreas H. Gomoll, M.D., Department of Orthopaedic Surgery, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, U.S.A. E-mail: gomolla@hss.edu

0749-8063/201813

https://doi.org/10.1016/j.arthro.2021.02.044

patients,[4] there remain significant limitations to the currently available treatment options for knee OA. Surgical intervention in the form of knee replacement surgery is increasingly common; the number of patients with OA undergoing knee replacement surgery is expected to rise from 680,150 Americans in 2010 to 1.28 million Americans in 2030.[5] However, concerns regarding the morbidity of the procedure and the increasing burden of potential revision procedures[6] suggest that it should be reserved for those with end-stage OA who have exhausted all other attempts at conservative management.

Therapy, bracing, weight loss, activity modification, oral medication, and intra-articular injections all play a significant role in the nonsurgical management of OA. Due to perceived and demonstrated limitations with the traditional injections of corticosteroids and hyaluronic acid, the field of orthobiologics has gained considerable attention in both the research community and the public. While an increasing number of studies have investigated the effectiveness of platelet-rich plasma for the treatment of OA,[7-9] the use of other orthobiologic treatments lacks high-quality supportive data on efficacy and safety.

One potential orthobiologic option for the nonoperative management of OA is the use of placental-derived tissues, which were first introduced in the early 1900s to treat burns, ulcers, and other nonhealing wounds,[10] such as corneal ulcers.[11] In fact, a weekly intradermal injection of placental-derived autolysate was evaluated in the late 1960s as a treatment for arthritis but did not result in a therapeutic use.[12,13] Recently, the use of placental-derived tissues has raised substantial interest again for the use in orthopaedic applications.[14-16] These products exist in several different formulations, with some containing morselized tissues (amnion, chorion, or both), cells from the amniotic fluid, amniotic fluid, or some combination of these components.[17] Placental tissues have been shown to contain several anti-inflammatory cytokines, growth factors, and inhibitors[18] that are hypothesized to reduce the inflammatory burden associated with OA.[19,20] One specific placental tissue product has been investigated in 2 prior studies. A 6-patient open-label, single-arm pilot study supported the safety of an amniotic suspension allograft (ASA), which contains amniotic membrane particulate and amniotic fluid cells,[21] but the study was not powered to demonstrate efficacy. A subsequent 200-patient multicenter randomized clinical trial reported greater improvements in patient-reported outcomes for ASA when compared to both hyaluronic acid (HA) and saline at 3 and 6 months.[22]

The current randomized controlled multicenter study investigated safety and efficacy of the use of ASA vs HA or saline for the treatment of symptomatic knee OA over a course of 12 months. The purpose of this study is to determine the efficacy of ASA compared to HA and saline at up to 12 months of follow-up through the use of patient-reported outcomes, immunoglobulin levels, and anti-human leukocyte antigen (HLA) levels. The hypothesis of this study was that there would be no significant differences in patient-reported outcomes (PROs), immunoglobulin levels, or anti-HLA levels between injections of ASA, HA, or saline at up to 12 months of follow-up.

Methods

This article is reporting the results from a prospective, multicenter, single-blinded, Good Clinical Practices randomized controlled trial (NCT number NCT02318511) that enrolled 200 adult patients with OA who met defined inclusion/exclusion criteria at 12 study sites in the United States. These patients were enrolled from June 2015 through July 2017 under a Western Institutional Review Board—approved protocol (20142125) after signing an informed consent form. Eligible patients included adults aged 18 years and older with a body mass index (BMI) less than 40 kg/m^2, a diagnosis of moderate knee OA defined by a Kellgren-Lawrence (KL) grade of 2 or 3, and a 7-day average pain score of 4 or greater on a scale of 1 to 10. All eligible female patients were abstinent, surgically sterilized, actively practicing an accepted contraceptive method, or postmenopausal. Exclusion criteria included regular use of anticoagulants, use of pain medication other than acetaminophen for conditions unrelated to OA of the index knee, use of pain medications less than 15 days prior to the injection, patients with a history of substance abuse, or patients who failed to agree not to take additional knee symptom—modifying drugs during the course of the study without reporting the medication use to the study team. Physical or knee-related treatment exclusion criteria included intra-articular injections with either corticosteroid or viscosupplementation in the index knee within 3 months, knee surgery on the index knee within 12 months or on the contralateral knee within 6 months, acute injury to the index knee within 3 months, or confirmed mechanical symptoms such as locking, intermittent block to range of motion, or loose body sensations (meniscal displacement or intra-articular loose body). Additional exclusion criteria included history of solid organ or hematologic transplantation, rheumatoid arthritis and other autoimmune disorders, current immunosuppressive treatment, infection requiring antibiotic treatment within 3 months, diagnosis of malignancy apart from treated basal cell cancer of the skin within the last 5 years, or workers' compensation patients. Female patients were excluded if they were pregnant or had a desire to become pregnant during the course of the study. The Consolidated Standards of Reporting Trials diagram illustrating the enrollment, allocation, and disposition of patients in the study is shown in Fig 1. Patients were randomly allocated 1:1:1 to 1 of 3 treatment groups: ASA, HA, or saline using block randomization across sites to treatment groups using sealed, opaque envelopes coded with an alpha-numeric identifier.

After enrollment, all patients had a baseline evaluation, which included standard baseline radiographs (standing anteroposterior and flexion posteroanterior, lateral, and Merchant views) to confirm moderate knee OA (KL 2 or 3), medical and knee history, physical examination, and blood draws for laboratory analyses, and completed the following PROs: EQ-5D-5L (global health), Knee Injury and Osteoarthritis Outcome Score (KOOS), Single Assessment Numerical Evaluation (SANE), visual analog scale (VAS) using a 150-mm scale, and the Tegner Activity Scale. Patients were blinded to their randomized treatment allocation, and all intra-articular (IA) injections were administered using unmarked syringes and vials. For this study, all patients received IA injections with 1 of 3 agents: ASA (2.0 mL ReNu diluted 1:1 with sterile normal saline; Organogenesis, Canton, MA), HA (Monovisc High Molecular Weight Hyaluronan; Anika Therapeutics, Boston, MA), or sterile normal saline. The final injection volume for all injections was 4 mL; injections were all prepared according to manufacturer's instructions. Patients' visits included baseline, treatment, and follow-up visits at 1 week, 6 weeks, 3 months, 6 months, and 12 months postinjection. If patients reported unacceptable pain at 3 months, they were considered treatment failures and withdrawn from the current study (Fig 1).

Radiographs were collected at baseline and 12 months. Blood draws were completed at baseline, 6 weeks, and 6 months for complete blood count, basic metabolic profile, C-reactive protein (CRP), immunoglobulin levels and anti-HLA responses. One vial from each blood draw was shipped to a central lab (Brigham and Women's Hospital Transplantation Research Center) directly from the study sites. Serum was isolated and stored from blood samples; at each time point (baseline, 6 weeks, and 6 months), the patients' serum samples were examined for the presence of anti-HLA antibodies using the One Lambda (West Hills, CA) LABScreen Mixed Class I and II (LSM12). Any positive or undefined samples were then further tested for Class I anti-HLA antibodies using the LABScreen Single Antigen HLA Class I assay (LS1A04). In brief, human serum was mixed with LABScreen-coated beads; each set of beads was read and a ratio calculated. This value was compared back to values obtained by the positive and negative controls supplied with the kit, and the samples were classified as positive, negative, or undefined.

In total, 296 patients were assessed for eligibility with n = 200 patients enrolled and randomized into the study; ASA, HA, and saline groups consisted of n = 68, 64, and 68 patients, respectively (Fig 1). Questionnaires were collected by research assistants or study staff, while the physical examination and assessments were performed by the site principal investigators. A single-blind (subject blind only) was in place and treatment efficacy was assessed using independently answered PROs to ensure nonprovider blinding did not introduce additional bias. If at 3 months the blinded patient reported inadequate pain relief from their treatment, they were considered a treatment failure and withdrawn from the study. Patients self-reported inadequate pain relief; this decision was made without the investigator. Patients' response to initial treatment was assessed out to 12 months, which is a follow-up from the data presented out to 6 months in Farr et al.[22]

All data analysis and statistics were structured and performed by an independent statistician, including all laboratory data, anti-HLA antibodies, PROs, and responder analysis. Two methods were used to address missing data: (1) last observation carried forward (LOCF) and (2) mixed effects model for repeated measures (MMRM) as sensitivity analyses. Using the LOCF model, if a patient dropped out of the study, the patient's last visit data were carried forward for analysis at 12 months (Table 1). The primary efficacy analysis using LOCF consisted of analysis of covariance in SAS PROC GLM of the change from baseline, which was accompanied by unadjusted contrasts between treatment group means, where the baseline values were included as the covariate.

In addition, using LOCF, a responder analysis was completed using the Outcome Measures in Arthritis Clinical Trials—Osteoarthritis Research Society International defined criteria. Briefly, patients were considered an OMERACT-OARSI simplified responder if they met the requirement for either high improvement or improvement.[23] A χ^2 test was run to determine significance between treatment groups, and P values $<.05$ were considered statistically significant. In addition, as a measure to address the missing data challenge, MMRM was employed in SAS PROC MIXED, where the baseline values were included as a covariate and treatment and visit were included as fixed factors. Interaction terms included baseline by visit and treatment by visit. Visit was the repeated factor within subject and an unstructured covariance was used. The MMRM estimate values and standard error were plotted (Figs 2, 3). MMRM analysis included all data for all patients until patient dropout, which included through month 3 for any patients who self-reported inadequate pain relief. Furthermore, the MMRM analysis did not incorporate LOCF.

To determine the proper sample size for this study, a power analysis was conducted using data from Roos and Lohmander.[24] based on detecting the minimal important difference of 8 to 10 points using the KOOS; difference = 8, standard deviation= 10, power = 0.9, and an $\alpha = 0.05$ was used, resulting in a minimum requirement of 34 patients per group. Assuming equal dropout rates in each group of 50% over the 1-year study results in 68 patients per group. A normality assumption is made for all groups along with the assumption that each group has the same common variance.

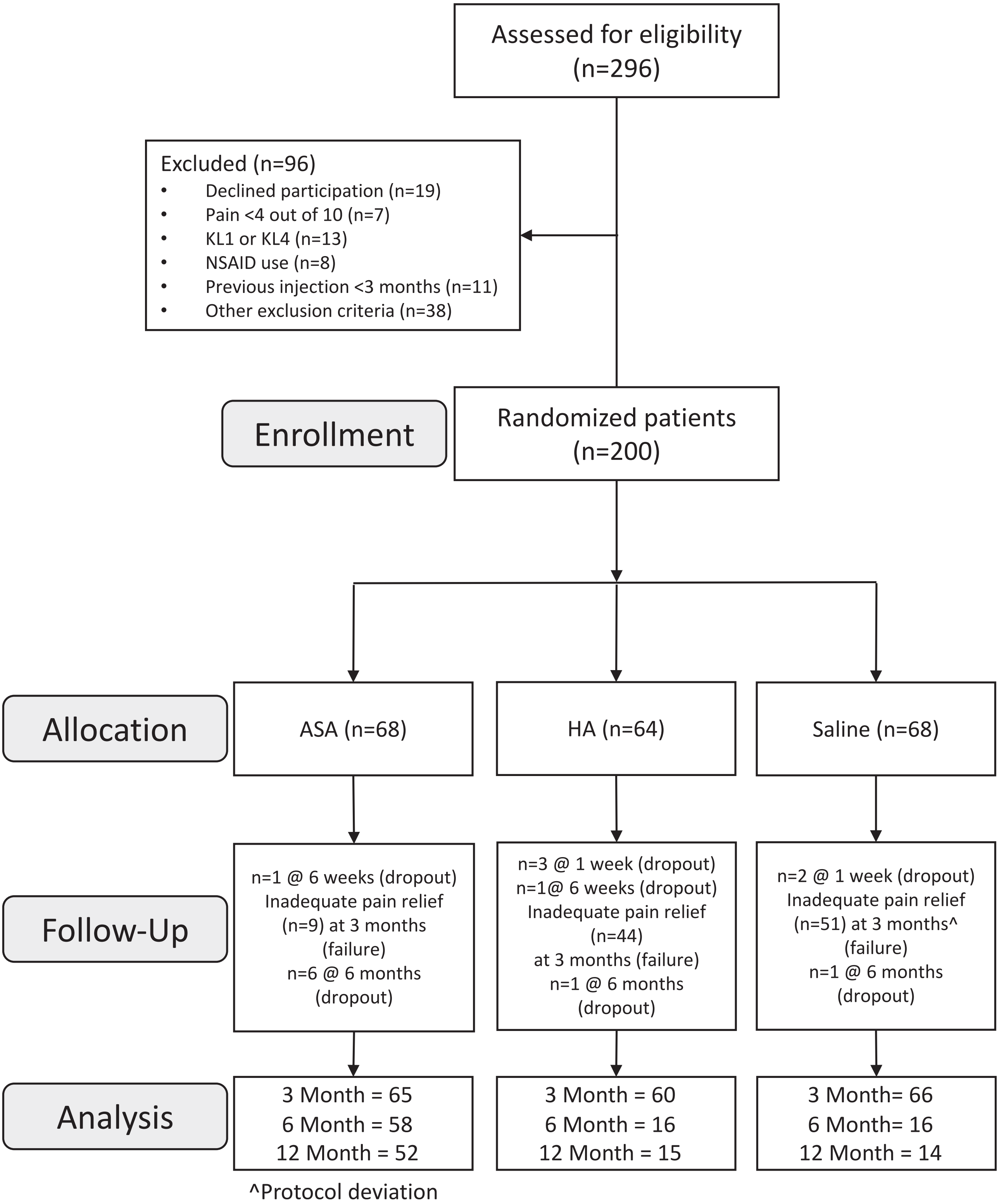

Fig 1. Consolidated Standards of Reporting Trials flow diagram used to describe the grouping and flow of patients throughout the clinical trial. ^ denotes protocol deviation at 6 months. (ASA, amniotic suspension allograft; HA, hyaluronic acid; KL, Kellgren-Lawrence; NSAID, nonsteroidal anti-inflammatory drug.)

Table 1. PRO Change From Baseline to 12 Months Using Last Observation Carried Forward (LOCF)

	KOOS Subsets					VAS			
	Pain	Symptoms	Activities of Daily Living	Sports and Recreation	Quality of Life	Overall Pain	Strenuous Work	Sedentary Work	Normal Daily Living
ASA	14.7 (21.1)	10.0 (14.4)	12.6 (19.9)	19.6 (27.6)	20.9 (24.2)	−32.9 (41.8)	−38.0 (46.1)	−13.7 (27.5)	−27.6 (36.5)
HA*	5.7 (16.5)	3.6 (12.3)	5.0 (15.5)	11.5 (21.4)	9.3 (16.2)	−9.8 (33.1)	−15.2 (35.0)	−2.7 (26.1)	−6.6 (32.7)
Saline†	7.1 (17.7)	1.7 (14.2)	7.3 (17.9)	10.5 (25.2)	10.9 (18.1)	−16.3 (36.5)	−22.8 (38.4)	−3.8 (33.9)	−14.1 (37.8)
Statistics	*0.0020*, 0.0065†*	*0.0080*, 0.0005†*	*0.0052*, 0.0339†*	*0.0173*, 0.0070†*	*0.0004*, 0.0005†*	*0.0002*, 0.0017†*	*0.0005*, 0.0057†*	*0.0046*, 0.0048†*	*0.0003*, 0.0066†*

Average (standard deviation) reported for each treatment group for the Knee Injury and Osteoarthritis Outcome Score (KOOS) and visual analog scale (VAS) subsets. *P* values were determined using an analysis of covariance.

ASA, amniotic suspension allograft; HA, hyaluronic acid.

*Denotes significance between ASA and HA.

†Denotes significance between ASA and saline.

Results

The patient population for this study in the ASA group consisted of 68 patients (33 females, 35 males) with a mean age of 55.9 ± 12.3 years and a mean BMI of 27.3 ± 5.0 kg/m^2. The HA group consisted of 64 patients (31 females, 33 males) with a mean age of 55.4 ± 11.0 years and a mean BMI of 28.2 ± 4.7 kg/m^2. The saline group consisted of 68 patients (31 females, 37 males) with a mean age of 54.9 ± 9.8 years and a mean BMI of 28.5 ± 4.2 kg/m^2. All patients in this study had either KL grade 2 or 3 OA based on the inclusion criteria, with KL grade 3 representing 54.4% of patients in the ASA group, 54.7% of patients in the HA group, and 61.8% of patients in the saline group.

Changes from baseline at 12 months post-treatment using LOCF for KOOS and VAS questionnaires are provided in Table 1. At 12 months, ASA-treated patients' KOOS scores improved 14.7 ± 21.1 points for pain, 10.0 ± 14.4 for symptoms, 12.6 ± 19.9 for activities of daily living (ADL), 19.6 ± 27.6 for sports and recreation, and 20.9 ± 24.2 for quality of life (QoL) (mean ± SD). VAS changes at 12 months from baseline are reported for overall pain, pain during strenuous work, pain during sedentary work, and pain during normal daily living (Table 1). The OMERACT-OARSI responder criteria were used to determine responders at 12 months. Using the OMERACT-OARSI simplified responder criteria, 63.2%, 35.9%, and 42.6% of patients in the ASA, HA, and saline treatment groups were considered responders, respectively ($P = .0045$). Using the high improvement criteria, 50.0%, 25.0%, and 25.0% of patients in the ASA, HA, and saline treatment groups were classified as high improvement ($P = .0018$).

Changes at 12 months from baseline following treatment using the MMRM are shown for KOOS subscales (Fig 2) and VAS subscales (Fig 3). At 12 months, KOOS pain improvement in the ASA group was 17.7 ± 2.5, while the KOOS ADL subscores improved by 14.6 ± 2.5 (mean ± standard error). ASA-treated patients also showed significant improvement in both the KOOS symptoms (11.2 ± 1.8) and KOOS QoL (25.1 ± 2.8) subscales ($P < .05$ for both). At 12 months, patients receiving ASA had significantly improved VAS scores in overall pain, strenuous pain, sedentary pain, and normal daily living subscales compared to both HA and saline groups. VAS overall pain improved −39.7 ± 4.2 from baseline, while VAS strenuous pain improved −47.0 ± 5.4 from baseline ($P < .05$ for both). Interestingly, when considering the duration of effect of ASA treatment, both KOOS and VAS scores are either comparable to scores at 3 or 6 months or continue to improve out to 12 months compared to HA and saline (Figs 2, 3).

The total number of patients who reported at least 1 treatment-emergent adverse event (TEAE) that met serious criteria was similar between the ASA (2.9%)

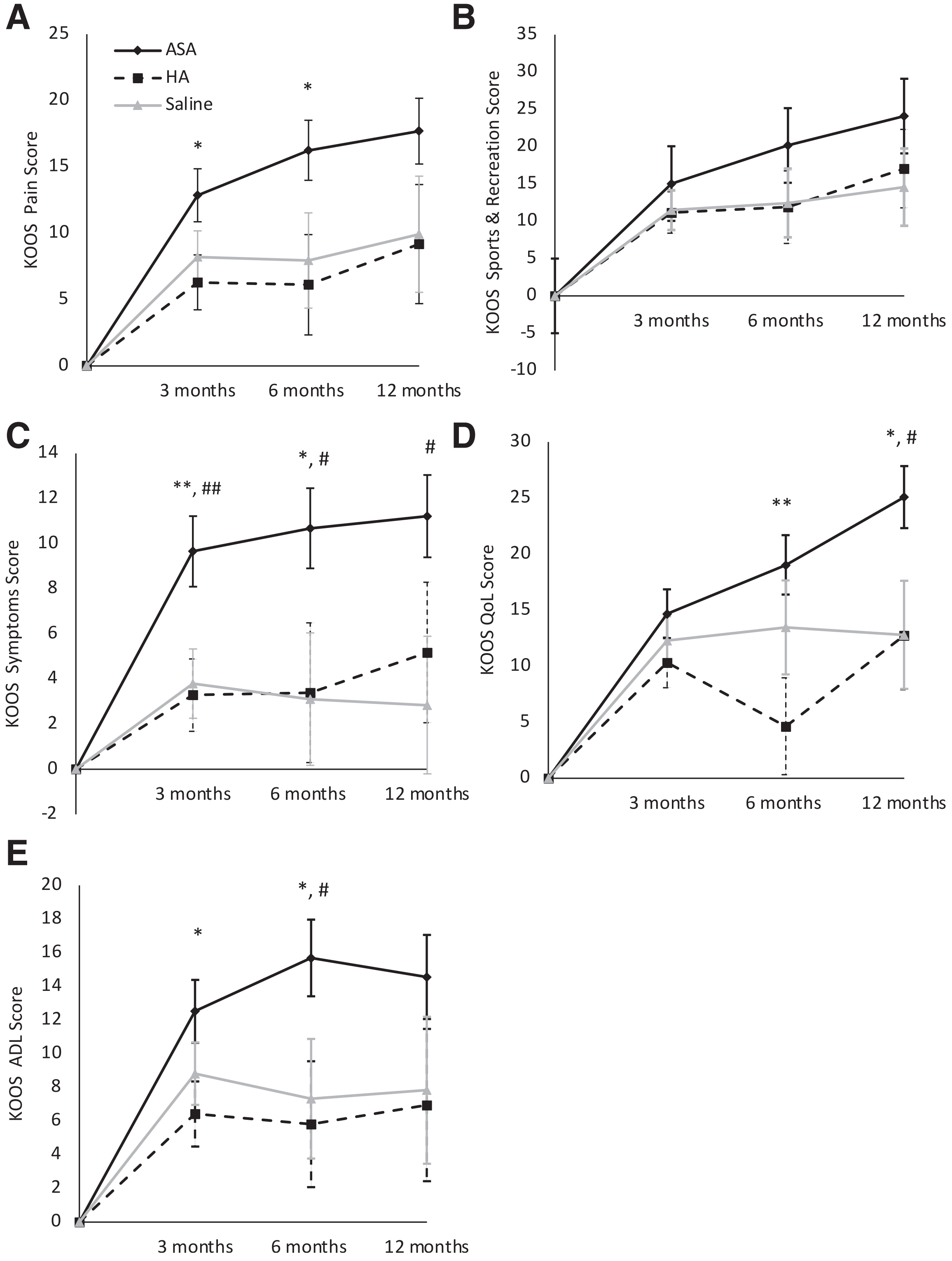

Fig 2. Knee Injury and Osteoarthritis Outcome Scores (KOOS) using Mixed effects model for repeated measures (MMRM). Average ± standard error reported for (A) KOOS pain, (B) KOOS sports and recreation, (C) KOOS symptoms, (D) KOOS quality of life (QoL), and (E) KOOS activities of daily living (ADL). *P* values were determined using PROC MIXED. $^{*}P < .05$, $^{**}P < .01$ for hyaluronic acid (HA) compared to amniotic suspension allograft (ASA); $^{\#}P < .05$, $^{\#\#}P < .01$ for saline compared to ASA.

and HA groups (3.1%), while the saline group had no reported TEAEs. Only 1 TEAE was considered to be related to the study treatment/procedure, and this occurred at week 1 in the HA arm. The event was knee stiffness (limited range of motion) and pain in the index knee with onset the day after treatment. The findings

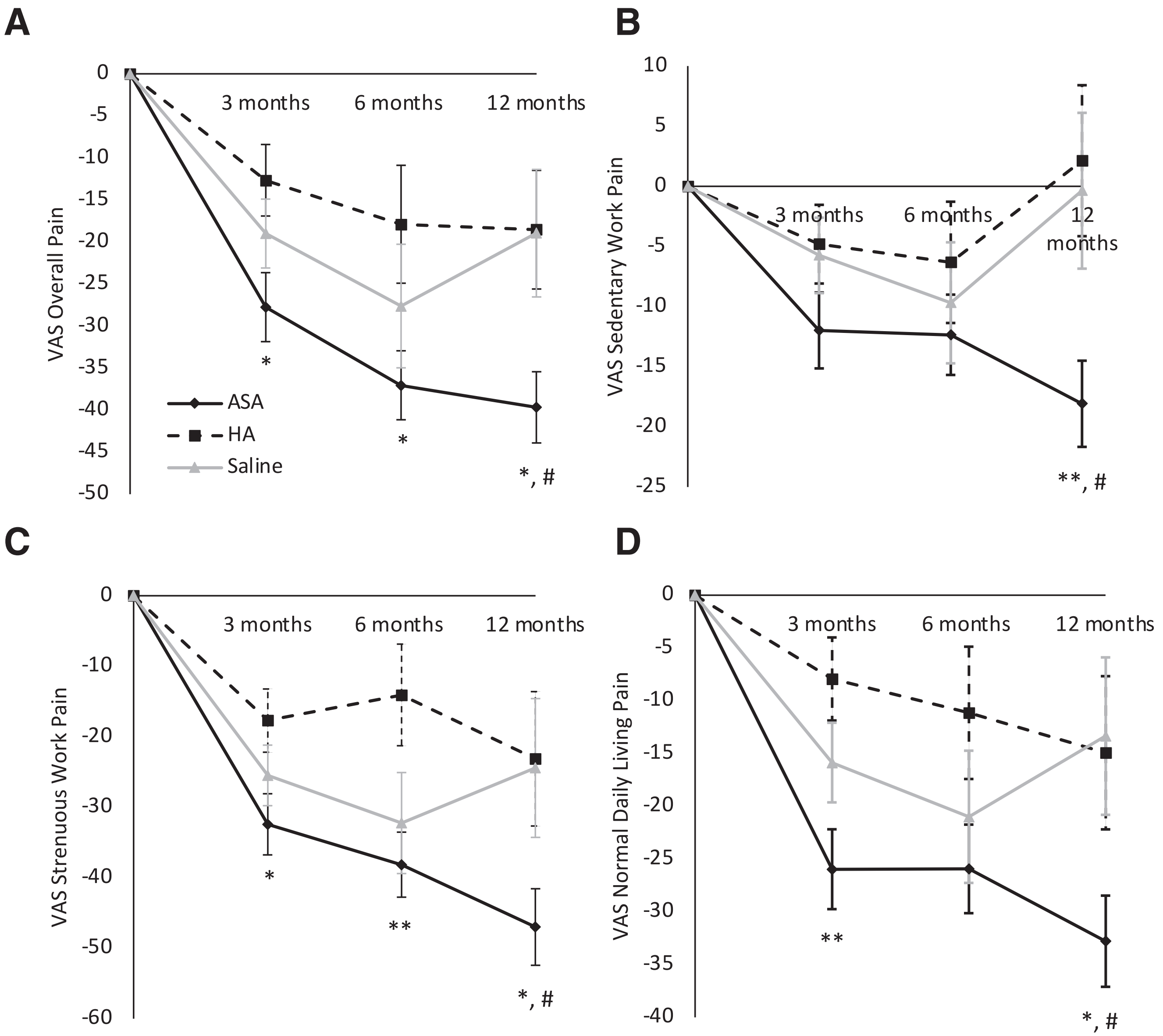

Fig 3. Visual analog scale (VAS) scores using mixed effects model for repeated measures (MMRM). Average ± standard error reported for (A) VAS overall pain, (B) VAS sedentary work pain, (C) VAS strenuous work pain, and (D) VAS normal daily living pain. *P* values were determined using PROC MIXED. *$P < .05$, **$P < .01$ for hyaluronic acid (HA) compared to amniotic suspension allograft (ASA); $^{\#}P < .05$ for saline compared to ASA.

were consistent with "pseudo septic reaction," which is a known associated risk with HA. The event involved hospitalization for aspirations, knee arthroscopy, and synovectomy.

There were no statistical differences in baseline radiologic parameters, including KL grade ($P = .6202$), worst compartment ($P = .2863$), and joint space narrowing ($P = .3065$) between treatment groups. The medial compartment demonstrated the highest proportions of worst compartment assessments across all treatment groups: ASA (64.7%), HA (65.6%), and saline (73.5%). Radiographs showed no significant changes between treatment groups with absolute joint space narrowing at 12 months ($P = .9031$) or change in joint space narrowing from baseline ($P = .9297$).

Testing of immunoglobulin (IgA, IgE, IgG, and IgM) levels was performed at baseline, 6 weeks, and 6 months. At baseline, no significant differences between cohorts were demonstrated. There were no significant differences between the HA or ASA groups at 6 weeks and 6 months. There was a small but statistically significant increase in IgE levels in the saline group compared to ASA at 6 months (Table 2, $P = .0014$). There were no significant differences in the CRP levels at any time point tested from baseline within or between treatment groups.

Table 2. Immunology Laboratory Values

	ASA			HA			Saline		
	Baseline (n = 64)	6 Weeks (n = 61)	6 Months (n = 49)	Baseline (n = 62)	6 Weeks (n = 57)	6 Months (n = 16)	Baseline (n = 63)	6 Weeks (n = 59)	6 Months (n = 15)
IgA (mg/dL)	187.8 ± 101.2	188.7 ± 100.6	185.9 ± 96.7	207.9 ± 90.9	208.0 ± 90.9	237.6 ± 101.9	183.2 ± 81.7	181.7 ± 77.8	192.6 ± 89.0
IgE (mg/dL)	90.6 ± 242.5	91.7 ± 263.1	71.8 ± 172.3*	71.5 ± 116.9	74.8 ± 120.7	81.9 ± 105.2	95.2 ± 187.2	90.0 ± 189.3	142.4 ± 248.0*
IgG (mg/dL)	981.4 ± 242.0	981.4 ± 246.1	951.8 ± 277.8	1039.0 ± 259.0	1016.6 ± 297.1	1101.2 ± 333.3	1024.9 ± 306.8	1032.7 ± 319.4	921.3 ± 282.5
IgM (mg/dL)	94.2 ± 73.3	94.2 ± 75.9	111.3 ± 121.1	100.8 ± 67.0	112.2 ± 115.2	110.7 ± 86.5	102.3 ± 54.6	101.4 ± 54.7	103.1 ± 52.6
CRP (mg/L)	2.2 ±2.3	2.2 ±2.2	2.7 ±3.2	2.9 ±3.0	2.4 ±2.2	1.9 ±2.1	3.0 ±3.5	2.80 ± 3.5	2.5 ± 2.7

Average ± standard deviation reported for IgA, IGE, IgG, IgM, and C-reactive protein (CRP) for all treatment groups at baseline, 6 weeks, and 6 months postinjection.
ASA, amniotic suspension allograft; HA, hyaluronic acid.
*Denotes $P < .05$ for saline compared to ASA; P values were determined using an analysis of covariance.

Furthermore, anti-HLA testing was conducted to determine whether IA injection with ASA, HA, or saline modified the presence of class I anti-HLA antibodies present in patients' serum at baseline, 6 weeks, and 6 months (Table 3). There were no significant differences between the proportion of patients with positive results at baseline between cohorts, but the highest frequency of patients demonstrating HLA antibodies at baseline was in the saline group (22.4%, $P = .3923$). At baseline, 6 weeks, and 6 months, the ASA group tested positive in 14.7% (10), 18.2% (12), and 25.0% (13) patients, respectively. Patients in the HA group had 14.5% (9, baseline), 26.2% (16, 6 weeks), and 28.0% (7, 6 months) positive responses, while the saline group had 22.4% (15, baseline), 30.3% (20, 6 weeks), and 19.0% (4, 6 months) positive patients. For all groups, there were small increases in the number of patients positive at 6 weeks relative to baseline, but these differences were not significantly different between treatment groups ($P = .2615$). The highest percentage of positives at 6 months was in the HA group (28.0%), followed by the ASA group (25.0%) and the saline group (19.0%); however, these differences were not significant. There was no apparent relationship between adverse events and development of anti-HLA antibodies.

Discussion

This systematic, multicenter, single-blinded study has shown efficacy using an IA injection with an amniotic product out to 12 months. Treatment of symptomatic knee OA with an ASA resulted in significant improvements out to 12 months in the KOOS subscales and VAS scores compared to HA and saline, highlighting the durability of the response of ASA treatment. In addition, within this study, we have extensively evaluated the safety profile of an IA injection of ASA. The number of adverse events reported for ASA was comparable to HA. Furthermore, there were no meaningful differences between groups for immunoglobulins, CRP, or anti-HLA serum levels. In addition, there were no differences between groups for radiographic measures in the index knee; however, 12-month follow-up may not be sufficient to draw robust conclusions about radiographic changes in OA.

Nonsurgical management for OA includes weight loss, exercise, physical therapy, and bracing to injections.[25-27] Current IA injection therapies include steroids,[26,28,29] HA,[30-32] platelet-rich plasma,[8,26,32] bone marrow aspirate concentrate,[8,26,33] adipose-derived mesenchymal stem cells,[26,34,35] autologous protein solution,[36] and saline.[37,38] Despite several preclinical and clinical trials utilizing injectable orthobiologic therapies, there remains debate about the efficacy of these treatments for knee OA. In this study, the focus was on the efficacy and safety outcomes of ASA out to

Table 3. Serum Anti-HLA Antibody Screening

	ASA, n (%)		HA, n (%)		Saline, n (%)		
	Negative	Positive	Negative	Positive	Nega/tive	Positive	*P* Value
Baseline	58 (85.3)	10 (14.7)	53 (85.5)	9 (14.5)	52 (77.6)	15 (22.4)	.3923
6 Weeks	54 (81.8)	12 (18.2)	45 (73.8)	16 (26.2)	46 (69.7)	20 (30.3)	.2615
6 Months	39 (75.0)	13 (25.0)	18 (72.0)	7 (28.0)	17 (81.0)	4 (19.0)	.7748

Number of patients for each category (positive/negative) and percentage of total patients tested reported for each treatment group at baseline, 6 weeks, and 6 months postinjection. *P* values determined using χ^2 test.

ASA, amniotic suspension allograft; HA, hyaluronic acid.

12 months post-treatment in comparison with an established modality (HA) and a control (saline).

Limited preclinical and clinical evidence supporting the use of placental-derived tissues is available,[39] with only 2 preclinical[40,41] and 3 clinical[21,22,42] studies published to date. Both preclinical studies used the rat medial meniscus transection model; Willett et al.[40] delivered micronized dehydrated human amnion/chorion membrane 24 hours following surgical induction, while Raines et al.[41] delivered particulated amniotic membrane/umbilical cord tissues 2 weeks following surgery. Study end points varied from 3 days to 28 days post-treatment, and the placental-derived tissues were shown to decrease cartilage degeneration compared to saline as demonstrated using histology and micro—computed tomography.[40,41] Clinically, Vines et al.[21] published a 6-patient pilot study in 2015 in patients with KL grade 3, which showed trends toward improvements in pain and function out to 12 months following a single IA injection of ASA.[21] This study was not powered for significance but led to the design of a multicenter, randomized, controlled trial with 200 patients comparing a single injection of ASA to HA and saline,[22] demonstrating improved efficacy of ASA compared to HA and saline at 3 and 6 months post-treatment using KOOS and VAS patient-reported outcomes.[2] In addition, a single-arm 20-patient clinical trial evaluating an amniotic membrane/umbilical cord particulate out to 24 weeks was recently published.[42] In this study, 11 patients failed to show a greater than 30% reduction in pain at 6 weeks and were provided with a second injection. Overall, Western Ontario and McMaster Universities Osteoarthritis Index pain and function scores significantly improved at all time points compared to baseline.[42]

In the present study, at 12 months, ASA patients reported an average change from baseline in the KOOS pain subscale of 14.3 using LOCF and 17.7 using MMRM. For KOOS ADL, changes from baseline were 12.61 using LOCF and 14.6 using MMRM. For VAS overall pain, the average change from baseline to 12 months for ASA was 32.9 mm using LOCF and 39.7 mm using MMRM (on recorded 150-mm scale).

The OMERACT-OARSI responder criteria were used as a way to assess how individual patients responded to their respective treatments. For patients to be a high improvement responder, they must have ≥50% improvement and absolute change of ≥20 points in pain or function, while improvement responders need ≥20% improvement and absolute change of ≥10 points in 2 of the 3 (pain, function, or QoL). The OMERACT-OARSI simplified criteria include anyone who is a high improvement or improvement responder. Of note, the absolute change mentioned above is consistent with the minimal important difference (MID) reported for the KOOS and VAS scales. Previous studies show that the MID for KOOS is 8 to 10 points,[24,43,44] while the MID for VAS is between 8 and 13 mm.[45,46] Using the OMERACT-OARSI responder analysis, individual responders were assessed using the MID of a 10-point change and 20% improvement for 2 of 3 of the defined variables (VAS pain, KOOS function, and KOOS QoL) or a high improvement responder with a minimum of 20 points and 50% improvement in pain or function.

While these results demonstrated improved patient responses to ASA, dropout from the study and uneven groups pose significant challenges in data analysis. To address this challenge, 2 different methods for dealing with missing data were employed: LOCF and MMRM. While imputation using LOCF may overestimate the effect of ASA due to carrying forward patients' last observation, because this study was focused on patients with mild to moderate OA with continued pain at 3 months, it is reasonable to assume that spontaneous recovery of pain and function to the point of washing out the findings presented is unlikely.[47] MMRM is based on the assumption that missing patients are random and that they would behave similarly to other patients in the same treatment group. Both LOCF and MMRM demonstrated robust durability of positive patient-reported outcomes in the ASA-treated patient group. Specifically, KOOS pain, symptoms, ADL, sports and recreation, and QoL scores improved within the ASA group not only from day 1 (initiation of ASA treatment) to 3 months but also from 3 months post-treatment to 12 months. In addition, at 12 months using the MMRM analysis, KOOS symptoms scores showed statistically significant differences between the ASA group and the HA group, while the KOOS QoL scores showed statistically significant differences between the ASA group and the HA and saline groups. At 12 months, using MMRM, there were significant differences between the ASA group and the HA and saline groups for the VAS scores (overall pain, strenuous work pain, sedentary work pain, and normal daily living pain). ASA-treated patients did not show worsening at 12 months from baseline in any of the clinical outcomes evaluated. In fact, efficacy end points attained at 3 months (the timepoint for assessment of the primary efficacy end point, KOOS pain) persisted out to both 6 and 12 months.

Limitations

This study had limitations: a single (patient-blinded) rather than double-blinded design since injector blinding was not possible due to obvious differences in the viscosity of the injections (HA vs saline/ASA). However, the risk of investigator bias due to single blinding was mitigated using patient-reported rather than investigator-reported outcomes for efficacy measures. In addition, ethical concerns over prolonged treatment with a placebo led to a study design allowing dropout at 3 months for those patients who reported unacceptable pain relief. This study design naturally resulted in more limited data sets for analysis at 6- and 12-month follow-up visits (Fig 1) due to dropout of patients at 3 months. Furthermore, the study inclusion and exclusion criteria are somewhat restrictive and may not accurately reflect the entire patient population that may receive the product. No hip to ankle alignment was recorded, and thus malignment may have been distributed unevenly across the 3 arms. However, the randomization and moderated numbers of enrollment should have decreased this effect. Standard of care for nonoperative OA management, including bracing, physical therapy, weight loss programs, and so on, was used per the physician's normal practice but was not globally harmonized throughout the study.

Conclusions

This randomized controlled trial of ASA vs HA and saline for the treatment of symptomatic knee OA demonstrated clinically meaningful improved outcomes with ASA over the control treatments out to 12 months post-injection. No concerning immunologic or adverse reaction to the ASA injection was identified with regards to severe adverse events, immunoglobulin, or anti-HLA levels.

Acknowledgments

This study was supported by Organogenesis. The authors thank statistician Tad Archembault, Ph.D. (Virtu Stat Lts), for his work on data analysis and statistics, and Anil Chandraker, M.D., and his laboratory for their work on the anti-HLA testing. The ASA study group includes Kavita Ahuja, M.D., C.R.C., Catherine Beckham, P.A.-C., James Borchers, M.D., David Shane Buggay, M.D., Jeffrey Clifton, M.D., Ricardo Colberg, M.D., Brian Cole, M.D., M.B.A., Jeffrey Davis, M.D., Kristen Davis, P.A., Jack Farr, M.D., David Flanigan, M.D., Samuel Goldstein, M.D., Andreas Gomoll, M.D., Kenneth Jaffe, M.D., Laith Jazrawi, M.D., Christopher Kaeding, M.D., Christian Lattermann, M.D., Robert Magnussen, M.D., Bert Mandelbaum, M.D., Rebecca McNair, C.R.N.P., Jose Ortega, M.D., Stephanie Pongracz, P.A., Seth Sherman, M.D., Beth Shubin Stein, M.D., Eric Strauss, M.D., Sabrina Strickland, M.D., Samuel Tabet, M.D., Nikhil Verma, M.D., Kathleen Weber, M.D., M.S., William Whitley, Pharm.D., Robert Wilson, M.D., Adam Yanke, M.D., Ph.D., and Kenneth Zaslav, M.D.

References

1. Cisternas MG, Murphy L, Sacks JJ, Solomon DH, Pasta DJ, Helmick CG. Alternative methods for defining osteoarthritis and the impact on estimating prevalence in a US population-based survey. *Arthritis Care Res (Hoboken)* 2016;68(5):574-580.
2. Murphy L, Schwartz TA, Helmick CG, et al. Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Rheum* 2008;59(9):1207-1213.
3. Lawrence RC, Felson DT, Helmick CG, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. *Arthritis Rheum* 2008;58(1):26-35.
4. Deshpande BR, Katz JN, Solomon DH, et al. Number of persons with symptomatic knee osteoarthritis in the US: Impact of race and ethnicity, age, sex, and *obesity*. *Arthritis Care Res (Hoboken)* 2016;68(12):1743-1750.
5. Sloan M, Sheth NP. *Projected volume of primary and revision total joint arthroplasty in the United States, 2030-2060.* Am Acad Orthop Surg, 2018;2060.
6. Maradit Kremers H, Larson DR, Crowson CS, et al. Prevalence of total hip and knee replacement in the United States. *J Bone Joint Surg Am* 2015;97(17):1386-1397.
7. Louis ML, Magalon J, Jouve E, et al. Growth factors levels determine efficacy of platelets rich plasma injection in knee osteoarthritis: A randomized double blind noninferiority trial compared with viscosupplementation. *Arthroscopy* 2018;34(5):1530-1540.e2.
8. Moatshe G, Morris ER, Cinque ME, et al. Biological treatment of the knee with platelet-rich plasma or bone marrow aspirate concentrates. *Acta Orthop* 2017;88(6):670-674.
9. Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. *Am J Sports Med* 2016;44(3):792-800.
10. Sabella N. Use of fetal membranes in skin grafting. *Med Rec NY* 1913;83:478-480.
11. Tseng SCG, Espana EM, Kawakita T, et al. How does amniotic membrane work? *Ocul Surf* 2004;2(3):177-187.
12. Livingston WS, Compton E. The Livingston placental autolysate: Effect upon rheumatoid and osteoarthritis. *Rocky Mt Med J* 1967;64(10):73-79.

13. Maxson T, Compton E. Controlled study of a new antiarthritic substance. *Ann Allergy* 1969;27(2):54-64.
14. Heckmann N, Auran R, Mirzayan R. Application of amniotic tissue in orthopedic surgery. *Am J Orthop* 2016;45(7):421-425.
15. Riboh JC, Saltzman BM, Yanke AB, Cole BJ. Human amniotic membrane-derived products in sports medicine. *Am J Sports Med* 2016;44(9):2425-2434.
16. McIntyre JA, Jones IA, Danilkovich A, Vangsness CT. The placenta: Applications in orthopaedic sports medicine. *Am J Sports Med* 2018;46(1):234-247.
17. Friel NA, de Girolamo L, Gomoll AH, Mowry KC, Vines JB, Farr J. Amniotic fluid, cells, and membrane application. *Oper Tech Sports Med* 2017;25(1):20-24.
18. McQuilling JP, Vines JB, Kimmerling KA, Mowry KC. Proteomic comparison of amnion and chorion and evaluation of the effects of processing on placental membranes. *Wounds Compend Clin Res Pract* 2017;29(6): E38-E42.
19. Goldring MB. Osteoarthritis and cartilage: The role of cytokines. *Curr Rheumatol Rep* 2000;2(6):459-465.
20. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011;7(1):33-42.
21. Vines J, Aliprantis A, Gomoll A, Farr J. Cryopreserved amniotic suspension for the treatment of knee osteoarthritis. *J Knee Surg* 2015;29(6):443-450.
22. Farr J, Gomoll AH, Yanke AB, Strauss EJ, Mowry KC. A randomized controlled single-blind study demonstrating superiority of amniotic suspension allograft injection over hyaluronic acid and saline control for modification of knee osteoarthritis symptoms. *J Knee Surg* 2019;1(212).
23. Pham T, van der Heijde D, Altman R, et al. OMERACT-OARSI Initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthr Cartil* 2004;12(5):389-399.
24. Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): From joint injury to osteoarthritis. *Health Qual Life Outcomes* 2003;1(2):64.
25. Taylor N. Nonsurgical management of osteoarthritis knee pain in the older adult. *Clin Geriatr Med* 2017;33(1):41-51.
26. Levy DM, Petersen KA, Scalley Vaught M, Christian DR, Cole BJ. Injections for knee osteoarthritis: Corticosteroids, viscosupplementation, platelet-rich plasma, and autologous stem cells. *Arthroscopy* 2018;34(5):1730-1743.
27. Taylor N. Nonsurgical management of osteoarthritis knee pain in the older adult. *Clin Geriatr Med* 2018;33(1):41-51.
28. Klocke R, Levasseur K, Kitas GD, Smith JP, Hirsch G. Cartilage turnover and intra-articular corticosteroid injections in knee osteoarthritis. *Rheumatol Int* 2018;38(3): 455-459.
29. McAlindon TE, LaValley MP, Harvey WF, et al. Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis. *JAMA* 2017;317(19):1967.
30. Altman RD, Manjoo A, Fierlinger A, Niazi F, Nicholls M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: A systematic review. *BMC Musculoskelet Disord* 2015;16(1):1-10.
31. Bert J, Kenney J, Sgaglione NA, et al. Viscosupplementation for osteoarthritis of the knee: A key opinion leader panel discussion. *J Manag Care Spec Pharm* 2018;24(6-a):S2-S8 (suppl).
32. Di Martino A, Di Matteo B, Papio T, et al. Platelet-rich plasma versus hyaluronic acid injections for the treatment of knee osteoarthritis: Results at 5 years of a double-blind, randomized controlled trial. *Am J Sports Med* 2019;47(2): 347-354.
33. Rodriguez-Fontan F, Piuzzi NS, Kraeutler MJ, Pascual-Garrido C. Early clinical outcomes of intra-articular injections of bone marrow aspirate concentrate for the treatment of early osteoarthritis of the hip and knee: A cohort study. *PM R* 2018;10(12):1353-1359.
34. McIntyre JA, Jones IA, Han B, Vangsness CT. Intra-articular mesenchymal stem cell therapy for the human joint: A systematic review. *Am J Sports Med* 2018;46(14): 3550-3563.
35. Spasovski D, Spasovski V, Baščarević Z, et al. Intra-articular injection of autologous adipose-derived mesenchymal stem cells in the treatment of knee osteoarthritis. *J Gene Med* 2018;20(1):e3002.
36. Kon E, Engebretsen L, Verdonk P, Nehrer S, Filardo G. Clinical outcomes of knee osteoarthritis treated with an autologous protein solution injection: A 1-year pilot double-blinded randomized controlled trial. *Am J Sports Med* 2018;46(1):171-180.
37. Altman RD, Devji T, Bhandari M, Fierlinger A, Niazi F, Christensen R. Clinical benefit of intra-articular saline as a comparator in clinical trials of knee osteoarthritis treatments: A systematic review and meta-analysis of randomized trials. *Semin Arthritis Rheum* 2016;46(2):151-159.
38. Saltzman BM, Leroux T, Meyer MA, et al. The therapeutic effect of intra-articular normal saline injections for knee osteoarthritis: A meta-analysis of evidence level 1 studies. *Am J Sports Med* 2017;45(11):2647-2653.
39. Hannon C, Yanke A, Farr J. Amniotic tissue modulation of knee pain—a focus on osteoarthritis. *J Knee Surg* 2019;32(1):26-36.
40. Willett NJ, Thote T, Lin AS, et al. Intra-articular injection of micronized dehydrated human amnion/chorion membrane attenuates osteoarthritis development. *Arthritis Res Ther* 2014;16(1):R47.
41. Raines AL, Shih M-S, Chua L, Su C-W, Tseng SCG, O'Connell J. Efficacy of particulate amniotic membrane and umbilical cord tissues in attenuating cartilage destruction in an osteoarthritis model. *Tissue Eng Part A* 2017;23(1-2):12-19.
42. Castellanos R, Tighe S. Injectable amniotic membrane/ umbilical cord particulate for knee osteoarthritis: A prospective, single-center pilot study. *Pain Med* 2019;20(11): 2283-2291.
43. Mills KAG, Naylor JM, Eyles JP, Roos EM, Hunter DJ. Examining the minimal important difference of patient-reported outcome measures for individuals with knee osteoarthritis: A model using the knee injury and osteoarthritis outcome score. *J Rheumatol* 2016;43(2):395-404.
44. Devji T, Guyatt GH, Lytvyn L, et al. Application of minimal important differences in degenerative knee disease outcomes: A systematic review and case study to inform

BMJ Rapid Recommendations. *BMJ Open* 2017;7(5):e015587.

45. Landorf KB, Radford JA, Hudson S. Minimal important difference (MID) of two commonly used outcome measures for foot problems. *J Foot Ankle Res* 2010;3(1):7.
46. Gallagher EJ, Liebman M, Bijur PE. Prospective validation of clinically important changes in pain severity measured on a visual analog scale. *Ann Emerg Med* 2001;38(6):633-638.
47. Arden N, Nevitt M. Osteoarthritis: Epidemiology. *Best Pract Res Clin Rheumatol* 2006;20(1):3-25.