STUDY SYNOPSIS

Protocol Number:	APSS-66-00
Title:	A Two-Phase, Multicenter, Randomized Study Comparing Autologous Protein Solution (APS) with Hyaluronic Acid (HA) Intra Articular Injections in Patients with Knee Osteoarthritis (OA)
Sponsor:	Zimmer Biomet
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 OA and associated symptoms.
Comparator Name:	Synvisc-One® (hylan G-F 20)
Comparator Description:	Synvisc-One (hylan G-F 20) is a sterile, nonpyrogenic, elastoviscous fluid containing hylans. Hylans are derivatives of hyaluronan (sodium salt of hyaluronic acid) and consist of repeating disaccharide units of N-acetylglucosamine and sodium glucoronate. Hylan A has an average molecular weight of approximately 6,000,000 daltons and hylan B is a hydrated gel. Hylan G-F 20 contains hylan A and hylan B (8.0 mg ± 2.0 mg per

	ml) in buffered physiological sodium chloride solution (pH 7.2 ± 0.3).
Comparator Intended Use:	Synvisc-One is only intended for intra-articular use by a physician to treat pain associated with osteoarthritis of the knee.
Study Center(s):	The study will be conducted at up to 15 investigative centers in Europe.
Planned Sample Size:	246 subjects will be randomized (1:1) into one of two treatment groups (APS and HA).
Study Population:	Patients with symptomatic OA in one knee, who have not been able to get satisfactory pain relief with prior treatment.
Study Objectives:	<u>Primary Objective</u> The primary objective of this study is to determine whether nSTRIDE APS is superior to HA with regard to the improvement in mean Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.1 pain score (change from baseline to 12 months post-injection). <u>Secondary Objectives</u> Secondary objectives of this study include determining whether nSTRIDE APS is superior to HA in improving WOMAC LK 3.1 function at 12 months (as evaluated using the percentage of subjects showing at least the minimal clinically important difference (MCID)), and WOMAC LK 3.1 pain at 12 months (as evaluated using the percentage of subjects showing at least the minimal clinically important difference (MCID)), OMERACT-OARSI responder rates at 12 months, analyzing WOMAC pain and function in only the KL-II Subgroup at 12 months, evaluating superiority of APS over HA in improving Numeric Rating Scale (NRS) pain at 12 months, assessment of the changes in WOMAC Pain scores over time (baseline through 12 months), and evaluation of the usage of rescue medication within 12 months. <u>Exploratory Objectives</u>

	Exploratory objectives of this study include determining whether nSTRIDE APS is superior to HA in improving patient reported outcomes including pain, function, stiffness, and quality of life in subjects with early to moderate symptomatic OA. A long-term follow-up phase will examine the superiority of nSTRIDE APS in the duration of the treatment effect, injection frequency, patient preferences, healthcare resource utilization, and associated costs. <u>Safety Objectives</u> Safety of nSTRIDE APS will be compared to HA 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 injection to HA in patients with symptomatic 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 HA. The primary efficacy measure will be pain as measured utilizing the WOMAC LK 3.1 scale; 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 (AEs). Anatomical changes will be evaluated by radiographs (X-ray). In the long-term follow-up phase (12 – 60 months), the study will evaluate treatment durability, patient preferences, and treatment cost effectiveness over time. During this long-term follow-up period, safety will be assessed by tracking the occurrence of AEs of interest only.

	During screening, potential subjects who provide informed consent will be assessed 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 (K-L) scale (assessed from radiographs). Subjects will also provide demographic and medication use information. Baseline X-ray[1] and an MRI[2] 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 Numeric Rating Scale (NRS) 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. An injection visit occurring between 28 and 32 days after the screening visit will be considered a minor protocol deviation. In the event that the injection procedure cannot be completed within 32 days of the screening visit, the subject will be screen-failed, but is eligible for immediate re-screening. The re-screening and injection visits can be combined, as long as the subject still qualifies per all

[1] Radiographs up to 24 months of follow-up will be sent to a core imaging laboratory for assessment of eligibility and therefore must always be performed following the criteria set in the Imaging Review Charter from Core Imaging Laboratory.

[2] Baseline MRIs will not be sent to a Core Imaging Laboratory and should be performed only after subject meets all other eligibility criteria. Also, previously taken MRI may be used if obtained up to 90 days prior to study treatment. Furthermore, in the event that an otherwise eligible subject is unable to undergo the MRI procedure due to significant safety risk (i.e. subject has pacemaker, documented diagnosis of claustrophobia, etc.), MRI does not need to be performed and this will be not be documented as a protocol deviation

	inclusion/ exclusion criteria. Baseline imaging acquisition and review are not required to be repeated. The Core Imaging laboratory will then be requested to re-issue an X-Ray eligibility report with the new subject ID. During the treatment visit, all subjects will have a blood draw, from which the APS will be prepared for injection. After all available joint fluid is aspirated and, according to randomization group assignment, approximately 2.5 milliliters (ml) of APS or 6.0 ml of HA will be injected into the joint. Needle placement in the joint may be verified using ultrasound (if standard of care). To ensure the subject will be blinded to the treatment assignment, the syringe (regardless of the study arm) shall be taped with opaque medical tape. The tape will be used to wrap the entire length of the syringe including the needle hub. To protect the double-blind, the treating health care professional and evaluating health care professional will be different individuals. Any AEs 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, 12, 18, 24, 30, 36, 48, and 60 months post injection. Subjects will be asked to abstain[3]

[3] During follow-up subjects will not be asked to abstain from low-doses of aspirin taken for cardio-protection. Long-term treatment with low doses of aspirin – usually max 100mg/day – only has an antiplatelet effect. Aspirin in low dose would not be enough to impact any pain sensations. Also, the low dose aspirin is supposed to be taken every day when taken for cardio-protection, so they should remain consistent on their regimen throughout the study. Aspirin taken prior to the follow-up visits will not be documented as a Protocol Deviation. Sites will document the low-dose of Aspirin in the patient medical file

	from analgesic use for 48 hours prior to assessments. They will complete the WOMAC, EQ-5D, and NRS for pain. An X-ray will be obtained at 12 months and annually thereafter to assess anatomical changes. Only radiograph images taken up to 24 months of follow-up will be transferred to the Core Imaging Laboratory for independent review. Image acquisition, transfer, and analysis procedures will be performed using validated, prospectively defined methods. All AEs that occur during the 12 month blinded study period will be recorded. In the long-term follow-up phase (12 – 60 months), only events of interest will be recorded. After each subject completes all 12-month follow-up evaluations, only subjects will be blinded to the individual treatment allocation resulting in single-blind design following 12-month follow-up time point. Subjects from both groups will be able to request additional injections of their originally assigned treatment as frequently[4] as needed, provided they did not experience any significant clinical concerns after previous treatment administrations and are benefiting from it, as determined by the investigator. During the long-term follow-up phase (12 – 60 months), subjects may also elect to cross over to the other treatment arm and receive additional injections of the other treatment as frequently as needed, provided they did not experience any significant clinical concerns after previous treatment administrations and are benefiting from it, as determined by the investigator. Subjects may only cross over from their originally assigned treatment group to the other treatment group one time during the study. Subjects seeking alternative invasive OA treatment in the index knee who do not wish to cross over or who have already crossed over will exit the study, and their selected treatment option will be recorded.

[4] Please refer to Synvisc One IFU for specified clinical use requirements.

Study Duration:	Maximum study duration per subject is 62 months: 60 months from treatment to last follow-up, and two additional months if the maximum visit window time is realized.
Inclusion Criteria:	1. Male or female at least 18 years of age at time of screening. 2. Willingness and ability to comply with study procedures and visit schedules and able to follow oral and written instructions. 3. A standing knee radiograph showing a K-L 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 Core 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 OA treatment (e.g. physical therapy, simple analgesics). 7. Signed an ethics committee-reviewed and approved informed consent form.
Exclusion Criteria:	1. Presence of clinically observed active infection or severe inflammation in the index knee joint or skin disease/breakdown or infection in the area of the planned injection site of the index knee. 2. Presence of symptomatic OA in the non-study knee at screening; 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; Human Immunodeficiency Virus (HIV), viral hepatitis; chondrocalcinosis, Paget's disease, or villonodular synovitis.

	4. Diagnosed with leukemia, known presence of metastatic malignant cells, or ongoing or planned chemotherapeutic treatment. 5. Disease of spine, hip or other lower extremity joints judged by the investigator to be contributing to the pain in the index knee (e.g. sciatica, nerve pain, hip OA). Note: Patients with contra-lateral knee replacement, 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. 6. 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). 7. Presence of surgical hardware or other foreign body intended to treat arthritis or cartilage-related pathology in the index knee. Note: this does not include small hardware (e.g. screws). 8. Presence of venous or lymphatic stasis in the index leg. 9. Orally administered systemic steroid use within 2 weeks prior to screening. 10. Planned/anticipated surgery of the index knee during the study period. 11. Major surgery (e.g. osteotomy) of the index knee within 12 months prior to screening. 12. Minor surgery (e.g. shaving or arthroscopy) of the index knee within 6 months prior to screening. 13. A history of local anesthetic allergy. 14. Use of systemic immunosuppressants within 6 weeks prior to screening. 15. 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[5]. 16. 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. 17. Pregnant or nursing mothers or women planning to become pregnant during the time they will be participating in the study. 18. Known hypersensitivity (allergy) to hyaluronan (sodium hyaluronate) preparations. 19. Previously documented failed treatment with nSTRIDE APS or Synvisc One. 20. Known drug or alcohol dependence currently or within the last year. 21. Use of any investigational drug or device within 30 days prior to screening. 22. Use of any investigational biologics 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) • 18 Month (± 28 days)

[5] Subjects taking low-doses of aspirin (maximum 100 mg/day) in **combination** with an ADP-receptor antagonist should be excluded from the study per exclusion criteria 15. A low doses of aspirin **alone** is not considered an exclusion criteria.

	• 24 Month (± 28 days) • 30 Month (± 28 days) • 36 Month (± 28 days) • 48 Month (± 28 days) • 60 Month (± 28 days) • Interim (when patient seeks additional intervention between regularly scheduled visits)
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>Numeric Rating Scale</u> The NRS is a validated measure of knee pain. The NRS is an 11 point Likert type scale anchored by 0 "no pain" and 10 "worst possible pain". Subjects rate their average pain over the last 48 hours. <u>Patient Preference Questionnaire</u> Patient preferences are concerned with determining patient-related factors such as patients' adherence to treatment, patients'

	satisfaction with treatment, and health outcomes. Patient preferences will be measured utilizing questions to elicit patients' preferences following their knee OA treatment. <u>Resource Utilization and Costing</u> Health economics will be evaluated throughout the study. Healthcare resource utilization for each subject will be recorded to capture information about healthcare costs following index treatment with nSTRIDE APS or HA and follow-up.
Imaging Assessment Tools:	<u>Radiographs</u> 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.
Primary Endpoint:	The primary endpoint will be the change in pain from baseline to 12 months following injection of nSTRIDE APS or HA, as measured by the WOMAC LK 3.1 pain subscale.
Primary Hypothesis for nSTRIDE Superiority Testing:	H_0: $\mu_{APS} = \mu_{HA}$ vs H_1: $\mu_{APS} \neq \mu_{HA}$ Where: H_0 is the Null Hypothesis The mean change in WOMAC Pain score from baseline to 12 months following an APS injection is equal to the mean change in WOMAC Pain score from baseline to 12 months following an HA injection. H_1 is the Alternative Hypothesis The mean change in WOMAC Pain score from baseline to 12 months following an APS injection is not equal to the mean change in WOMAC Pain score from baseline to 12 months following an HA injection. μ_{APS} is the mean change in WOMAC Pain from baseline to 12 months for nSTRIDE APS group.

	μ_{HA} is the mean change in WOMAC Pain from baseline to 12 months for HA group.
Secondary Endpoints	• The subjects showing a minimum clinically important difference (MCID) in the WOMAC Function subscale • The subjects showing a minimum clinically important difference (MCID) in the WOMAC Pain subscale • Change in function as measured by the WOMAC function scale (baseline to 12 months) • Percentage of subjects achieving clinical success as defined by OMERACT-OARSI Responder Criteria (1) and including restricted and rescue medication usage • Change in pain as measured by the WOMAC pain scale (baseline to 12 months) in KL-II subgroup • Change in function as measured by the WOMAC function scale (baseline to 12 months) in KL-II subgroup • The effects of rescue and restricted medication on changes in WOMAC pain (baseline to 12 months) • Change in pain as measured by the NRS pain scale (baseline to 12 months) • Change in WOMAC LK 3.1 Pain subscale over time
Exploratory Endpoints:	The following exploratory endpoints will be assessed at all post-injection time points: • Change in pain as measured by NRS pain scale • Percentage of subjects achieving clinical success as defined by OMERACT-OARSI Responder Criteria (1) • Change in WOMAC LK 3.1 pain (excluding 12-month time point), function and stiffness subscale scores • Change in overall WOMAC LK 3.1 score • Change in quality of life and global assessment as measured by the EQ-5D-3L and EQ-VAS • The effects of rescue and restricted medication on changes in WOMAC pain (baseline to 12 months)

	The following secondary endpoints will be assessed until the 24-month follow-up: • Joint Space Narrowing (JSN) • Correlation of JSN to change in NRS pain score • Relationship between JSN and OMERACT-OARSI status • Change in K-L grade The following endpoints will be assessed at all long-term follow-up time points (months 12 - 60): • Percentage of patients achieving treatment success from initial injection (i.e. patients who have not received additional injection, cross-over, or study exit for other invasive treatment and have clinically meaningful improvement in WOMAC Pain score[6]) • Percentage of patients achieving treatment success from originally assigned treatment (i.e. patients who have not crossed over or exited the study due to dissatisfaction with symptom relief and have clinically meaningful improvement in WOMAC Pain score) • Percentage of patients achieving success in each treatment arm (i.e. patients with clinically meaningful improvement in WOMAC Pain score, inclusive of originally assigned and cross-over patients) • Average number of cumulative interventions per patient • Time from the initial injection (nSTRIDE APS or HA) to subsequent injection(s), cross-over, or study exit for other invasive treatment • Resource utilization for index knee OA.

[6] Clinically meaningful improvement is considered to be an improvement of at least 20% from baseline

	The following endpoint will be assessed annually in the long-term follow-up phase for patients that have crossed over: • Percentage of patients preferring each treatment due to: 1. Greater pain relief 2. Longer lasting pain relief 3. Greater improvement in function 4. Longer lasting improvement in function 5. Fewer post-injection side effects
Safety Endpoints	• Occurrence of all AEs up to 12 month time-point and occurrence of AEs of interest across all post-operative time points

This Clinical Investigation Plan is written in accordance with ISO14155:2011 “Clinical Investigation of medical devices for human subjects – Good clinical practice” (40). The Adverse Events sections is written in accordance with the ISO14155:2020 “Clinical Investigation of medical devices for human subjects – Good clinical practice” (41).