Cryopreserved Amniotic Suspension for the Treatment of Knee Osteoarthritis

Jeremy B. Vines, MS[1,2] Antonios O. Aliprantis, MD, PhD[3] Andreas H. Gomoll, MD, PhD[4] Jack Farr, MD[5]

[1] Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, Alabama
[2] NuTech Medical—Research and Development, Birmingham, Alabama
[3] Division of Rheumatology, Immunology and Allergy, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts
[4] Department of Orthopaedic Surgery, Brigham and Women's Hospital, Boston, Massachusetts
[5] OrthoIndy—Cartilage Restoration Center, Greenwood, Indiana

Address for correspondence Jack Farr, MD, OrthoIndy—Cartilage Restoration Center, Greenwood, IN 46143 (e-mail: jfarr@orthoindy.com).

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Abstract

There are few treatment options for symptomatic knee osteoarthritis (OA). Human amniotic suspension allografts (ASA) have anti-inflammatory and chondroregenerative potential and thus represent a promising treatment strategy. In anticipation of a large, placebo-controlled trial of intra-articular ASA for symptomatic knee OA, an open-label prospective feasibility study was performed. Six patients with Kellgren–Lawrence grades 3 and 4 tibiofemoral knee OA were administered a single intra-articular ASA injection containing cryopreserved particulated human amnion and amniotic fluid cells. Patients were followed for 12 months after treatment. No significant injection reactions were noted. Compared with baseline there were (1) no significant effect of the ASA injection on blood cell counts, lymphocyte subsets, or inflammatory markers and (2) a small, but statistically significant increase in serum IgG and IgE levels. Patient-reported outcomes including International Knee Documentation Committee, Knee Injury and Osteoarthritis Outcome, and Single Assessment Numeric Evaluation scores were collected throughout the study and evaluated for up to 12 months. Overall, this study demonstrates the feasibility of a single intra-articular injection of ASA for the treatment of knee OA and provides the foundation for a large placebo-controlled trial of intra-articular ASA for symptomatic knee OA.

Keywords

- osteoarthritis
- viscosupplementation
- amnion
- stem cells
- allograft

Osteoarthritis (OA) is the leading cause of chronic disability in the United States,[1] affecting more than 27 million people annually. The pathophysiology of OA is multifactorial and has been recently reviewed.[2–4] The initiating event is thought to be an insult to the articular cartilage related to injury, biomechanics, genetics, or prior joint inflammation. Furthermore, as individuals age, decreased proteoglycan and water within cartilage increases its susceptibility to degradation.[5] The cartilage degradation products promote release of inflammatory cytokines, which increase the expression of degradative enzymes. A vicious cycle is then established wherein cytokines, abnormal biomechanics, and catabolic enzymes propagate the remodeling processes.[3,4] Therapies that suppress inflammation and promote regenerative pathways may arrest this cycle and thus hold promise for the treatment of this disease.

Currently, there are few effective treatments for OA, and none prevent disease progression. Medications that reduce pain, such

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as acetaminophen, opiates, and nonsteroidal anti-inflammatory drugs (NSAIDs), provide only temporary relief and frequently lead to adverse events, especially in the patient population suffering from OA.[6] Intra-articular corticosteroids are also commonly used; however, the therapeutic effect is small and benefits may be short term.[7] In an attempt to combat proteoglycan loss and improve joint lubrication, hyaluronic acid (HA), the main source of proteoglycans within the synovial fluid, is injected into the joint space. However, studies have demonstrated a modest clinical benefit of HA injections.[8–10] This could be because although HA may increase cartilage water content, improve joint cushioning, and decrease inflammation,[11,12] it is unclear if it interrupts the degenerative process.

Over the past decade, some molecular targets have been identified as mediators of OA. These targets include inflammatory cytokines and pathways, such as interleukin-1 (IL-1) and complement,[2,4] growth factors including transforming growth factor-β (TGF-β), and enzymes, such as matrix metalloproteinases and aggrecanases.[3,4] While some of these targets are promising, they may yield therapies with high risk-to-benefit ratios. For example, inhibition of IL-1 and complement may predispose to infection and blockade of TGF-β may cause autoimmunity.[13,14] Thus, a safe and effective therapeutic strategy for OA is a major unmet medical need.

Human amniotic suspension allografts (ASA) contain human amniotic membrane (HAM) and human amniotic fluid–derived cells (HAFCs). HAM and HAFCs have documented use in multiple clinical scenarios, including the treatment of burns,[15] ulcers,[16] foot and ankle wounds,[17] and orthopedic applications such as in arthrotomies and atrophic arthritis dating back as far as 1938.[18] HAM and HAFCs exhibit properties that may help alleviate the symptoms of OA and prevent disease progression. First, amniotic tissues contain anti-inflammatory factors such as IL-10 and IL-1 receptor antagonist, as well as tissue inhibitors of matrix metalloproteinases 1, 2, 3, and 4.[19–21] Second, much like bone marrow and adipose-derived mesenchymal stem cells (MSCs), when introduced to inflammatory cytokines, HAFCs upregulate anti-inflammatory pathways including IL-10, indoleamine 2,3,-dioxygenase, TGF-β1, and soluble human leukocyte antigen G5.[22–24] Third, HAM contains a high content of HA as well as small amounts of proteoglycans.[25,26] Lastly, HAFCs have the capacity to differentiate into chondrocytes.[27–29] These observations have led to the investigational use of human amnion–derived tissues as a carrier in autologous chondrocyte implantation.[25] It is this combination of anti-inflammatory and chondroregenerative properties that make HAM and HAFCs a promising option for OA patients. Thus, in anticipation of a larger, blinded trial, an open-label pilot study was conducted to assess the feasibility of an intra-articular injection of ASA for knee OA, and to gather preliminary information on safety and efficacy.

Methods and Materials

Trial Design and Disposition

This was an open-label pilot study of cryogenically preserved ASA delivered by a single intra-articular injection for the alleviation of moderate to severe OA. The primary goal of this study was to assess the feasibility of injection of ASA for the treatment of knee OA. Secondary goals included assessing safety and obtaining preliminary efficacy data. The trial was conducted in compliance with current good clinical practice standards and in accordance with the principles set forth under the Declaration of Helsinki in its latest revised version (2004). The study was performed at Orthopaedics Indianapolis (Indianapolis, IN) under a protocol approved by the Western Institutional Review Board (WIRB). Each patient who participated in the study signed an IRB-approved informed consent form.

Patients

Six patients that met the following inclusion and exclusion criteria were enrolled. Inclusion criteria included age 18 years or over and radiographic Kellgren–Lawrence (KL) grade 3 or 4 tibiofemoral knee OA. Tibiofemoral knee OA KL grade was determined using standing, weight-bearing anteroposterior, and Rosenberg (flexion, weight bearing) X-ray radiographs. For the purposes of this study, KL grade 3 OA was defined by the presence of definite joint space narrowing with KL grade 4 exhibiting marked joint space narrowing. Notable exclusion criteria included a history of diabetes mellitus, morbid obesity (defined as a body mass index of 40 or greater), rheumatoid arthritis or other autoimmune disorders, organ transplantation, NSAID use 15 days prior, or corticosteroid or viscosupplementation injections within the previous 3 months. Individuals with symptoms of meniscal displacement as defined by locking, intermittent block to range of motion or loose body sensation, or surgery within either 6 or 12 months on the contralateral or index knee, respectively, were also excluded.

Study Treatment

Patients received a commercially available single, intra-articular injection of a cryogenically preserved ASA consisting of particulated HAM and HAFCs. The ASA evaluated in this study was processed in a GMP (good manufacturing practice)-compliant facility in accordance with all applicable standards of the Food and Drug Administration and the American Association of Tissue Banks. Both the HAM and amniotic fluid components were obtained during elective cesarean section from consenting donors without donor pooling. Two milliliters of the ASA (ReNu, NuTech Medical, Birmingham, AL) was thawed, volume expanded with sterile 0.9% saline to 4 mL, and injected into the knee via a superior lateral approach using standard of care sterile injection technique. Patients were assessed pretreatment and prospectively evaluated at 1 and 2 weeks, and at 3, 6, and 12 months posttreatment.

Safety

Safety assessments were performed at each follow-up visit and included recording new medical diagnoses, vital signs, and a standard physical examination of the index joint. Routine laboratory data were obtained at baseline and at every follow-up visit and included a comprehensive metabolic profile, creatinine (Cr) and liver function

tests, and a complete blood count. Inflammatory markers, including C-reactive protein (CRP) and an erythrocyte sedimentation rate (ESR), were obtained at baseline, 2 weeks, and 6 months. T, B, and natural killer (NK) cell lymphocyte subsets were measured at baseline, 2 weeks, and 3 months. Serum was assayed for IgG, IgA, IgM, and IgE levels at baseline, 3 months, and 12 months.

Patient-Reported Outcomes

Patient-reported outcomes (PROs) were collected at baseline and at every follow-up visit. Knee pain was assessed using a Single Assessment Numeric Evaluation (SANE) scale and the pain (P) subscale of the Knee Injury and Osteoarthritis Outcome Score (KOOS) assessment.[30] Functional assessments were performed using the International Knee Documentation Committee (IKDC) scale, and the KOOS adjusted daily living (ADL), quality of life (QOL), and sports and recreation (SR) subscales.[31] The symptoms (S) subscale of the KOOS assessment was also determined. An overall KOOS score ($KOOS_5$) was obtained by averaging each of the KOOS subscale assessment scores.[32,33]

Statistical Methods

PRISM (Graphpad, La Jolla, CA) was used for all statistical analyses. For general and immunologic laboratory data, a repeated measure one-way analysis of variance was performed to assess significant differences between values at each time point relative to baseline. Due to the small patient population assessed, it was determined that statistical testing would not be appropriate for PRO data.

Results

Baseline Patient Data

Six patients with KL grade 3 or 4 tibiofemoral OA were recruited for a single injection of ASA containing a cryopreserved suspension of particulated HAM and HAFCs. Age, gender, baseline values for ESR and CRP, and baseline $KOOS_5$, IKDC, and SANE scores are provided in ►**Table 1**.

Adverse Events

The injections all proceeded without immediate complication. Two of the six patients experienced a transient increase in pain in the injected knee that resolved by the 2-week visit. None of the six patients developed infection, or acute or chronic inflammatory reactions, such as effusion or stiffness, in the injected knee. None of the six patients developed any new medical diagnoses during the 12-month follow-up period.

General and Immunologic Laboratory Panels

Throughout the 12 months of follow-up, compared with baseline values, no significant differences in hematocrit, white blood cell count, platelet count, Cr, CRP, or ESR were identified (►**Table 2**). A nonstatistically significant reduction in CRP was observed between the baseline and the 2-week and 6-month time points. Given the potential immunosuppressive effects of MSCs,[32,33] immunologic phenotyping was done with available clinical laboratory tests for lymphocyte subsets (T, B, and NK cell), as well as for immunoglobulin (IgG, IgA, IgE, IgM) levels (►**Table 3**). No changes were observed between baseline and the 2-week, 3-month, and 12-month visits in the fraction of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ T-cells, $CD19^+$ B-cells, and $CD56^+$ NK cells within the lymphocyte gate in peripheral blood. While total IgA, IgE, and IgM levels did not change between 0 and 3 months, a statistically significant 5 to 20% increase ($p < 0.05$) in total IgG was observed. At the 12-month time point, statistically significant increases ($p < 0.05$) in IgG and IgE of ~15% were observed from baseline for both. None of the IgG levels rose above the normal reference range. One patient had an elevated IgE level above the normal reference range; however, this elevation was also present at the baseline measure. Given the increased IgG levels at 3 months, a serum protein electrophoresis was checked at the 12-month visit. One of the six patients had a positive IgG lambda monoclonal band at the lower limit of detection.

Patient-Reported Outcomes

All six patients were assessed at baseline, 1 week, 2 weeks, 3 months, 6 months, and 1 year using both patient-reported

Table 1 Baseline patient characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (y)	55	65	55	44	58	55
Sex	F	F	F	M	M	F
OA KL grade	3	3	3	3	3	3
ESR (mm/h)	15	13	15	10	1	11
CRP (mg/dL)	13.3	6.52	13.3	2.15	0.71	18.2
$KOOS_5$	39.2	52.2	39.2	46.8	ND	35.2
IKDC	44.83	54.02	44.83	37.93	ND	29.89
SANE	50	65	50	20	70	ND

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IKDC, International Knee Documentation Committee scale; KL, Kellgren–Lawrence grade; KOOS, Knee Injury and Osteoarthritis Outcome Score assessment; OA, osteoarthritis; SANE, Single Assessment Numeric Evaluation scale.

Table 2 General laboratory parameters

	Baseline	2 wk	3 mo	6 mo	1 y
	Mean (range)	Mean difference from baseline (± 95% CI)			
Cr (mg/dL)	1.03 (0.7–1.5)	0.07 (± 0.1)	0 (± 0.05)	0.12 (± 0.08)	−0.03 (± 0.07)
HCT (%)	45.12% (40.6–54.3%)	−1.3% (± 2.92%)	−2.55% (± 1.27%)	−2.13% (± 1.97%)	0.88% (± 2.61%)
WBC (cells/$mm^3 \times 10^3$)	8.22 (6.5–11.8)	−1.58 (± 1.64)	−0.87 (± 1.33)	−0.98 (± 1.29)	−0.98 (± 1.65)
PLT (cells/$mm^3 \times 10^3$)	241.17 (179–275)	−5 (± 29.27)	1.5 (± 18.89)	3 (± 15.42)	−20.17 (± 27.41)
ESR (mm/h)	13.17 (1–29)	4.17 (± 7.29)	ND	0.83 (± 5.05)	ND
CRP (mg/L)	7 (0.71–18.2)	−3.07 (± 4.45)	ND	−1.13 (± 1.26)	ND

Abbreviations: CI, confidence interval; Cr, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HCT, hematocrit; PLT, platelet count; WBC, white blood cell count.

Table 3 Immunologic parameters

	Baseline	2 wk	3 mo	1 y
	Mean (range)	Mean difference from baseline (± 95% CI)		
$CD3^+$ T cells (% of lymphocytes)	71.5 (57–89%)	−0.17% (± 2.79%)	0% (± 2.26%)	2.83% (± 3.37%)
$CD3^+CD4^+$ T cells (% of lymphocytes)	53.67% (49–60%)	2% (± 3.08%)	−0.17% (± 1.63%)	2.17% (± 1.18%)
$CD3^+CD8^+$ T cells (% of lymphocytes)	17.5% (8–38%)	−2.33% (± 2.61%)	−0.17% (± 1.55%)	0.5% (± 2.25%)
$CD19^+$ B cells (% of lymphocytes)	11.5% (7–17%)	1.17% (± 1.47%)	1.5% (± 1.58%)	1.5% (± 2.25%)
$CD56^+$ NK cells (% of lymphocytes)	12% (3–18%)	−0.83% (± 1.55%)	0.17% (± 1.47%)	−0.83% (± 2.84%)
IgA (mg/dL)	164.17 (107 − 246)	ND	24.5 (± 12.97)	23.83 (± 23.71)
IgG (mg/dL)	749.83 (668–840)	ND	120.83 (± 67.06)[a]	119.33 (± 78.44)[a]
IgM (mg/dL)	120.67 (38–282)	ND	−7 (± 5.99)	6.33 (± 17.2)
IgE (mg/dL)	33 (14–57)	ND	0 (± 2.86)	5.5 (± 4.13)*

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IgE, immunoglobulin E; NK, natural killer.
[a]$p < 0.05$.

pain and functional measures. These included the P, ADL, QOL, S, and SR subscales of the KOOS and an overall KOOS subscale average ($KOOS_5$) (►**Fig. 1**), as well as IKDC (►**Fig. 2**) and SANE (►**Fig. 3**) scores. At the baseline evaluation, two patients did not complete the PRO surveys for KOOS, IKDC, and SANE (►**Tables 1** and **4**), and at the 2-week time point, one patient did not complete the KOOS survey. For this reason, we elected to display averages across all patients for each time point as opposed to determining the relative change compared with baseline as was done for the general and immunologic laboratory parameters. The figures (►**Figs. 1–3**) and scores described below reflect all available patient data. Reference data for ►**Figs. 1** to **3**, including individual PRO data for all time points across all patients, are reported in ►**Table 4**. The $KOOS_5$ outcome score increased from a baseline of 43.35 to 70.23 by the 1-year time point. The IKDC assessment increased from an average score of 41.7 at baseline to 63.4 at 6 months.[31] This improvement was maintained at the 1-year time point with an average of 64.4. SANE scores[30] increased from an average of 51.25 at baseline to 87.3 at 6 months. This improvement was maintained at the 1-year time point, with an average score of 85.8. Due to the small patient population assessed, it was determined that statistical testing would not be appropriate for PRO data.

Discussion

We achieved our primary goal to demonstrate the feasibility of an intra-articular injection of cryogenically preserved human ASA for patients suffering from knee OA. At a single site, six patients were recruited based on our inclusion and exclusion criteria, and injected with the ASA without difficulty or immediate complications. No acute or chronic inflammatory reactions were observed in the injected knee, and patients were followed with a clinical exam, multiple PRO measures, and periodic laboratory monitoring. Based on this feasibility study, a multicenter randomized controlled trial to compare intra-articular cryogenically preserved human ASA to both placebo and an HA derivative for the treatment of symptomatic knee OA is planned.

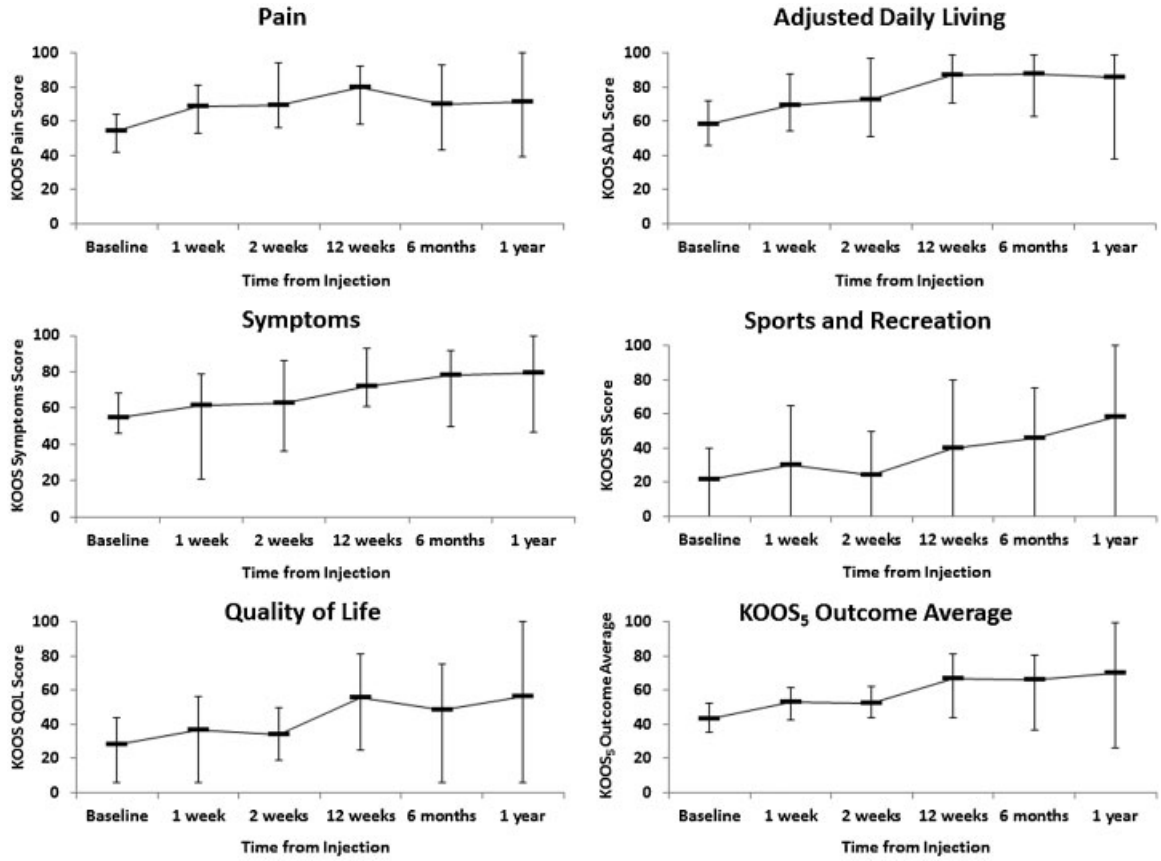

Fig. 1 Average KOOS subscale scores over time. All KOOS subscales including ADL, QOL, SR, S, and P were obtained at baseline and at each follow-up visit. An overall KOOS score ($KOOS_5$) was obtained by averaging together all KOOS subscale assessment scores. Data represent the average ± minimum and maximum values for all available data.

The small size of this feasibility study, lack of a control group, and large placebo effects seen in knee OA trials[34] preclude any interpretation regarding efficacy of the investigational agent for symptomatic knee OA. However, the observed improvements in the KOOS and its associated subscales, as well as the IKDC and SANE scores, would be substantial if they are reproducible in the controlled trial and differ from placebo and HA by a clinically significant margin.

No concerning changes were observed in this small number of patients in renal function, blood cell counts, or lymphocyte subsets. While patients exhibited statistically significant increases in IgG and IgE relative to baseline, none of the values increased above the reference range of the laboratory, except for the level of IgE in one patient who had a baseline elevation in this immunoglobulin class. One of the six patients had a positive IgG lambda monoclonal band at

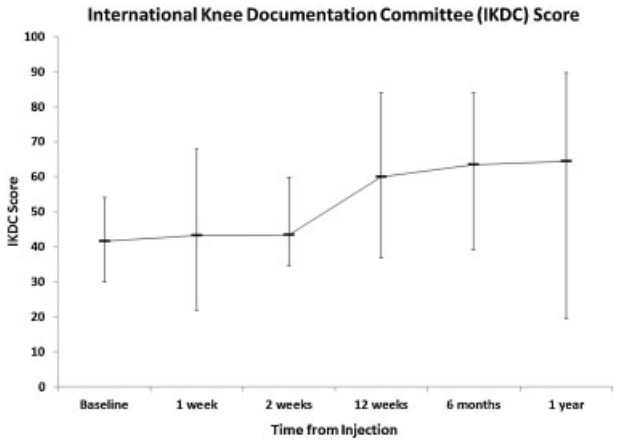

Fig. 2 Average IKDC scores over time. In order to assess functional outcomes, an IKDC assessment was performed at baseline and at each follow-up visit. Data represent the average ± minimum and maximum values for all available data.

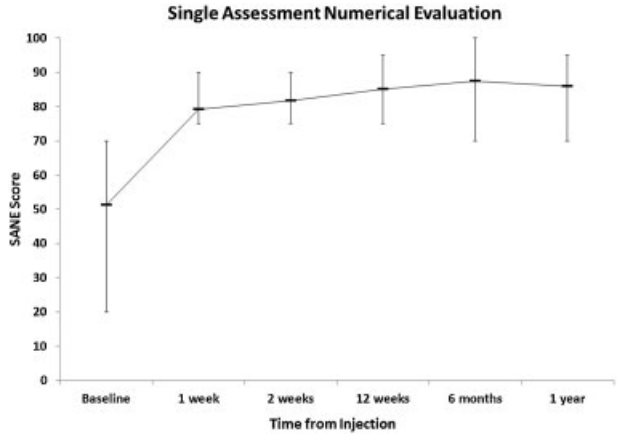

Fig. 3 Average SANE scores over time. To assess perceived pain, a SANE score was obtained at each follow-up visit. Data represent the average ± minimum and maximum values for all available data.

Table 4 Patient-reported outcome reference data

		Baseline	1 wk	2 wk	12 wk	6 mo	1 y
KOOS P	Patient 1	53	53	61	67	75	75
	Patient 2	N/A	58	67	58	57	39
	Patient 3	N/A	72	69	86	43	64
	Patient 4	64	75	56	83	89	100
	Patient 5	42	72	N/A	92	93	93
	Patient 6	58	81	94	92	64	57
KOOS S	Patient 1	46	57	61	61	81	83
	Patient 2	N/A	71	68	64	50	47
	Patient 3	N/A	21	36	64	92	92
	Patient 4	50	71	64	79	86	100
	Patient 5	54	79	N/A	93	83	81
	Patient 6	68	71	86	71	78	75
KOOS ADL	Patient 1	53	60	65	74	82	93
	Patient 2	N/A	54	65	71	63	38
	Patient 3	N/A	78	85	94	99	99
	Patient 4	63	65	51	87	96	99
	Patient 5	46	71	N/A	99	99	97
	Patient 6	72	88	97	96	87	87
KOOS SP	Patient 1	0	5	5	25	45	65
	Patient 2	N/A	0	0	0	0	0
	Patient 3	N/A	65	50	80	75	95
	Patient 4	40	45	50	75	70	100
	Patient 5	15	5	N/A	10	30	20
	Patient 6	30	60	15	50	55	70
KOOS QOL	Patient 1	44	56	50	69	75	81
	Patient 2	N/A	31	19	25	13	6
	Patient 3	N/A	50	44	81	69	88
	Patient 4	44	44	38	56	63	100
	Patient 5	19	31	N/A	63	63	44
	Patient 6	6	6	19	38	6	19
$KOOS_5$	Patient 1	39	46	48	59	72	79
	Patient 2	N/A	43	44	44	37	26
	Patient 3	N/A	57	57	81	76	88
	Patient 4	52	60	52	76	81	100
	Patient 5	35	52	N/A	71	74	67
	Patient 6	47	61	62	69	58	62
IKDC	Patient 1	45	39	34	55	68	75
	Patient 2	N/A	22	43	37	39	20
	Patient 3	N/A	68	60	84	84	90
	Patient 4	54	51	39	56	74	82
	Patient 5	30	45	N/A	62	67	62
	Patient 6	38	36	41	66	49	59

Table 4 (*Continued*)

		Baseline	1 wk	2 wk	12 wk	6 mo	1 y
SANE	Patient 1	N/A	75	80	80	70	70
	Patient 2	65	75	75	75	90	90
	Patient 3	50	75	85	85	100	90
	Patient 4	20	90	90	95	90	80
	Patient 5	70	75	75	85	89	95
	Patient 6	N/A	85	85	90	85	90

Abbreviations: ADL, adjusted daily living; IKDC, International Knee Documentation Committee scale; KOOS, Knee Injury and Osteoarthritis Outcome Score; P, pain subscale; QOL, quality of life subscale; S, symptom subscale; SANE, Single Assessment Numeric Evaluation scale; SR, sports and recreation subscale.

the lower limit of detection at the 12-month time point. However, given the low level of this monoclonal protein, and the fact that an ASA is likely to contain many alloantigens and thus elicit a polyclonal response, it was felt to be unrelated to the study agent. The observed increase in IgG levels is not unexpected, as similar studies involving administration of high doses of MSCs, as well as other allograft matrices, have found that they are likely to produce a humoral response. However, this humoral response does not appear to promote inflammation or other concerning reactions.[35,36] As an example, in one study patients who had been implanted with frozen, cortical bone grafts exhibited IgG levels that had increased by 12 weeks following surgery without modification to any of the other studied parameters including CRP, C3 complement factor, rheumatoid factor, and other immunoglobulins, demonstrating that humoral responses did not result in systemic inflammation.[37] Whether the increase in immunoglobulins in this and previous studies reflects a nonspecific polyclonal response or a polyclonal humoral reaction against specific ASA antigens is unknown.

If specific polyclonal immunological responses occur in patients that receive ASA, the dominant antigens are currently unclear. The investigational agent is biphasic, containing both cellular and a growth factor/matrix-rich components. Although the concentration of viable cells within this raw, unprocessed amniotic fluid preparation is relatively low,[38,39] effective standard cryogenic preservation methodology[40] and the enhanced expansionary capabilities of these cells compared with other adult-derived stem cell types, such as bone marrow–derived MSCs,[41,42] make their final concentration in vivo difficult to determine. Thus, polymorphic cell surface and intracellular molecules are one potential source of alloantigens. ASA also contains particulated HAM, which is a reservoir for growth factors, extracellular matrix proteins,[19–21,25,26,43,44] and other potential alloantigens. Currently, the relative contribution of each component to the current application, with regard to potential efficacy or immunological responses, is unclear. Elucidating the mechanism of action of ASA components for knee OA will be an important future direction. Furthermore, while the changes in serum immunoglobulins we observed do not appear to be of clinical concern, a potential specific humoral reaction to ASA, either after primary administration or upon retreatment, will need to be monitored in future studies, as these responses may impact efficacy and tolerability.

Conclusion

Overall, this open-label pilot study indicates that a single intra-articular injection of ASA is feasible in patients with knee OA. In this small cohort, intra-articular ASA was safe and did not result in laboratory changes consistent with immunosuppression or inflammation. A larger, placebo-controlled trial is underway to evaluate the efficacy and safety of intra-articular ASA for the treatment of symptomatic knee OA.

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