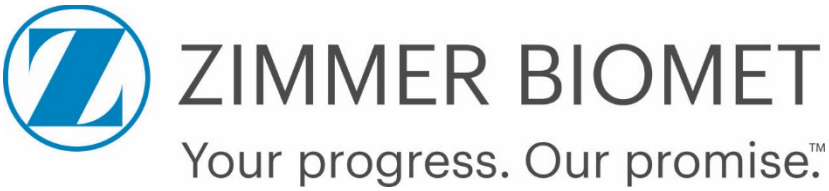

CLINICAL STUDY PROTOCOL

Protocol Title:	A Multicenter, Double-Blind, Randomized, Saline-Controlled Study of a Single, Intra-Articular Injection of Autologous Protein Solution in Patients with Knee Osteoarthritis
Short Title:	Saline-Controlled Study of nSTRIDE APS for Knee Osteoarthritis
Protocol Number:	APSS-44-00
Study Sponsor:	Biomet Biologics, LLC 56 East Bell Drive Warsaw, IN 46581 USA
Protocol Version:	Version 5.0
Version Date:	06 August 2019

SPONSOR PROTOCOL APROVAL PAGE

The signatories have read and understood the clinical protocol and agree to conduct the clinical investigation in compliance with this protocol:

Protocol approved by Scientific Affairs:
Ann Blanton
Clinical Project Lead (Study Manager), Biologics
Zimmer Biomet

Ann Blanton (Aug 9, 2019)

Signature Date

Protocol approved by Research:
Jennifer Woodell-May, Ph.D.
Associate Director of Research, Biologics
Zimmer Biomet

Jennifer WOODELL-MAY (Aug 9, 2019)

Signature Date

Version 5.0, 06 August 2019

INVESTIGATOR PROTOCOL AGREEMENT PAGE

I agree:

- To assume responsibility for the proper conduct of the study at this site and supervise all testing of the device involving human subjects
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by the Sponsor.
- To ensure that the requirements for obtaining informed consent from each subject are met.
- Not to implement any changes to the protocol without written agreement from the Sponsor and prior review and written approval from my institutional review board except where necessary to eliminate an immediate hazard to patients.
- That I am thoroughly familiar with the appropriate use of the study device, as described in this protocol and any other information provided by the Sponsor.
- That I am aware of, and will comply with, good clinical practice (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the investigational device and have been trained on their study-related duties and functions as described in the protocol.

Signature: _____ Date: _____

Name (print): _____
Investigator

STUDY CONTACT INFORMATION

Responsibility	Name and Address	Phone/E-mail Contact
Study Sponsor	Joel Higgins Senior Director, Biologics Zimmer Biomet 56 East Bell Drive Warsaw, IN 46582 USA	**Phone** Office: 574.372.1734 Mobile: 574.527.6964 **Email** joel.higgins@zimmerbiomet.com
Sponsor Project Manager	Ann Blanton Clinical Project Lead, Biologics Zimmer Biomet 56 East Bell Drive Warsaw, IN 46582 USA	**Phone** Office: 574.371.3095 Mobile: 574.253.5556 **Email** ann.blanton@zimmerbiomet.com
Sponsor Regulatory Affairs Manager	Susan Mack Regulatory Affairs Manager, Biologics and Sports Medicine Zimmer Biomet 56 East Bell Drive Warsaw, IN 46582 USA	**Phone** Office: 574.372.6894 Mobile : 765.490.0521 **Email** susan.mack@zimmerbiomet.com
Monitoring Lead	Christian Barille Lead Clinical Research Associate IMARC Research, Inc. 22560 Lunn Road Strongsville, OH 44149 USA	**Phone** Office: 440.801.1540 Mobile: 440.823.3898 **Email** cbarille@imarcresearch.com

STUDY SYNOPSIS

Protocol Number:	APSS-44-00
Title:	A Multicenter, Double-Blind, Randomized, Saline-Controlled Study of a Single, Intra-Articular Injection of Autologous Protein Solution in Patients with Knee Osteoarthritis
Sponsor:	Biomet Biologics, A Zimmer Biomet Company
Name of Product:	nSTRIDE APS Kit
Device Description:	The nSTRIDE autologous protein solution (APS) Kit with anticoagulant citrate dextrose solution, formula A (ACD-A) is a self-contained, sterile-packaged, single-use device designed to concentrate anti-inflammatory cytokines and growth factors from whole blood. The device system is to be used at the point of care to create an autologous solution. This device system consists of two parts: the nSTRIDE Cell Separator and the nSTRIDE Concentrator. The nSTRIDE Cell Separator separates the cellular components from plasma and red blood cells in whole blood. The cell solution is then loaded into the nSTRIDE Concentrator, which uses filtration through polyacrylamide beads to concentrate the cytokines in the injectable output.
Intended Use:	The nSTRIDE APS Kit is designed to be used for the safe and rapid preparation of autologous protein solution (APS) from a small sample of blood at the patient's point of care. The APS is to be injected intra-articularly for the treatment of knee osteoarthritis and associated symptoms or knee pain associated with osteoarthritis.
Study Center(s):	The study will be conducted at up to 30 investigative centers in the United States.
Planned Sample Size:	332 subjects will be randomized (1:1) into one of two treatment groups (APS and Saline).
Study Population:	Patients with symptomatic osteoarthritis (OA) in one knee, who have not been able to get satisfactory pain relief with prior treatment.
Study Objectives:	**Primary Objective** The primary objective of this study is to determine whether nSTRIDE APS is superior to a saline with regard to the mean improvement from baseline to 12 months in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK

	3.1 pain score. **Secondary and Exploratory Objectives** Secondary objectives of this study include determining whether nSTRIDE APS is superior to saline in improving the WOMAC function subscale score (as evaluated using the percentage of subjects showing at least the minimal clinically important difference (MCID)), and the WOMAC pain subscale score (as evaluated using the percentage of subjects showing at least the minimal clinically important difference (MCID)), OMERACT-OARSI responder rates, analyzing WOMAC pain and function in only the KL-II Subgroup, evaluating superiority of APS over saline in improving Visual Analog Scale (VAS) pain, assessment of the changes in WOMAC Pain scores over time, and evaluation of the usage of rescue and restricted medication. Additionally, this study will evaluate change in joint morphology and APS output characteristics. Safety of nSTRIDE APS will be compared to saline following intra-articular knee injections in subjects with early to moderate symptomatic OA.
Study Design and Procedures:	The study will compare the efficacy of nSTRIDE APS to saline in patients with early to moderate symptomatic osteoarthritis (OA) in one knee, who have failed at least one prior conservative OA therapy (e.g. physiotherapy, simple analgesics). This will be done using a double-blind, multicenter, randomized, controlled trial (RCT) with study subjects receiving either a single injection of nSTRIDE APS or saline. The primary efficacy measure will be pain as measured on the WOMAC LK 3; other measures of efficacy will include function, stiffness, and quality of life. In addition to clinical efficacy measures, safety will be assessed by tracking adverse events. Anatomical changes will be evaluated by magnetic resonance imaging (MRI) and radiographs (X-ray), and key factors in baseline blood and APS will be characterized. Laboratory characterization procedures will be performed using validated, prospectively defined methods. During the screening process, potential subjects will provide informed consent and then be screened for eligibility. Screening will consist of meeting all inclusion and exclusion criteria, including a WOMAC LK 3.1 pain subscale score ≥ 9 and ≤ 19 and by providing objective physiological evidence of OA using the Kellgren-Lawrence scale (assessed from normal radiographs). Subjects will also provide demographics and

	medication use information as part of this process. Baseline X-ray (if needed) and MRI will be collected. Within 28 days of the screening visit, subjects will return to the clinic for treatment. Subjects will complete the baseline outcomes measurements including the EuroQol questionnaire (EQ-5D), and visual analog scale (VAS) for knee pain. The WOMAC score recorded at the screening visit will serve as the baseline (pre-injection) WOMAC score, and the WOMAC questionnaire will not be re-administered prior to treatment. During the treatment visit, all subjects will have a blood draw, from which the APS will be prepared for injection and for laboratory characterization. The nSTRIDE APS Kits will be processed for all subjects in both treatment groups. After all available joint fluid is aspirated and, according to randomization group assignment, approximately 2.5 milliliters (ml) of APS or saline will be injected into the joint. Needle placement in the joint will be verified using ultrasound. A blinding sleeve covering the contents of the syringe will mask treatment assignment from the subject and the injecting physician. Any adverse events associated with the blood draw and/or injection procedure will be recorded. All subjects will be instructed to refrain from exceeding the pre-injection level of activity for 14 days. Efficacy and safety will be assessed at 1, 3, 6, and 12 months post injection. Concomitant treatment for OA will be standardized to be oral acetaminophen (e.g. Tylenol) for all subjects during the study. Subjects will be asked to abstain from analgesic use for 48 hours prior to assessments. They will complete the WOMAC, EQ-5D, and VAS for pain. An X-ray and MRI will be obtained at 12 months to assess anatomical changes. All images will be transferred to the central core lab for independent review. Image acquisition, transfer, and analysis procedures will be performed using validated, prospectively defined methods. After each subject completes all 12 month follow-up evaluations, individual treatment allocation will be unblinded; subjects from both groups will be permitted to enter a one month open-label repeat treatment phase if they have had no major safety events due to the first injection. One month after the second injection, subjects will complete the WOMAC, EQ-5D, and VAS for pain.

Study Duration:	Maximum study duration per subject is 16 months: 12 months from treatment to last follow-up, 1 additional month for follow-up after second injection, and 3 months if the maximum visit window time is realized.
Inclusion Criteria:	1. Male or female ≥ 21 and ≤ 80 years old at time of screening. 2. Willingness and ability to comply with the study procedures and visit schedules and ability to follow oral and written instructions. 3. A standing radiograph of the knee showing a Kellgren-Lawrence grade of 2 to 4 and an absence of severe osteoarthritis (defined as advanced stage osteoarthritis, including large osteophytes, chronic fractures or bone remodeling, severe deformity or bone attrition, and/or bone-on-bone contact indicative of severe osteoarthritis/full thickness cartilage loss), as confirmed by the central imaging laboratory. 4. Body mass index ≤ 40 kg/m^2. 5. A WOMAC LK 3.1 pain subscale total score ≥ 9 and ≤ 19. 6. Has undergone at least one prior conservative osteoarthritis treatment (e.g. physical therapy, simple analgesics). 7. Signed an institutional review board approved informed consent.
Exclusion Criteria:	1. Presence of clinically observed active infection in the index knee. 2. Presence of symptomatic osteoarthritis in the non-study knee; if unclear then the WOMAC LK 3.1 pain sub-scale for the non-index knee must be ≤ 5.0. 3. Diagnosed with rheumatoid arthritis, Reiter's syndrome, psoriatic arthritis, gout, ankylosing spondylitis, or arthritis secondary to other inflammatory diseases; HIV, viral hepatitis; chondrocalcinosis, Paget's disease, or villonodular synovitis. 4. Clinically symptomatic patellofemoral chondromalacia (i.e. knee pain in the anterior knee only) or diagnosis of isolated patellofemoral OA. 5. Diagnosed with leukemia, known presence of metastatic malignant cells, or ongoing or planned chemotherapeutic treatment. 6. Disease of spine, hip or other lower extremity joints judged by the investigator to be contributing to the pain in the index knee (i.e. sciatica, nerve pain, hip OA). Note: Patients with knee replacement at the contra-

	lateral knee or hip replacement in either hip may be enrolled provided there is sufficient pain relief after knee replacement or hip replacement that analgesics are not required. 7. Untreated symptomatic injury of the index knee (e.g., acute traumatic injury, anterior cruciate ligament injury, clinically symptomatic meniscus injury characterized by mechanical issue such as locking or catching). 8. Any condition other than OA of the index knee which, in the opinion of the investigator, affects the ability to ambulate to a sufficient degree to interfere with the assessment of the safety and treatment effects of the study injection. 9. Presence of surgical hardware or other foreign body intended to treat arthritis or cartilage-related pathology in the index knee. 10. Previous cartilage repair procedure on the injured cartilage surface (i.e., microfracture, osteoarticular transfer system (OATS) and autologous chondrocyte implantation (ACI)) of the index knee. 11. Arthroscopy or open surgery of the index knee within 6 months of screening. 12. Intra-articular steroid injection in the index knee within 3 months of screening. 13. Intra-articular hyaluronic acid injection in the index knee within 6 months of screening. 14. Other intra-articular therapy in the index knee within 6 months prior to screening. 15. Orally administered systemic steroid use within 2 weeks of screening. 16. Planned/anticipated surgery of the index knee during the study period. 17. A history of local anesthetic allergy. 18. Use of systemic immunosuppressants within 6 weeks of screening. 19. Currently on anticoagulant therapy, such as Warfarin, vitamin K antagonists, direct thrombin inhibitors, or factor Xa inhibitors or on potent anti-platelet therapy, such as GPIIb-IIIa antagonists, Par-1 antagonists or dual anti-platelet therapy, i.e. an ADP receptor antagonist in combination with aspirin. 20. Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and

	treatment effects of the study injection. 21. Skin breakdown at the knee where the injection is planned to take place. 22. Pregnant or nursing mothers or women planning on getting pregnant during the time they will be participating in the study. 23. Known drug or alcohol dependence currently or within the last year. 24. Participated in any investigational drug or device trial within 30 days prior to screening. 25. Participated in any investigational biologic trial within 60 days prior to screening.
Schedule of Visits:	• Screening • Procedure (within 28 days of screening) • 1 Month (± 7 days) • 3 Month (± 14 days) • 6 Month (± 14 days) • 12 Month (± 28 days) • (Optional) Second Injection (within 14 days of 12 Month visit) • Second Injection Follow-Up (if needed) will occur within 1 month of second injection (± 7 days)
Clinical Assessment Tools:	<u>The Western Ontario and McMaster Universities Osteoarthritis Index using the Likert scale, Version 3.1:</u> The WOMAC LK 3.1 questionnaire is a validated tool used for assessing knee pain, stiffness, and function. The WOMAC LK 3.1 has 24 items; 5 items assessing knee pain, 2 items assessing knee stiffness, and 17 items assessing physical function. Each item is answered on a 5-point Likert scale, with grading from 0 (none or never) to 4 (extreme or always). A higher score indicates worse pain, stiffness, or functional limitation. <u>The EuroQol-5 Dimensions</u> The EuroQol-5 Dimensions (EQ-5D) is a validated instrument which assesses an individual's current health status and heath related quality of life. The EQ-5D-3L descriptive component assesses five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression over three levels of severity. The EQ visual analogue scale (EQ VAS) assesses the respondent's self-rated overall health state on a scale from 0 (worst imaginable health state) to 100 (best imaginable health state). <u>Visual Analog Scale (VAS) for knee pain</u>

	The VAS is a validated measure of knee pain. The VAS is a nominal 100 mm line anchored by 0 “no pain” and 100 “worst possible pain”. Subjects rate their average pain over the last 24 hours by indicating their level of pain with a slash mark through the line.
Imaging Assessment Tools:	Radiographs Standing posterior-anterior (PA) fixed flexion knee radiographs (X-rays) are used to assess structural features of the joint, including joint space width, and presence or absence of subchondral sclerosis, subchondral cysts, and osteophytes. X-ray assessments will be performed by a core imaging laboratory. Magnetic Resonance Imaging Magnetic Resonance Imaging (MRI) has been shown to be sensitive to structural changes in cartilage. Specifically, quantitative assessment via T2 MR relaxation mapping is used to detect early compositional changes in cartilage. In addition, semi-quantitative assessment via MRI Osteoarthritis Knee Score (MOAKS), is used to evaluate the following: • Subchondral Bone • Cartilage • Meniscus • Peri-Articular Features Magnetic Resonance Imaging (MRI) assessments will be performed by a core imaging laboratory.
Laboratory Characterization:	Each subject will have an additional nSTRIDE APS Kit processed for laboratory characterization. The APS output from the additional kit, along with a baseline blood sample and synovial fluid (if available), will be sent to a core laboratory for analysis. Analyses will include quantification of cellular content and key cytokines and growth factors. Laboratory characterization procedures will be performed using validated, prospectively defined methods.
Primary Endpoint:	The primary endpoint will be the change in pain from baseline to 12 months following injection of nSTRIDE APS or saline, as measured by the WOMAC LK 3.1 pain subscale.
Primary Hypothesis for nSTRIDE Superiority	The primary hypothesis to be tested is that the mean improvement in WOMAC pain subscale score (baseline to 12 months) in the APS group will be greater than that of the saline group. The primary hypothesis described below will be tested along

Testing:	with the secondary hypotheses using a pre-specified order of hypotheses. These tests will be performed at the 0.05 level. If the first null hypothesis is rejected, the second test will be performed at the 0.05 level. If the first hypothesis is not tested significantly, the second test, and all subsequent tests, will be performed as exploratory analyses. A two-tailed independent sample T-test will be used to test the primary endpoint. The hypothesis will be: H_0: $\mu_{APS} = \mu_{Control}$ Versus H_A: $\mu_{APS} \neq \mu_{Control}$ Where: μ_{APS} = mean change in WOMAC Pain from baseline to 12 months in the APS group, and $\mu_{control}$ = mean change in WOMAC Pain from baseline to 12 months in the control group. The impact of the usage of APS on function will be evaluated as part of the primary endpoint; however, no formal statistical test will be done as a part of the study success criteria. A qualitative assessment of the changes in WOMAC Function over time for APS and Saline will be discussed, in order to ensure that the impact of the treatment on function is neutral or positive. The following descriptive analyses will be performed for this assessment of function: (1) A graphical examination of the changes in WOMAC Function for APS and Saline over time, including means and standard error bars. (2) Descriptive statistics for the mean changes in WOMAC Function over time, separately for each treatment group. These will include mean, median, standard deviation, minimum, maximum, and 95% confidence intervals. For each analysis, it is expected that the changes in WOMAC function will remain neutral or increase over time within the APS group, and also that the mean changes in WOMAC Function over time will be at least nominally better for the APS group than for Saline. A finding of nSTRIDE APS superiority on the mean improvement in WOMAC LK 3.1 Pain along with no corresponding deterioration in WOMAC Function will be considered evidence of nSTRIDE APS efficacy, and the device will be considered efficacious for the treatment of knee pain

	associated with osteoarthritis.
Secondary Endpoints:	Secondary objectives of this study include determining whether nSTRIDE APS is superior to saline with regard to the endpoints shown below. These tests will be performed if the primary null hypothesis has been rejected. If the primary null hypothesis is not rejected, these analyses will be conducted as exploratory analyses only. These secondary hypotheses will be tested using a fixed-sequence procedure, constructed using a pre-specified order of hypotheses, which will be tested in the order below. Subsequent to the rejection of the primary null hypothesis, these tests will be performed at the 0.05 level for each consecutive test until one hypothesis is tested not significantly, after which all subsequent tests will not be performed as secondary analyses, but instead will be performed as exploratory analyses only.

Order of Testing	Secondary Outcomes
1	WOMAC MCID Function Responder rate
2	WOMAC MCID Pain Responder rate
3	Mean WOMAC Function Δ (12 Month minus Baseline)
4	OMERACT-OARSI Responder / Non-responder (12 Month)
5	Mean WOMAC Pain Δ (12 Month minus Baseline) in K-L II Subgroup
6	Mean WOMAC Function Δ (12 Month minus Baseline) in K-L II Subgroup
7	Use of rescue medication (acetaminophen) use (for index knee OA) over time
8	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of rescue medication within 48 hours of the 12 month visit for knee OA as a covariate
9	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of rescue medication within 48 hours of the 12 month visit for any reason as a covariate
10	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of restricted medication within 48 hours of the 12 month visit for knee OA as a covariate
11	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of restricted medication within 48 hours of the 12 month visit for any reason as a covariate
12	Mean VAS Pain Δ (12 Month minus Baseline)
13	Mean WOMAC Pain changes over time within treatment

Exploratory Endpoints:	Exploratory outcomes include assessment of change in quality of life, pain, function, and stiffness from baseline to all post-injection time points. Exploratory analyses will also include evaluation of changes in joint morphology, determined with MRI images and X-ray images, as well as analyses related to cell/cytokine content of blood and APS.

Table of Contents

ABBREVIATIONS AND TERMS

ACD-A	Anticoagulant Citrate Dextrose Solution-Formula A
ACI	Autologous chondrocyte implantation
ACS	Autologous conditioned serum
ADE	Adverse device effect
ADP	Adenosine diphosphate
AE	Adverse event
APS	Autologous protein solution
BMI	Body mass index
CRF	Case report form
EDC	Electronic Data Capture
EQ-5D	EuroQol – 5 Dimensions
FDA	Food and Drug Administration (US)
GPIIb-IIIa	Glycoprotein IIb and IIIa
GCP	Good clinical practice
HA	Hyaluronic acid
ICF	Informed consent form
IGF-1	Insulin-like growth factor 1
IL-1β	Interleukin-1 beta
IL-1ra	IL-1 receptor antagonist
IRB	Institutional Review Board
JSN/JSW	Joint Space Narrowing/Joint Space Width
K-L	Kellgren-Lawrence
ml	Milliliter
MMP	Matrix metalloproteinase
MOAKS	MRI Osteoarthritis Knee Score
MRI	Magnetic Resonance Imaging
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OATS®	Ostechondral Autograft Transfer System
OMERACT-OARSI	Outcome Measures in Rheumatology – Osteoarthritis Research Society International
PA	Posterior-Anterior
Par-1	Protease-activated receptor 1
sIL-1RII	Soluble form of IL-1 receptor II
sTNF-RI,sTNF-RII	Soluble forms of TNFα receptor I and receptor II
SAE	Serious adverse event
SAP	Statistical analysis plan
TGF-β1	Transforming growth factor beta 1
TNFα	Tumor necrosis factor alpha
UADE	Unanticipated adverse device effect
VAS	Visual Analog Scale
WBC	White blood cell
WOMAC LK 3.1	Western Ontario and McMaster Universities Osteoarthritis index using the Likert scale, Version 3.1

1 INTRODUCTION

Osteoarthritis (OA) is a degenerative and disabling articulating joint disease that affects both younger, more active patients (e.g., patients with trauma or who have prolonged participation in highly demanding sports) and the elderly (1-4). The disease is progressive and debilitating, eventually resulting in pain that may be so severe that restive sleep is impossible, along with life-altering loss of function.

Surgical intervention is clinically successful, and widely used, in treating severe degenerative OA; however, treatment modalities for less advanced OA are associated with varying—and often disappointing—rates of success. Current treatment options include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroid injections, and hyaluronic acid (HA) injections. Although these treatments can relieve pain temporarily for some OA patients, they do not address the biological mechanisms causing the disease (5). Most are palliative. They mask symptoms.

Osteoarthritis causes chronic pain, cartilage degradation and loss, detrimental subchondral bone remodeling, and varying degrees of synovial inflammation. OA pain is a complex response resulting from the interplay between inflammation, anatomic pathology, innervation of articular cartilage, nerve sensitization, and psychological factors. Inflammation associated with OA results in joint stiffness and pain. Patients may experience local warmth, tenderness, and effusion (6). Although OA is classified as a non-inflammatory disease, inflammation is implicated in many symptoms and in OA progression. Pro-inflammatory cytokines are involved in OA development (7-12). These cytokines include interleukin-1 (IL-1), tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and interleukin-8 (IL-8). These proteins are integral in the initiation and maintenance of inflammation by mediating cell-to-cell interactions. Evidence exists that these factors are out-of-balance in the OA knee (13). Of these cytokines, IL-1 has been proposed as playing a key role (14-16). The cytokines associated with inflammation in OA, primarily interleukin-1 beta (IL-1β) and TNFα, are also implicated in cartilage matrix breakdown (17-19). These cytokines induce cells in the joint to produce matrix metalloproteinases (MMPs) that in turn are responsible for cartilage matrix degradation (20). The interactions between chondrocytes in articular cartilage, synovium, IL-1 and TNFα result in a positive feedback loop that increases inflammation and cartilage breakdown further and is associated with cartilage repair attenuation. IL-1, TNFα, IL-6, and IL-8, along with nerve growth factor, can also lead to nerve sensitization and stimulation (21).

Because both IL-1β and TNFα play important roles in inflammation and cartilage breakdown, inhibition of these cytokines may limit inflammation and matrix degradation. Consequently, inhibition of these proteins may constitute an effective OA therapy. The anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1ra), a competitive IL-1 receptor antagonist, blocks the signaling activity of IL-1 (and has no signal-inducing activity itself) (22-24). Soluble forms of the IL-1 cell receptor (sIL-1R) can bind with IL-1, reducing IL-1 biologic activity by preventing it from binding to surface receptors on the cells (25). Moreover, soluble forms of the cell

receptors for TNFα, known as sTNF-RI and sTNF-RII, can bind to TNFα, preventing TNF-α surface receptor binding and thus inhibiting cell signaling (26).

These anti-inflammatory cytokines are present systemically, but not in the concentrations or locations that may be clinically beneficial in the treatment of knee OA. Autologous Protein Solution (APS), produced using the nSTRIDE APS Kit, contains concentrated levels of anti-inflammatory cytokines, including IL-1ra, sIL-1RII, sTNF-RI, and sTNF-RII. APS also contains concentrated levels of anabolic cytokines associated with cartilage genesis, including insulin-like growth factor 1 (IGF-I) and transforming growth factor β1 (TGF-β1) (27). Balancing these cytokines by autologous conditioned serum (ACS) injection has been explored. ACS is an autologous acellular plasma serum containing proteins. ACS contains up-regulated levels of anti-inflammatory cytokines and has shown promise when compared to hyaluronic acid (HA) and saline for OA treatment (28). APS builds upon knowledge gained through ACS studies. It is designed to halt and potentially reverse the OA disease process by rebalancing cytokine activity.

In summary, inflammatory and catabolic cytokines are strongly implicated in the OA degenerative process. Inhibiting their action may be beneficial clinically. Anti-inflammatory and anabolic cytokines found in and concentrated from whole blood may reduce or reverse the degenerative process. Processing whole blood using the nSTRIDE APS Kit can substantially increase anti-inflammatory and anabolic cytokine concentrations. Thus, the introduction of concentrated levels of anti-inflammatory and anabolic cytokines in a targeted fashion with APS yields a more favorable intra-articular environment. A likely result is the reduction and potentially the reversal of the degenerative impact of out-of-balance inflammatory and catabolic cytokines present in the OA knee.

The study proposed here builds upon decades of research into the causes of osteoarthritis. This study is designed to determine whether rebalancing of cytokines with APS prepared using the nSTRIDE APS Kit will, as suggested by OA literature, finally yield an effective treatment for early to moderate osteoarthritis of the knee that targets the causes of this painful and debilitating disease.

1.1 Preclinical Studies

1.1.1 Mechanism of Action Cell Assays

The proposed mechanism of action is a process of reducing OA-related upregulated inflammatory cytokines by introducing cytokines that inhibit inflammatory cytokine activity. APS has been shown to reduce production of proteins associated with inflammation and pain responses. In an *in vitro* model of inflammation and pain reduction, APS was incubated with IL-1-stimulated macrophages. APS decreased IL-8 production from these activated macrophages (29). Another study examined the effect of APS on cartilage degradation. APS inhibited IL-1- and TNFα-induced chondrocyte production of MMP-13, a known degradation enzyme of cartilage (30). Further, inhibition of MMP-13 activity, specifically glycosaminoglycan release, was also exhibited in cartilage explant cultures stimulated with IL-1 and APS (31). These

cell assay studies demonstrate that APS can inhibit deleterious enzyme production, consistent with the proposed mechanism of action.

1.1.2 Cytokine Concentration Study

Analysis of APS samples and baseline whole blood samples from 105 OA patients revealed that the APS device concentrates IL-1ra and other anti-inflammatory cytokines 3 to 5 fold over that of whole blood baseline concentrations (27).

1.1.3 Equine Clinical Study

An animal efficacy study was performed to evaluate the ability of APS, prepared using the nSTRIDE APS Kit, to alleviate OA pain in a randomized, blinded study of 40 horses. Between April and December 2011, 20 horses were injected with APS, and 20 horses were injected with saline. Lameness was evaluated blindly by a single trained investigator at 1 week and 2 weeks post-injection. A force plate analysis was completed 2 weeks post-injection. At 3 months and 12 months post-injection, the owners completed a survey.

At 2 weeks post-injection, lameness and quantitative force plate mean results were both significantly improved relative to the pre-treatment baseline, and APS results were statistically superior to saline-treated horse results. Similarly, owners reported significant improvements in lameness at 3 months and 12 months in the APS-treated horses. No related adverse events (AEs) were reported. Nor were there differences in joint swelling, as measured by joint circumference, between the APS- and saline-treated animals. There were also no changes from baseline in total protein, total white blood cells (WBCs), or percent neutrophils in joint fluid aspirated from the APS-treated group at 2 weeks post-injection. This study provided safety and efficacy data showing that APS reduces pain and improves function in horses with OA (32).

1.2 Clinical Experience

An open-label feasibility study of a single intra-articular injection of APS in subjects with osteoarthritis of the knee was conducted at Saint Anna Hospital, Geldrop, The Netherlands ((33;34), NCT01773226). The primary study objective was to assess safety. Nine of 11 subjects (seven male) reported 22 AEs (total). There were no deaths or serious adverse events. The investigator deemed every AE to be unrelated to the device. All were rated ‘mild’ in severity. The most frequent AEs were joint effusion (n=9) and arthralgia (n=5). These were most likely related to the injection procedure and not to the device per se. One subject withdrew from the study subsequent to continued knee pain.

WOMAC scores improved significantly by the second week post-injection and continued to improve as the study progressed. By 12 weeks, 80% of both physicians and subjects rated the condition under investigation as ‘very much’ or ‘much’ improved as determined by the Clinician and Patient Global Impression Change Scale. At 26 weeks follow-up, the OMERACT-OARSI high pain responder criteria were met by 8 of 11 subjects (73%). At final follow-up, mean WOMAC pain reduced by 72% (89% in the 8 responders). WOMAC stiffness and function scores improved

by 53% and by 68%, respectively. After study completion, a long-term analysis was performed at an average of 78 weeks (18 months) after subjects were enrolled. Six of the 11 subjects returned WOMAC and Patient Global Impression-Change (PGI-C) questionnaires and reported pain reduction from baseline measures. The data presented here suggest that the treatment is safe and shows a complication profile that is mild and consistent with similar treatments. A single injection of APS for treatment of early to moderate knee osteoarthritis led to symptom improvement over the study course.

After completion of the open-label feasibility study, a multicenter, prospective, randomized, double-blind, saline-controlled trial was conducted at three enrolling centers in Europe ((35), NCT02138890). A total of 46 patients with unilateral OA (Kellgren-Lawrence 2 or 3) knee pain were randomized into two groups. Group 1 (31 patients) received a single ultrasound-guided injection of APS, and Group 2 (15 patients) received a single saline injection. Patient reported outcomes and adverse events were collected at 2 weeks, 1, 3, 6, and 12 months post-injection. The patients and evaluators were blinded to the treatment allocation, and the outcome was evaluated through VAS, WOMAC, and KOOS scores. Imaging evaluation was also performed with X-Ray and MRI before and after the treatment (12 months and 3 and 12 months, respectively).

The demographics were similar between the groups. The change from baseline to 12 months in WOMAC pain score was 65% in Group 1 and 41% in Group 2 ($p = 0.02$). Additionally, VAS pain improvement was 49% in Group 1 and 13% in Group 2 ($p = 0.07$). WOMAC function change from baseline to 12 months was 55% in Group 1 and 45% in Group 2 ($p = 0.38$). The safety profile was also positive, with no significant differences in frequency, severity, or relatedness of adverse events between groups. No procedure- or device-related serious adverse events were reported.

This pilot study provides evidence to support the safety and clinical effectiveness of a single intra-articular injection of this novel autologous therapy. Long-term follow-up is ongoing, and this positive results obtained against saline has been used to plan this confirmatory trial that will be conducted to further substantiate these findings against those offered by other treatments for knee OA.

An additional open-label study is ongoing in the United States (NCT02262364). Recruitment of the planned 10 patients has been completed, and follow-up is ongoing. All patients have completed follow-up through 6 months. There have been no unanticipated adverse device effects reported to date. A single serious adverse event has been reported, and it was unrelated to the procedure or the device.

1.3 Device Description

The nSTRIDE APS Kit is manufactured by Biomet Biologics, LLC, 56 East Bell Drive, P.O. Box 587, Warsaw, IN 46581. The nSTRIDE APS Kit contains two polymer blood processing devices and a 30 milliliter (ml) vial of Anticoagulant Citrate Dextrose Solution-Formula A (ACD-A). The first of the two devices is the nSTRIDE

Cell Separator. It is a plastic tube containing a tuned-density buoy and separates cellular components of whole blood when appropriately cycled in a Biomet Biologics centrifuge. The resulting cell solution is further processed by the second of the two devices, the nSTRIDE Concentrator. This device is a plastic tube containing polyacrylamide beads to further concentrate the cell suspension and produce an injectable output, the APS. The nSTRIDE APS Kit is a self-contained, sterile-packaged, single-use and disposable device used at the point of care to produce APS. There are no anticipated changes in the device design during this trial.

Component Name	Part Number
nSTRIDE APS Kit	800-3000US
nSTRIDE Cell Separator (GPS III)	800-1003-01
nSTRIDE Concentrator	800-3000-02
nSTRIDE APS Kit IFU	01-50-1497
Anticoagulant Citrate Dextrose Solution-Formula A	01-09-9289
Anticoagulant Citrate Dextrose Solution-Formula A IFU	01-05-1496

1.3.1 Indications

The nSTRIDE APS Kit is designed to be used for the safe and rapid preparation of autologous protein solution (APS) from a small sample of blood at the patient's point of care. The APS is to be injected intra-articularly for the treatment of knee pain associated with osteoarthritis.

1.3.2 Contraindications

Do not inject APS in the knees of patients having knee joint infections or skin diseases or infections in the area of the injection site.

nSTRIDE APS Kit is not for use in patients with systemic inflammatory conditions.

nSTRIDE APS Kit is not intended for use in patients with leukemia, metastatic malignant cells or who are receiving chemotherapeutic treatment.

1.3.3 Use and Training

Appropriate investigative site personnel will be trained on nSTRIDE APS Kit processing. The investigator or other personnel who will be administering treatment should have the appropriate medical training to give intra-articular knee injections. Specifics on preparation of APS using the nSTRIDE APS Kit are given in Section 2.6.2.3 – APS Preparation.

1.4 Current Study Rationale

OA is a degenerative disease characterized by chronic pain, cartilage degradation, cartilage loss, subchondral bone remodeling, and varying degrees of synovial inflammation mediated by increased pro-inflammatory and catabolic proteins. Currently available OA therapies address the symptoms, but none is known to address the underlying pathology of this disease. Analysis of APS, prepared using the nSTRIDE APS Kit, clearly shows multiple fold increases (relative to whole blood) of anti-inflammatory and anabolic proteins. *In vitro* and non-human animal study results are consistent with the idea that APS can—in a targeted area—inhibit

deleterious proteins, increase beneficial proteins, delay cartilage breakdown, and deliver reduction in OA pain and improvement in joint functionality. Moreover, preliminary clinical trial results in humans provide evidence that these benefits extend to human use as well.

Whole blood-derived products such as ACS and platelet-rich plasma have a substantial history of clinical use with no report of any serious adverse events (SAEs). Collectively, (1) the demonstrated safety profile, (2) the evidence of clinical utility, and (3) the potential for disease modification by APS, justify continuing human clinical trials of the nSTRIDE APS Kit for OA treatment. Evidence shows that a pivotal human trial is a safe and reasonable next step in validating APS, prepared using the nSTRIDE APS Kit, as a safe and effective minimally invasive pain-reducing therapy for OA, one that *in vitro* findings suggest may have the potential to reverse the disease process.

1.5 Study Objectives

The primary objectives of this study are to determine whether nSTRIDE APS is superior to a saline with regard to the mean improvement from baseline to 12 months in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.1 pain subscale score.

The impact of the usage of APS on function will be evaluated as part of the primary endpoint; however, no formal statistical test will be done as a part of the study success criteria. A qualitative assessment of the changes in WOMAC Function over time for APS and Placebo will be discussed, in order to ensure that the impact of the treatment on function is neutral or positive. The following descriptive analyses will be performed for this assessment of function:

(1) A graphical examination of the changes in WOMAC Function for APS and Placebo over time, including means and standard error bars.
(2) Descriptive statistics for the mean changes in WOMAC Function over time, separately for each treatment group. These will include mean, median, standard deviation, minimum, maximum, and 95% confidence intervals.

Secondary and exploratory objectives of this study include determining whether nSTRIDE APS is superior to saline in improving patient-reported outcomes including function, pain, stiffness, and quality of life in subjects with early to moderate symptomatic OA.

Additionally, this study will evaluate change in joint morphology and APS output characteristics. Safety of nSTRIDE APS will be compared to saline following intra-articular knee injections in subjects with early to moderate symptomatic OA.

2 INVESTIGATIONAL PLAN

2.1 Study Design

This is a multicenter, double blind, randomized, saline–controlled, prospective evaluation of a single APS injection. The study duration for each subject will be 12 to 16 months. A total of 332 patients will be enrolled. These patients will meet specific inclusion and exclusion criteria but can be generally characterized as patients with painful unilateral knee osteoarthritis who have not been able to get satisfactory pain relief with previous conservative treatment.

2.2 Inclusion Criteria

Subjects must meet all inclusion criteria to be eligible for study enrollment.

1. Male or female ≥ 21 and ≤ 80 years old at time of screening.
2. Willingness and ability to comply with the study procedures and visit schedules and ability to follow oral and written instructions.
3. A standing radiograph of the knee showing a Kellgren-Lawrence grade of 2 to 4 and an absence of severe osteoarthritis (defined as advanced stage osteoarthritis, including large osteophytes, chronic fractures or bone remodeling, severe deformity or bone attrition, and/or bone-on-bone contact indicative of severe osteoarthritis/ full thickness cartilage loss), as confirmed by the central imaging laboratory.
4. Body mass index (BMI) ≤ 40 kg/m^2.
5. A WOMAC LK 3.1 pain subscale total score ≥ 9 and ≤ 19.
6. Has undergone at least one prior conservative osteoarthritis treatment (e.g. physical therapy, simple analgesics).
7. Signed an institutional review board approved informed consent.

2.3 Exclusion Criteria

Subjects must not meet any of the following exclusion criteria to be eligible for study enrollment.

1. Presence of clinically observed active infection in the index knee.
2. Presence of symptomatic osteoarthritis in the non-study knee; if unclear then the WOMAC LK 3.1 pain sub-scale for the non-index knee must be ≤ 5.0.
3. Diagnosed with rheumatoid arthritis, Reiter's syndrome, psoriatic arthritis, gout, ankylosing spondylitis, or arthritis secondary to other inflammatory diseases; HIV, viral hepatitis; chondrocalcinosis, Paget's disease, or villonodular synovitis.
4. Clinically symptomatic patellofemoral chondromalacia (i.e. knee pain in the anterior knee only) or diagnosis of isolated patellofemoral OA.
5. Diagnosed with leukemia, known presence of metastatic malignant cells, or ongoing or planned chemotherapeutic treatment.
6. Disease of spine, hip or other lower extremity joints judged by the investigator to be contributing to the pain in the index knee (i.e. sciatica, nerve pain, hip OA). Note: Patients with knee replacement at the contra-lateral knee or hip replacement in either hip may be enrolled provided there is sufficient pain

relief after knee replacement or hip replacement that analgesics are not required.

7. Untreated symptomatic injury of the index knee (e.g., acute traumatic injury, anterior cruciate ligament injury, clinically symptomatic meniscus injury characterized by mechanical issue such as locking or catching).
8. Any condition other than OA of the index knee which, in the opinion of the investigator, affects the ability to ambulate to a sufficient degree to interfere with the assessment of the safety and treatment effects of the study injection.
9. Presence of surgical hardware or other foreign body intended to treat arthritis or cartilage-related pathology in the index knee.
10. Previous cartilage repair procedure on the injured cartilage surface (i.e., microfracture, Ostechondral Autograft Transfer System (OATS®) and autologous chondrocyte implantation (ACI) of the index knee.
11. Arthroscopy or open surgery of the index knee within 6 months of screening.
12. Intra-articular steroid injection in the index knee within 3 months of screening.
13. Intra-articular hyaluronic acid injection in the index knee within 6 months of screening.
14. Other intra-articular therapy in the index knee within 6 months prior to screening.
15. Orally administered systemic steroid use within 2 weeks of screening.
16. Planned/anticipated surgery of the index knee during the study period.
17. A history of local anesthetic allergy.
18. Use of systemic immunosuppressants within 6 weeks of screening.
19. Currently on anticoagulant therapy, such as Warfarin, vitamin K antagonists, direct thrombin inhibitors, or factor Xa inhibitors or on potent anti-platelet therapy, such as glycoprotein IIb and IIIa (GPIIb-IIIa) antagonists, protease – activated receptor 1 (Par-1) antagonists or dual anti-platelet therapy, i.e. an adenosine diphosphate (ADP) receptor antagonist in combination with aspirin.
20. Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and treatment effects of the study injection.
21. Skin breakdown at the knee where the injection is planned to take place.
22. Pregnant or nursing mothers or women planning on getting pregnant during the time they will be participating in the study.
23. Known drug or alcohol dependence currently or within the last year.
24. Participated in any investigational drug or device trial within 30 days prior to screening.
25. Participated in any investigational biologic trial within 60 days prior to screening.

2.4 Eligibility for Re-Screening

Given the binary nature and time-dependency of many of the eligibility criteria of this study, there are times when a patient may not initially meet the inclusion/exclusion criteria but may eventually become eligible due to elapsed time and/or lifestyle modification or resolution of a pre-existing condition.

For the following parameters, a patient may be re-screened immediately upon resolution of the issue leading to the initial screen failure:

- Age; patient was initially < 21 years of age but is now ≥ 21 years of age
- Inability to comply with the study protocol; patient previously met all study inclusion and no exclusion criteria but was unable to complete MRI or receive injection within 28 day window due to scheduling conflicts
- BMI; patient initially had BMI > 40 kg/m^2 but now has BMI ≤ 40 kg/m^2
- Active infection of index knee; patient had active infection of the index knee which has since resolved
- Symptomatic OA in the non-study knee; patient has had a knee replacement in the non-study knee and is now fully recovered from the surgery and rehabilitation
- Untreated symptomatic injury of the index knee; patient had untreated symptomatic injury of the index knee which has since been successfully treated
- Previous treatment of index knee; patient was previously within the 3 month window (steroid injection) or the 6 month window (arthroscopy, open surgery, hyaluronic acid injection, other intra-articular injection) of previous index knee treatment but now is not
- Orally administered systemic steroid use within the past 2 weeks; patient was previously within the 2 week window of systemic oral steroid use but now is not
- Use of systemic immunosuppressants within the past 6 weeks; patient was previously within the 6 week window of immunosuppressant use but now is not
- Skin breakdown at the knee where the injection is planned to take place; patient previously had skin breakdown but the skin is now intact
- Pregnant or nursing mother; woman who was previously pregnant or nursing is either no longer pregnant or no longer nursing and does not plan to become pregnant or nurse during the study
- Trial participation; patient was previously within the 30 day (drug or device) or 60 day (biologic) window of clinical trial participation but now is not

Given that OA is a degenerative condition, there are times when a patient may not initially be eligible because their OA or associated symptoms are not severe enough but, over time, the condition may become more severe, thereby making the patient eligible for participation in this study. For the following parameters, a patient may be re-screened upon failure of a newly administered OA therapy, provided that the therapy and subsequent failure are documented in the patient's medical records:

Note: Failure of a regularly administered oral or topical medication requires at least 6 weeks of use with inadequate improvement of symptoms.

- Kellgren-Lawrence score; initial Kellgren-Lawrence score was 0 or 1, but there is now reason to believe that the joint degeneration due to the OA condition is more severe
- WOMAC score; initial WOMAC pain score of the index knee was not high enough, but there is now reason to believe that the pain due to the OA condition is more severe
- No documented prior conservative OA treatment; initial screening indicated that previous conservative treatment had not been attempted, but the patient has now failed one or more conservative treatments

If the patient has failed screening for a reason other than any of those listed above, then the patient will not be eligible for re-screening.

If a patient is re-screened, then the screening process should be done all over again, beginning with the informed consent, and a new subject ID should be assigned.

2.5 Concomitant Treatment and Medication

2.5.1 Allowable Medications/Nonpharmacological Therapies

The following medications and nonpharmacological therapies may be taken or used throughout the study:

- Any treatment for a pre-existing condition or for an AE, outside of the study indication, that is not listed as restricted
- Aspirin for cardio-protection at a maximum stable dose of 100 mg per day provided the dose was stabilized over 3 months prior to study entry
- Glucosamine, chondroitin sulfate, or avocado/soya extracts if initiated and consistent prior to study entry
- Physical therapy for the index knee if the program was initiated and consistent prior to study entry
- Bracing of the index knee if initiated and consistent prior to study entry
- Acetaminophen for analgesic treatment of breakthrough OA pain of the index knee (see Section 2.5.3) or other sources of pain that may arise (not to exceed a daily dose of 3000 mg)

2.5.2 Restricted Medications/Nonpharmacological Therapies

Patients will be advised that participation in the study will require them to abstain from certain medications and therapies. The following medications and nonpharmacological therapies should not be taken or used beginning immediately upon enrollment (after signing informed consent) until subject reaches the end of the study:

- Oral nonsteroidal anti-inflammatory drugs (NSAIDs)
- Topical NSAIDs (including plasters and patches) applied to the index knee
- Other topical pain therapies applied to the index knee (e.g., capsaicin, lidocaine, heat patches)

- Orally administered systemic corticosteroids
- Intra-articular corticosteroids administered to the index knee
- Intra-articular hyaluronic acid administered to the index knee
- Narcotics
- Centrally acting medications for analgesia

2.5.3 Allowed Concomitant OA Medication

Concomitant treatment for OA will be standardized to be oral acetaminophen (e.g. Tylenol) for all subjects during the study. During the screening period (i.e., prior to the procedure visit), subjects may take acetaminophen as needed to a maximum of maximum of 3000 mg per day. Use of acetaminophen must be discontinued 48 hours prior to the procedure visit and each subsequent scheduled visit.

The use of acetaminophen for other types of pain or illness during the study (e.g., toothache, headache, fever) should also be recorded on the Follow-Up Visit form.

Beginning at the procedure visit through the end of the study, each subject will be provided with a sufficient quantity of acetaminophen tablets at each scheduled clinic visit. Subjects will be asked to report the frequency of tablet consumption since the previous visit at each follow-up visit.

2.6 Study Assessments and Procedures

This table summarizes clinical study assessments, procedures, and information collected on case report forms (CRFs)

Procedure	Screening	Procedure	1 Month	3 Months	6 Months	12 Months	Optional APS Injection	Optional 2nd Inj 1 Month F/U
		Within 28 days of completed Screening	±7 days	±14 days	±14 days	±28 days	Within 14 days of 12 Month visit	± 7 days
Informed consent	X							
Demographics	X							
Knee radiograph	X[1]					X		
MRI	X					X		
Inclusion/exclusion criteria	X							
Pregnancy test (as applicable)	X						X	
WOMAC LK 3.1	X		X	X	X	X		X
Medication use	X	X	X	X	X	X		X
Blood Draw and Injection Procedure		X					X	
Needle placement ultrasound		X						
Sample preparation and shipment		X						
EQ-5D		X	X	X	X	X		X
VAS knee pain		X	X	X	X	X		X
Adverse events (as applicable)		X	X	X	X	X	X	X
Study Exit[2]						X[2]		X[2]

[1] If qualifying, posterior-anterior fixed flexion radiographs were collected within the previous six months, they may be used

[2] Study exit form should be completed one time only, per subject, when any subject exits a study. It is anticipated that subjects will exit after the 12 month primary follow-up or after the repeat injection 1 month follow-up

2.6.1 Subject Recruitment and Screening

Documented Institutional Review Board (IRB) approval of the protocol and informed consent form (ICF), and a fully executed clinical trial agreement must be obtained prior to subject recruitment.

Subjects will be recruited from the population of individuals with knee OA who have failed at least one prior conservative OA treatment. Subjects must meet the requirements of inclusion and exclusion criteria at screening.

The investigator will keep a log listing all patients who are screened and consented and a subject identification log listing subjects who were enrolled into the study.

The principal investigator or officially designated site personnel will explain the study procedures, set expectations, and go over the informed consent with the patient. The patient will be given an opportunity to discuss the study with one of the investigators, including medical aspects of their disease and the study treatment. The patient will be allowed sufficient time to think about participation in the study and to discuss the study with family, friends, their primary care provider and/or an independent physician. The patient will sign the ICF only when they are satisfied they understand the requirements and want to participate in the study. The patient will sign and date the ICF before any study-required screening procedures occur.

During the screening visit, the following information will be obtained and, as appropriate, recorded in the subject study record and CRFs for all subjects:

- Signed and dated original ICF
- Demographics and Medication Use
- A standing radiograph of the knee confirming a Kellgren-Lawrence grade of 2 to 4 and an absence of severe osteoarthritis. If a subject has qualifying knee X-rays performed within 6 months of screening, then they may be used without repeating the radiograph. Radiographs will be sent to a central imaging laboratory for confirmation of eligibility.
- Pregnancy test (as applicable)
- WOMAC LK 3.1 questionnaire for index knee
- WOMAC LK 3.1 pain scale for non-index knee (as applicable)
- Inclusion and exclusion criteria

Patients who sign the ICF, satisfy the inclusion and exclusion criteria, and agree to the conditions of the study will be eligible to enter the study.

Subjects will be scheduled for an MRI which will be completed prior to injection procedure.

Subjects will be reminded to abstain from restricted medications and therapies as detailed in Section 2.5.

Subjects will be advised to maintain a stable lifestyle with regard to physical activity during the study.

2.6.2 Injection Procedure Visit

During the injection procedure visit, but prior to APS preparation and injection, baseline evaluations will be performed, including:

- EuroQol 5-Dimensions (EQ-5D)
- Visual Analog Scale for knee pain (VAS)
- Medication Use

2.6.2.1 Blood Draws

Specific instructions on how to complete the blood draws, prepare APS for injection, and prepare APS, whole blood, and synovial fluid for shipment for this study will be provided in a separate laboratory processing and shipping instructions document (to be provided separately at a later date).

Note: in total, 3 samples (2 x 60 ml, 1 x 12 ml) of blood will be drawn to produce two APS volumes and 1 whole blood volume. For subjects randomized to the APS treatment group, one (processed) APS volume will be used for treatment. A second (processed) APS volume will be used for analytical testing, and one whole blood sample will be used for analytical testing. For subjects randomized to the saline treatment group, one (processed) APS volume will be used to determine the volume of saline for injection and will be used for analytical testing. A second (processed) APS volume will be used for analytical testing, and one whole blood sample will be used for analytical testing.

If it is not possible to draw the volume of blood required for injection (1 x 60 ml), then that subject should be withdrawn from the study and should not be randomized. Multiple attempts may be made to draw the required volume of blood, provided that the safety of the subject is never compromised. If the volume required for injection is obtained, but the additional volume required for laboratory analysis cannot be drawn, then sample preparation and shipping for analysis should be done according to the instructions provided in the separate laboratory processing and shipping instructions document.

2.6.2.2 Randomization

After the blood draw, subjects will undergo a 1:1 randomization to determine their treatment group. Details regarding Randomization can be found in Section 5.6.1.

2.6.2.3 APS Preparation

Two nSTRIDE APS Kits should be processed for each subject as described below. If a second nSTRIDE APS Kit cannot be processed due to an inability to draw enough blood, fill the second nSTRIDE Cell Separator with water or saline equivalent to the volume of blood being processed in the first nSTRIDE Cell Separator (approximately 60 ml) to act as a counterbalance. Similarly, put water or saline in the second nSTRIDE Concentrator (approximately 6 ml) to act as a counterbalance. Alternatively, if only one nSTRIDE APS Kit is being processed in the centrifuge, then the appropriate nSTRIDE counterbalances may be used.

To process the nSTRIDE APS Kit, using standard aseptic technique, draw 5 ml of ACD-A into a 60 ml syringe. Attach the syringe to an 18-gauge butterfly apheresis needle and prime with ACD-A. Draw 55 ml of whole blood into the syringe and gently mix. This will produce 60 ml of anticoagulated blood. Inject the 60 ml of anticoagulated whole blood into the nSTRIDE Cell Separator. Place the nSTRIDE Cell Separator into the centrifuge with another nSTRIDE Cell Separator so that they counterbalance one another, and run for 15 minutes at 3200 RPM. After centrifugation, using a 30 ml syringe, remove the plasma and discard appropriately. Then, using a 10 ml syringe, extract 2 ml of the cell solution and suspend the cells by vigorously shaking the syringe and nSTRIDE Cell Separator for 30 seconds while the nSTRIDE Cell Separator and corresponding syringe are attached to one another. Extract the remaining cell solution into a syringe. Detach the syringe from the nSTRIDE Cell Separator and inject the cell solution into the upper chamber of the nSTRIDE Concentrator (which contains polyacrylamide beads). Turn the paddle to mix the cell solution with the beads. Place the nSTRIDE Concentrators into the centrifuge so that they counterbalance one another and process for 2 minutes at 2000 RPM.

Using a new 10 ml syringe, extract the final APS product (final APS volume will be approximately 2.5 ml for each device processed).

The first APS preparation should be used for the treatment injection. In the case of a subject randomized to the saline group, the first APS volume should be used to determine the volume of saline to be prepared for injection. The second APS preparation is reserved for testing at the central laboratory. If the subject is randomized to the saline group, then after using the first APS preparation to identify the quantity of saline to be injected, it should be prepared for shipment to the core laboratory for analysis along with the second APS preparation.

Record each APS volume on the Sample Processing Form. A volume of at least 1 ml is required for injection. If a volume of less than 1 ml is obtained from the nSTRIDE APS Kit, then do not inject this APS, and use the Sample Processing Form to record that the nSTRIDE APS Kit did not function as expected due to low output volume. In this circumstance, the second APS volume may be used for the treatment injection. If a volume of less than 1 ml is obtained from both processed kits, then do not perform the injection procedure. The injection procedure may be rescheduled, and additional kits may be used.

For further details of preparing APS using the nSTRIDE APS Kit, consult the product package insert in Supplement 1, training material, or contact the sponsor directly.

If at any point the blood and/or intermediate cell solution and/or APS is transferred from one facility location to another, then the syringe shall be capped with a sterile syringe cap or a sterile capped needle prior to transferring the syringe from one location to another.

2.6.2.4 Injection Preparation and Blinding

Once the APS has been prepared, the delegated unblinded member of the study staff shall prepare a syringe for injection. If the subject has been randomized to the

nSTRIDE APS group, then the syringe containing APS output labeled for injection shall be placed in the blinding sleeve and provided to the injecting physician. If the subject has been randomized to the Saline group, then the syringe containing APS output labeled for injection shall be used to determine the volume of saline to draw up into a new 10 ml syringe. The syringe containing the appropriate volume of saline (equal to the volume of the APS) shall be placed in the blinding sleeve and provided to the injecting physician. Additional details regarding Blinding can be found in Section 5.6.2.

2.6.2.5 Injection Procedure

Administer the contents of the masked syringe (approximately 2.5 ml) as a single injection into the joint. The procedure for the injection involves the following steps:

1. Clean the injection area with an antiseptic solution.
2. Apply a local anesthetic on the injection site (optional). If local anesthetic is used, then a topical anesthetic, such as ethyl chloride, is recommended. **Under no circumstances may the anesthetic be injected intra-articularly.**
3. Position needle into the intra-articular space, and confirm needle placement with an ultrasound image.
4. Attach an empty syringe to the needle, aspirate all available joint fluid (aspiration volume must be recorded). Retain the aspirated joint fluid for shipping to the core laboratory.
5. Transfer the masked syringe that contains the injection solution (APS or saline) to the needle positioned at the injection site.
6. Inject all contents of the masked syringe into the synovial space of the joint.

Alternate technique:

Confirm if there is any available joint fluid for aspiration via palpation or ultrasound.

<u>If no fluid is available for aspiration, proceed as follows</u>:

1. Clean the injection area with an antiseptic solution.
2. Apply a local anesthetic on the injection site (optional). If local anesthetic is used, then a topical anesthetic, such as ethyl chloride, is recommended. **Under no circumstances may the anesthetic be injected intra-articularly.**
3. Attach needle (18-22 gauge) to masked syringe containing injection solution (APS or saline), and position needle into the intra-articular space, confirming needle placement with an ultrasound image.
4. Inject all contents of the masked syringe into the synovial space of the joint.

<u>If synovial fluid is available for aspiration, proceed as follows</u>:

1. Clean the injection area with an antiseptic solution.
2. Apply a local anesthetic on the injection site (optional). If local anesthetic is used, then a topical anesthetic, such as ethyl chloride, is recommended. **Under no circumstances may the anesthetic be injected intra-articularly.**

3. Position needle, attached to an empty syringe, into the intra-articular space, and aspirate all available joint fluid (aspiration volume must be recorded). Retain the aspirated joint fluid for shipping to the core laboratory.
4. Position needle (18-22 gauge), attached to the masked syringe containing injection solution (APS or saline), into the intra-articular space, confirming needle placement with an ultrasound image.
5. Inject all contents of the masked syringe into the synovial space of the joint.

A clear ultrasound image should be taken to document needle placement in the synovial space.

The injector may choose the position of the knee (e.g., extended or bent) and the approach for the injection (e.g., medial or lateral). These selections must be recorded.

Any adverse events associated with the blood draw or joint aspiration and injection procedure will be recorded. Additionally, any device processing issues will be recorded.

Before discharge subjects will be instructed not to exceed their pre-injection physical activity level for 14 days post-injection. Subjects will also be instructed to contact their physician's office if they intend to increase their activity level substantially while they are study subjects.

2.6.2.6 Sample Preparation and Shipping

After the blood draw, device processing, and joint fluid aspiration, the samples will be prepared and shipped to a central laboratory for characterization. Methods for sample preparation, shipping, and analysis will be specified in a separate laboratory processing and shipping instructions document.

2.6.3 Follow-up Visits

Follow-up assessment visits will be at the following intervals following the injection:

- 1 Month (±7 days)
- 3 Months (±14 days)
- 6 Months (±14 days)
- 12 Months (±28 days)

Follow-up windows are provided for reference only. Out of window visits will not be considered protocol deviations.

The following assessments and information will be collected at all follow-up visits:

- WOMAC LK 3.1
- EQ-5D
- VAS knee pain scale
- Medication use
- Adverse events

At the 12-month follow-up visit, a standing radiograph and MRI of the index knee will be acquired. Image acquisition, transfer, and analysis procedures will be performed using validated, prospectively defined methods.

2.6.4 Second Injection Procedure (Optional)

After each subject completes the 12 month follow-up visit assessments, the subject and study personnel may be unblinded to the treatment allocation. At that time, if they have had no major safety events due to the first injection, then the subject may choose to enter the repeat injection phase and receive an injection of APS, regardless of their original randomization allocation. If the subject prefers not to receive a second injection, then they will exit the study. If the subject chooses to receive a second injection, then it may be administered immediately following the completion of 12 month follow-up activities. Alternatively, a separate injection visit can be scheduled within 14 days of the 12 month follow-up visit. Prior to the second injection, a urine pregnancy test will be administered, where applicable. A positive test will render the subject ineligible for injection.

The second injection will not require an ultrasound image. The injection method/approach will be documented on the Procedure Form. The procedure for the injection involves the following steps:

1. Clean the injection area with an antiseptic solution.
2. Apply a local anesthetic on the injection site (optional). If local anesthetic is used, then a topical anesthetic, such as ethyl chloride, is recommended. **Under no circumstances may the anesthetic be injected intra-articularly.**
3. Position needle into the intra-articular space (ultrasound image is not required).
4. Attach an empty syringe to the needle, aspirate and discard all available joint fluid. (Aspiration volume must be recorded)
5. Transfer the syringe that contains the APS to the needle positioned at the injection site.
6. Inject all contents of the syringe into the synovial space of the joint.

The injector may choose the position of the knee (e.g., extended or bent) and the approach for the injection (e.g., medial or lateral). These selections must be recorded.

Any adverse events associated with the blood draw or joint aspiration and injection procedure will be recorded. Additionally, any device processing issues will be recorded.

2.6.5 Second Injection Follow-up Assessment (If Applicable)

One month (±7 days) after the second injection, a follow-up visit will be completed. The following assessments or information will be collected at this follow-up visit:

- WOMAC LK 3.1
- EQ-5D
- VAS knee pain scale

- Medication use
- Adverse events
- Study Exit form

2.7 Subject Withdrawal

Subjects may withdraw from the study at any time, for any reason or no reason, without jeopardizing their medical care. The investigative site will attempt to determine and document the reason(s) for discontinuation on the Study Exit form. Any subject who does not return for a scheduled follow-up evaluation will be contacted by telephone to determine the cause for the missed appointment, and an attempt to re-schedule the visit will be made. A subject will be withdrawn from the study (lost to follow-up) after a minimum of two unsuccessful attempted contacts were made. All subjects withdrawing from the study during the follow-up phase but prior to completion of the 12 month endpoint will be asked to voluntarily return for a final follow-up.

Subjects may be withdrawn by the investigator prior to receiving an injection for the following reasons:

- If it is not possible to withdraw the required amount of blood for nSTRIDE APS Kit processing (60 milliliters), then the subject will be withdrawn from the study and will not receive the injection. This will ensure that all treated subjects receive the appropriate treatment volume.
- If, on the day of injection, it is determined by the investigator that the subject is no longer an appropriate candidate for injection (e.g., due to development of infection of the index joint) then the subject will be withdrawn from the study and will not receive the injection.

Subjects who are withdrawn from the study prior to receiving an injection will be replaced.

The investigator, the IRB, and the sponsor have the right to discontinue a subject for the following reasons:

- Occurrence of unacceptable risk to the subjects enrolled in the study
- Insufficient compliance with the protocol by the subject
- AEs intolerable to the subject
- Participation in another clinical investigation that interferes with this study

2.8 Description of Assessments and CRFs

All data to be collected is described below. Sample CRFs may be found in Supplement 2.

2.8.1 Demographics

Demographic information collected will include gender, ethnicity, age, height and weight.

2.8.2 Medication Use

Information regarding the subject's allowable OA medication use, as well as use of acetaminophen for breakthrough pain, will be collected.

2.8.3 Knee Radiograph

A standing posterior-anterior (PA) fixed flexion radiograph of the index knee will be acquired. Joint space width (JSW) and joint space narrowing (JSN), as well as Kellgren-Lawrence grade, will be measured according to validated, prospectively defined techniques described in a signed charter produced by the Imaging Core Laboratory. This charter will specify the details of radiograph acquisition, transfer, and analysis techniques. All X-rays will be transferred to the Imaging Core Laboratory for independent review. Radiographs will be taken at the time of screening, or up to 6 months prior to screening, and at the 12 Month Follow-Up Visit.

2.8.4 Magnetic Resonance Imaging

MRI will be used to quantitatively assess cartilage changes via T2 relaxation mapping. If centers do not have T2 relaxation mapping capability, they will be exempt from submitting the T2-related sequences to the Imaging Core Laboratory. In addition, semi-quantitative assessment via MRI Osteoarthritis Knee Score (MOAKS) will be used to evaluate tissues including the subchondral bone, cartilage, meniscus, and peri-articular features. All MRIs will be transferred to the Imaging Core Laboratory for independent review. Image acquisition, transfer, and analysis procedures will be performed under the direction of a signed charter, using validated, prospectively defined methods. MRI will be taken at screening, following confirmation of eligibility, and at the 12 Month Follow-Up Visit.

In the event that an otherwise eligible subject is unable to undergo the MRI procedures at screening and at the 12 Month Follow-Up Visit due to significant safety risk (i.e. subject has pacemaker), the Sponsor has determined that the MRI assessment shall be waived. This will not result in a protocol deviation.

2.8.5 Pregnancy Test

A urine assay will be completed, as applicable, to determine whether the subject is pregnant. The pregnancy test will be done for any woman less than one year post-menopausal who is sexually active and is not actively using contraceptives.

2.8.6 WOMAC LK 3.1 Questionnaire

The WOMAC LK 3.1 questionnaire is a validated tool used for assessing knee pain, stiffness, and function. The WOMAC LK 3.1 has 24 items; 5 items assessing knee pain, 2 items assessing knee stiffness, and 17 items assessing physical function. Each item is answered on a 5-point Likert scale, with grading from 0 (none or never) to 4 (extreme or always). A higher score indicates worse pain, stiffness, or functional limitation.

2.8.7 EQ-5D

The EuroQol-5 Dimensions (EQ-5D) is a validated instrument which assesses an individual's current health status and heath related quality of life. The EQ-5D-3L descriptive component assesses five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression over three levels of severity. The EQ visual analogue scale (EQ VAS) assesses the respondent's self-rated overall health state on a scale from 0 (worst imaginable health state) to 100 (best imaginable health state).

2.8.8 VAS Pain

The Visual Analog Rating Scale is a validated measure of knee pain. The VAS is a nominal 100 mm scale anchored by 0 "no pain" and 100 "worst possible pain". Subjects rate their average pain over the last 24 hours by marking a slash at the point in the scale line representing their level of pain.

2.8.9 Injection Procedure

Subject will receive an injection of APS or Saline. For further information see Section 2.6.2.

2.8.10 Ultrasound

The initial APS injection will be completed with a clear ultrasound image captured of the injection site with the needle in position.

2.8.11 Laboratory Characterization

Whole blood and APS will be characterized by a central laboratory for cellular content (red blood cells, total and differential white blood cells, and platelets) and key cytokine and growth factor concentrations (IL-1ra, IL-1β, sIL-1RII, sTNF-RII, TNFα, IGF-I, TGF-β1). Synovial fluid will also be sent to the core laboratory for evaluation. Laboratory characterization procedures will be performed using validated, prospectively defined methods.

2.8.12 Adverse Events

The Adverse Event form will document all adverse events reported to or identified by site personnel including but not limited to onset, duration, severity and relatedness to the device.

2.9 Study Completion

Each subject will exit the study upon completion of the 12 month follow-up visit or, if the subject elects to enter the repeat injection phase and receive an additional injection of APS, then they will exit the study upon completion of the 1 Month Follow-Up Visit after the second injection. The Study Exit form will be used to document study completion.

This clinical investigation will be considered completed subsequent to the last subject last visit and after all final reports have been submitted to the Food and Drug Administration (FDA) and the governing IRB(s).

2.10 Study Endpoints

2.10.1 Primary Endpoint

The primary objective of this study is to determine whether nSTRIDE APS is superior to saline with regard to mean improvement in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.1 pain score (change from baseline to 12 months post-injection.
Further detail is given in Section 9.1 and in the Statistical Analysis Plan (SAP) for this study.

2.10.2 Secondary Endpoints

The secondary endpoint hypothesis tests will be performed at the 0.05 level. If any hypothesis is not tested significantly, all subsequent tests will be performed as exploratory analyses.

Secondary endpoints are as follows:

Order of Testing	Secondary Outcomes
1	WOMAC MCID Function Responder rate
2	WOMAC MCID Pain Responder rate
3	Mean WOMAC Function Δ (12 Month minus Baseline)
4	OMERACT-OARSI Responder / Non-responder (12 Month)
5	Mean WOMAC Pain Δ (12 Month minus Baseline) in K-L II Subgroup
6	Mean WOMAC Function Δ (12 Month minus Baseline) in K-L II Subgroup
7	Use of rescue medication (acetaminophen) use (for index knee OA) over time
8	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of rescue medication within 48 hours of the 12 month visit for knee OA as a covariate
9	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of rescue medication within 48 hours of the 12 month visit for any reason as a covariate
10	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of restricted medication within 48 hours of the 12 month visit for knee OA as a covariate
11	Mean WOMAC Pain Δ (12 Month minus Baseline) with Usage of restricted medication within 48 hours of the 12 month visit for any reason as a covariate

12	Mean VAS Pain Δ (12 Month minus Baseline)
13	Mean WOMAC Pain changes over time within treatment

2.10.3 Exploratory Endpoints

Exploratory outcomes include assessment of change from baseline to each time point for quality of life, WOMAC Pain, Stiffness, and Function, and VAS pain score as well as the OMERACT-OARSI criteria.

Exploratory analyses will also include evaluation of changes in joint morphology, determined with MRI images and X-ray images, as well as analyses of whole blood and APS cell/cytokine concentrations.

Exploratory endpoints are listed in the Statistical Analysis Plan (SAP) (Supplement 3). All analyses of exploratory endpoints will be conducted at $\alpha = 0.05$ and will not be adjusted for multiple comparisons as these are exploratory outcomes.

3 PROTOCOL DEVIATION REPORTING AND MANAGEMENT

Protocol violations will be tracked and reported by the sponsor.

Protocol violations identified by the investigative site will be documented on the appropriate CRF. Protocol violations identified during monitoring visits will be documented on the appropriate CRF and discussed with the investigator. Where necessary a corrective action will be implemented.

Protocol deviations will be classified into two severity categories:

- Minor deviations: violations that do not impact and do not have the potential to impact patient safety or scientific validity of the primary endpoint. Examples include isolated instances of missing laboratory values or study assessments.
- Major deviations: violations that impact patient safety or have the potential to do so, or have the potential to impact scientific validity of the primary endpoint. Examples include no ICF prior to procedure or inclusion of a subject who clearly did not meet eligibility criteria.

4 ADVERSE EVENT REPORTING AND MANAGEMENT

All AEs reported to or identified by investigative center personnel occurring during the study period and after the treatment procedure will be documented. AEs will have the onset and resolution date(s) listed (when known), will have their management and outcome documented (if possible), and will be assessed for severity, relatedness, and whether they were anticipated. SAEs will be described in a narrative in the final study report.

Anticipated AEs in this trial include, but are not limited to, those associated with any aspiration procedure or blood draw procedure including pain, bleeding, bruising, infection, deep venous thrombosis, scar tissue formation, thrombotic complications, or nerve/nervous system damage. Anticipated AEs associated with the injection procedure include worsening of knee pain and/or function, effusion, and infection.

4.1 Definitions

Adverse Event

An AE is any untoward medical occurrence in a subject receiving an investigational medical device which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational device, whether or not related to the investigational device.

Any AE that occurs during or after the blood draw will be recorded.

Serious Adverse Event

A serious adverse event (SAE) is an AE that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Adverse Device Effect

An adverse device effect (ADE) is an AE whose causal relationship with the investigational device is determined to be likely or definite. The following are identified as possible examples of ADEs:

- Injection site reaction – swelling, redness, burning, itching at the injection site – to a degree that is atypical of an intra-articular aspiration and injection procedure
- Knee arthralgia – severe pain in the knee joint
- Knee joint ache – to suffer dull, continued pain
- Knee joint inflammation – a localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function
- Knee joint effusion – the escape of fluid from the blood vessels or lymphatics into the knee joint
- Knee arthrosis – degenerative disease of the knee joint – beyond what is considered to be normal OA progression

Unanticipated Adverse Device Effect

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device; if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

4.2 Adverse Event Assessments

All AEs reported to or identified by investigative center personnel will be assessed by the investigator and recorded in the patient's study chart and on the Event Form, including but not limited to the following:

- Observed or volunteered problems
- Physical signs and symptoms
- Medical condition which occurs during the study, having been absent at baseline
- Medical condition present at baseline, which appears to worsen during the study

All AEs will be documented on the Adverse Event Form regardless of whether the medical/clinical event is associated with the use of the investigational device.

Each AE record must include a description of the event, date of onset, date of resolution (when known), severity, action taken, relationship to study device and seriousness criteria. Each AE must be recorded separately.

An outcome which may be expected to occur following any joint aspiration and injection procedure (e.g. transient pain at the injection site, mild swelling of the joint) should not be classified as an adverse event unless it is considered to be more severe or of longer duration or otherwise more pronounced than is typical.

Negative responses on follow-up questionnaires which are intended to evaluate clinical efficacy will not be recorded as adverse events.

A worsening of index knee osteoarthritis (i.e. joint space narrowing observed on X-ray) which is considered by the investigator to be part of the normal progression of the disease will not be recorded as an adverse event.

Severity will be assessed by the investigator using the following definitions:

Mild: Aware of sign or symptom, but easily tolerated

Moderate: Discomfort enough to cause interference with usual activity

Severe: Incapacitating with inability to work or do usual activity

Relationship to the study device will be assessed by the investigator using the following definitions:

Definitely Not: Evidence exists that the AE definitely has a cause other than the study device (e.g., pre-existing condition or underlying disease, intercurrent illness, or concomitant medication) and does not meet any other criteria listed.

Unlikely: A temporal relationship exists between the event onset and preparation/administration of the investigational device (or comparator). Although the AE may appear unlikely to be related to the investigational device, it cannot be ruled out with certainty; and/or the event cannot be readily explained by the patient's clinical state or concomitant therapies.

Likely: A temporal relationship exists between the event onset and preparation/administration of the investigational device (or comparator) and appears with some degree of certainty to be related based on the known

mechanism of action of the device. It cannot be readily explained by the patient's clinical state or concomitant therapies.

Definitely: Strong evidence exists that the investigational device caused the AE. There is a temporal relationship between the event onset and preparation/administration of the investigational device (or comparator). There is strong mechanistic evidence that the event was caused by the investigational device. The patient's clinical state and concomitant therapies have been ruled out as a cause.

4.3 Adverse Event Reporting

All subjects will be evaluated for AEs. All AEs will be evaluated beginning with onset, and evaluation will continue until the last day of the study, until recovery is noted, or until the investigator determines that the subject's condition is stable, whichever is earlier. The investigator will take all appropriate and necessary therapeutic measures required for resolution of the AE. Any medication necessary for the treatment of an AE must be recorded on the Adverse Event Form. If the medication necessary for treatment is on the restricted medication list, a Protocol Deviation form must also be completed. If more than one distinct AE occurs, each event should be recorded separately. The worsening of an adverse event should not be recorded as a new AE but as a continuation of an ongoing AE. Based on the information reported for the AE, if the sponsor or the sponsor's agent determines that the reported AE is an unanticipated adverse *device* effect, then it shall be reported as an unanticipated adverse device effect as directed in Section 4.5 – Unanticipated Adverse Device Effect Reporting.

4.4 Serious Adverse Event Reporting

All SAEs that occur during the study, including death, ***must be reported to the sponsor within 24 hours*** of occurrence or of the time the investigator becomes aware of the event by telephone, fax, email, or any other modality. All information on the AE event form must be made available to the sponsor as early as possible. Based on the information reported for the SAE, if the sponsor or the sponsor's agent determines that the reported SAE is an unanticipated adverse *device* effect, then it shall be reported as an unanticipated serious adverse device effect as directed in Section 4.5 - Unanticipated Adverse Device Effect Reporting.

4.5 Unanticipated Adverse Device Effect Reporting

The investigator shall submit a report of any unanticipated adverse device effect occurring during an investigation to the sponsor and to the reviewing IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event.

5 RISK EVALUATION

This study is designed to confirm the efficacy and safety of the nSTRIDE APS Kit and will weigh the benefits of the nSTRIDE APS Kit with regard to treatment of knee osteoarthritis and associated symptoms, or knee pain associated with osteoarthritis, against potential risks detected by the occurrence of adverse events.

The nSTRIDE APS Kit has a favorable risk profile. The injectable APS is processed from the patient's own whole blood using the nSTRIDE APS Kit. If properly processed then the risk of any immunogenic reactions, disease transmission or adverse interactions with systemic drugs is minimal. Because APS is delivered intra-articularly, there is localized anatomical impact allowing for the precise monitoring of adverse effects. There is little, if any, systemic introduction. Therefore, safety concerns with APS may be primarily related to the injection process. The most likely adverse effects are pain and swelling at the injection site. Such reactions typically resolve in one or two days with no treatment or minimal treatment. More serious complications of intra-articular injections include, but are not limited to, local infections, nerve damage, and deep vein thrombosis, all of which are extremely rare, and none of which have been reported in any previous clinical investigation of the nSTRIDE APS Kit (35;36).

5.1 Potential Risks to Study Subjects

Subjects in this study are exposed to potential risks associated with aspiration of joint fluid, a blood draw, and solution injection into the knee. These include pain, bleeding, bruising, infection, deep venous thrombosis, scar tissue formation, thrombotic complications and nerve damage. These risks are not unique to this study and may occur with any aspiration, blood draw, or joint injection procedure. Potential risks associated with the injection of the APS include worsening of pain and/or knee function, effusion, and infection. Mixing up blood and/or APS samples from multiple donors presents a risk of injecting the APS produced from one patient into another patient. This would be associated with a possible inflammatory reaction or disease transmission to the patient receiving the injection. There are no known specific risks of the investigational device itself.

5.2 Methods to Minimize Risks

Several procedures have been incorporated into this protocol to protect study subjects and to detect any injection site reactions or other AEs. Rigorous inclusion/exclusion criteria ensure that any patient who may be at increased risk is not enrolled in the study (e.g., patients with a systemic inflammatory condition). Appropriate investigative center personnel will be thoroughly trained on the processing of the nSTRIDE APS Kit.

Sticker labels are included in each kit to identify the subject associated with each syringe, device and specimen. The use of these labels will reduce the risk of sample mix-up. Additionally, only one subject's blood will be processed in the centrifuge at a time.

5.3 Medical Monitor

An independent medical monitor will be available to adjudicate all serious adverse events and unanticipated adverse device effects with respect to severity and relatedness within 10 working days from the time the sponsor is made aware of the event. The medical monitor will have the authority to suspend or terminate the

investigation pursuant to Section 5.4. Sponsor study personnel may choose to have any AE independently reviewed by the medical monitor at their discretion.

5.4 Investigation Suspension or Termination

The Investigator, the IRB, and the Sponsor may suspend or terminate the investigation at any/all investigational sites at any time if they believe:

- There is unacceptable risk to the subjects enrolled in the study
- A decision on the part of the sponsor to suspend or discontinue testing, evaluation, or development of the product (subjects already enrolled would be followed until they complete the study)
- Failure of the investigator to enroll patients into the study at an acceptable rate
- Failure of the investigator to comply with the protocol or appropriate regulations, especially with respect to subject safety
- Site personnel knowingly submit false information from the investigative site to the sponsor, study monitor, IRB, or regulatory authority

The sponsor should be notified immediately if any one of these conditions is present. The sponsor will determine the appropriate course of action taking into account medical assessments, regulatory mandates and ethical considerations. If the clinical investigation is suspended or terminated then the sponsor, in coordination with the IRB and investigator, will comply with any necessary action regarding investigation, documentation, and reporting.

5.5 Potential Benefits of the Procedure

The potential benefit of APS is the treatment of knee OA and associated symptoms, or the treatment of knee pain associated with OA. This study also explores other potential benefits, such as knee function restoration and anatomical improvement within the joint.

5.6 Bias Minimization

5.6.1 Randomization

The randomization plan will be produced using SAS v 9.2 for Windows or similar software. Balanced, blocked randomization (1:1, APS: Saline) will be implemented. In the event that a screen failure occurs post-randomization, and no study treatment was given, randomization will not be reassigned and in this case will not count toward the overall sample size. Randomization will continue with the next case enrolled until the minimum sample size is reached in both treatment groups. Randomization will be stratified by site, and each site will receive separate randomization plans using random predetermined block sizes that will remain undisclosed to the sites. The randomization file will be uploaded into the electronic data capture (EDC) system. Once the subject is enrolled into the EDC and has been identified as eligible for randomization, the randomization allocation will be visible

within the EDC and viewable only by the unblinded research associate. The subject will be treated according to the contents of the displayed randomization allocation.

5.6.2 Blinding

This is a double-blind study with subjects and evaluators blinded to treatment. Throughout the course of the study, whether subjects receive APS or saline treatment, they will be cared for in the same manner in order to blind them from their treatment. Although saline control subjects will not receive APS treatment, they will undergo a blood draw, as is required for nSTRIDE APS Kit processing, in order to protect the blinding of the study. The nSTRIDE APS Kits will be processed identically for subjects randomized to both treatment groups. For subjects allocated to the saline treatment group, an identically sized treatment syringe will be loaded with a volume of saline equal to the volume of APS output from the first nSTRIDE APS Kit by the unblinded research associate. The unblinded research associate will cover the syringe to mask the contents, regardless of the treatment allocation, prior to presenting it to the injecting clinician. The clinician will perform the injection procedure, and capture an ultrasound image showing needle placement within the joint, taking care not to reveal the contents of the syringe. To further protect the blind, any recorded information specifically related to APS treatment will be kept in an “unblinded” file, separate from the rest of the study data, and will only be available to the unblinded research associate. Blinded evaluators will not have access to the unblinded file.

Study-related communications between the unblinded research associate and the blinded evaluators will be limited to prevent breaking the blind. Study-related communications should be limited in scope to the subject’s pre-existing conditions prior to treatment or to adverse events following treatment. The unblinded research associate will not play a role in any follow-up visit study procedures, and unblinded personnel will be strictly forbidden from discussing treatment allocation with subjects and clinical observers.

The subject’s randomization allocation will be viewable in the EDC by the unblinded research associate only. This allocation will only be communicated to blinded study personnel if a serious adverse event occurs and information as to which treatment the subject received is essential for the medical treatment of the subject and adverse event reporting. All sites and the sponsor will keep a record of the communication of the randomization allocation of any subject.

6 CONTROL OF INVESTIGATIONAL DEVICE

All study devices will be provided by the sponsor at no charge. Documentation of receipt, disposition, and return of all investigational device materials must be maintained by the investigator or his/her designee on a device accountability log. It is the investigator’s responsibility to ensure that all investigational devices are kept in a secure location, with access limited to individuals authorized by the investigator. The device will be shipped with a confirmation of receipt form. Once signed, copies of the confirmation of receipt form should be maintained by the sponsor in the Trial Master File and in the Investigator’s files.

The nSTRIDE APS Kit with ACD-A can be stored at room temperature. The investigator must ensure that the study devices are used only for eligible study subjects who are appropriately enrolled in the study.

At the conclusion of each procedure, and after recording all requested information on the Procedure Form, the investigator will discard any remaining investigational material which was prepared (using an acceptable disposal method for products potentially contaminated with blood).

After completion of the treatment period of the study, all unopened devices will be returned to the sponsor in the original containers or will be destroyed at the study site by the study monitor. The monitor will complete an accountability form to document the action taken.

The device accountability records must be readily available for inspection by the study monitor, independent auditor, and any regulatory authority inspector. The study monitor will reconcile device supplies as part of the routine monitoring visit.

6.1 Description and Control of Saline Comparator

The saline comparator will be provided by the sponsor. The comparator will be 0.9% sodium chloride solution. The saline solution will be stored and administered in a consistent manner with the investigational device.

7 DATA COLLECTION, HANDLING AND RETENTION

7.1 Source Documentation

Source documentation for this study will be maintained to capture the course of treatment and to substantiate the integrity of the trial data. Source documentation will include, but is not limited to, worksheets, hospital and/or clinic or office records documenting subject visits including study and other treatments or procedures, medical history and physical examination information, imaging results, device accountability records, medical consultations and laboratory results and reports.

7.2 Case Report Forms

Sample CRFs are provided in Supplement 2.

Data for this clinical trial will be collected and documented on the subject's CRFs. CRFs provided may appear in paper or electronic form. Only authorized study site personnel or subjects will complete CRFs as appropriate to the specific CRF. CRFs must be reviewed and signed by the investigator or their documented designees. This may be done electronically within the electronic data capture system. Because there is a potential for errors, inaccuracies, and misinterpretation in the process of transcribing data onto CRFs (whether paper or electronic), the following documents must be available at all times for inspection and comparison to the CRFs by the study monitor where applicable.

- Data query forms
- Originals or photocopies/certified copies of all relevant records and reports

- Copies of test results and reports
- Other records as listed in 21 CFR Part 812.140.

Only trained study site personnel may complete and sign (or authorize) the forms.

7.3 Electronic Data Entry

When using electronic data handling or remote electronic trial data systems, the sponsor or the sponsor's representative will:

- Ensure and document that the electronic data processing system(s) conforms to the sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation)
- Maintain SOPs for using these systems
- Ensure that systems are designed to permit changes to the data in such a way that the data changes are documented and there is no deletion of any edited or entered data (i.e., maintains an audit trail)
- Maintain a security system to prevent unauthorized access to the data and to be able to uniquely identify individuals who access the data entry system
- Maintain a list of individuals who are authorized to make data changes
- Maintain adequate backup of the data

7.4 Records Retention

Study documents will be retained by the investigator for a period of 2 years after the latter of the following two dates:

- The date on which the investigation is terminated or completed
- The date the records are no longer required for purposes of supporting a regulatory application

8 REPORTING

8.1 Data Reporting

The investigator shall provide an interim progress report to the sponsor, the monitor, and the reviewing IRB annually following IRB approval.

The investigator shall, within 3 months after termination or completion of the study or the investigator's part of the study, submit a final report to the sponsor and reviewing IRB.

8.2 Other Reporting

The investigator shall provide all reports in accordance with 21 CFR Part 812.150(a) including but not limited to:

- Unanticipated adverse device effects

- Withdrawal of IRB approval
- Major deviations from the investigative plan
- Use of an investigational device without informed consent

An investigator shall provide accurate, complete, and current information about any aspect of the study upon request by the Sponsor, the reviewing IRB, or FDA.

9 STATISTICAL ANALYSIS

The complete SAP is provided in Supplement 3. Briefly, the planned analyses are described below.

9.1 Primary Efficacy Analysis

The primary objectives of this study are to determine whether nSTRIDE APS is superior to a saline with regard to the mean improvement from baseline to 12 months in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.1 pain subscale score.

The primary hypothesis will be tested along with the secondary hypotheses listed in the Statistical Analysis Plan using a conventional fixed-sequence procedure, constructed using a pre-specified order of hypotheses. These tests will be performed at the 0.05 level. If the first null hypothesis is rejected, the second test will be performed at the 0.05 level. If the first null hypothesis is not rejected, the second test, and all subsequent tests, will be performed as exploratory analyses. Since the order of the tests is fixed a priori, and the second hypothesis is tested only if the previous hypothesis has been rejected, the principle of closed testing implies that no adjustment to control the familywise error rate is necessary. The Fixed-Sequence Method is described as an acceptable method for addressing the multiplicity problem in Section IV.C.5 of the FDA draft guidance entitled, “Guidance for Industry – Multiple Endpoints in Clinical Trials”.

If the primary hypothesis is not tested significantly, all subsequent secondary tests will be performed as exploratory analyses only.

A two-tailed independent sample T-test will be used to test the primary endpoint. The hypothesis will be:

H_0: $\mu_{APS} = \mu_{Control}$
Versus
H_A: $\mu_{APS} \neq \mu_{Control}$

Where:
μ_{APS} = mean change in WOMAC Pain from baseline to 12 months in the APS group, and
$\mu_{control}$ = mean change in WOMAC Pain from baseline to 12 months in the control group.

The impact of the usage of APS on function will be evaluated as part of the primary endpoint; however, no formal statistical test will be done as a part of the study success criteria. A qualitative assessment of the changes in WOMAC Function over time for APS and Placebo will be discussed, in order to ensure that the impact of the treatment on function is neutral or positive. The following descriptive analyses will be performed for this assessment of function:

(3) A graphical examination of the changes in WOMAC Function for APS and Placebo over time, including means and standard error bars.
(4) Descriptive statistics for the mean changes in WOMAC Function over time, separately for each treatment group. These will include mean, median, standard deviation, minimum, maximum, and 95% confidence intervals.

For each analysis, it is expected that the changes in WOMAC function will remain neutral or increase over time within the APS group, and also that the mean changes in WOMAC Function over time will be at least nominally better for the APS group than for Saline.

A finding of nSTRIDE APS superiority on the mean improvement in WOMAC LK 3.1 Pain along with no corresponding deterioration in WOMAC Function will be considered evidence of nSTRIDE APS efficacy, and the device will be considered efficacious for the treatment of knee pain associated with osteoarthritis.

9.2 Demographics and Medication Use

Analyses will be done to determine whether randomization succeeded in creating groups that were balanced with regards to key baseline characteristics. Planned tests aimed at determining this are specified in the statistical analysis plan. All tests will be under a null hypothesis of no difference between treatment groups.

A time-course tabulation showing rescue medication (acetaminophen) use (for index knee OA) by treatment group will be generated. In addition the percentages of subjects requiring rescue medication for their index knee OA during the course of follow-up (up to 12 months) will be compared for APS vs. Control using a Fisher's Exact test.

9.3 Subject Reported Outcomes

Subject reported outcome measures, WOMAC LK 3.1, EQ-5D, and VAS knee pain, will be summarized and thoroughly characterized with the appropriate descriptive statistics including error measures. Statistics may include mean, mode, median, range, inter-quartile range, minimum, maximum, frequency, cumulative frequency percentage and cumulative percentage. Results will be presented in a narrative and graphically.

These measures will also be analyzed using inferential statistics. These analyses are described in detail in the SAP.

9.4 Safety Analysis

The safety profile of APS will be characterized. AEs will be standardized using the Medical Dictionary for Regulatory Activities. Characterization will include narratives of all SAEs and descriptive statistics including AE incidence overall and per subject, AE severity, AE device relatedness, AE duration, and AE onset for each treatment group. AEs will also be used to characterize the safety of a repeat APS injection.

10 STUDY MONITORING AND QUALITY CONTROL

The investigators will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s), providing direct access to the trial site and to source data/documents upon request. Clinical trials sponsored by Biomet Biologics are conducted under standard operating procedures. The sponsor's staff and/or representatives will closely monitor the conduct of the clinical investigation so that any questions and problems that may arise can be promptly resolved. Such monitoring will also ensure that the clinical investigation is conducted in accordance with this clinical investigation plan, including all amendments, with good clinical practice (GCP) guidelines, and with International Standards Organization (ISO) 14155 stipulations. Monitoring will involve visits by the sponsor's representative to the investigational centers to verify good management of subjects and the clinical investigation devices, to observe procedures and to audit the clinical investigation for quality control purposes (to check device accountability and supplies, presence of required documents, informed consent and to compare CRFs with source data). There will also be frequent telephone contact and written communication between the monitor and clinical investigators. The Monitoring Plan is presented in Supplement 4.

11 DEVICE LABELING

The device package insert is provided in Supplement 1.

12 ETHICAL CONSIDERATIONS

12.1 Code of Conduct

The investigator will ensure that the clinical study is conducted in accordance with:

- The Protocol
- Regulatory and reviewing IRB requirements
- ISO 14155 and GCP

12.2 Regulatory Approval

As an Investigational Device Exemption and premarket approval study this investigation will be conducted under an approved IDE application from FDA.

12.3 Institutional Review Board Approval and Protocol Changes

The Investigator must obtain IRB approval before the study is initiated at his or her site. A copy of the written IRB approval and a copy of the IRB approved ICF should be provided to the sponsor. A list of the IRB members (including their Institution

affiliations, gender makeup, and occupations); or a statement from the IRB specifying that the membership complies with applicable regulations, including but not limited to 21 CFR Part 56, is to be provided to the sponsor by the investigator.

Any protocol changes must be discussed and approved by the sponsor in writing unless the change is made to assure the safety of the subject. In the non-emergent setting, after agreement on the changes has been reached, an amendment to the protocol will be provided by the sponsor for submission to the IRB for review and approval prior to initiation of the change. Any change made emergently must be documented in the subject's medical record and reported to the sponsor within the time period required by local SOPs and applicable regulations.

12.4 Informed Consent

Prior to the performance of any study-specific procedures, subjects will be provided with an informed consent form (ICF) and patient information sheet and be given ample opportunity to review the ICF and ask questions. If the subject agrees to participate in the study, then the subject must sign and date the ICF. The Investigator or designee must also sign and date the ICF. A copy of the ICF should be given to the subject.

The specific ICF used must have current IRB approval at time of use. A HIPAA statement must be included in the ICF or provided as a separate document.

Subjects will be informed of new information learned during the study, if any, which may affect the subject’s decision to continue participation in the study.

An Informed Consent Log will be completed to document the existence of the signed ICF. The log will contain: Subject ID, date ICF signed, and the version signed. The monitor will initial and date the log once the executed ICF has been reviewed. Signed ICF (or copies) are to be maintained in the study file and must be available for verification by monitors and inspectors.

13 LIST OF SUPPLEMENTS

Supplement 1 – Package Insert
Supplement 2 – Sample Case Report Forms
Supplement 3 – Statistical Analysis Plan
Supplement 4 – Monitoring Plan

14 REFERENCES

(1) Buckwalter JA, Martin JA. Sports and osteoarthritis. Curr Opin Rheumatol 2004 Sep;16(5):634-9.

(2) Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. Eur J Appl Physiol 2007 Mar;99(4):443-7.

(3) Lequesne MG, Dang N, Lane NE. Sport practice and osteoarthritis of the limbs. Osteoarthritis Cartilage 1997 Mar;5(2):75-86.

(4) Neidhart M, Muller-Ladner U, Frey W, et al. Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. Osteoarthritis Cartilage 2000 May;8(3):222-9.

(5) Brzusek D, Petron D. Treating knee osteoarthritis with intra-articular hyaluronans. Curr Med Res Opin 2008 Dec;24(12):3307-22.

(6) Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. Rheumatology (Oxford) 2005 Jan;44(1):7-16.

(7) Abramson SB, Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. Rheumatology (Oxford) 2002 Sep;41(9):972-80.

(8) Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology 2002;39(1-2):237-46.

(9) Goldring MB. Osteoarthritis and cartilage: the role of cytokines. Curr Rheumatol Rep 2000 Dec;2(6):459-65.

(10) Goldring MB. The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models. Connect Tissue Res 1999;40(1):1-11.

(11) Pelletier JP, McCollum R, Cloutier JM, Martel-Pelletier J. Synthesis of metalloproteases and interleukin 6 (IL-6) in human osteoarthritic synovial membrane is an IL-1 mediated process. J Rheumatol Suppl 1995 Feb;43:109-14.:109-14.

(12) Vuolteenaho K, Moilanen T, Hamalainen M, Moilanen E. Effects of TNFalpha-antagonists on nitric oxide production in human cartilage. Osteoarthritis Cartilage 2002 Apr;10(4):327-32.

(13) Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res 2004 Oct;(427 Suppl):S27-S36.

(14) Chambers MG, Bayliss MT, Mason RM. Chondrocyte cytokine and growth factor expression in murine osteoarthritis. Osteoarthritis Cartilage 1997 Sep;5(5):301-8.

(15) Pelletier JP, Faure MP, DiBattista JA, et al. Coordinate synthesis of stromelysin, interleukin-1, and oncogene proteins in experimental osteoarthritis. An immunohistochemical study. Am J Pathol 1993 Jan;142(1):95-105.

(16) Verbruggen G. Chondroprotective drugs in degenerative joint diseases. Rheumatology (Oxford) 2006 Feb;45(2):129-38.

(17) Goldring MB. Anticytokine therapy for osteoarthritis. Expert Opin Biol Ther 2001 Sep;1(5):817-29.

(18) Goldring MB, Otero M, Tsuchimochi K, Ijiri K, Li Y. Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. Ann Rheum Dis 2008 Dec;67 Suppl 3:iii75-82.:iii75-iii82.

(19) Goldring MB. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. Best Pract Res Clin Rheumatol 2006 Oct;20(5):1003-25.

(20) Shlopov BV, Gumanovskaya ML, Hasty KA. Autocrine regulation of collagenase 3 (matrix metalloproteinase 13) during osteoarthritis. Arthritis Rheum 2000 Jan;43(1):195-205.

(21) Niissalo S, Hukkanen M, Imai S, Tornwall J, Konttinen YT. Neuropeptides in experimental and degenerative arthritis. Ann N Y Acad Sci 2002 Jun;966:384-99.:384-99.

(22) Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. Annu Rev Immunol 1998;16:27-55.:27-55.

(23) Cominelli F, Pizarro TT. Interleukin-1 receptor antagonist: a "novel" acute phase protein with antiinflammatory activities. J Clin Invest 1997 Jun 15;99(12):2813.

(24) Dinarello CA. The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. N Engl J Med 2000 Sep 7;343(10):732-4.

(25) Symons JA, Young PR, Duff GW. Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses

affinity for IL-1 receptor antagonist. Proc Natl Acad Sci U S A 1995 Feb 28;92(5):1714-8.

(26) Van Zee KJ, Kohno T, Fischer E, et al. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. Proc Natl Acad Sci U S A 1992 Jun 1;89(11):4845-9.

(27) O'Shaughnessey K, Klaassen MA, Kaeding C, Lattermann C, Farr J, Hoeppner J, et al. Examining the cytokine profiles of whole blood and autologous protein solution of patients with osteoarthritis: preliminary results. 2012; 2012.

(28) Baltzer AW, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthritis Cartilage 2009 Feb 1;17(2):152-60.

(29) O'Shaughnessey KM, Panitch A, Woodell-May JE. Blood-derived anti-inflammatory protein solution blocks the effect of IL-1beta on human macrophages in vitro. Inflamm Res 2011 Oct;60(10):929-36.

(30) Woodell-May J, Matuska A, Oyster M, et al. Autologous protein solution inhibits MMP-13 production by IL-1beta and TNFalpha-stimulated human articular chondrocytes. J Orthop Res 2011 Sep 15;29:1320-6.

(31) Matuska A, O'Shaughnessey KM, King WJ, Woodell-May JE. Autologous solution protects bovine cartilage explants from IL-1á- and TNFá- induced cartilage degradation. Journal of Orthopaedic Reseaarch 2013;31(12):1929-35.

(32) Bertone AL, Ishihara A, Zekas LJ, et al. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in horses. American Journal of Veterinary Research 2014 Feb 1;75(2):141-51.

(33) King WJ, van der Weegen W, Van DR, et al. White blood cell concentration correlates with increased concentrations of IL-1ra and improvement in WOMAC pain scores in an open-label safety study of autologous protein solution. J Exp Orthop 2016 Dec;3(1):9-0043.

(34) van Drumpt RA, van der Weegen W, King WJ, Toler K, Macenski M. Safety and treatment effectiveness of a single autologous protein solution injection in patients with knee osteoarthritis. BioResearch Open Access 2016;5.1:261-8.

(35) Zimmer Biomet. A Multicenter, Double-Blind, Randomized, Placebo [Saline]-Controlled Pilot Study of a Single, Intra-Articular Injection of

Autologous Protein Solution in Patients with Osteoarthritis of the Knee. 2016.

(36) Kwon D.R., Park GY. Intra-Articular Injections for the Treatment of Osteoarthritis: Focus on the Clinical Use of Several Regimens. In: Chen Q., editor. Osteoarthritis - Diagnosis, Treatment and Surgery. InTech; 2012. p. 67-101.

15 PROTOCOL REVISION HISTORY

Version	Description of Change	Sections
V 1.0	Original Protocol submitted to FDA (Dated 2016-06-28)	
V 2.0	Updated Study Contact Information with relevant personnel	Study Contact Information
	Clarified concomitant OA treatment. Updated to reflect changes to SAP with regard to the fixed sequential testing of the primary and secondary endpoints. Revised summary of exploratory endpoints. Increased sample size from 246 to 332 subjects.	Study Synopsis
	Removed extraneous reference to ultrasound guidance.	Use and Training (Section 1.3.3)
	Specified version of WOMAC to be used. (i.e. LK 3.1)	Study Objectives (Section 1.5)
	Increased sample size from 246 to 332 subjects.	Study Design (Section 2.1)
	Added immediate re-screening criterion for inability to comply with the protocol (i.e. screen failure in which screening to injection window closes prior to MRI or injection, but subject otherwise remains eligible).	Eligibility for Re-Screening (Section 2.4)
	Added the Pregnancy Test requirement to the Optional 2nd Injection column of the clinical study assesment table.	Study Assessments and Procedures (Section 2.6)
	Added alternative injection technique to allow for knee exam/ palpation or ultrasound to be used in order to determine if synovial fluid is available for aspiration, per standard of care.	Injection Procedure (Section 2.6.2.5)
	Added the Pregnancy Test requirement prior to the optional 2nd Injection procedure.	Second Injection Procedure (Optional) (Section 2.6.4)
	Added MRI waiver detail for subjects at significant safety risk.	Magnetic Resonance Imaging Section (2.8.4)
	Updated to reflect changes to SAP with regard to the fixed sequential testing of the primary study hypotheses.	Primary Endpoints (Section 2.10.1)
	Updated to reflect changes to SAP with regard to the fixed sequential testing of the secondary study hypotheses.	Secondary Endpoints (Section 2.10.2)
	Updated to reflect changes to SAP with regard to the exploratory outcomes assessments.	Exploratory Endpoints (Section 2.10.3)
	Updated to reflect changes to SAP with regard to the fixed sequential testing of the primary study hypotheses.	Primary Efficacy Analysis

		(Section 9.1)
	Updated to reflect changes to SAP to clarify the analysis of baseline data and medication use.	Demographics and Medication Use (Section 9.2)
	Updated to reflect the location of the inferential statistics detail.	Subject Reported Outcomes (Section 9.3)
V 2.1	Updated intended use to be the treatment of knee pain associated with osteoarthritis.	Study Synopsis, Indications (Section 1.3.1)
	Updated primary objective/endpoint to be WOMAC pain score, rather than WOMAC pain and function scores.	Study Synopsis, Study Objectives (Section 1.5), Primary Endpoint (Section 2.10.1), Primary Efficacy Analysis (Section 9.1)
	Updated primary hypothesis to reflect one primary test (pain) rather than two (pain and function).	Study Synopsis
	Updated secondary endpoints to include mean WOMAC function change as the first priority secondary outcome to test.	Study Synopsis, Secondary Endpoints (Section 2.10.2)
	Updated criteria for major and minor deviations to reflect one primary endpoint (pain) rather than two (pain and function)	Protocol Deviation Reporting and Management (Section 3)
	Updated the potential benefit of APS to be treatment of knee OA pain. Updated other porential benefits that are explored in this study.	Potential Benefits of the Procedure (Section 5.5)
	Updated criteria for evidence of nSTRIDE APS efficacy.	Primary Efficacy Analysis (Section 9.1)
V 3.0	Updated intended use to be the treatment of knee osteoarthritis and associated symptoms or knee pain associated with osteoarthritis.	Study Synopsis, Indications (Section 1.3.1)
	Updated primary objective/endpoint to be WOMAC pain (mean improvement) and function scores (MCII responder rate), rather than WOMAC pain score alone.	Study Synopsis, Study Objectives (Section 1.5), Primary Endpoint (Section 2.10.1), Primary Efficacy Analysis (Section 9.1)
	Updated primary hypothesis to reflect two sequential primary tests (pain and then function) rather than one (pain only).	Study Synopsis
	Updated the potential benefit of APS to be treatment of knee OA and associated symptoms or knee pain associated with OA. Updated other potential benefits that are explored in this study.	Potential Benefits of the Procedure (Section 5.5)
	Updated criteria for evidence of nSTRIDE APS efficacy.	Primary Efficacy Analysis (Section 9.1)
V 4.0	Updated intended use to be the treatment of knee pain associated with osteoarthritis.	Study Synopsis, Indications (Section 1.3.1)
	Updated primary objective/endpoint to be WOMAC pain score, rather than WOMAC pain and function scores. Added additional detail regarding the assessment of trends on	Study Synopsis, Study Objectives (Section 1.5), Primary Endpoint

	WOMAC Function	(Section 2.10.1), Primary Efficacy Analysis (Section 9.1)
	Updated secondary endpoints to reflect (1) inclusion of test of WOMAC MCID Function as a secondary endpoint, and (2) moving some exploratory endpoint to secondary in order to better comply with the FDA Analgesics Indications guidance document (https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm384691.pdf).	Study Synopsis, Secondary Endpoints (Section 2.10.2)
V 5.0	Updated secondary endpoints to include (1) test of WOMAC MCID Pain as a secondary endpoint, and (2) specifically define the analyses of the impact of rescue and restricted medications on the primary outcome.	Study Synopsis, Secondary Endpoints (Section 2.10.2)