nSTRIDE® Autologous Protein Solution Kit

Scientific Narrative
70% Improvement in Osteoarthritic Knee Pain at 2 years following a Single Injection

70, #
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Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease characterized by loss of cartilage and underlying bony changes.¹ Osteoarthritis of the knee affects the entire joint, including cartilage, ligaments, bone, and joint lining.² Driven by underlying inflammation, pain is the most common reason patients seek treatment for OA, which negatively impacts quality of life.³ ⁴ Osteoarthritis of the knee is 1 of 5 leading causes of disability among non-institutionalized adults in the United States.¹ The worldwide prevalence of radiographically confirmed symptomatic knee OA is 3.8% (with similar rates across Europe), with lifetime risk of symptomatic OA of the knee as high as 45% in the United States.⁶ ⁷ Standard of care has focused on palliative efforts designed to reduce pain, as well as address inflammation, increase mobility, and slow disease progression. Patients who have failed non-pharmacologic or simple analgesic treatments may receive intra-articular injections of hyaluronic acid (HA). A more recent investigational option is platelet-rich plasma (PRP), an injectable concentration of platelets and associated growth factors used to promote healing. However, currently available conservative and minimally invasive therapies fail to significantly delay or prevent disease progression, and surgical intervention to restore joint integrity and function may be required.⁸ ¹¹ Rates of total knee replacement are increasing worldwide, with more than 700,000 procedures performed in 2010 in the United States alone.¹² However, although patients with OA of the knee account for more than 90% of total knee replacement surgeries, only a minority of patients with disabling OA are willing to consider total knee replacement.¹³ There are currently no disease-modifying treatments approved for the management of OA, and there is a growing treatment gap for patients with moderate to severe OA who refuse or are not candidates for surgical intervention.¹⁰ ¹¹ This underscores the need for newer therapies that address the underlying disease process and delay or preclude the need for arthroplasty. As understanding of the underlying pathophysiology of OA at the molecular level grows, more targeted treatment options may meet this need. Notably, OA is associated with an increase in catabolic pro-inflammatory cytokines, which ultimately contribute to cartilage matrix breakdown.¹⁴ ¹⁷ Targeting this pro-inflammatory/anti-inflammatory imbalance may offer hope for patients with OA.

The nSTRIDE APS Kit produces an autologous protein solution (APS), which is designed for the treatment of knee OA and uses the patient’s own blood to concentrate anti-inflammatory cytokines and anabolic growth factors.¹⁶ ¹⁷ The point-of-care preparation is administered as an intra-articular injection, addressing the imbalance of cytokines in the joint by offering anti-inflammatory effects and healing at the site of injury, and potentially delaying or avoiding the need for surgery. The nSTRIDE APS Kit represents an opportunity for Zimmer Biomet to apply its established technology and unique expertise in autologous therapies to meet a pressing unmet medical need in OA of the knee and offer patients and their caregivers an effective option for the management of this degenerative disorder.
This narrative will review:

- The burden of OA of the knee
- How overall pathophysiology is founded on changes occurring at the molecular level
- Medical and surgical treatments currently used in OA of the knee, and their respective efficacy and limitations
- The scientific basis for targeted therapy in OA
- The role of the nSTRIDE APS Kit in providing an output that can be delivered point-of-care to decrease inflammation and promote cartilage health
The Burden of OA of the Knee

Osteoarthritis of the knee is a degenerative joint condition characterized by wearing down of the articular cartilage that provides cushioning at the ends of the femur and tibia. This tissue breakdown commonly leads to pain, swelling, and stiffness.\(^1\) Diagnosis may be based on clinical, radiographic, and/or pathologic criteria.\(^3\)

The global prevalence of radiographically confirmed symptomatic knee OA is estimated at 3.8%, ranging from 1.8% in males in Southeast Asia to 9.8% in females in Oceania.\(^6\) Prevalence of knee OA peaks around 50 years of age, with the United States and Europe reporting a prevalence of 14.1% and 22.8% in men and women ≥45 years of age, respectively.\(^18,19\) The lifetime risk of developing symptomatic knee OA has been reported to be as high as 45%. Patients who are overweight or obese and those with a history of joint injury are more likely to suffer from OA of the knee, with two-thirds of obese patients likely to develop symptomatic knee OA in their lifetime.\(^7\)

Osteoarthritis of the knee is 1 of 5 leading causes of disability among non-institutionalized adults in the United States. Adults with knee OA miss more than 13 days of work per year due to health problems. The annual direct and indirect cost of OA per patient is $5700 per year in the United States, with an estimated job-related cost of $3.4 to $13.2 billion per year.\(^1\) In Europe, the direct and indirect cost of OA per patient ranges from €1330 to €10 452, depending on the country and the treatment approach taken.\(^20\)

The Underlying Pathophysiology of OA of the Knee

The knee, the largest and strongest joint in the body,\(^21\) is the site of convergence of the femur, tibia, and patella. A healthy knee joint is cushioned by articular cartilage and the meniscus (Figure 1). In OA, articular cartilage wears away, becoming frayed and rough and decreasing the protective space between the bones. This bone-on-bone articulation results in pain and the formation of osteophytes. Common signs and symptoms of OA of the knee include inflammation, swelling, deformity, tenderness, crepitus (joint cracking or popping), and pain. Patients with OA of the knee may experience acute symptom flares and/or a chronic worsening of signs and symptoms over time.\(^3,21\)

![Figure 1. Anatomy of the Healthy and Osteoarthritic Knee](image)
Pain is the most common reason patients with OA seek treatment. The pain impacts quality of life, producing negative physiologic, immunologic, and psychological sequelae. Patients with musculoskeletal disorders report among the lowest health-related quality of life (HRQoL), with patients with OA of the knee reporting lower scores on every HRQoL parameter compared with age-matched norms, including physical, psychological, social, and cognitive functioning, as well as overall well-being.

Pain and inflammation are driven by a complex signaling cascade of cytokines and resulting cartilage matrix breakdown. Cells that promote inflammation are recruited to the site of insult, cytokine genes are up-regulated, and innervation is stimulated by nerve growth factor, thus compounding pain sensations.

Within cartilage remodeling, chondrocytes, the cellular component of cartilage, participate in catabolic (inducing cartilage matrix degradation) and anabolic (driving chondrocytes to increase cartilage matrix synthesis) activities. In healthy individuals, there is a steady-state equilibrium between pro-inflammatory/anti-inflammatory cytokines and anabolic growth factors. This maintains the structural and functional integrity of the cartilage extracellular matrix. When a degenerative imbalance develops, chondrocyte activity is disturbed and a net loss of cartilage matrix components results.

While both pro-inflammatory and anti-inflammatory cytokines are present in osteoarthritic joint tissue, it is the balance that ultimately determines the extent of cartilage damage (Figure 2). The pro-inflammatory cytokines over-represented in OA include interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-α (TNF-α). IL-1 and TNF-α are also catabolic and stimulate chondrocytes to produce metalloproteinases (MMPs), which degrade matrix collagen. A positive-feedback loop is created as collagen degradation increases IL-1 and TNF-α production, which stimulates additional MMP production (Figure 3).
Risk factor modification

Risk factor modification is an approach to management of patients with OA of the knee that represents the most conservative of options, which range from noninvasive to invasive (Figure 4). Risk factor modification measures include maintaining a healthy body weight by participating in low-impact physical activity and consuming a healthy diet, avoiding joint injury (eg, sports, work trauma), and addressing structural misalignment or muscle weakness. Some risk factors that contribute to OA development are not modifiable, however, including gender (females are at higher risk), age (OA is more prevalent in older populations), and race (OA is less prevalent among some Asian populations). In addition, some individuals have a higher genetic predisposition to developing OA.1,23

Currently Available Treatment Options for OA of the Knee

Risk Factor Modification
Weight management
Exercise

Inflammation and Pain Reduction
Nonsteroidal anti-inflammatory drugs
Topical agents
Hyaluronic acid
Experimental autologous therapies

Surgical Intervention
Cartilage repair techniques
Osteotomy
Joint replacement

Figure 3. OA Pain and Inflammation-Signaling Cascade

Figure 4. Overview of Treatment Options for OA of the Knee
Topical and oral pain relievers

Palliative care that addresses pain and inflammation is a mainstay of OA treatment. These interventions are designed to reduce pain and inflammation and increase mobility. Pain relievers such as acetaminophen may address mild pain but likely do not have an impact on inflammation or swelling. Nonsteroidal anti-inflammatories (NSAIDs) have also been shown to be effective in OA pain relief; however, they are associated with risk of stomach bleeding. In addition, some COX-2 inhibitors, a form of NSAID, were taken off the market due to risk of heart attack. For most patients, pharmacologic intervention alone is not sufficient, and a multimodal approach, including physical therapy, should be considered.

Intra-articular injections

Intra-articular injections of corticosteroids ease pain and stiffness by decreasing inflammation. However, the duration of the effect is variable, generally 3 months, and over time and with repeated injections, soft tissue damage may occur, potentially accelerating the course of the disease. In addition, a small subset of patients may experience a cortisone flare reaction or may develop Cushing syndrome.

For patients whose pain is not controlled with standard palliative agents, intra-articular injection with hyaluronic acid (HA) is available. In healthy subjects, hyaluronan is produced by chondrocytes and synovial cells within the joint and acts as a lubricant and shock absorber. HA may be injected directly into the fluid of the knee joint and is thought to improve the lubricating properties of the synovial fluid, reduce pain, and improve mobility.

Hyaluronic acid is approved by the US Food and Drug Administration for the treatment of pain associated with OA of the knee. The global market for HA is expected to reach nearly $2.5 billion by 2017, with the majority of use focused on knee joints. Clinical trial outcomes have been contradictory. A recent study of 588 subjects reported only a modest difference in pain reduction from baseline using HA injections compared with intra-articular injections of saline (53% vs 38%). Given these modest outcomes, the American Academy of Orthopaedic Surgeons (AAOS) does not recommend the use of HA for patients with symptomatic OA of the knee. In addition, the Osteoarthritis Research Society International (OARSI) guidelines, citing inconsistent conclusions and conflicting results, categorize HA as “uncertain” for knee-only OA.

As described above, modifying risk factors, over-the-counter and prescription pain relievers, and intra-articular injections have not proven effective for a large percentage of patients, with many achieving only short-term relief from pain. In an effort to address the need for more successful therapies, specific molecules in the pain and inflammation cascade have been explored as therapeutic targets.
Autologous cellular therapies
Autologous cellular therapies are at the forefront of regenerative medicine. Such treatment options include blood and bone marrow aspirate and concentration, dehydrated human amnion/chorion, PRP, and nSTRIDE APS. Although none of these products has been approved for the treatment of OA of the knee in the US, many are currently under development.

Platelet-rich plasma
Whole blood comprises several cell types suspended in a liquid component. Access to these cells may represent a critical step in targeting the drivers of inflammation and cartilage breakdown in OA. When centrifuged, whole blood can be separated into 3 layers: plasma (growth factors, sugars, clotting factors, immunoglobulins), buffy coat (leukocytes [white blood cells] and platelets), and erythrocytes (red blood cells) (Figure 5).

Platelets, found in the buffy coat, play a significant role in tissue repair. In healthy patients, tissue injury prompts activation of the wound-healing cascade, which triggers inflammation, leading to aggregation of platelets at the site of injury. These platelets release growth factors that promote healing, including:

- Transforming growth factor beta (TGF-β)
- Platelet-derived growth factor (PDGF)
- Basic fibroblast growth factor (bFGF)
- Epidermal growth factor (EGF)
- Vascular endothelial growth factor (VEGF)

It has been hypothesized that concentrating and activating platelets and returning them to the patient at the site of insult may promote healing, reduce pain, improve function, limit inflammation, and stimulate cartilage regeneration. Platelet-rich plasma is blood plasma that has been enriched with platelets. To prepare PRP, whole blood is removed from the patient and centrifuged until the plasma layer is approximately 10% the volume of the original sample. Depending on the centrifugation process, PRP products may concentrate platelets to 3 to 9 times baseline concentration. The preparation is immediately injected into the joint under aseptic conditions, often using ultrasound guidance. PRP is contraindicated in patients with thrombocytopenia or other platelet dysfunction.

Although PRP has been used successfully in other disease states (eg, autologous bone grafting, lateral epicondylitis, tendinopathy), varying clinical outcomes have been reported in OA. Some studies report significantly better pain reduction, higher Knee Society Scores (KSS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores, higher response rates, and improved efficacy compared with HA. However, other studies report no difference between PRP and HA. The AAOS does not recommend for or against the use of PRP for patients with symptomatic OA of the knee, and the OARSI guidelines do not include mention of PRP. No PRP is approved or cleared by the US Food and Drug Administration to treat OA.
Surgical intervention

While the aforementioned conservative and minimally invasive therapies offer hope to some patients, these treatments often fail to significantly delay or prevent disease progression, and costly surgical intervention is required. Surgical interventions may range from cartilage repair techniques to partial or complete joint replacement. Each method has a distinct risk-benefit profile that must be considered in light of each patient’s needs.

Cartilage repair techniques aim to restore the articular surface. Cartilage repair represents a unique challenge, however, as articular cartilage has a poor healing capacity, and the moving joint presents a hostile healing environment. A number of reconstructive techniques have been examined, including abrasion chondroplasty, microfracture, osteochondral autograft transplant surgery (OATS) or osteochondral allograft transplant surgery (OALT), and autologous chondrocyte implantation (ACI) (Table 1).

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<tr>
<th>Technique</th>
<th>Description and Details</th>
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<tr>
<td>Abrasion</td>
<td>Utilizes arthroscopic debridement</td>
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<td>Chondroplasty</td>
<td>Typically reserved for very small lesions</td>
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<td>Microfracture</td>
<td>Stimulates the underlying bone marrow</td>
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<td></td>
<td>Lesion is scraped and a tapered awl is used to produce microfractures, which result in formation of blood clots that contain mesenchymal stem cells, which induce repair</td>
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<tr>
<td></td>
<td>Associated with short-term functional improvement; however, long-term functional deterioration has been observed</td>
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<td>OATS</td>
<td>Multiple plugs of hyaline cartilage and underlying subchondral bone are removed from an unaffected, non–weight bearing area of the knee and used as implants at the site of defect</td>
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<tr>
<td></td>
<td>OATS is an autograft and therefore live cells are preserved</td>
</tr>
<tr>
<td></td>
<td>Topography of the donor site does not match that of the recipient site, which may affect biomechanics</td>
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<tr>
<td></td>
<td>Clinical studies report conflicting results, with some demonstrating positive long-term follow-up and others indicating that OATS is inferior to other surgical procedures</td>
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<tr>
<td>OALT</td>
<td>Similar to OATS, but utilizes tissue from a cadaver rather than tissue from the patient</td>
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<tr>
<td></td>
<td>OALT is an allograft with donor tissue and therefore lacks living bone cells</td>
</tr>
<tr>
<td></td>
<td>Improves topography and biomechanics, as donor and recipient sites can be matched</td>
</tr>
<tr>
<td></td>
<td>Use of cadaver tissue associated with risk of rejection and viral transmission, as well as the potential for limited tissue availability</td>
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<tr>
<td>ACI</td>
<td>A 2-step procedure involving 1) arthroscopy of healthy articular cartilage and culture of the resulting chondrocytes; 2) debridement of the lesion, coverage by a periosteal flap, and implantation of the new cells into the defect</td>
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<td>While some clinical studies have demonstrated superiority to abrasion and microfracture techniques, others have shown no longer-term difference</td>
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Table 1. Cartilage Repair Techniques for OA of the Knee
The OARSI guidelines recommend that patients with knee OA who do not achieve adequate pain relief and functional improvement from nonpharmacologic and pharmacologic treatment should be considered for joint replacement surgery. Several surgical options are available to patients, including high tibial osteotomy (HTO), unicompartmental knee arthroplasty (UKA), and total knee arthroplasty (TKA) (Figure 6). The goal of surgical treatment is to reduce pain, restore function, and improve quality of life.

High tibial osteotomy is an invasive procedure for patients with moderate unicompartmental knee OA that requires significant bone reshaping to transfer the load away from the diseased medial compartment and toward the unaffected lateral compartment. Osteotomy means “cutting of the bone.” During HTO, the tibia (shin bone) is cut and reshaped to relieve pressure on the knee joint. The aim is to slow medial disease progression and delay the need for UKA or TKA. HTO is associated with the potential for significant complications and prolonged rehabilitation, with patient outcomes deteriorating over time due to disease progression. HTO is typically reserved for young (18-44 years), active patients with a life expectancy exceeding the expected survival of a knee prosthesis. Older age (≥60 years) is considered a contraindication to HTO and a strong predictor of poor prognosis. Unicompartmental knee arthroplasty is indicated for patients with OA restricted to 1 knee compartment. Ideally, the patient presents with a low body mass index (BMI), a correctable deformity, and no fixed flexion contractures and is willing to avoid strenuous physical activity. During UKA, only the damaged compartment is replaced with synthetic material. UKA is associated with fewer complications than HTO and is less invasive than TKA. Compared with TKA, however, pain relief tends to be less predictable and there is the potential for additional surgery if OA progresses to the other compartment.

In addition, the anabolic growth factors insulin-like growth factor (IGF-1) and TGF-β1 are also concentrated to levels in excess of those found in native whole blood. These anti-inflammatory cytokines and anabolic growth factors work to correct the imbalance in OA. They simultaneously inhibit IL-1 and TNF-α, catabolic pro-inflammatory targets, and stimulate cartilage matrix synthesis. Lastly, hepatocyte growth factor (HGF) is known to protect tissue from inflammatory damage. HGF inhibits transcription and translation of pro-inflammatory genes and proteins, resulting in reduced pain and inflammation.

The unique composition of APS, produced using the nSTRIDE APS Kit, addresses the cytokine and growth factor imbalance observed in OA and offers patients the potential to modify the course of their disease, reduce pain and inflammation and promote cartilage health.
The standard of care in the surgical management of end-stage multicompartment knee OA, and some unicompartmental cases, is total knee replacement (TKA). During TKA, diseased bone and cartilage are removed and replaced with an artificial joint made of synthetic materials. TKA has been shown to restore joint function and improve HRQoL. TKA is not typically recommended for patients under 65 years, since younger, more active patients are at higher risk for needing future revision surgery. Unfortunately, patient expectations do not always align with outcomes. Whereas 85% of patients state that they expect to be pain free after TKA, only 43% report complete absence of pain after surgery.\(^4\)

Rates of total knee replacement are increasing worldwide, with more than 700,000 procedures performed in 2010 in the United States alone.\(^1\) The Swedish Knee Arthroplasty Registry reported that total knee replacement rates doubled between 2000 and 2010. While direct comparisons of total knee replacement rates between countries are limited owing to lack of data, the 2007-2009 estimated rates of primary knee replacement for all diagnoses per 100,000 people varied between 9 in Romania, 98 in France, 188 in Germany, and 213 in the United States. Interestingly, although patients with OA of the knee account for more than 90% of total knee replacement surgeries in the United States, only a minority of patients with disabling OA are willing to consider total knee replacement.\(^1\)

In the United States, it is estimated that each total knee replacement procedure costs $20,000. This results in an annual cost of $13 billion.\(^1\) In view of the limited life span of prosthetic implants, the progressive nature of OA, the aging population, and the increasing rates of obesity, the rate of revision surgery is expected to rise, in conjunction with an increase in associated expense and morbidity.\(^4\)

Given the limitations of currently available treatment modalities, there is a need for new therapies that provide long-term pain relief, address the underlying disease process, and delay or preclude the need for arthroplasty.

**Targeting OA of the Knee With the nSTRIDE APS Kit**

As described above, there are no disease-modifying treatments available for the management of OA, and currently available agents and modalities may lack long-term efficacy, can be costly and invasive, and often fail to meet patient expectations. Targeted therapy that addresses the catabolic pro-inflammatory/anti-inflammatory cytokine and anabolic growth factor imbalance in OA offers hope to patients as it may provide pain relief and may promote cartilage health.

The nSTRIDE APS Kit is a cell-concentration system designed to isolate anti-inflammatory cytokines and anabolic growth factors from whole blood so that they can be reintroduced to the body at the site of insult. Compared with PRP, APS provides a higher concentration of white blood cells and plasma proteins (Figure 7).
Whereas PRP formulations contain high concentrations of all platelet growth factors, APS is enriched for specific growth factors and cytokines that inhibit multiple inflammatory signaling pathways, thereby addressing the pro-inflammatory/anti-inflammatory imbalance observed in OA and promoting cartilage health (Figures 8 and 9).
The inclusion of a white blood cell fraction in PRP products has been the topic of some debate. White blood cells are known to produce pro-inflammatory cytokines, and some researchers have hypothesized that the presence of leukocytes in a PRP preparation would therefore increase the inflammatory response. However, white blood cells are also responsible for the production of anti-inflammatory antagonists. The presence of a higher white blood cell concentration in APS allows for enrichment of IL-1 receptor antagonist (IL-1ra) and soluble forms of TNF-α cell receptors (sTNF-RI, sTNF-RII), 2 cytokines critical to the anti-inflammatory and pain-reducing effect of APS.

Of particular interest are the anti-inflammatory cytokines, each present in APS in a concentration well above that found in native whole blood (Table 2). Each of these cytokines inhibits a different inflammatory signaling pathway, ultimately correcting the imbalance and contributing to the restoration of collagen matrix stability.

| Interleukin-1 receptor antagonist (IL-1ra) | Binds IL-1 receptors, blocking the signaling activity of IL-1 and IL-1ra has no known agonist function itself |
| Soluble forms of the IL-1 cell receptor (sIL-1R) | Binds IL-1, preventing it from binding a surface receptor that would lead to cell signaling |
| Soluble forms of TNF-α cell receptors (sTNF-RI, sTNF-RII) | Binds TNF-α, preventing it from binding a surface receptor that would lead to cell signaling |

Table 2. Role of IL-1ra, sIL-1R, and sTNF-Rs in Preventing Tissue Destruction

Figure 9. nSTRIDE APS Addresses Cytokine Imbalance in OA
In addition, the anabolic growth factors insulin-like growth factor (IGF-1) and TGF-β1 are also concentrated to levels in excess of those found in native whole blood. These anti-inflammatory cytokines and anabolic growth factors work to correct the imbalance in OA. They simultaneously inhibit IL-1 and TNF-α, catabolic pro-inflammatory targets, and stimulate cartilage matrix synthesis.15

Lastly, hepatocyte growth factor (HGF) is known to protect tissue from inflammatory damage. HGF inhibits transcription and translation of pro-inflammatory genes and proteins, resulting in reduced pain and inflammation.56,57

The unique composition of APS, produced using the nSTRIDE APS Kit, addresses the cytokine and growth factor imbalance observed in OA and offers patients the potential to modify the course of their disease, reduce pain and inflammation and promote cartilage health.
nSTRIDE® Scientific Narrative

Preclinical studies
The nSTRIDE APS Kit has been widely studied in preclinical trials, animal models, and human studies. In preclinical studies, APS reduced pro-inflammatory cytokines up-regulated in OA. When incubated with macrophages stimulated with IL-1ß, APS decreased the effect of IL-1ß and limited the expression of the pro-inflammatory cytokines IL-8 and TNF-α. In a second study, APS reduced production of enzymes involved in cartilage degradation. APS was observed to inhibit IL-1ß– and TNF-α–induced chondrocyte production of MMP-13, a known cartilage degradation enzyme. And finally, APS demonstrated a chondroprotective effect by reducing glycosaminoglycan release in bovine cartilage explant cultures stimulated with IL-1 and stimulating chondrocyte proliferation in the explant culture model. Notably, APS significantly outperformed PRP in a chondrocyte cell assay.

Animal studies
The nSTRIDE APS Kit has been studied in rats, horses, and dogs. In an athymic rat model, intra-articular injection of APS was not associated with any toxic effects. In addition, an athymic rat meniscal tear study was performed to examine the disease-modifying potential of APS. Transection of the meniscus at the narrowest point followed by 1 week of load-bearing creates a well-characterized degenerative model that can be categorized histologically into zones of mild, moderate, and severe OA. Compared to a single intra-articular injection of saline, a single injection of APS statistically improved cartilage degradation histological scores in the medium OA zone as well as in the overall total joint score. This study demonstrated that treatment with APS is significantly beneficial in rat meniscal tear-induced OA as determined by evaluation of knee histopathology.

In a randomized, observer-blinded study of 40 client-owned horses with naturally occurring OA, subjects were randomized 1:1 to receive injection with either nSTRIDE APS or saline control. The primary endpoint of the study was blinded lameness evaluation at 2 weeks post-treatment. Secondary efficacy endpoints included force plate analysis at 2 weeks and 3 month and 12 month owner surveys. Safety was evaluated by blood hematology and chemistry, joint inflammation evaluations, and 3 month and 12 month owner surveys.
nSTRIDE APS significantly improved lameness at 1 and 2 weeks (Figure 10). The nSTRIDE APS group had a significantly greater number of horses with sound gait or improved gait at weeks 1 and 2 compared with the control group. The range of joint motion without signs of pain and the signs-of-pain score on flexion in the nSTRIDE APS-treated group were significantly improved at days 4 to 14, both compared with baseline and compared with the control group. Conversely, the range of joint motion and the signs-of-pain score on flexion in the control group were significantly worse on days 4 to 14 compared with baseline.\textsuperscript{48}

Client-evaluated subjective grades of lameness, comfort at rest in the stall, and comfort at turnout were all significantly improved at 12 week and 52 week follow-up with nSTRIDE APS treatment (Figure 11). No adverse events associated with APS injection were reported.\textsuperscript{48}

A prospective, multipractice, evaluator-blinded study randomized 19 dogs with stifle or elbow OA to receive a single injection of nSTRIDE APS or saline. Baseline demographics were similar between the treatment and control groups. Dogs treated with nSTRIDE APS experienced a significant decrease in pain by week 12 (compared with baseline), as measured by the Canine Brief Pain Inventory (CBPI) and the Hudson visual analogue scale (HVAS; both completed by dog owners). Overall, nSTRIDE APS-treated dogs improved in more subcategories of the CBPI and HVAS at week 12 compared with control dogs. While no significant differences in gait velocity were reported between groups or across time, a significant improvement in lameness was noted in nSTRIDE APS-treated dogs at week 12.\textsuperscript{63}
Human studies

APS-Affected Patient Study

The APS-Affected Patient Study included 105 human subjects with radiographic OA. It was designed to determine if nSTRIDE APS could be successfully prepared across a broad population of OA patients. nSTRIDE APS was prepared from each patient and analyzed. Patient metrics were collected, including demographic information, medical history, medication records, and Knee Injury and Osteoarthritis Outcome Score (KOOS) surveys. The KOOS knee survey is a patient-administered questionnaire that assesses symptoms (eg, swelling, grinding), stiffness, pain, function, and quality of life over the past week. Cytokine and growth factor concentrations in whole blood and APS were measured using enzyme-linked immunosorbent assay. Anti-inflammatory cytokines were preferentially increased compared with pro-inflammatory cytokines in APS from 98% of patients. APS contained high concentrations of IL-1ra, sIL-1RII, sTNF-RI, and sTNF-RII. Analysis of 82 patient metrics indicated that no single patient metric was strongly correlated with cytokine concentration in APS. Therefore, the authors concluded that APS can be prepared from a broad population of OA patients.

APSS-11-00

APSS-11-00 was the first in-human trial with nSTRIDE APS. This open-label, feasibility study enrolled 11 patients with moderate to marked OA of the knee, who were followed for 6 months. Endpoints included scores on the WOMAC, clinical global impression (CGI), and patient global impression (PGI) scales, as well as safety, knee examination, and cytokine analysis. Among enrolled patients, the mean age was 57.5 years and the mean BMI was 26.6 kg/m². In all, 26 adverse events were reported, with no adverse events related to the treatment. Of these events, 23 were classified as mild, and 2 required treatment (oral pain medication). There were 2 adverse events related to and 8 possibly related to the procedure. No serious adverse events were observed. Efficacy outcomes reported a 72% reduction in WOMAC pain at 6 months (n=10) and 89% reduction in 73% (8/11) who were Outcomes Measures in Rheumatology-Osteoarthritis Research Society International (OMERACT-OARSI) high responders. Significant improvement in WOMAC pain, stiffness, and function subscales was demonstrated with nSTRIDE APS treatment at every time point studied (Figure 12).
The OMERACT-OARSI responder criteria for OA clinical trials is based on a combination of absolute and relative change of pain, function, and global patient assessment. The OMERACT-OARSI high responder criteria were applied, and 8 of 11 subjects were responders at 12 and 26 weeks post-treatment. Clinicians and subjects were asked to grade improvement on a 7-point scale ranging from very much worse to very much improved. The CGI indicated more than 50% of patients were much improved or very much improved by week 2 and 80% of patients were much improved or very much improved by week 26. The PGI was consistent with clinician reports, with more than 50% and 80% of patients reporting much improved or very much improved by weeks 2 and 26, respectively.

A secondary analysis was performed to identify characteristics of APS that may correlate with improved WOMAC pain scores or OMERACT-OARSI responder status after treatment with an intra-articular injection of APS. White blood cell (WBC) and cytokine concentrations were measured from the APS. Linear regression analyses were performed on the blood components of APS with subject outcomes. The WBC concentration in APS was significantly ($p<0.05$) and strongly ($R^2 > 0.7$) correlated with IL-1ra in APS but not significantly correlated with IL-1β. The ratio of IL-1ra to IL-1β in APS was significantly correlated with improved WOMAC pain scores 1 week and 6 months post-injection. The correlations between the IL-1ra:IL-1β ratio and WBC concentration in a subject’s APS and their WOMAC pain scores provides initial confirmation of the mechanism of action of APS (Figure 13). The outcomes of APSS-11-00 suggest that nSTRIDE APS is effective in a substantial percentage of the target population, yielding significant improvements in pain, stiffness, and function, and with subjects reporting substantial improvements in their condition. Treatment with nSTRIDE APS demonstrated a favorable safety profile and was well tolerated.

Figure 12. WOMAC Pain Subscale

\[\text{Figure 12. WOMAC Pain Subscale}\]
Long-term follow-up was conducted after the initial study period. The mean WOMAC pain score was 11.8 ± 1.5 at baseline and 4.2 ± 3.3 at the 18-month time point (n=6). This corresponded to a 64.4% improvement in knee pain. The mean WOMAC stiffness and function scores also had significant improvements of 58.3% (p=0.03) and 61.0% (p<0.01), respectively. Two subjects rated their knee OA condition as “Very Much Improved” and 4 subjects rated it as “Much Improved” compared to baseline status. Finally, 5 of 6 subjects met the OMERACT-OARSI high pain responder status 18 months post-treatment.

**PROGRESS I: APSS-22-00**

Ten patients with unilateral knee OA were enrolled in this prospective, single-site, open-label pilot study to evaluate safety and effectiveness of the nSTRIDE APS Kit, used to prepare APS from a sample of the patient’s blood. The single site has obtained institutional review board approval, enrollment is complete, and the 12-month follow-up is ongoing. Clinical outcome measures include the KOOS, numeric rating scale pain assessment, patient global assessment (PGA), CGI, and WOMAC Index. There have been 23 adverse events and 1 serious adverse event (not related to the procedure or the device) reported in enrolled subjects, with 15 unanticipated adverse events and 1 unanticipated serious adverse event. There have been no unanticipated adverse device effects. To date, the WOMAC pain score and WOMAC function score have both decreased compared to baseline measurements.

**PROGRESS II: APSS-33-00**

In this study, 46 patients with unilateral OA (Kellgren-Lawrence 2 or 3) knee pain were randomized into 2 groups at 3 institutions. Group 1 (31 patients) received a single ultrasound-guided injection of nSTRIDE APS, and Group 2 (15 patients) received a single saline injection. Patient-reported outcomes and adverse events at 2 weeks, 1, 3, 6, and
12 months post-injection were collected. The patients and evaluators were blinded to the treatment allocation, and the outcome was evaluated through visual analogue scale (VAS), WOMAC, and KOOS scores. Imaging evaluation was also performed with X-ray and magnetic resonance imaging before and after the treatment (12 months and 3-12 months, respectively). The demographics were similar between the groups. The average change from baseline to 12 months in WOMAC pain score was 65% in Group 1 and 41% in Group 2 (\( p = 0.02 \)) (Figure 14). Additionally, average VAS pain improvement was 49% in Group 1 and 13% in Group 2 (\( p = 0.06 \)). Average WOMAC function change from baseline to 12 months was 57% in Group 1 and 44% in Group 2 (\( p = 0.24 \)). 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 APS. Long-term follow-up is ongoing, and these positive results obtained against saline have been used to plan a confirmatory trial that will be conducted to further substantiate these findings against those offered by other treatments for knee OA.\(^{68}\)

\[\text{Figure 14. Percentage Change from Baseline in WOMAC Pain Score}^{68}\]
The nSTRIDE APS Kit in Everyday Use

Step-by-step instructions for processing the nSTRIDE APS Kit are presented in Figure 15. The nSTRIDE APS Kit with ACD-A is a self-contained, single-use device, sterile packaged for use at point of care. The nSTRIDE APS Kit comprises 2 multicompartmental plastic tubes. The first tube, the nSTRIDE Cell Separator, contains a tuned-density buoy, which sequesters white blood cells and platelets in a small fraction of plasma. The second tube, the nSTRIDE Concentrator, contains polyacrylamide beads, which desiccate the product via filtration. The nSTRIDE APS Kit processes the patient’s own blood. It is autologous, not a synthetic therapy, and does not require cell culture. Once prepared, the final APS output is administered via intra-articular injection (Figure 16).

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Figure 15. nSTRIDE APS Kit Preparation Process

- Load nSTRIDE Cell Separator
- Centrifuge
- Prepare Cell Solution
- Load nSTRIDE Concentrator
- Centrifuge
- Extract APS

Figure 16. Intra-articular Injection of nSTRIDE APS
Summary

Osteoarthritis of the knee is a degenerative joint disease characterized by inflammation and loss of cartilage matrix. Numerous nonpharmacologic, pharmacologic, and surgical options are available for patient management, but they have been associated with a lack of long-term efficacy, risk of complication, significant recovery time, high cost, and failure to meet patient expectations. As described earlier, there are currently no disease-modifying treatments available for the management of OA, and there is a growing need for treatment that addresses pain and inflammation at its source while also promoting cartilage health.

Osteoarthritis is associated with an increase in catabolic pro-inflammatory cytokines, which contributes to cartilage matrix breakdown. The nSTRIDE APS Kit aims to target this imbalance by using the patient’s own blood to concentrate anti-inflammatory cytokines and anabolic growth factors, returning them to the site of insult. The point-of-care preparation is administered as an intra-articular injection, offering anti-inflammatory effects, pain reduction, and promoting cartilage health. The nSTRIDE APS Kit is an attractive therapeutic option for patients, surgeons, and the healthcare system as it is effectively designed to deliver an APS output that targets the source of OA symptoms and promotes cartilage health, potentially delaying costly and painful surgery. The nSTRIDE APS Kit, through ongoing and future clinical studies, may also demonstrate disease-process modification, potentially leading to longer and improved pain relief for patients who have failed conventional conservative therapies.
References


As measured by WOMAC pain scores reported by patients continuing follow-up through 2 years (n = 22)
Results may vary. Not all patients are candidates for this product and/or procedure. Zimmer Biomet does not practice medicine. The treating surgeon is responsible for determining the appropriate treatment, techniques(s), and product(s) for each individual patient.

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