



# 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

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## Abstract

Placental-derived tissues are a known source of anti-inflammatory and immune modulating factors. Published pilot data on amniotic suspension allograft (ASA) for the treatment of osteoarthritis (OA) demonstrated safety and trends for improved pain and function. A multicenter randomized controlled trial was designed to evaluate the efficacy of symptom modulation with ASA compared with saline and hyaluronic acid (HA) in subjects with knee OA. A total of 200 subjects were randomized 1:1:1 to ASA, HA, or saline, with subjects blinded to their allocation. Changes from baseline of patient-reported outcomes (PROs)—EQ-5D-5L, Knee Osteoarthritis Outcome Score (KOOS), visual analog scale (VAS), Tegner, and Single Assessment Numerical Evaluation (SANE)—were compared between groups. Patients reporting unacceptable pain at 3 months were considered treatment failures and withdrawn from the study. Statistical analysis was completed by comparing changes in PROs from baseline to 3 and 6 months for all groups. Comparison of demographics between treatment groups showed no significant differences between groups. Patients reporting unacceptable pain at 3 months in each group were ASA (13.2%), HA (68.8%), and saline (75%). Patients receiving ASA demonstrated significantly greater improvements from baseline for

## Keywords

- ▶ osteoarthritis
- ▶ amniotic membrane
- ▶ nonoperative management
- ▶ injection

\* For details of the ASA Study Group members, please refer the Acknowledgments section provided at the end of the article

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overall pain (VAS), KOOS pain, and KOOS-activities of daily living scores compared with those in the HA group (3 months) and both groups (6 months). ASA patients had significantly greater improvements in KOOS symptom scores compared with HA and saline at 3 and 6 months, respectively. OMERACT-OARSI responder rates for ASA, HA, and saline groups were 69.1, 39.1, and 42.6%, respectively ( $p = 0.0007$ ). Subjects receiving ASA treatment showed greater improvements in PROs and fewer patients reported unacceptable pain compared with HA and saline. The evidence presented in this Level I Randomized Controlled Trial suggests that ASA injection is an effective treatment for the nonoperative management of symptomatic knee OA.

Osteoarthritis (OA) currently affects ~30.8 million people in the United States alone,<sup>1</sup> and the volume of knee replacement surgery performed for symptomatic knee OA is predicted to rise significantly in the future.<sup>2,3</sup> While knee replacement surgery has proven benefits, delaying or avoiding surgery altogether appears desirable from both a medical and health care system perspective. However, conservative treatment options for patients must be appropriate and effective for their pain and functional limitations. Furthermore, delaying a primary knee replacement will potentially reduce the number that ultimately go on to costly revision total knee arthroplasty (TKA). In addition, previous studies have shown that patients with Kellgren–Lawrence (KL) grades 2 and 3 have significantly worse outcomes following TKA than patients with KL grade 4,<sup>4,5</sup> further supporting the concept of providing symptomatic relief for patients with OA that is symptomatic but not yet end-stage.

A conservative management program has been recommended by the American Academy of Orthopaedic Surgeons (AAOS) Clinical Practice guidelines (CPG)<sup>6</sup>; however, these treatment options are limited. Approaches include nonsteroidal anti-inflammatory drugs, physical therapy, hyaluronic acid (HA), and corticosteroids, among others.<sup>7–9</sup> Intra-articular (IA) injections of corticosteroids have been utilized for years in the treatment of symptomatic knee OA with short-term effectiveness (1 week–3 months)<sup>10</sup>; however, in a study by McAlindon et al, repeated injections over 2 years demonstrated small but potentially concerning long-term detrimental effects.<sup>11</sup> Similarly, despite reasonable clinical experience with various HA formulations, the AAOS has recently recommended against the use of HA in their AAOS CPGs since overall improvements were reported not to meet the minimum clinically important improvement thresholds.<sup>6</sup>

Despite appropriate conservative care efforts, a significant number of patients will remain symptomatic. These patients may not be candidates for total knee replacement due to having disease that is not end-stage yet, medical frailty, obesity, young age, high activity level, or simply because they wish to avoid surgery. For this subset of patients, a new category of injectable “biologic” medications has recently become available. While not formally defined, this group of injectables includes platelet-rich plasma (PRP), bone marrow aspirate concentrate (BMAC), adipose-derived stromal vascular fraction, allogeneic placental products, and others. Placental products include amniotic tissues, which overall

have demonstrated safety and efficacy in other medical applications, such as treatment of corneal and diabetic foot ulcers. While increasingly popular, there remains a lack of high-level, peer-reviewed studies on the efficacy of amniotic tissue for the treatment of symptomatic knee OA.

In an initial pilot clinical study evaluating an amniotic suspension allograft (ASA) for OA, six patients with KL grade 3 knee OA were enrolled.<sup>12</sup> Subjects had IA injections of 2 mL of cryopreserved ASA. Patients were followed at 1 week, 2 weeks, 3 months, 6 months, and 12 months postinjection; no adverse events were reported. There were trends in improvement in patient-reported outcomes (PROs), but a formal statistical analysis was not feasible due to the small sample size. The patients were monitored at multiple time points for safety with white blood cell count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and an immunologic panel with no concerning changes identified. These results prompted the undertaking of a larger study to evaluate the efficacy of an ASA injection in patients with symptomatic OA of the knee.

The current patient-blinded, randomized controlled multicenter study investigated the use of ASA, which contains amniotic fluid cells and amniotic membrane particulate, for the treatment of knee OA symptoms. In this study, the use of a single IA injection of ASA was compared with a single IA injection of HA or saline. The hypothesis of this study was that there would be no difference in patients receiving injections of ASA, HA, or saline with respect to pain and function at 3 and 6 months.

## Materials and Methods

### Study Population

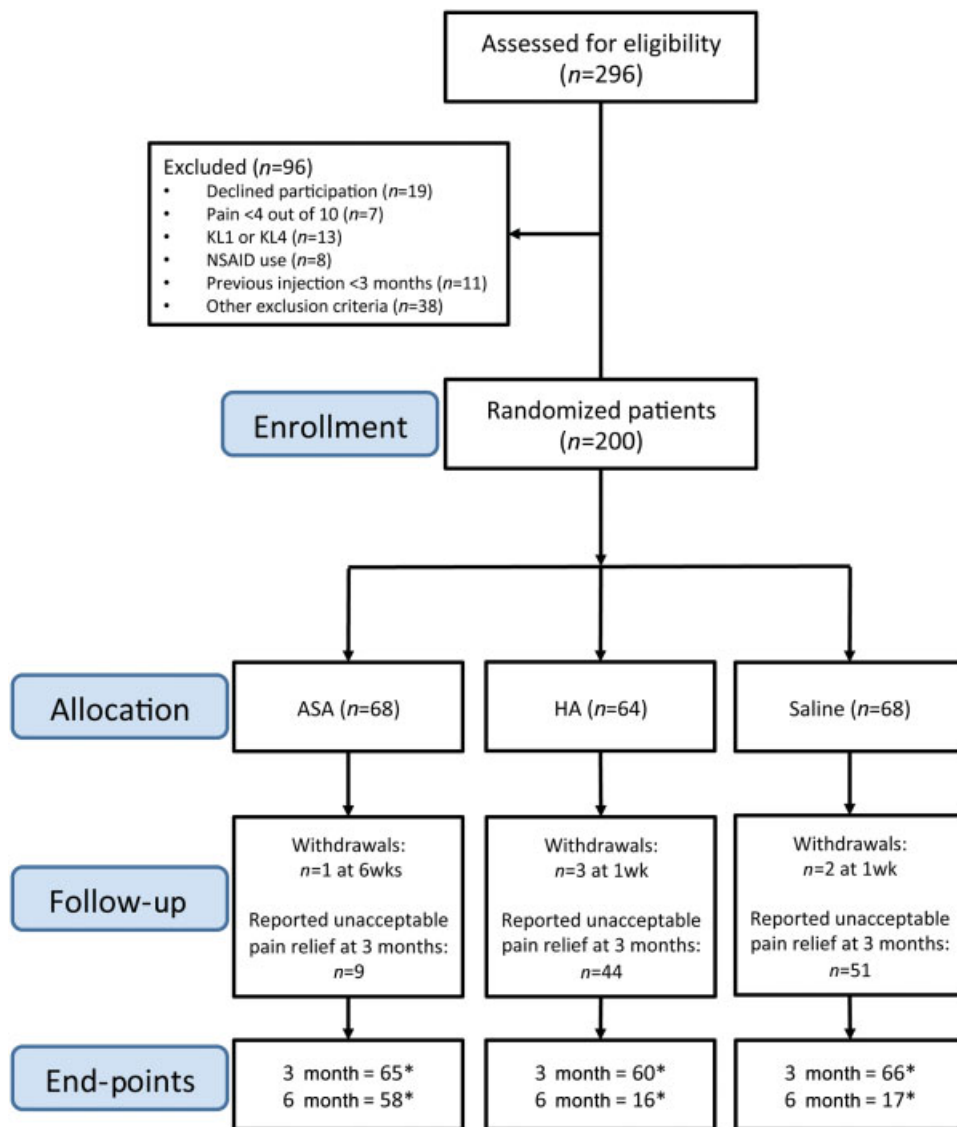
This multicenter patient-blinded, randomized, controlled clinical trial had a target enrollment of 200 adult patients who met defined inclusion and exclusion criteria at 12 study sites in the United States (NCT02318511). Eligible patients included adults older than 18 years with a body mass index (BMI)  $< 40 \text{ kg/m}^2$ , diagnosed with moderate knee OA (grade 2 or 3 on the KL score), and a 7-day average pain score of 4 or greater on a scale of 1 to 10. Female patients were abstinent, actively practicing an accepted contraceptive method, surgically sterilized, or postmenopausal. Exclusion criteria included patients who had taken pain medication  $< 15$  days prior to the injection, receive pain medicine other than

acetaminophen for conditions unrelated to OA of the index knee, regularly use anticoagulants, history of substance abuse, or failure to agree not to take additional knee symptom-modifying drugs during the course of the study without reporting the use to the study team. Physical or IA injection exclusion criteria included frank mechanical symptoms such as locking, intermittent block to range of motion, or loose body sensations (meniscal displacement or IA loose body), corticosteroid or viscosupplementation injection into the index knee within 3 months, knee surgery on index knee within 12 months or on contralateral knee within 6 months, or acute injury to the knee within 3 months. Additional exclusion criteria included workers' compensation patients, history of solid organ or hematologic transplantation, rheumatoid arthritis and other autoimmune disorders, current immunosuppressive treatment, diagnosis of nonbasal cell malignancy within preceding 5 years, or infection requiring

antibiotic treatment within the preceding 3 months. Female patients were excluded if they were pregnant or had a desire to become pregnant during the study.

To determine the appropriate sample size, a power analysis was conducted using data from Roos and Lohmander.<sup>13</sup> To detect a minimal important difference (MID) of 8 to 10 points using the Knee injury and Osteoarthritis Outcome Score (KOOS) with three treatment groups, a power of 0.9, and  $\alpha = 0.05$ , sample size per group was calculated at 32. To account for dropouts, the final sample size was increased to at least 61 per group. The CONSORT chart illustrating the enrollment of patients is shown in ► Fig. 1.

All patients had moderate knee OA on standardized baseline radiographs (standing AP and flexion PA, lateral and Merchant views) (KL score of 2 or 3). Patients were randomly assigned to treatment groups using sealed opaque envelopes coded with an  $\alpha$ -numeric identifier to ensure



\* Last observation carried forward (LOCF) used for analysis

**Fig. 1** Consolidated Standards of Reporting Trials flow diagram used to describe the grouping and flow of patients in the trial. ASA, amniotic suspension allograft; HA, hyaluronic acid; NSAID, nonsteroidal anti-inflammatory drug.

consecutive allocation of envelopes. Block randomization across sites to ensure even distribution of groups occurred at a 1:1:1 allocation to one of the three treatment arms: ASA, HA, or saline.

### Study Design

After enrollment, patients underwent a baseline evaluation, including standard plain radiographs, medical history, knee history and physical examination, blood draws for laboratory analysis, and the following PROs: EQ-5D-5L, KOOS, Tegner Activity Scale, visual analog scale (VAS), and Single Assessment Numerical Evaluation (SANE). Subjects were blinded to their randomized allocation, and the IA injection was completed using unmarked syringes and vials. For this study, patients received one of three IA injections: ASA (2.0-mL ReNu diluted 1:1 with sterile normal saline, Organogenesis, Canton, MA), HA (Monovisc, Anika Therapeutics, Boston, MA), or sterile normal saline. For all injections, the final volume injected was 4 mL; injections were prepared according to packaging instructions.

Patients underwent a baseline visit, treatment visit, and follow-up visits at 1 week, 6 weeks, 3 months, and 6 months postinjection. If patients reported unacceptable pain at

3 months and requested unblinding, they were considered treatment failures and withdrawn from the current study.

### Data and Statistical Analysis

All data analysis and statistics were structured and performed by an independent statistician, including data describing the patient's overall disease state (affected side, most symptomatic compartment, KL grade, and joint space narrowing); mean values and standard deviations are reported. For this study, PROs collected included EQ-5D-5L, KOOS, SANE, Tegner, and VAS. Mean values for all PRO subsets for baseline, 3 months, and 6 months are reported in ►Table 1.

Subject disposition was evaluated for all groups. For this evaluation, two categories were considered: (1) those who were withdrawn from the study due to unacceptable pain at 3 months and (2) withdrawals before 3 months for reasons other than pain. A chi-square test was used to compare the three groups for both categories with a  $p$ -value < 0.05 considered to be statistically significant (►Table 2). Additional analysis was performed to compare subjects who reported unacceptable pain with those who did not at 3 months,

**Table 1** PRO average values at baseline, 3 months, and 6 months

		ASA			HA			Saline		
		Baseline	3 mo	6 mo	Baseline	3 mo	6 mo	Baseline	3 mo	6 mo
EQ-5D-5L		$n = 68$	$n = 65$	$n = 58$	$n = 64$	$n = 60$	$n = 16$	$n = 68$	$n = 66$	$n = 17$
	Mobility	2.2	1.9	1.6	2.4	2.1	1.7	2.3	2.0	1.4
	Self-care	1.3	1.2	1.1	1.3	1.3	1.1	1.4	1.3	1.1
	Activities	2.3	1.8	1.6	2.4	2.1	1.9	2.3	2.0	1.7
	Pain	2.8	2.3	2.1	2.9	2.6	2.4	2.9	2.5	1.9
	Anxiety	1.4	1.2	1.1	1.5	1.5	1.3	1.4	1.4	1.1
	Health Today	78.04	83.74	83.89	81.09	82.38	82.81	78.66	78.17	86.00
KOOS										
	Pain	56.70	69.41	73.99	53.17	61.95	66.51	52.87	62.21	74.58
	Symptoms	46.11	56.85	57.95	46.84	50.65	50.69	47.06	51.27	56.94
	Activities of daily living	65.34	77.46	81.57	61.09	69.84	75.20	60.89	70.82	80.39
	Sports and recreation	32.89	47.69	55.60	28.40	42.02	48.75	28.07	40.86	55.93
	Quality of life	33.94	48.62	54.24	31.67	43.26	43.80	26.86	40.37	55.91
SANE										
		50.58	69.94	75.71	50.38	64.78	74.13	47.15	63.76	78.88
Tegner										
		4.1	3.9	4.0	4.1	4.1	3.9	3.9	3.7	4.3
VAS										
	Overall pain	76.38	47.87	36.82	75.69	61.08	49.72	81.02	58.31	23.88
	Strenuous work pain	97.89	66.08	57.50	99.54	80.42	71.75	104.86	77.12	42.25
	Sedentary work pain	34.88	24.38	22.25	40.21	32.75	27.63	42.31	34.28	8.97
	Normal daily living pain	62.86	36.56	34.13	63.64	53.14	47.00	67.91	50.07	17.75

ASA, amniotic suspension allograft; HA, hyaluronic acid.

Note: Average reported for patient-reported outcomes (PROs) from each treatment group, including EQ-5D-5L, Knee Injury and Osteoarthritis Outcome Score (KOOS), Single Assessment Numerical Evaluation (SANE), Tegner Activity Levels, and visual analog scale (VAS).

**Table 2** Subject disposition

		ASA (n = 68)	HA (n = 64)	Saline (n = 68)	Statistics		
Withdrawals before 3-mo visit		1 (1.5%)	3 (4.7%)	2 (2.9%)	$p = 0.5561$		
Reported unacceptable pain at 3 mo		9 (13.2%)	44 (68.8%)	51 (75.0%)	$p < 0.0001$		
Subjects reporting unacceptable pain	3 mo change from baseline: KOOS					3 mo change: VAS	
	Pain	Symptoms	Activities of daily living	Sports and recreation	Quality of life	Overall pain	
No	n = 96	17.64 (1.91)	9.99 (1.50)	16.62 (1.87)	22.49 (2.55)	21.05 (2.09)	-38.76 (3.82)
Yes	n = 104	0.16 (1.89)	-0.04 (1.50)	2.15 (1.85)	1.08 (2.54)	4.21 (2.08)	-0.83 (3.71)
Statistics		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

ASA, amniotic suspension allograft; HA, hyaluronic acid.

Note: Numbers of patients who reported unacceptable pain at 3 months for all groups (n [% of enrolled]) or withdrew early from the study (n [% of enrolled]). A chi-squared test was run to determine significance and p values reported. Average change from baseline for KOOS scores and overall pain (VAS) for subjects reporting unacceptable pain at 3 months and those who did not. p-Values were determined using an analysis of covariance (ANCOVA) with baseline as a covariate and treatment and unacceptable pain status as factors. LSMEANS (standard error) are shown.

focusing on changes from baseline to 3 months with regard to KOOS subsets and VAS overall pain (► **Table 2**). To determine significance between subjects who withdrew and subjects who continued in the study, p values were determined using an analysis of covariance (ANCOVA) with baseline as a covariate and treatment and unacceptable pain status as factors. LSMEANS (standard error) are shown.

Due to the study design, patients' results were carried forward using a single imputation model for missing data. Last observation carried forward (LOCF) has been used in several clinical studies<sup>14,15</sup> and was deemed appropriate here due to the nonrandomness of missing data; most missing data were because of dropout due to an inadequate response to the assigned study injection. Inclusive of all previously mentioned PRO subsets, 17 variables were identified and analysis of each was completed by calculating the change from baseline at follow-up time points (3 or 6 months) for all groups. The primary efficacy analysis consisted of ANCOVA in the PROC GLM of the change from baseline accompanied by uncorrected contrasts between treatment group means, where the baseline was included as the covariate at 3 and 6 months.

Responder analysis was conducted using the criteria defined by the OMERACT-OARSI international set of responder criteria.<sup>16</sup> Briefly, subjects were considered a responder in the OMERACT-OARSI simplified criteria if they met either the requirement for high improvement or improvement. For high improvement, subjects must have a  $\geq 50\%$  decrease in pain or increase in function and an absolute change of  $\geq 20$  points. For improvement, subjects must have at least two of the following: (1) improvement in pain  $\geq 20\%$  and absolute change  $\geq 10$ , (2) improvement in function  $\geq 20\%$  and absolute change  $\geq 10$ , and/or (3) improvement in patients' global assessment  $\geq 20\%$  and absolute change  $\geq 10$ . A chi-square test was run to determine significance and p values  $< 0.05$  were considered statistically significant.

## Results

The ASA treatment group consisted of 68 patients (33 females and 35 males) with a mean age of  $55.9 \pm 12.3$  years and a mean BMI of  $27.3 \pm 5.0$  kg/m<sup>2</sup>. The HA treatment group consisted of 64 patients (31 females and 33 males) with a mean age of  $55.4 \pm 11.0$  years and a mean BMI of  $28.2 \pm 4.7$  kg/m<sup>2</sup>, and the normal saline group consisted of 68 patients (31 females and 37 males) with a mean age of  $54.9 \pm 9.9$  years and a mean BMI of  $28.5 \pm 4.2$  kg/m<sup>2</sup>. The medial compartment was the worst affected in most patients, with the lateral and patellofemoral compartments representing between 11.8 and 21.2% of patients depending on group ( $p = 0.3569$ ). KL 2 and 3 grade subjects were included in the study, with KL 2 subjects representing 45.6, 45.3, and 38.2% of the ASA, HA, and saline groups, respectively ( $p = 0.6202$ ). Baseline values for joint space narrowing were reported as  $2.67 \pm 1.26$ ,  $3.00 \pm 1.17$ , and  $2.85 \pm 1.23$  mm for ASA, HA, and saline, respectively ( $p = 0.3065$ ).

Average PRO values for all patients at baseline, 3 months, and 6 months are reported (► **Table 1**). Patients who reported unacceptable pain relief at 3 months, and therefore were withdrawn from the study, were distributed as follows: ASA 9 patients (13.2%), HA 44 patients (68.8%), and saline 51 patients (75%) (► **Table 2**;  $p < 0.0001$ ). In addition, rates of withdrawal before 3 months for reasons other than pain were 1 (1.47%), 3 (4.69%), and 2 (2.94%) for ASA, HA, and saline, respectively (► **Table 2**;  $p = 0.5561$ ). Overall, larger improvements were seen for patients who did not report unacceptable pain than for patients who did (► **Table 2**;  $p < 0.0001$ ). In subjects who reported acceptable pain relief, increases from baseline to 3 months were  $17.64 \pm 1.91$  and  $9.99 \pm 1.50$  for KOOS pain and symptoms; for subjects who reported unacceptable pain relief, scores changed by  $0.16 \pm 1.89$  and  $-0.04 \pm 1.50$ , respectively ( $p < 0.0001$  for the difference between the groups reporting acceptable

versus unacceptable pain relief, for both KOOS subscales). Similar findings were seen for activities of daily living (ADL), sports and recreation, quality-of-life subscales, and VAS overall pain scores (►Table 2;  $p < 0.0001$ , for all).

Change from baseline for all PROs at 3 and 6 months is reported for all groups (►Supplementary Tables 1 and 2). At 3 months posttreatment, patients in the ASA group reported significant improvements in the EQ-5D-5L Pain and Anxiety subsets compared with the HA group (pain  $p = 0.0422$ ; anxiety  $p = 0.0485$ ; ►Supplementary Table 1), and significant improvement in the Overall Health Today subset compared with the saline group ( $p = 0.0141$ ). At 6 months, the ASA treatment group had significant improvement in the mobility (HA  $p = 0.0059$ ; saline  $p = 0.0310$ ), activities (HA  $p = 0.0431$ ; saline  $p = 0.0417$ ), pain (HA  $p = 0.0028$ ; saline  $p = 0.0091$ ), and the Health Today subsets (HA  $p = 0.0296$ ; saline  $p = 0.0090$ ).

Examining the KOOS PROs at 3 months, the ASA treatment group showed significantly greater improvements in pain (►Fig. 2A) and ADL (►Fig. 2C) scores compared with the HA group (pain  $p = 0.0282$ ; ADL  $p = 0.0245$ ), and significantly greater improvements in the symptoms (►Fig. 2B) score compared with both groups (HA  $p = 0.0075$ ; saline  $p = 0.0098$ ). At 6 months, the ASA group showed significantly greater improvement compared with HA in the sports and recreation (S&R; ►Fig. 2D) and quality of life (QOL; ►Fig. 2E) subsets (S&R  $p = 0.0343$ ; QOL  $p = 0.0050$ ), and significantly greater improvement compared with both groups in the pain (HA  $p = 0.0014$ ; saline  $p = 0.0086$ ; ►Fig. 2A), symptoms (HA  $p = 0.0047$ ; saline  $p = 0.0005$ ; ►Fig. 2B), and ADL subsets (HA  $p = 0.0016$ ; saline  $p = 0.0132$ ; ►Fig. 2C).

Evaluating differences in SANE PROs between the groups at 3 months, there were no significant differences in changes from baseline; however, at 6 months, the ASA treatment group had significantly greater improvements in scores compared with HA ( $p = 0.0229$ ) and saline ( $p = 0.0395$ ) (►Supplementary Table 1). The Tegner Activity Scale did not show any statistically significant differences between groups at 3- or 6-months posttreatment (►Supplementary Table 1).

Using the VAS, we evaluated pain in the following categories: overall pain, pain during strenuous work, pain during sedentary work, and pain during normal daily living (►Supplementary Table 2, ►Fig. 3). The ASA group had significantly greater improvements in overall pain (decreased pain scores) ( $p = 0.0072$ ; ►Fig. 3A), strenuous work ( $p = 0.0320$ ; ►Fig. 3B), and normal daily living ( $p = 0.0015$ ; ►Supplementary Table 2) compared with the HA group at 3 months. At 6 months, ASA treatment resulted in significantly greater improvements in pain scores compared with both HA and saline (HA  $p = 0.0002$ ; saline  $p = 0.0074$ ), and improved strenuous work ( $p = 0.0009$ ) and normal daily living ( $p = 0.0013$ ) scores compared with the HA group. There were no significant differences between treatment groups when comparing pain during sedentary work at 3 or 6 months (►Supplementary Table 2).

A responder analysis was conducted at 6 months utilizing the OMERACT-OARSI criteria. At 6 months, ASA, HA, and saline responder rates for the OMERACT-OARSI simplified criteria were 69.1, 39.1, and 42.6%, respectively ( $p = 0.0007$ ; ►Fig. 4).

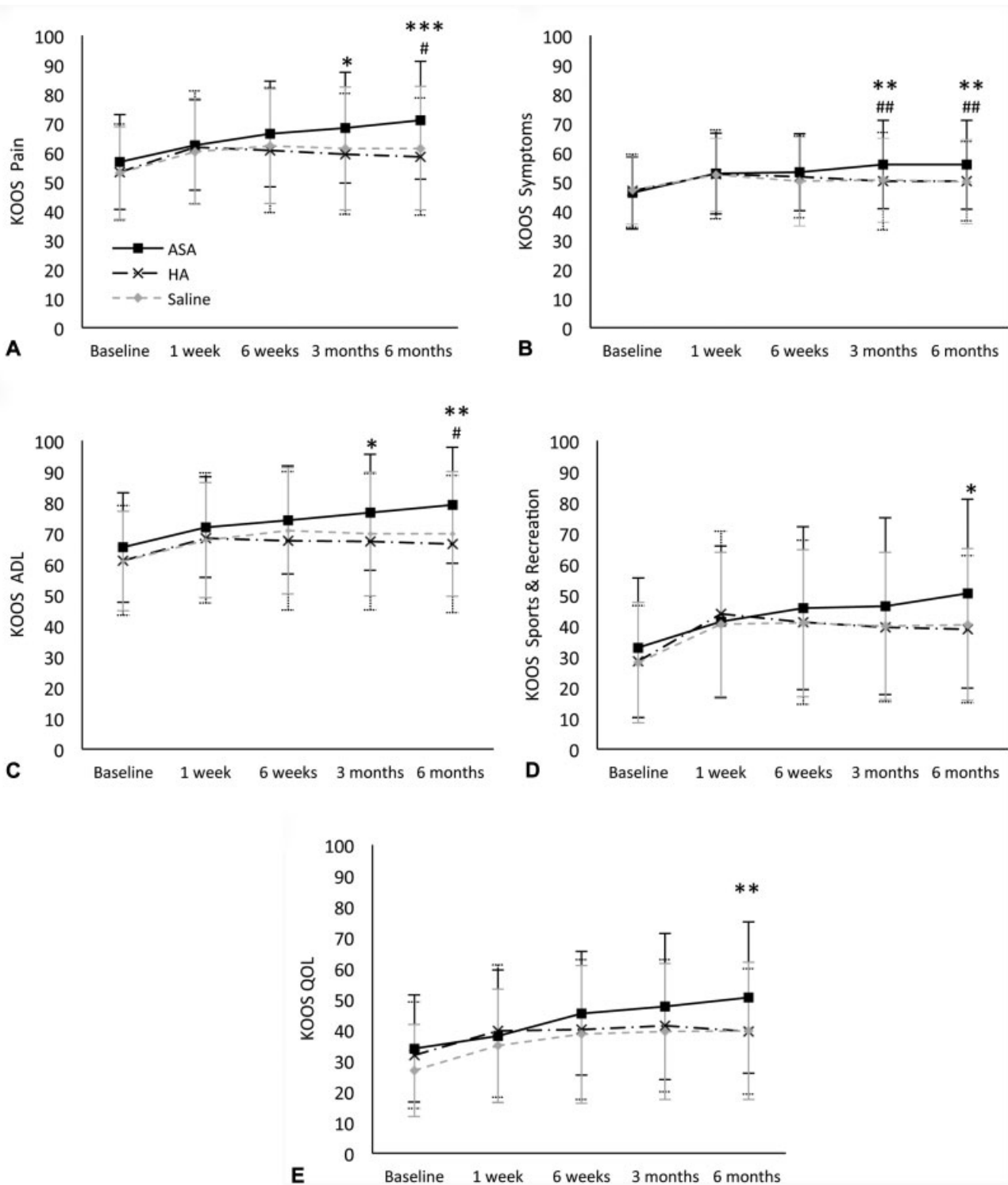
Rates for high improvement and improvement for ASA were also significantly greater than those for the saline and HA groups ( $p = 0.0003$  and  $p = 0.0020$ , respectively; ►Fig. 4).

## Discussion

This is the first randomized controlled trial (RCT) to evaluate the efficacy of ASA for the treatment of knee OA. This study found that the results of treatment with ASA were superior to HA and placebo in this setting. We found significant differences between ASA and HA at 3 months, including EQ-5D-5L pain and anxiety subsets; KOOS pain, symptoms, and ADL subsets; and VAS scores for overall pain, pain during strenuous work, and pain during normal daily living. In addition, ASA was significantly better than saline at 3 months in the KOOS symptoms subsets. At 6 months posttreatment, changes from baseline showed greater improvement for ASA than both HA and saline for several scores including EQ-5D-5L mobility, activities, pain, and health today subsets; KOOS pain, symptoms, and ADL; SANE scores; and VAS overall pain. In addition, responder analysis at 6 months showed a significantly greater responder rate for ASA (69.1%) compared with HA (39.1%) and saline (42.6%) groups. Interestingly, for the ASA group, responder rates at 3 and 6 months were 52.9 and 69.1%, respectively, suggesting delayed onset of action and continued improvement for some patients between 3 and 6 months. In the OARSI-OMERACT responder validation paper by Pham et al using these criteria to evaluate an IA knee OA specific drug, similar percentages were seen for the active and placebo groups (70.6 and 43.6%, respectively) as reported in this study.<sup>16</sup> This is the first study of a placental-derived injection for OA; however, these results compare favorably to other studies evaluating single IA injections, including a high-molecular-weight HA (58.9% for HA, 51.2% for saline at 26 weeks)<sup>17</sup> and a cross-linked HA (61.0% for HA, 54.6% for saline at 13 weeks).<sup>18</sup>

IA injections for OA include a variety of therapies including steroids,<sup>11,19,20</sup> HA,<sup>19,21,22</sup> PRP,<sup>23-25</sup> bone marrow aspirate concentrate (BMAC),<sup>24,26</sup> adipose-derived mesenchymal stem cells (AD-MSCs),<sup>27</sup> and autologous protein solution (APS),<sup>28</sup> with varying levels of supporting evidence. In this study, we focused on HA and saline as comparators to ASA for treatment of OA. HA is a polysaccharide that was approved by the FDA as a "device" to provide viscosupplementation to the joint.<sup>19</sup> Although current science suggests a potential pharmacologic impact that extends beyond the simple effects of joint lubrication,<sup>21</sup> these studies are limited. In one systematic review, low-molecular-weight HA formulations were found to have more inconsistent results, while high-molecular-weight HA showed improved pain relief and function scores following three injections compared with conservative care or placebo groups.<sup>19</sup> AAOS CPG meta-analysis notes that high-molecular-weight HAs were more effective than formulations with lower molecular weights, with 9 of 12 statistically significant placebo-controlled pain outcomes utilizing HA formulations of at least 2.4 million Daltons.<sup>6</sup>

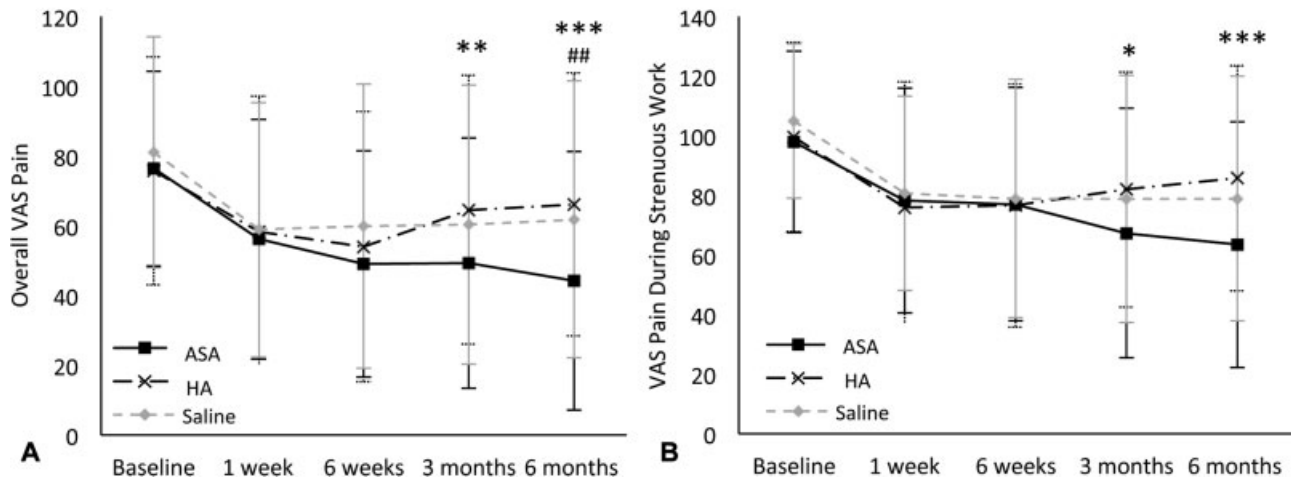
Many studies, including the current investigation, use IA saline injection as control for RCTs. However, recent reviews



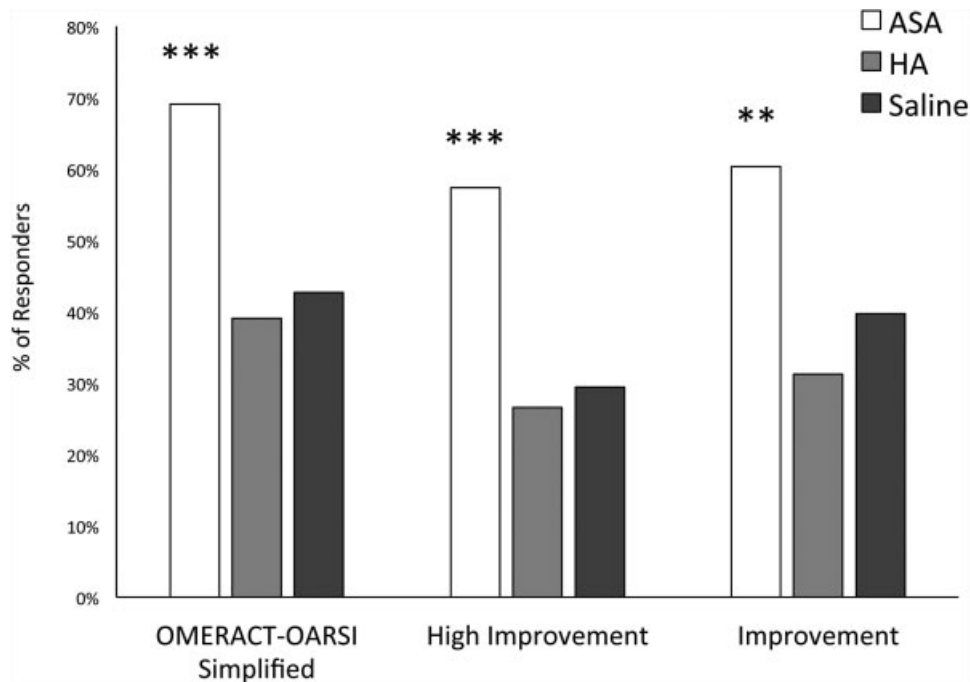
**Fig. 2** Knee Injury and Osteoarthritis Outcome Scores (KOOS). Average  $\pm$  standard deviation reported for (A) KOOS pain, (B) KOOS symptoms, (C) KOOS activities of daily living (ADL), (D) KOOS sports and recreation, and (E) KOOS quality of life (QOL). *p* Values were determined using an analysis of covariance. \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$ , for HA compared with ASA; # denotes  $p < 0.05$ , ### denotes  $p < 0.001$ , for saline compared with ASA. ASA, amniotic suspension allograft.

of the published literature suggest potential benefits of saline that extend beyond a pure placebo effect.<sup>29,30</sup> It has been hypothesized that saline may exert beneficial effects by reducing proinflammatory cells and molecules and provide temporary pain relief.<sup>29</sup> In a meta-analysis by Saltzman et al, 14 placebo cohorts over 13 studies were evaluated.<sup>30</sup> Their

results showed statistically significant improvements in VAS scores at 3 and 6 months, and statistically significant improvements at 6 months for WOMAC scores. These studies suggest that IA saline may not technically be a placebo and that instead, saline injection results in a clinically meaningful improvement in PROs for up to 6 months. In this study,



**Fig. 3** Visual analog scale (VAS) scores. Average  $\pm$  standard deviation reported for (A) overall VAS pain and (B) VAS pain during strenuous work. *p*-Values were determined using an analysis of covariance. \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$ , \*\*\* denotes  $p < 0.001$  for HA compared with ASA; ## denotes  $p < 0.01$  for saline compared with ASA. ASA, amniotic suspension allograft; HA, hyaluronic acid.



**Fig. 4** Responder analysis. Responders in each group at 6 months were calculated using the OMERACT-OARSI criteria. At 6 months, responder categories, including OMERACT-OARSI simplified, high improvement, and improvement, were reported. A chi-squared test was run to determine significance; \*\* denotes  $p < 0.01$  and \*\*\* denotes  $p < 0.001$ . ASA, amniotic suspension allograft; HA, hyaluronic acid.

significant improvements over saline were shown for patients treated with ASA at 3 and 6 months.

For KOOS scores, the MID has been reported as an 8- to 10-point change.<sup>13,31,32</sup> In the current study, among patients treated with HA, the change in baseline for KOOS subsets scores, including pain, symptoms, and ADL, at 3 and 6 months were below this MID, while sports and recreation, and quality-of-life values achieved MID (**Supplementary Table 1**). Patients who received ASA achieved the MID for all KOOS subsets (**Supplementary Table 1**). For VAS scores, the MID has been reported as 8 to 13 points<sup>33,34</sup>; HA changes from baseline values were within the MID range for overall

pain and pain during strenuous work only, while patients receiving ASA achieved the MID range for all the VAS subsets (**Supplementary Table 1**).

Amniotic tissue has been investigated for various applications in wound healing, such as diabetic foot and corneal ulcers. It contains factors that upregulate anabolic and anti-inflammatory pathways relative to those that are catabolic and proinflammatory. Anabolic growth factors identified in amniotic tissue include transforming growth factor- $\alpha$  (TGF- $\alpha$ ), TGF- $\beta$ , basic fibroblast growth factor, interleukin-4 (IL-4), IL-6, IL-8, IL-10, tissue inhibitors of metalloproteinases 1 (TIMP-1), TIMP-2, and TIMP-4, epidermal growth factor, and



platelet-derived growth factor (PDGF)-AA and -BB.<sup>35</sup> Improvements in the anti-inflammatory balance in the OA knee could be affected through upregulation of proteins, such as IL-10 and IL-1 receptor antagonist (IL-1Ra or IRAP).<sup>36</sup> Amniotic tissue is also known to upregulate TIMPs, which inhibit matrix metalloproteinases.<sup>37</sup> A unique molecule of the amniotic membrane has high-density core proteins bonded to HA (hyaluronic acid-binding proteins or HABPs); in addition, AM has free HA. Both may improve joint homeostasis through anti-inflammatory and antifibrotic properties. Last, amniotic tissues also contain PDGF and fibroblast growth factor-18 (FGF-18), which some studies have shown to exhibit chondroprotective properties.<sup>38</sup>

As of this writing, there are two preclinical studies published on the use of amniotic tissue for OA. In a Lewis rat medial meniscus transection model of OA, Willett et al reported significant reduction in cartilage damage, including less erosions, cartilage attenuation, and focal defects in those receiving dehydrated human amnion/chorion membrane.<sup>39</sup> The same Lewis rat OA model was utilized to study the effects of human cryopreserved, particulate amniotic membrane/umbilical cord (AM/UC).<sup>40</sup> The authors reported significant reduction in cartilage degeneration and improvement in the OARSI histological joint score of rats receiving AM/UC.

Vines et al reported a clinical study on the effect of ASA (identical to the one used in this study) on symptoms of knee OA.<sup>12</sup> This was an open-label pilot safety study of six patients with advanced knee OA (KL grade 3 or 4). All subjects had IA injections with 2 mL of ASA. As this was a first in-man study, each patient was closely monitored with an immunologic panel, as well as white blood cell count, CRP, and ESR levels. No clinically significant changes in these parameters were noted and no adverse events were reported throughout the 12-month study. While there were trends of improvement in PROs, (SANE, International Knee Documentation Committee, and KOOS), this safety study was not powered to allow for meaningful statistical analysis.

Our study has limitations; the design was single-blinded, rather than double-blinded. While the initial design considered double-blinding, this was abandoned due to the obvious differences in viscosity between the injectates (saline, HA, ASA) that made blinding of the injector impossible. However, the primary outcome parameters were patient-reported, thus reducing or eliminating the influence of an unblinded investigator. Due to ethical concerns of requiring patients reporting unacceptable pain control to continue with the study, withdrawal was allowed at 3 months, limiting subsequent data recording. However, using the LOCF technique, an accepted method in similar trials, in this setting carried forward a poor result that is unlikely to improve over time. Due to varying HA formulations (molecular weight, cross linking, etc.), results and conclusions of this study may not be applicable to other HA products.

To address potential bias of two investigators with financial involvement with ASA, a multicenter design was employed as well as limiting subjects enrollment at these sites. Furthermore, an independent statistician was used to perform post hoc subanalyses for bias. The first included

analyses of the primary efficacy variables included in the analysis; to do this, a site grouping that pooled two sites (J.F. and A.H.G.) compared with the remaining sites was defined as a categorical factor with two levels. This factor was then included in the primary efficacy analysis as a factor and as an interaction factor with randomized treatment. The primary analysis was an ANCOVA with baseline score as a continuous covariate and with treatment as a factor. The investigation for bias included the primary efficacy variables, including KOOS Pain Score, the KOOS Function (ADL) score, and VAS Pain score. The interaction of treatment and the site grouping (pooled [J.F. and A.H.G.] vs. other sites) demonstrated interaction *p* values > 0.40 in all cases, indicating no discernable response by treatment for the different site groups. A second analysis centered on the rates of withdrawal due to inadequate response to the primary injection at 3 months between the pooled two sites (J.F. and A.H.G.) compared with the remaining study sites. Evaluation of the percentage of patients withdrawn in pooled sites compared with remaining sites showed no significant differences. In sum, there were no significant differences in the two sites with potential investigator bias (J.F. and A.H.G.) compared with the remaining sites as measured by primary outcome measures and withdrawal rate, indicating no significant outcome bias by these investigators.

In conclusion, this randomized single-blinded controlled multicenter trial of ASA demonstrated both statistically significant and clinically meaningful improvements in symptoms of knee OA that exceeded those in the control groups of saline and HA.

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#### Conflict of Interest

Dr Farr reports grants and personal fees from Organogenesis during the conduct of the study and personal fees from Organogenesis outside the submitted work. Dr Gomoll reports grants and personal fees from Organogenesis, grants and personal fees from Vericel, personal fees from Moximed, and grants and personal fees from JRF, outside the submitted work. Dr Yanke reports grants from Organogenesis, during the conduct of the study; grants and other from Arthrex, Inc.; grants and other from JRF Ortho, outside the submitted work. Dr Strauss reports grants from Organogenesis during the conduct of the study and personal fees from Organogenesis outside the submitted work, personal fees from Vericel, and personal fees from Joint Restoration Foundation, outside the submitted work. Dr Mowry reports that she is an employee of Organogenesis Inc.

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